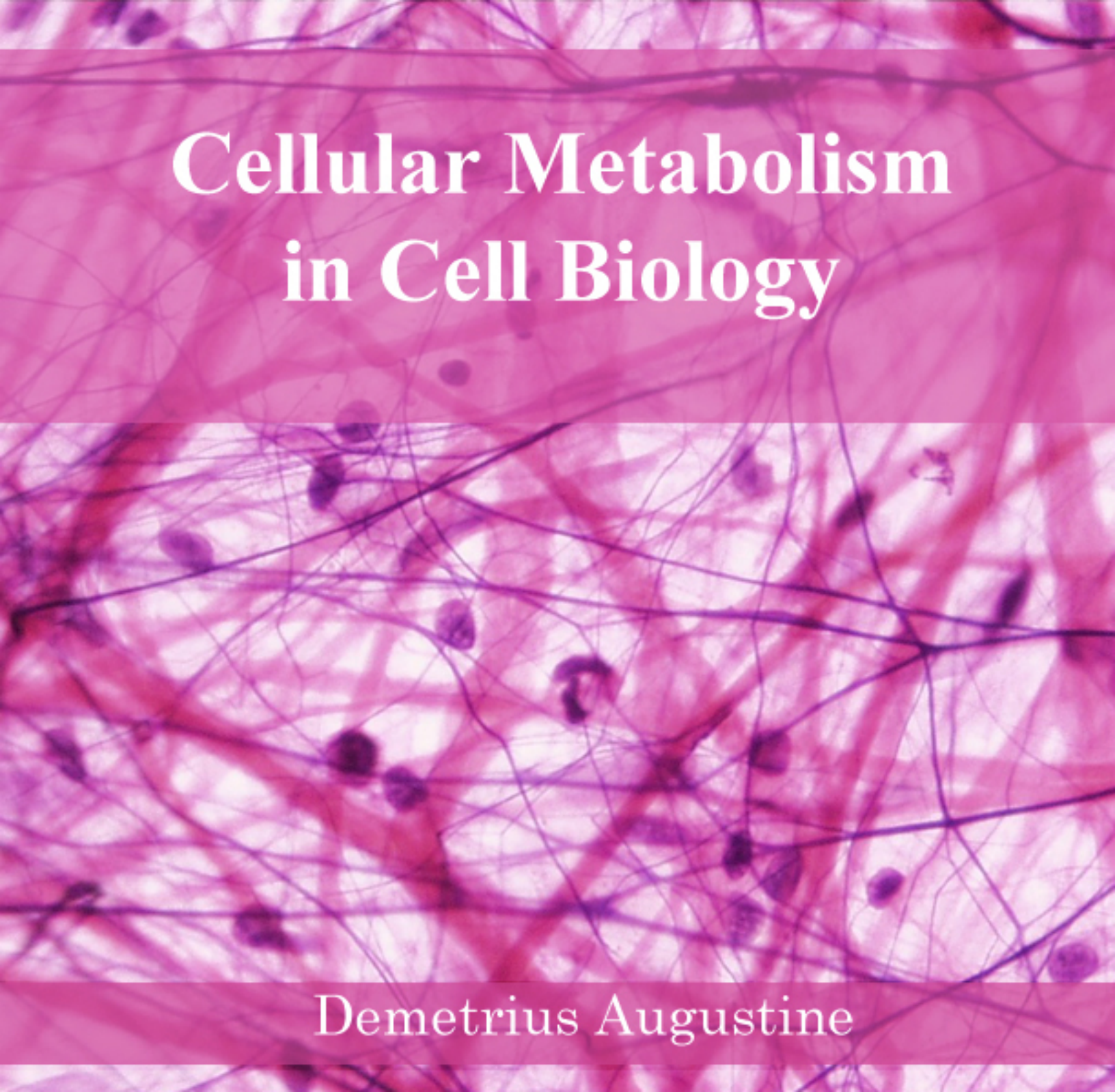
Cellular Metabolism in Cell Biology



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Metabolic Pathway

In biochemistry, **metabolic pathways** are series of chemical reactions occurring within a cell. In each pathway, a principal chemical is modified by a series of chemical reactions. Enzymes catalyze these reactions, and often require dietary minerals, vitamins, and other cofactors in order to function properly. Because of the many chemicals (a.k.a. "metabolites") that may be involved, metabolic pathways can be quite elaborate. In addition, numerous distinct pathways co-exist within a cell. This collection of pathways is called the metabolic network. Pathways are important to the maintenance of homeostasis within an organism. Catabolic (break-down) and Anabolic (synthesis) pathways often work interdependently to create new biomolecules as the final end-products.

A metabolic pathway involves the step-by-step modification of an initial molecule to form another product. The resulting product can be used in one of three ways:

- To be used immediately, as the end-product of a metabolic pathway
- To initiate another metabolic pathway, called a flux generating step
- To be stored by the cell

A molecule called a substrate enters a metabolic pathway depending on the needs of the cell and the availability of the substrate. An increase in concentration of anabolic and catabolic intermediates and/or end-products may influence the metabolic rate for that particular pathway.

Overview

Each metabolic pathway consists of a series of biochemical reactions that are connected by their intermediates: the products of one reaction are the substrates for subsequent reactions, and so on. Metabolic pathways are often considered to flow in one direction. Although all chemical reactions are technically reversible, conditions in the cell are often such that it is thermodynamically more favorable for flux to flow in one direction of a

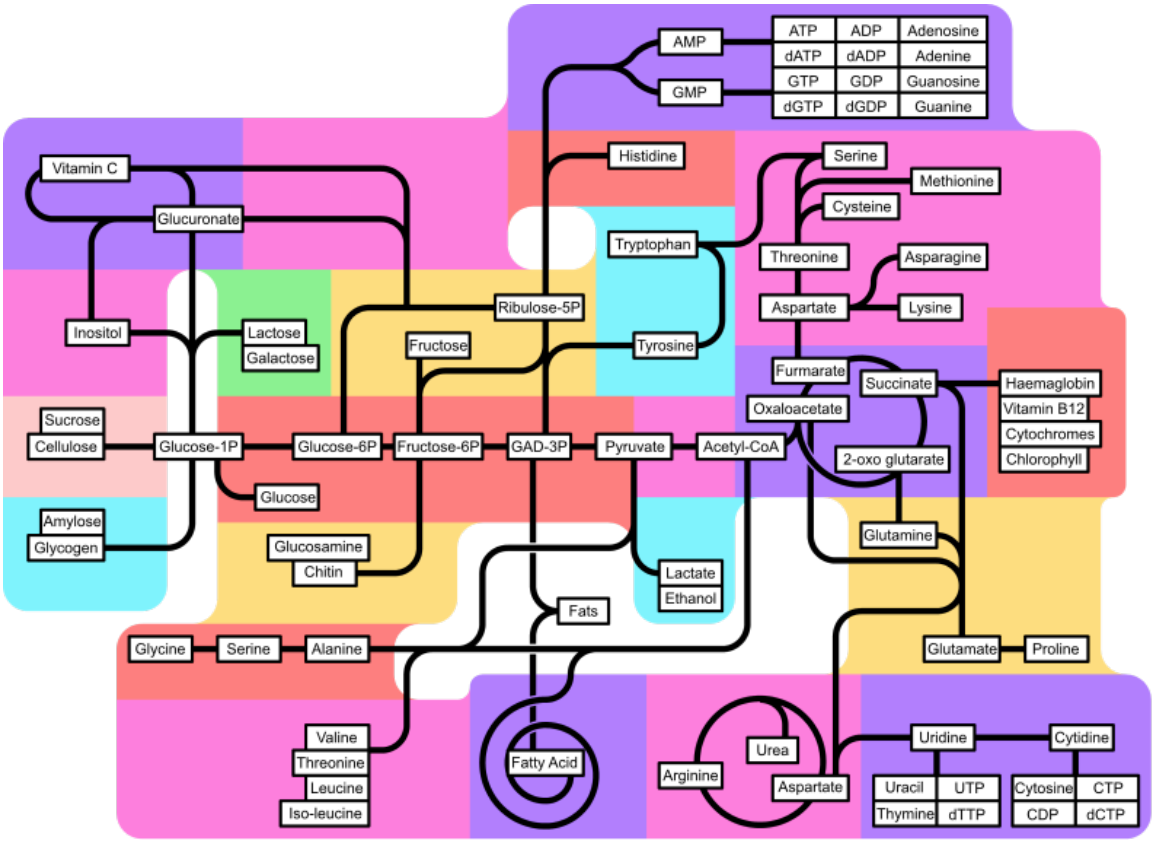
reaction. For example, one pathway may be responsible for the synthesis of a particular amino acid, but the breakdown of that amino acid may occur via a separate and distinct pathway. One example of an exception to this "rule" is the metabolism of glucose. Glycolysis results in the breakdown of glucose, but several reactions in the glycolysis pathway are reversible and participate in the re-synthesis of glucose (gluconeogenesis).

- Glycolysis was the first metabolic pathway discovered:

1. As glucose enters a cell, it is immediately phosphorylated by ATP to glucose 6-phosphate in the irreversible first step.
2. In times of excess lipid or protein energy sources, certain reactions in the glycolysis pathway may run in reverse in order to produce glucose 6-phosphate which is then used for storage as glycogen or starch.

- Metabolic pathways are often regulated by feedback inhibition.
- Some metabolic pathways flow in a 'cycle' wherein each component of the cycle is a substrate for the subsequent reaction in the cycle, such as in the Krebs Cycle.
- Anabolic and catabolic pathways in eukaryotes often occur independently of each other, separated either physically by compartmentalization within organelles or separated biochemically by the requirement of different enzymes and co-factors.

Major metabolic pathways



Cellular respiration

Several distinct but linked metabolic pathways are used by cells to transfer the energy released by breakdown of fuel molecules into ATP and other small molecules used for energy (e.g. GTP, NADPH, FADH).

These pathways occur within all living organisms in some form:

1. Glycolysis
2. Aerobic respiration and/or Anaerobic respiration
3. Citric acid cycle / Krebs cycle
4. Oxidative phosphorylation

Other pathways occurring in (most or) all living organisms include (but are not limited to):

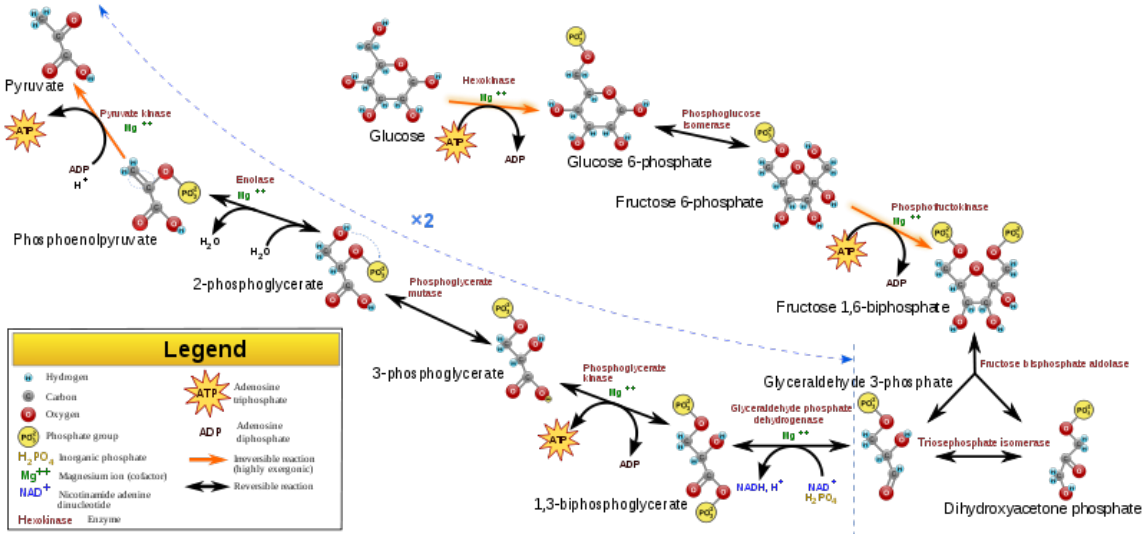
- Fatty acid oxidation (β-oxidation)
- Gluconeogenesis
- Amino acid metabolism
- Urea cycle / Nitrogen metabolism
- Nucleotide metabolism
- Glycogen synthesis / Glycogen storage
- Pentose phosphate pathway (hexose monophosphate shunt)
- Porphyrin synthesis (or heme synthesis) pathway
- Lipogenesis
- HMG-CoA reductase pathway

Synthesis of energetic compounds from non-living matter:

- Photosynthesis (plants, algae, cyanobacteria)
- Chemosynthesis (some bacteria)

Chapter- 2

Glycolysis



Glycolysis overview

Glycolysis (from *glycose*, an older term for glucose + *-lysis* degradation) is the metabolic pathway that converts glucose $C_6H_{12}O_6$, into pyruvate, $CH_3COCOO^- + H^+$. The free energy released in this process is used to form the high-energy compounds ATP (adenosine triphosphate) and NADH (reduced nicotinamide adenine dinucleotide).

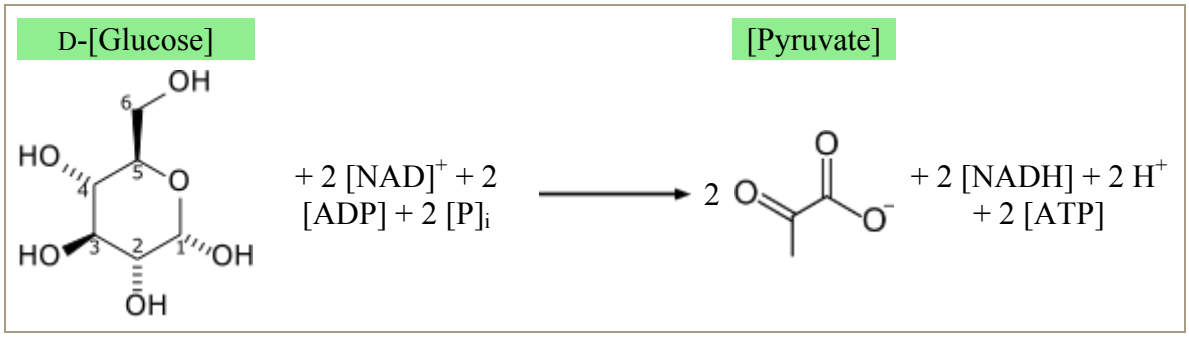
Glycolysis is a definite sequence of ten reactions involving ten intermediate compounds (one of the steps involves two intermediates). The intermediates provide entry points to glycolysis. For example, most monosaccharides, such as fructose, glucose, and galactose, can be converted to one of these intermediates. The intermediates may also be directly useful. For example, the intermediate dihydroxyacetone phosphate (DHAP) is a source of the glycerol that combines with fatty acids to form fat.

Glycolysis is thought to be the archetype of a universal metabolic pathway. It occurs, with variations, in nearly all organisms, both aerobic and anaerobic. The wide occurrence of glycolysis indicates that it is one of the most ancient known metabolic pathways.

The most common type of glycolysis is the *Embden-Meyerhof-Parnas pathway (EMP pathway)*, which was first discovered by Gustav Embden, Otto Meyerhof and Jakub Karol Parnas. Glycolysis also refers to other pathways, such as the *Entner–Doudoroff pathway* and various heterofermentative and homofermentative pathways. However, the discussion here will be limited to the Embden-Meyerhof pathway.

Overview

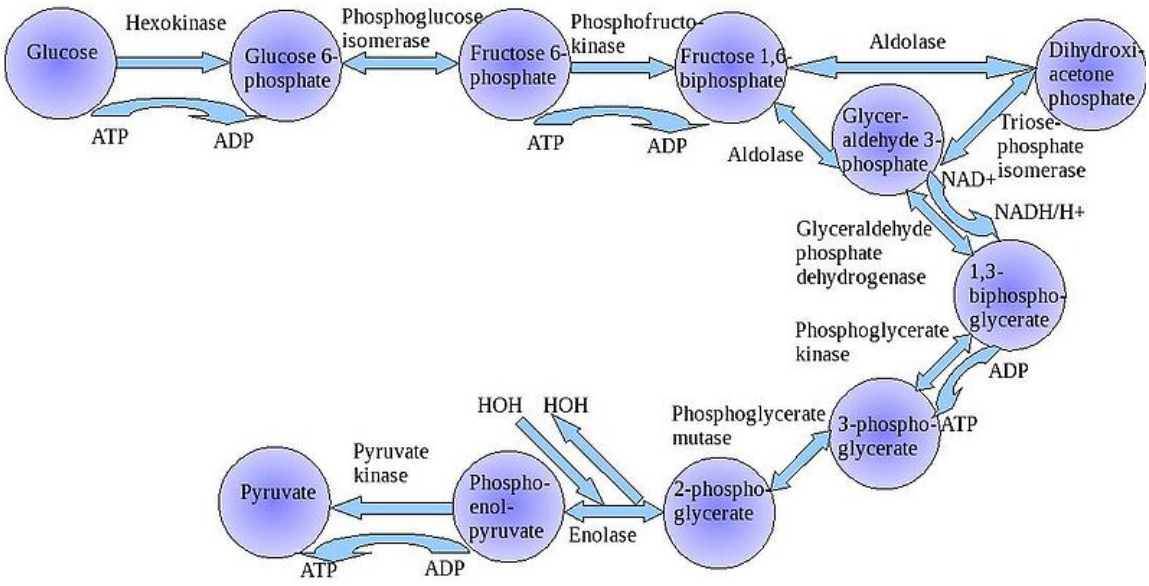
The overall reaction of glycolysis is:



The use of symbols in this equation makes it appear unbalanced with respect to oxygen atoms, hydrogen atoms, and charges. Atom balance is maintained by the two phosphate (P_i) groups:

- each exists in the form of a hydrogen phosphate anion (HPO_4^{2-}), dissociating to contribute 2 H^+ overall
- each liberates an oxygen atom when it binds to an ADP (adenosine diphosphate) molecule, contributing 2 O overall

Charges are balanced by the difference between ADP and ATP. In the cellular environment, all three hydroxy groups of ADP dissociate into $-O^-$ and H^+, giving ADP^{3-}, and this ion tends to exist in an ionic bond with Mg^{2+}, giving $ADPMg^-$. ATP behaves identically except that it has four hydroxy groups, giving $ATPMg^{2-}$. When these differences along with the true charges on the two phosphate groups are considered together, the net charges of -4 on each side are balanced.



Glycolysis

For simple anaerobic fermentations, the metabolism of one molecule of glucose to two molecules of pyruvate has a net yield of two molecules of ATP. Most cells will then carry out further reactions to 'repay' the used NAD^+ and produce a final product of ethanol or lactic acid. Many bacteria use inorganic compounds as hydrogen acceptors to regenerate the NAD^+.

Cells performing aerobic respiration synthesize much more ATP, but not as part of glycolysis. These further aerobic reactions use pyruvate and NADH + H^+ from glycolysis. Eukaryotic aerobic respiration produces approximately 34 additional molecules of ATP for each glucose molecule, however most of these are produced by a vastly different mechanism to the substrate-level phosphorylation in glycolysis.

The lower-energy production, per glucose, of anaerobic respiration relative to aerobic respiration, results in greater flux through the pathway under hypoxic (low-oxygen) conditions, unless alternative sources of anaerobically-oxidizable substrates, such as fatty acids, are found.

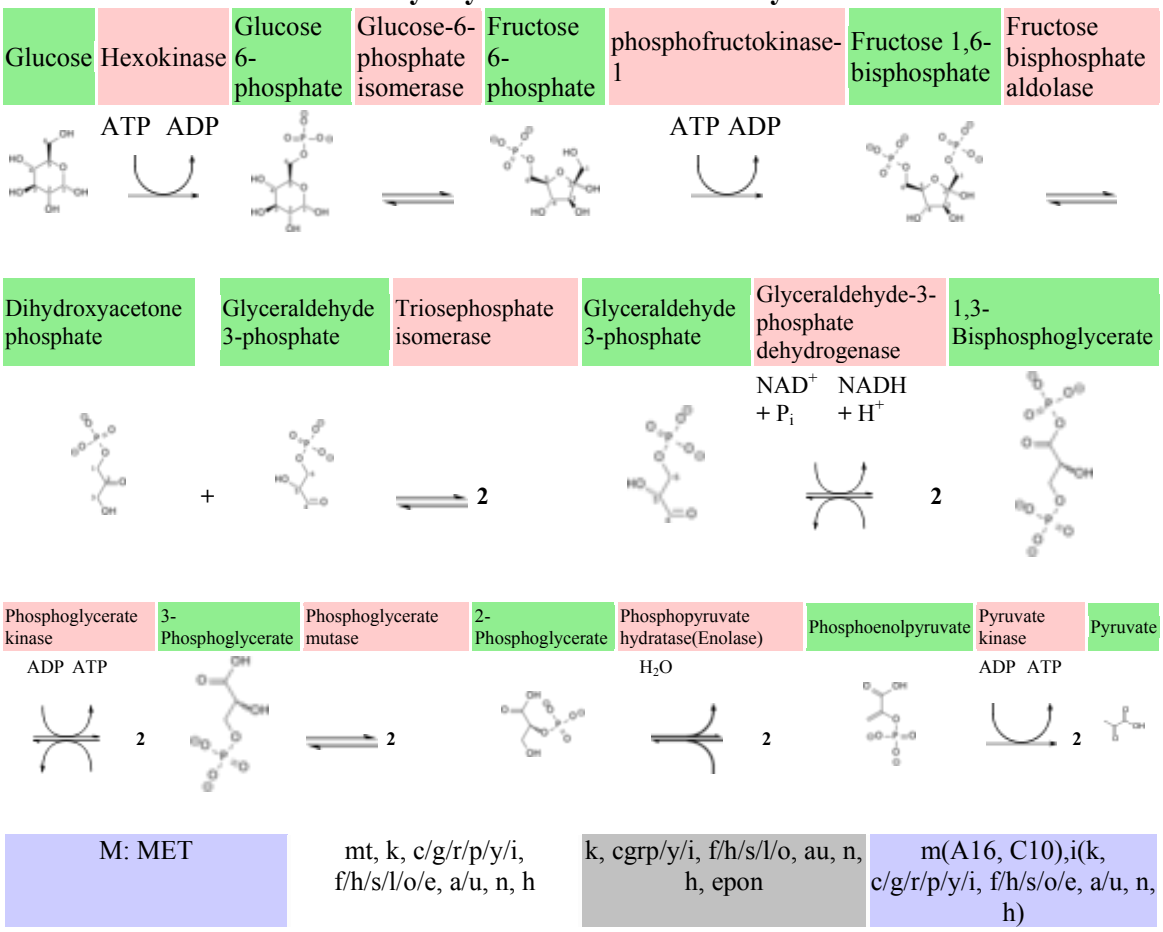
Elucidation of the pathway

In 1860, Louis Pasteur discovered that microorganisms are responsible for fermentation. In 1897, Eduard Buchner found that *extracts* of certain cells can cause fermentation. In 1905, Arthur Harden and William Youngalong with Nick Sheppard determined that a heat-sensitive high-molecular-weight subcellular fraction (the enzymes) and a heat-insensitive low-molecular-weight cytoplasm fraction (ADP, ATP and NAD^+ and other cofactors) are required together for fermentation to proceed. The details of the pathway were eventually determined by 1940, with a major input from Otto Meyerhof and some years later by Luis Leloir. The biggest difficulties in determining the intricacies of the

pathway were due to the very short lifetime and low steady-state concentrations of the intermediates of the fast glycolytic reactions.

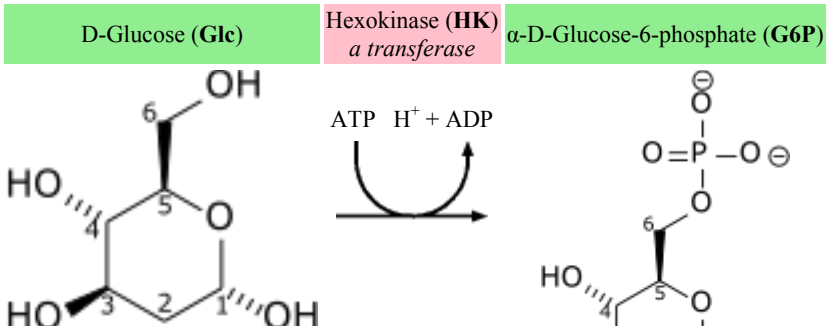
Sequence of reactions

Glycolysis Metabolic Pathway



Preparatory phase

The first five steps are regarded as the preparatory (or investment) phase, since they consume energy to convert the glucose into two three-carbon sugar phosphates (G3P).

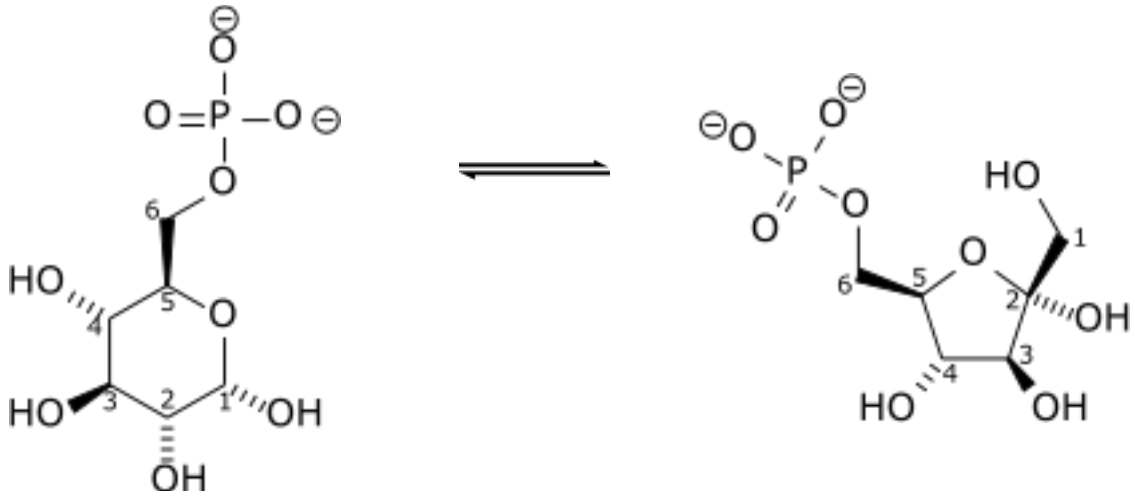


The first step in glycolysis is phosphorylation of glucose by a family of enzymes called hexokinases to form glucose 6-phosphate (G6P). This reaction consumes ATP, but it acts to keep the glucose concentration low, promoting continuous transport of glucose into the cell through the plasma membrane transporters. In addition, it blocks the glucose from leaking out - the cell lacks transporters for G6P. Glucose may alternatively be from the phosphorolysis or hydrolysis of intracellular starch or glycogen.

In animals, an isozyme of hexokinase called glucokinase is also used in the liver, which has a much lower affinity for glucose (K_m in the vicinity of normal glycemia), and differs in regulatory properties. The different substrate affinity and alternate regulation of this enzyme are a reflection of the role of the liver in maintaining blood sugar levels.

Cofactors: Mg^{2+}

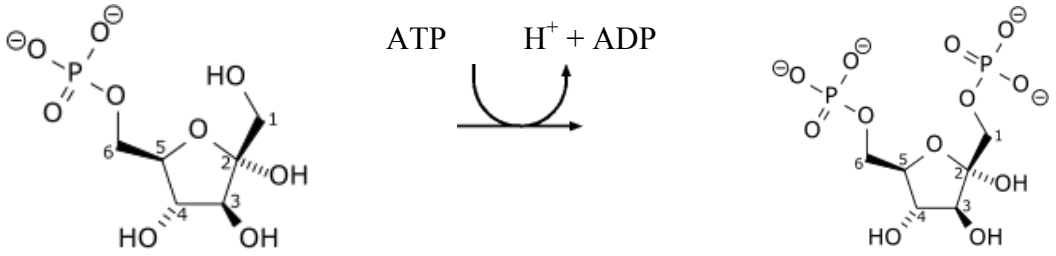
α-D-Glucose 6-phosphate (**G6P**)	Phosphoglucose isomerase *an isomerase*	β-D-Fructose 6-phosphate (**F6P**)



G6P is then rearranged into fructose 6-phosphate (F6P) by glucose phosphate isomerase. Fructose can also enter the glycolytic pathway by phosphorylation at this point.

The change in structure is an isomerization, in which the G6P has been converted to F6P. The reaction requires an enzyme, phosphohexose isomerase, to proceed. This reaction is freely reversible under normal cell conditions. However, it is often driven forward because of a low concentration of F6P, which is constantly consumed during the next step of glycolysis. Under conditions of high F6P concentration, this reaction readily runs in reverse. This phenomenon can be explained through Le Chatelier's Principle.

β-D-Fructose 6-phosphate (**F6P**)	phosphofructokinase (**PFK-1**) *a transferase*	β-D-Fructose 1,6-bisphosphate (**F1,6BP**)

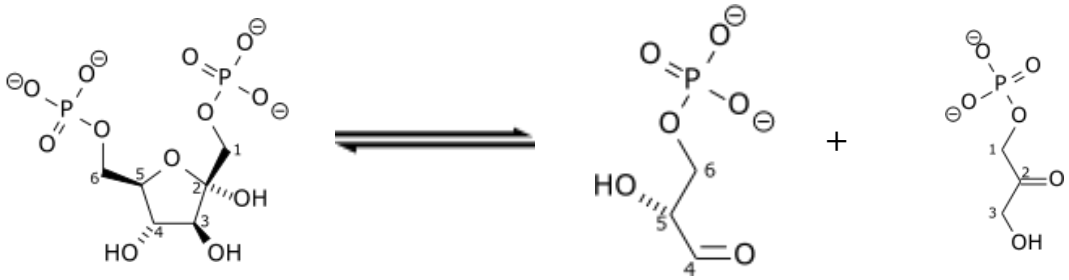


The energy expenditure of another ATP in this step is justified in 2 ways: The glycolytic process (up to this step) is now irreversible, and the energy supplied destabilizes the molecule. Because the reaction catalyzed by Phosphofructokinase 1 (PFK-1) is coupled to the hydrolysis of ATP, an energetically favorable step, it is, in essence, irreversible, and a different pathway must be used to do the reverse conversion during gluconeogenesis. This makes the reaction a key regulatory point. This is also the rate-limiting step.

The same reaction can also be catalysed by pyrophosphate-dependent phosphofructokinase (**PFP** or **PPi-PFK**), which is found in most plants, some bacteria, archea, and protists, but not in animals. This enzyme uses pyrophosphate (PPi) as a phosphate donor instead of ATP. It is a reversible reaction, increasing the flexibility of glycolytic metabolism. A rarer ADP-dependent PFK enzyme variant has been identified in archaean species.

Cofactors: Mg^{2+}

β-D-Fructose 1,6-bisphosphate (**F1,6BP**)	fructose bisphosphate aldolase (**ALDO**) *a lyase*	D-glyceraldehyde 3-phosphate (**GADP**)	Dihydroxyacetone phosphate (**DHAP**)



Destabilizing the molecule in the previous reaction allows the hexose ring to be split by aldolase into two triose sugars, dihydroxyacetone phosphate, a ketone, and glyceraldehyde 3-phosphate, an aldehyde. There are two classes of aldolases: class I aldolases, present in animals and plants, and class II aldolases, present in fungi and bacteria; the two classes use different mechanisms in cleaving the ketose ring.

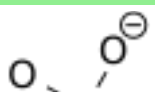
Dihydroxyacetone phosphate (**DHAP**)	triosephosphate isomerase (**TPI**) *an isomerase*	D-glyceraldehyde 3-phosphate (**GADP**)

Triosephosphate isomerase rapidly interconverts dihydroxyacetone phosphate with glyceraldehyde 3-phosphate (**GADP**) that proceeds further into glycolysis. This is advantageous, as it directs dihydroxyacetone phosphate down the same pathway as glyceraldehyde 3-phosphate, simplifying regulation.

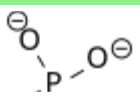
Pay-off phase

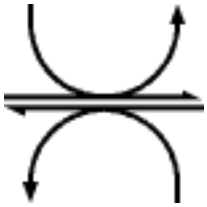
The second half of glycolysis is known as the pay-off phase, characterised by a net gain of the energy-rich molecules ATP and NADH. Since glucose leads to two triose sugars in the preparatory phase, each reaction in the pay-off phase occurs twice per glucose molecule. This yields 2 NADH molecules and 4 ATP molecules, leading to a net gain of 2 NADH molecules and 2 ATP molecules from the glycolytic pathway per glucose.

glyceraldehyde 3-phosphate (**GADP**)	glyceraldehyde phosphate dehydrogenase (**GAPDH**) *an oxidoreductase*	D-1,3-bisphosphoglycerate (**1,3BPG**)



$NAD^+ + P_i \quad NADH + H^+$



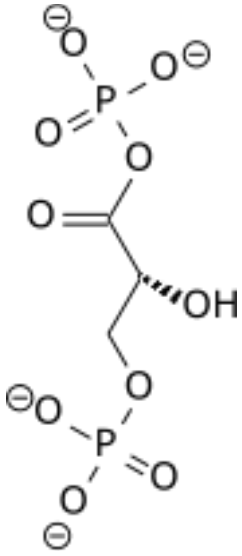


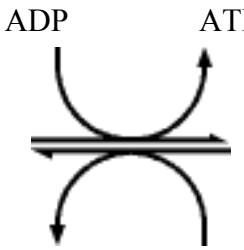
The triose sugars are dehydrogenated and inorganic phosphate is added to them, forming 1,3-bisphosphoglycerate.

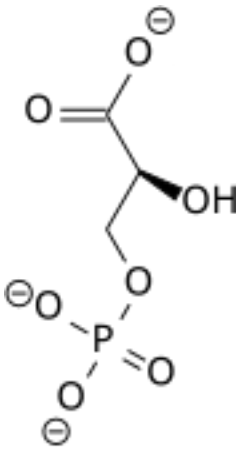
The hydrogen is used to reduce two molecules of NAD^+, a hydrogen carrier, to give NADH + H^+ for each triose.

Hydrogen atom balance and charge balance are both maintained because the phosphate (P_i) group actually exists in the form of a hydrogen phosphate anion (HPO_4^{2-}), which dissociates to contribute the extra H^+ ion and gives a net charge of -3 on both sides.

1,3-bisphosphoglycerate (**1,3-BPG**)	phosphoglycerate kinase (**PGK**) *a transferase*	3-phosphoglycerate (**3-P-G**)







phosphoglycerate kinase (**PGK**)

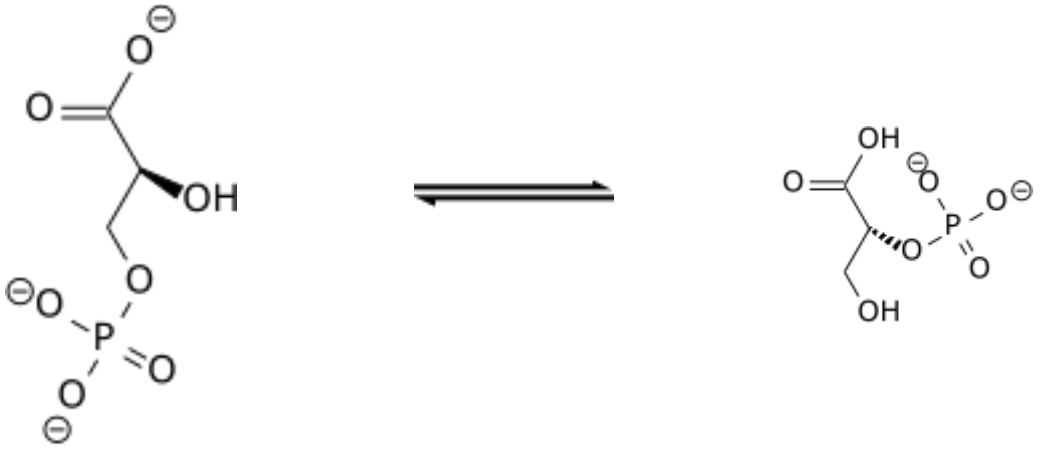
This step is the enzymatic transfer of a phosphate group from 1,3-bisphosphoglycerate to ADP by phosphoglycerate kinase, forming ATP and 3-phosphoglycerate. At this step, glycolysis has reached the break-even point: 2 molecules of ATP were consumed, and 2

new molecules have now been synthesized. This step, one of the two substrate-level phosphorylation steps, requires ADP; thus, when the cell has plenty of ATP (and little ADP), this reaction does not occur. Because ATP decays relatively quickly when it is not metabolized, this is an important regulatory point in the glycolytic pathway.

ADP actually exists as $ADPMg^-$, and ATP as $ATPMg^{2-}$, balancing the charges at -5 both sides.

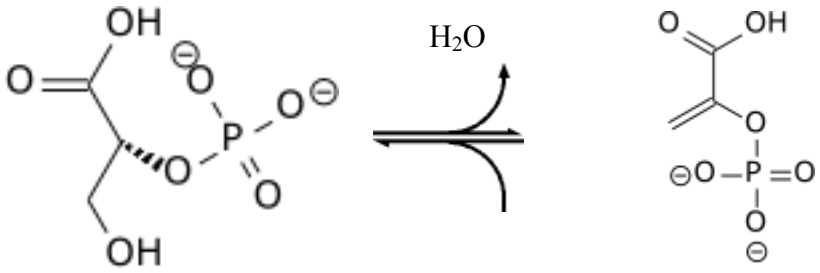
Cofactors: Mg^{2+}

3-phosphoglycerate (**3PG**)	phosphoglycerate mutase (**PGM**) *a mutase*	2-phosphoglycerate (**2PG**)



Phosphoglycerate mutase now forms 2-phosphoglycerate.

2-phosphoglycerate (**2PG**)	enolase (**ENO**) *a lyase*	phosphoenolpyruvate (**PEP**)

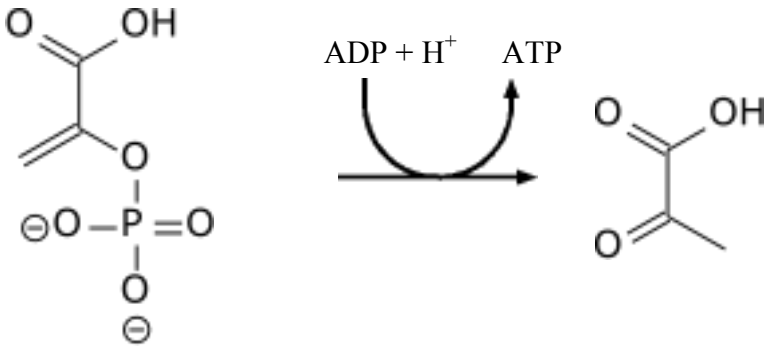


enolase (**ENO**)

Enolase next forms phosphoenolpyruvate from 2-phosphoglycerate.

Cofactors: 2 Mg^{2+}: one "conformational" ion to coordinate with the carboxylate group of the substrate, and one "catalytic" ion that participates in the dehydration.

phosphoenolpyruvate (**PEP**)	pyruvate kinase (**PK**) *a transferase*	pyruvate (**Pyr**)



A final substrate-level phosphorylation now forms a molecule of pyruvate and a molecule of ATP by means of the enzyme pyruvate kinase. This serves as an additional regulatory step, similar to the phosphoglycerate kinase step.

Cofactors: Mg^{2+}

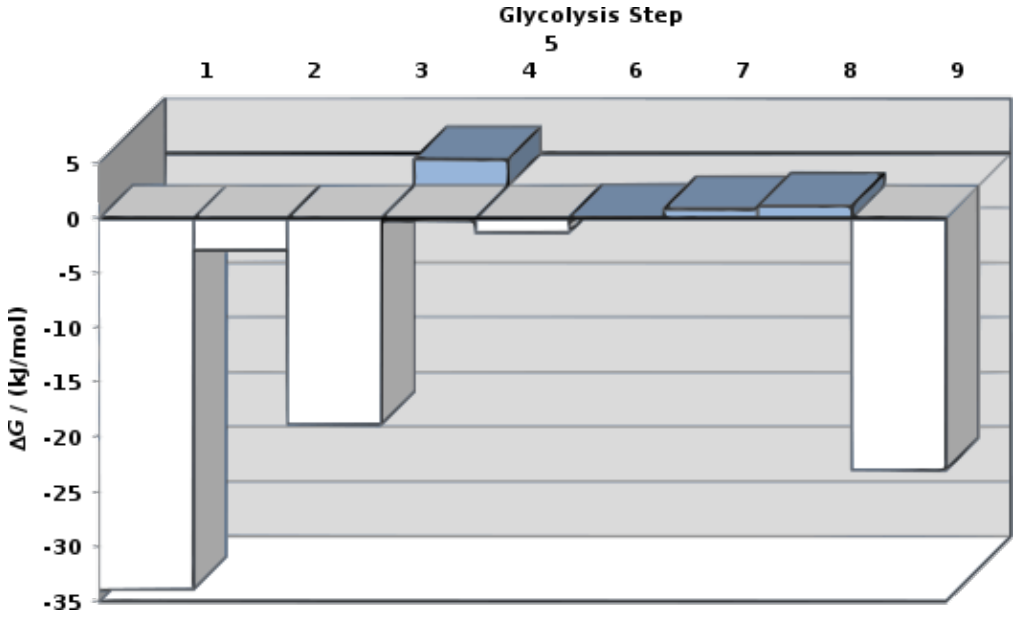
Regulation

Glycolysis is regulated by slowing down or speeding up certain steps in the glycolysis pathway. This is accomplished by inhibiting or activating the enzymes that are involved. The steps that are regulated may be determined by calculating the change in free energy, ΔG, for each step. If a step's products and reactants are in equilibrium, then the step is assumed to not be regulated. Since the change in free energy is zero for a system at equilibrium, *any step with a free energy change near zero is not being regulated*. If a step is being regulated, then that step's enzyme is not converting reactants into products as fast as it could, resulting in a build-up of reactants, which would be converted to products if the enzyme were operating faster. Since the reaction is thermodynamically favorable, the change in free energy for the step will be negative. *A step with a large negative change in free energy is assumed to be regulated.*

Free energy changes

Concentrations of metabolites in erythrocytes

Compound	Concentration / m*M*
glucose	5.0
glucose-6-phosphate	0.083
fructose-6-phosphate	0.014
fructose-1,6-bisphosphate	0.031
dihydroxyacetone phosphate	0.14
glyceraldehyde-3-phosphate	0.019
1,3-bisphosphoglycerate	0.001
2,3-bisphosphoglycerate	4.0
3-phosphoglycerate	0.12
2-phosphoglycerate	0.03
phosphoenolpyruvate	0.023
pyruvate	0.051
ATP	1.85
ADP	0.14
P_i	1.0



The change in free energy for each step of glycolysis estimated from the concentration of metabolites in a erythrocyte.

The change in free energy, ΔG, for each step in the glycolysis pathway can be calculated using $\Delta G = \Delta G^{\circ\prime} + RT\ln Q$, where Q is the reaction quotient. This requires knowing the concentrations of the metabolites. All of these values are available for erythrocytes, with the exception of the concentrations of NAD^+ and NADH. The ratio of NAD^+ to NADH is approximately 1, which results in these concentrations canceling out in the reaction quotient. (Since NAD^+ and NADH occur on opposite sides of the reactions, one will be in the numerator and the other in the denominator.)

Using the measured concentrations of each step, and the standard free energy changes, the actual free energy change can be calculated.

Change in free energy for each step of glycolysis

Step	Reaction	$\Delta G^{\circ\prime}$ / (kJ/mol)	ΔG / (kJ/mol)
1	glucose + ATP^{4-} → glucose-6-phosphate^{2-} + ADP^{3-} + H^+	-16.7	-34
2	glucose-6-phosphate^{2-} → fructose-6-phosphate^{2-}	1.67	-2.9
3	fructose-6-phosphate^{2-} + ATP^{4-} → fructose-1,6-bisphosphate^{4-} + ADP^{3-} + H^+	-14.2	-19
4	fructose-1,6-bisphosphate^{4-} → dihydroxyacetone phosphate^{2-} + glyceraldehyde-3-phosphate^{2-}	23.9	-0.23
5	dihydroxyacetone phosphate^{2-} → glyceraldehyde-3-phosphate^{2-}	7.56	2.4
6	glyceraldehyde-3-phosphate^{2-} + P_i^{2-} + NAD^+ → 1,3-bisphosphoglycerate^{4-} + NADH + H^+	6.30	-1.29
7	1,3-bisphosphoglycerate^{4-} + ADP^{3-} → 3-phosphoglycerate^{3-} + ATP^{4-}	-18.9	0.09
8	3-phosphoglycerate^{3-} → 2-phosphoglycerate^{3-}	4.4	0.83
9	2-phosphoglycerate^{3-} → phosphoenolpyruvate^{3-} + H_2O	1.8	1.1
10	phosphoenolpyruvate^{3-} + ADP^{3-} + H^+ → pyruvate$^-$ + ATP^{4-}	-31.7	-23.0

From measuring the physiological concentrations of metabolites in a erythrocyte it seems that about seven of the steps in glycolysis are in equilibrium for that cell type. Three of the steps — the ones with large negative free energy changes — are not in equilibrium and are referred to as *irreversible*; such steps are often subject to regulation.

Step 5 in the figure is shown behind the other steps, because that step is a side-reaction that can decrease or increase the concentration of the intermediate glyceraldehyde-3-phosphate. That compound is converted to dihydroxyacetone phosphate by the enzyme triose phosphate isomerase, which is a catalytically perfect enzyme; its rate is so fast that the reaction can be assumed to be in equilibrium. The fact that ΔG is not zero indicates that the actual concentrations in the erythrocyte are not accurately known.

Biochemical logic

The existence of more than one point of regulation indicates that intermediates between those points enter and leave the glycolysis pathway by other processes. For example, in the first regulated step, hexokinase converts glucose into glucose-6-phosphate. Instead of continuing through the glycolysis pathway, this intermediate can be converted into glucose storage molecules, such as glycogen or starch. The reverse reaction, breaking down, e.g., glycogen, produces mainly glucose-6-phosphate; very little free glucose is formed in the reaction. The glucose-6-phosphate so produced can enter glycolysis *after* the first control point.

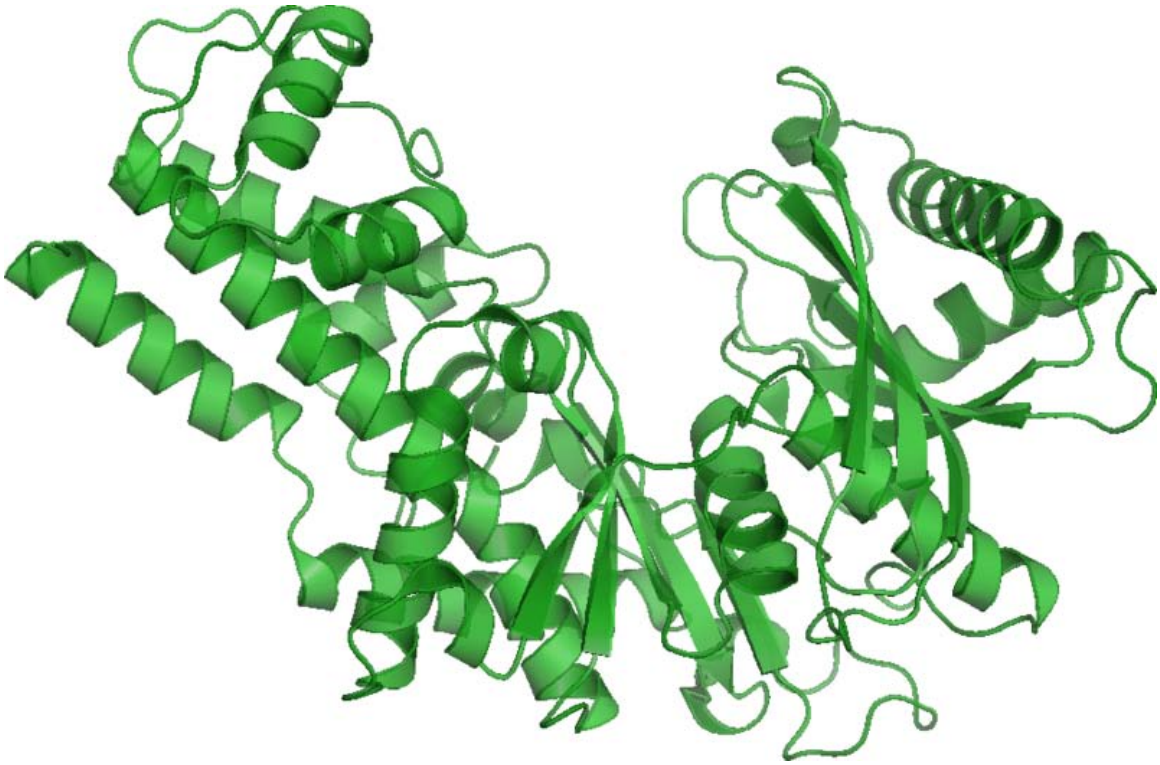
In the second regulated step (the third step of glycolysis), phosphofructokinase converts fructose-6-phosphate into fructose-1,6-bisphosphate, which then is converted into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. The dihydroxyacetone phosphate can be removed from glycolysis by conversion into glycerol-3-phosphate, which can be used to form triglycerides. On the converse, triglycerides can be broken down into fatty acids and glycerol; the latter, in turn, can be converted into dihydroxyacetone phosphate, which can enter glycolysis *after* the second control point.

Regulation

The three regulated enzymes are hexokinase, phosphofructokinase, and pyruvate kinase.

The flux through the glycolytic pathway is adjusted in response to conditions both inside and outside the cell. The rate in liver is regulated to meet major cellular needs: (1) the production of ATP, (2) the provision of building blocks for biosynthetic reactions, and (3) to lower blood glucose, one of the major functions of the liver. When blood sugar falls, glycolysis is halted in the liver to allow the reverse process, gluconeogenesis. In glycolysis, the reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase are effectively irreversible in most organisms. In metabolic pathways, such enzymes are potential sites of control, and all three enzymes serve this purpose in glycolysis.

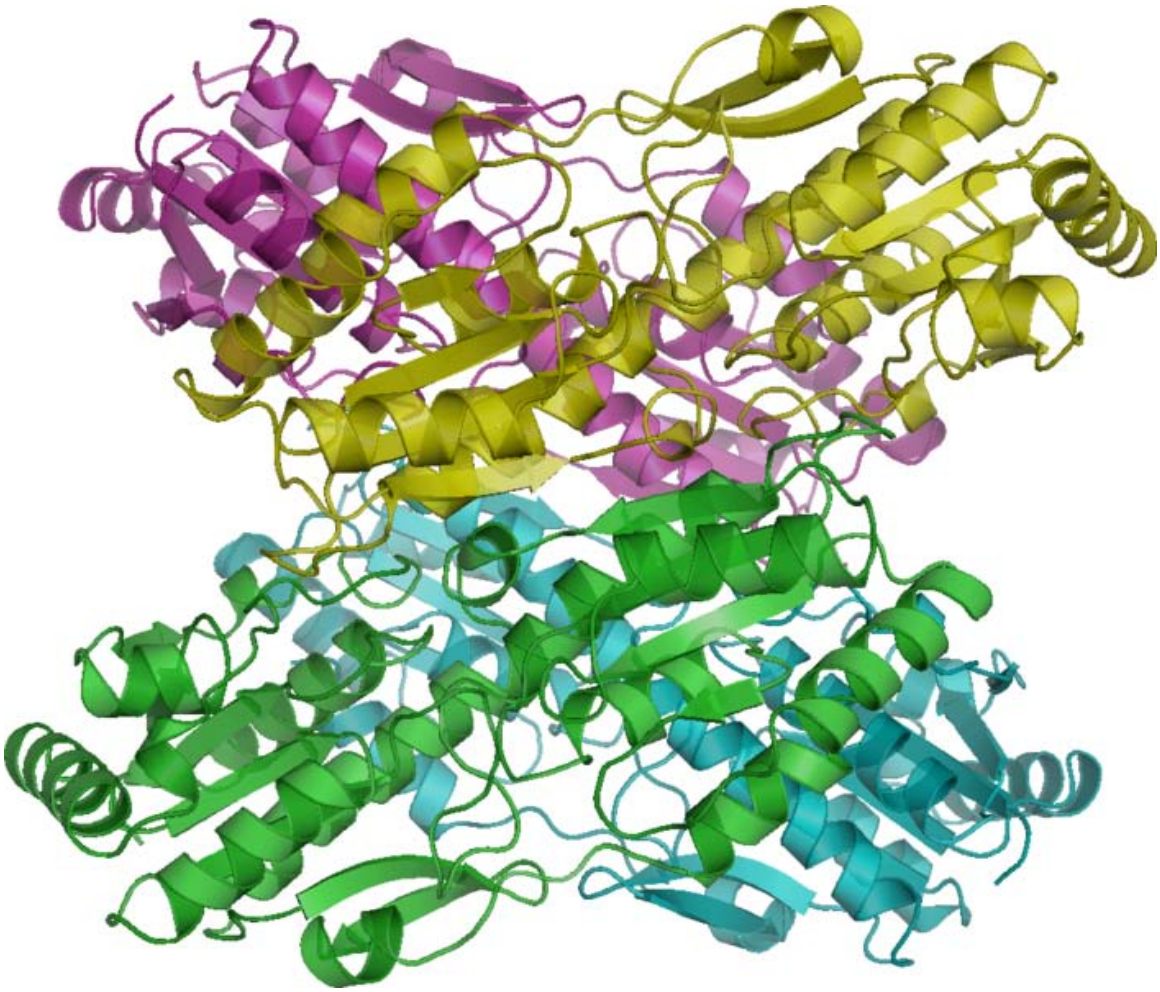
Hexokinase



Yeast hexokinase B. PDB 1IG8.

In animals, regulation of blood glucose levels by the liver is a vital part of homeostasis. In liver cells, extra G6P (glucose-6-phosphate) may be converted to G1P for conversion to glycogen, or it is alternatively converted by glycolysis to acetyl-CoA and then citrate. Excess citrate is exported to the cytosol, where ATP citrate lyase will regenerate acetyl-CoA and OAA. The acetyl-CoA is then used for fatty acid synthesis and cholesterol synthesis, two important ways of utilizing excess glucose when its concentration is high in blood. Liver contains both hexokinase and glucokinase; the latter catalyses the phosphorylation of glucose to G6P and is not inhibited by G6P. Thus, it allows glucose to be converted into glycogen, fatty acids, and cholesterol even when hexokinase activity is low. This is important when blood glucose levels are high. During hypoglycemia, the glycogen can be converted back to G6P and then converted to glucose by the liver-specific enzyme glucose 6-phosphatase. This reverse reaction is an important role of liver cells to maintain blood sugars levels during fasting. This is critical for brain function, since the brain utilizes glucose as an energy source under most conditions.

Phosphofructokinase



Bacillus stearothermophilus phosphofructokinase. PDB 6PFK.

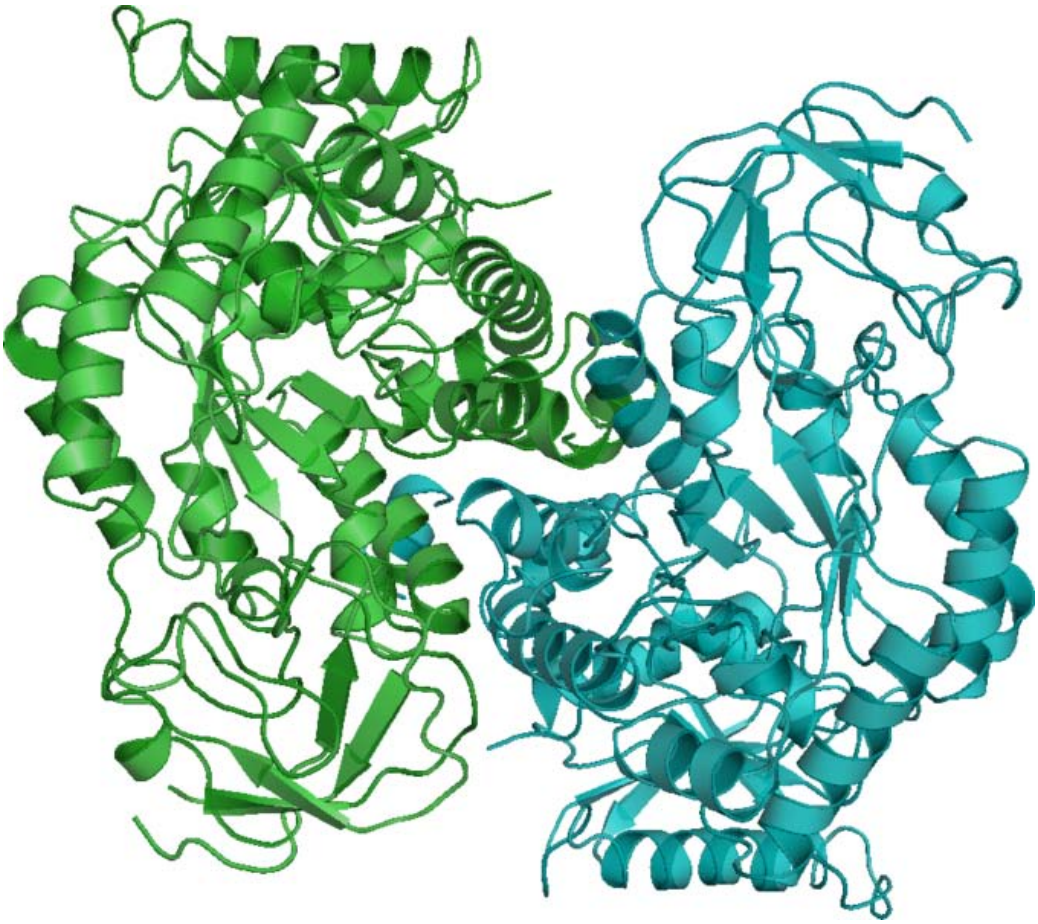
Phosphofructokinase is an important control point in the glycolytic pathway, since it is one of the irreversible steps and has key allosteric effectors, AMP and fructose 2,6-bisphosphate (F2,6BP).

Fructose 2,6-bisphosphate (F2,6BP) is a very potent activator of phosphofructokinase (PFK-1), which is synthesised when F6P is phosphorylated by a second phosphofructokinase (PFK2). In liver, when blood sugar is low and glucagon elevates cAMP, PFK2 is phosphorylated by protein kinase A. The phosphorylation inactivates PFK2, and another domain on this protein becomes active as fructose 2,6-bisphosphatase, which converts F2,6BP back to F6P. Both glucagon and epinephrine cause high levels of cAMP in the liver. The result of lower levels of liver fructose-2,6-bisphosphate is a decrease in activity of phosphofructokinase and an increase in activity of fructose 1,6-bisphosphatase, so that gluconeogenesis (in essence, "glycolysis in reverse") is favored. This is consistent with the role of the liver in such situations, since the response of the liver to these hormones is to release glucose to the blood.

ATP competes with AMP for the allosteric effector site on the PFK enzyme. ATP concentrations in cells are much higher than those of AMP, typically 100-fold higher, but the concentration of ATP does not change more than about 10% under physiological conditions, whereas a 10% drop in ATP results in a 6-fold increase in AMP. Thus, the relevance of ATP as an allosteric effector is questionable. An increase in AMP is a consequence of a decrease in energy charge in the cell.

Citrate inhibits phosphofructokinase when tested *in vitro* by enhancing the inhibitory effect of ATP. However, it is doubtful that this is a meaningful effect *in vivo*, because citrate in the cytosol is utilized mainly for conversion to acetyl-CoA for fatty acid and cholesterol synthesis.

Pyruvate kinase



Yeast pyruvate kinase. PDB 1A3W.

This enzyme catalyzes the last step of glycolysis, in which pyruvate and ATP are formed. Regulation of this enzyme is discussed in the main topic, pyruvate kinase.

Post-glycolysis processes

The overall process of glycolysis is:

glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 pyruvate + 2 NADH + 2 H^+ + 2 ATP + 2 H_2O

If glycolysis were to continue indefinitely, all of the NAD^+ would be used up, and glycolysis would stop. To allow glycolysis to continue, organisms must be able to oxidize NADH back to NAD^+.

Fermentation

One method of doing this is to simply have the pyruvate do the oxidation; in this process, the pyruvate is converted to lactate (the conjugate base of lactic acid) in a process called lactic acid fermentation:

pyruvate + NADH + H^+ → lactate + NAD^+

This process occurs in the bacteria involved in making yogurt (the lactic acid causes the milk to curdle). This process also occurs in animals under hypoxic (or partially-anaerobic) conditions, found, for example, in overworked muscles that are starved of oxygen, or in infarcted heart muscle cells. In many tissues, this is a cellular last resort for energy; most animal tissue cannot maintain anaerobic respiration for an extended length of time.

Some organisms, such as yeast, convert NADH back to NAD^+ in a process called ethanol fermentation. In this process, the pyruvate is converted first to acetaldehyde and carbon dioxide, then to ethanol.

Lactic acid fermentation and ethanol fermentation can occur in the absence of oxygen. This anaerobic fermentation allows many single-cell organisms to use glycolysis as their only energy source.

Anaerobic respiration

In the above two examples of fermentation, NADH is oxidized by transferring two electrons to pyruvate. However, anaerobic bacteria use a wide variety of compounds as the terminal electron acceptors in cellular respiration: nitrogenous compounds, such as nitrates and nitrites; sulfur compounds, such as sulfates, sulfites, sulfur dioxide, and elemental sulfur; carbon dioxide; iron compounds; manganese compounds; cobalt compounds; and uranium compounds.

Aerobic respiration

In aerobic organisms, a complex mechanism has been created to use the oxygen in air as the final electron acceptor of respiration.

- First, pyruvate is converted to acetyl-CoA and CO_2 within the mitochondria in a process called pyruvate decarboxylation.
- Second, the acetyl-CoA enters the citric acid cycle, also known as Krebs Cycle, where it is fully oxidized to carbon dioxide and water, producing yet more NADH.
- Third, the NADH is oxidized to NAD^+ by the electron transport chain, using oxygen as the final electron acceptor. This process creates a "hydrogen ion gradient" across the inner membrane of the mitochondria.
- Fourth, the proton gradient is used to produce a large amount of ATP in a process called oxidative phosphorylation.

Intermediates for other pathways

Here we, concentrates on the catabolic role of glycolysis with regard to converting potential chemical energy to usable chemical energy during the oxidation of glucose to pyruvate. However, many of the metabolites in the glycolytic pathway are also used by anabolic pathways, and, as a consequence, flux through the pathway is critical to maintain a supply of carbon skeletons for biosynthesis.

In addition, not all carbon entering the pathway leaves as pyruvate and may be extracted at earlier stages to provide carbon compounds for other pathways.

These metabolic pathways are all strongly reliant on glycolysis as a source of metabolites:

- Gluconeogenesis
- Lipid metabolism
- Pentose phosphate pathway
- Citric acid cycle, which in turn leads to:
    - Amino acid synthesis
    - Nucleotide synthesis
    - Tetrapyrrole synthesis

From an anabolic metabolism perspective, the NADH has a role to drive synthetic reactions, doing so by directly or indirectly reducing the pool of NADP+ in the cell to NADPH, which is another important reducing agent for biosynthetic pathways in a cell.

Glycolysis in disease

Genetic diseases

Glycolytic mutations are generally rare due to importance of the metabolic pathway, this means that the majority of occurring mutations result in an inability for the cell to respire, and therefore cause the death of the cell at an early stage. However, some mutations are

seen with one notable example being Pyruvate kinase deficiency, leading to chronic hemolytic anemia.

Cancer

Malignant rapidly-growing tumor cells typically have glycolytic rates that are up to 200 times higher than those of their normal tissues of origin. This phenomenon was first described in 1930 by Otto Warburg and is referred to as the Warburg effect. The Warburg hypothesis claims that cancer is primarily caused by dysfunctionality in mitochondrial metabolism, rather than because of uncontrolled growth of cells. A number of theories have been advanced to explain the Warburg effect.

This high glycolysis rate has important medical applications, as high aerobic glycolysis by malignant tumors is utilized clinically to diagnose and monitor treatment responses of cancers by imaging uptake of 2-^{18}F-2-deoxyglucose (FDG) (a radioactive modified hexokinase substrate) with positron emission tomography (PET).

There is ongoing research to affect mitochondrial metabolism and treat cancer by reducing glycolysis and thus starving cancerous cells in various new ways, including a ketogenic diet.

Alzheimer's disease

Disfunctioning glycolysis or glucose metabolism in fronto-temporo-parietal and cingulate cortices has been associated with Alzheimer's disease, probably due to the decreased amyloid β (1-42) (Aβ42) and increased tau, phosphorylated tau in cerebrospinal fluid (CSF)

Alternative nomenclature

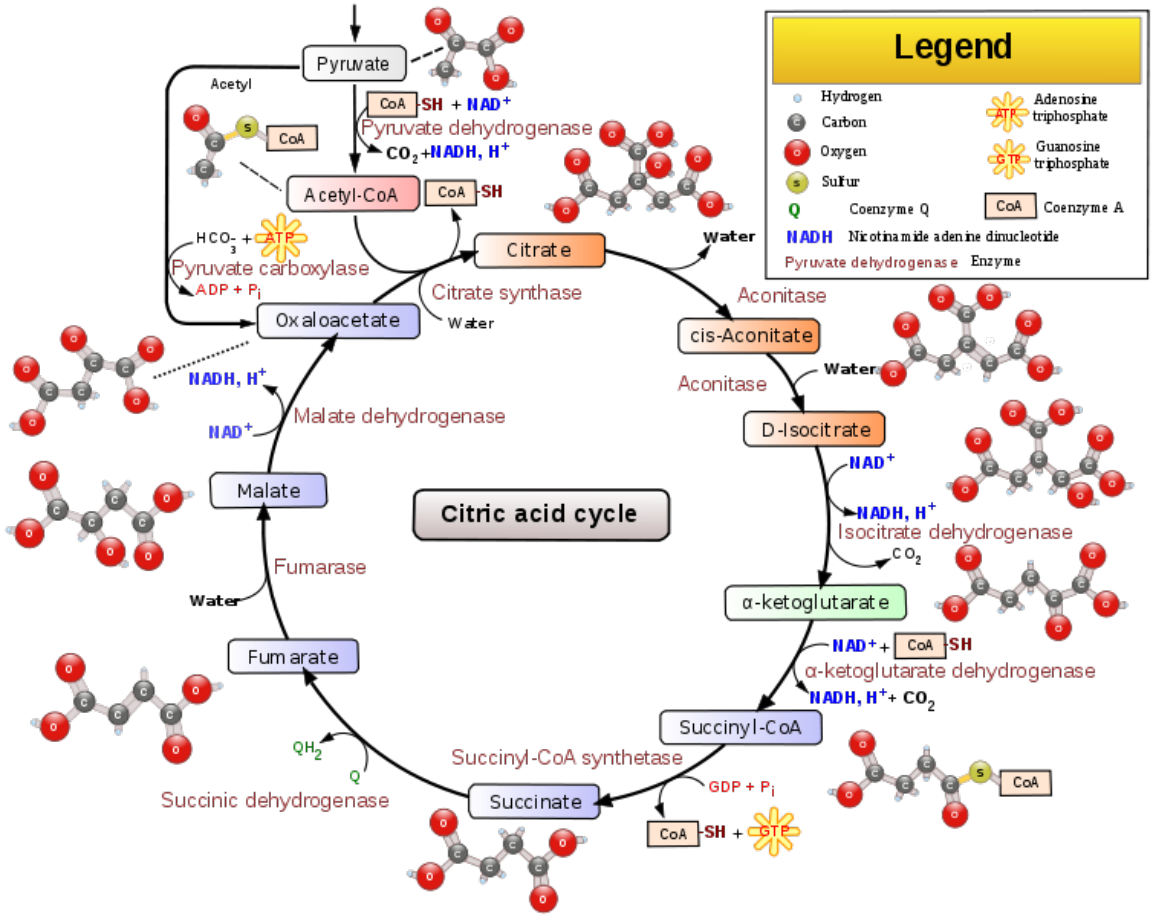
Some of the metabolites in glycolysis have alternative names and nomenclature. In part, this is because some of them are common to other pathways, such as the Calvin cycle.

	Given Names		Alternative names	Alternative nomenclature
1	glucose	**Glc**	dextrose	
3	fructose 6-phosphate	**F6P**		
4	fructose 1,6-bisphosphate	**F1,6BP**	fructose 1,6-diphosphate	**FBP**, **FDP**, **F1,6DP**
5	dihydroxyacetone phosphate	**DHAP**	glycerone phosphate	
6	glyceraldehyde 3-phosphate	**GADP**	3-phosphoglyceraldehyde	**PGAL**, **G3P**, **GALP**,**GAP**,**TP**
7	1,3-bisphosphoglycerate	**1,3BPG**	glycerate 1,3-bisphosphate,	**PGAP**, **BPG**, **DPG**

			glycerate 1,3-diphosphate, 1,3-diphosphoglycerate	
8	3-phosphoglycerate	**3PG**	glycerate 3-phosphate	**PGA**, **GP**
9	2-phosphoglycerate	**2PG**	glycerate 2-phosphate	
10	phosphoenolpyruvate	**PEP**		
11	pyruvate	**Pyr**	pyruvic acid	

Chapter- 3

Citric Acid Cycle



Overview of the citric acid cycle

The **citric acid cycle** — also known as the **tricarboxylic acid cycle** (**TCA cycle**), the **Krebs cycle**, or the **Szent-Györgyi-Krebs cycle** — is a series of enzyme-catalysed chemical reactions, which is of central importance in all living cells that use oxygen as

part of cellular respiration. In eukaryotic cells, the citric acid cycle occurs in the matrix of the mitochondrion. The components and reactions of the citric acid cycle were established by seminal work from Albert Szent-Györgyi and Hans Krebs.

In aerobic organisms, the citric acid cycle is part of a metabolic pathway involved in the chemical conversion of carbohydrates, fats and proteins into carbon dioxide and water to generate a form of usable energy. Other relevant reactions in the pathway include those in glycolysis and pyruvate oxidation before the citric acid cycle, and oxidative phosphorylation after it. In addition, it provides precursors for many compounds including some amino acids and is therefore functional even in cells performing fermentation.

A simplified view of the process

- The citric acid cycle begins with the transfer of a two-carbon acetyl group from acetyl-CoA to the four-carbon acceptor compound (oxaloacetate) to form a six-carbon compound (citrate).
- The citrate then goes through a series of chemical transformations, losing two carboxyl groups as CO_2. The carbons lost as CO_2 originate from what was oxaloacetate, not directly from acetyl-CoA. The carbons donated by acetyl-CoA become part of the oxaloacetate carbon backbone after the first turn of the citric acid cycle. Loss of the acetyl-CoA-donated carbons as CO_2 requires several turns of the citric acid cycle. However, because of the role of the citric acid cycle in anabolism, they may not be lost, since many TCA cycle intermediates are also used as precursors for the biosynthesis of other molecules.
- Most of the energy made available by the oxidative steps of the cycle is transferred as energy-rich electrons to NAD^+, forming NADH. For each acetyl group that enters the citric acid cycle, three molecules of NADH are produced.
- Electrons are also transferred to the electron acceptor Q, forming QH_2.
- At the end of each cycle, the four-carbon oxaloacetate has been regenerated, and the cycle continues.

Steps

Two carbon atoms are oxidized to CO_2, the energy from these reactions being transferred to other metabolic processes by GTP (or ATP), and as electrons in NADH and QH_2. The NADH generated in the TCA cycle may later donate its electrons in oxidative phosphorylation to drive ATP synthesis; $FADH_2$ is covalently attached to succinate dehydrogenase, an enzyme functioning both in the TCA cycle and the mitochondrial electron transport chain in oxidative phosphorylation. $FADH_2$, therefore, facilitates transfer of electrons to coenzyme Q, which is the final electron acceptor of the reaction catalyzed by the Succinate:ubiquinone oxidoreductase complex, also acting as an intermediate in the electron transport chain.

The citric acid cycle is continuously supplied with new carbon in the form of acetyl-CoA, entering at step 1 below.

	Substrates	Products	Enzyme	Reaction type	Comment
1	Oxaloacetate + Acetyl CoA + H_2O	Citrate + CoA-SH	Citrate synthase	Aldol condensation	rate-limiting stage (irreversible), extends the 4C oxaloacetate to a 6C molecule
2	Citrate	*cis*-Aconitate + H_2O	Aconitase	Dehydration	reversible isomerisation
3	*cis*-Aconitate + H_2O	Isocitrate		Hydration	
4	Isocitrate + NAD^+	Oxalosuccinate + NADH + H^+	Isocitrate dehydrogenase	Oxidation	generates NADH (equivalent of 2.5 ATP)
5	Oxalosuccinate	α-Ketoglutarate + CO_2		Decarboxylation	irreversible stage, generates a 5C molecule
6	α-Ketoglutarate + NAD^+ + CoA-SH	Succinyl-CoA + NADH + H^+ + CO_2	α-Ketoglutarate dehydrogenase	Oxidative decarboxylation	irreversible stage, generates NADH (equivalent of 2.5 ATP), regenerates the 4C chain (CoA excluded)
7	Succinyl-CoA + GDP + P_i	Succinate + CoA-SH + GTP	Succinyl-CoA synthetase	substrate-level phosphorylation	or ADP→ATP instead of GDP→GTP, generates 1 ATP or equivalent
8	Succinate + ubiquinone (Q)	Fumarate + ubiquinol (QH_2)	Succinate dehydrogenase	Oxidation	uses FAD as a prosthetic group (FAD→$FADH_2$ in the first step of the reaction) in the enzyme, generates the equivalent of 1.5 ATP
9	Fumarate + H_2O	*L*-Malate	Fumarase	H_2O addition (*hydration*)	
10	*L*-Malate + NAD^+	Oxaloacetate + NADH + H^+	Malate dehydrogenase	Oxidation	generates NADH (equivalent of 2.5 ATP)

Mitochondria in animals, including humans, possess two succinyl-CoA synthetases: one that produces GTP from GDP, and another that produces ATP from ADP. Plants have the type that produces ATP (ADP-forming succinyl-CoA synthetase). Several of the enzymes in the cycle may be loosely-associated in a multienzyme protein complex within the mitochondrial matrix.

The GTP that is formed by GDP-forming succinyl-CoA synthetase may be utilized by nucleoside-diphosphate kinase to form ATP (the catalyzed reaction is GTP + ADP → GDP + ATP).

Products

Products of the first turn of the cycle are: *one GTP (or ATP), three NADH, one QH_2, two CO_2*.

Because two acetyl-CoA molecules are produced from each glucose molecule, two cycles are required per glucose molecule. Therefore, at the end of two cycles, the products are: two GTP, six NADH, two QH_2, and four CO_2

Description	Reactants	Products
The sum of all reactions in the citric acid cycle is:	Acetyl-CoA + 3 NAD^+ + Q + GDP + P_i + 2 H_2O	→ CoA-SH + 3 NADH + 3 H^+ + QH_2 + GTP + 2 CO_2
Combining the reactions occurring during the pyruvate oxidation with those occurring during the citric acid cycle, the following overall pyruvate oxidation reaction is obtained:	Pyruvate ion + 4 NAD^+ + Q + GDP + P_i + 2 H_2O	→ 4 NADH + 3 H^+ + QH_2 + GTP + 3 CO_2
Combining the above reaction with the ones occurring in the course of glycolysis, the following overall glucose oxidation reaction (excluding reactions in the respiratory chain) is obtained:	Glucose + 10 NAD^+ + 2 Q + 2 ADP + 2 GDP + 4 P_i + 2 H_2O	→ 10 NADH + 10 H^+ + 2 QH_2 + 2 ATP + 2 GTP + 6 CO_2

The above reactions are balanced if P_i represents the $H_2PO_4^-$ ion, ADP and GDP the ADP^{2-} and GDP^{2-} ions, respectively, and ATP and GTP the ATP^{3-} and GTP^{3-} ions, respectively.

The total number of ATP obtained after complete oxidation of one glucose in glycolysis, citric acid cycle, and oxidative phosphorylation is estimated to be between 30 and 38. A recent assessment of the total ATP yield with the updated proton-to-ATP ratios provides an estimate of 29.85 ATP per glucose molecule.

Regulation

Although pyruvate dehydrogenase is not technically a part of the citric acid cycle, its regulation is included here.

The regulation of the TCA cycle is largely determined by substrate availability and product inhibition. NADH, a product of all dehydrogenases in the TCA cycle with the exception of succinate dehydrogenase, inhibits pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and also citrate synthase. Acetyl-coA inhibits pyruvate dehydrogenase, while succinyl-CoA inhibits succinyl-CoA synthetase and citrate synthase. When tested in vitro with TCA enzymes, ATP inhibits citrate synthase and α-ketoglutarate dehydrogenase; however, ATP levels do not change more than 10% in vivo between rest and vigorous exercise. There is no known allosteric mechanism that can account for large changes in reaction rate from an allosteric effector whose concentration changes less than 10%.

Calcium is used as a regulator. It activates pyruvate dehydrogenase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. This increases the reaction rate of many of the steps in the cycle, and therefore increases flux throughout the pathway.

Citrate is used for feedback inhibition, as it inhibits phosphofructokinase, an enzyme involved in glycolysis that catalyses formation of fructose 1,6-bisphosphate,a precursor of pyruvate. This prevents a constant high rate of flux when there is an accumulation of citrate and a decrease in substrate for the enzyme.

Recent work has demonstrated an important link between intermediates of the citric acid cycle and the regulation of hypoxia-inducible factors (HIF). HIF plays a role in the regulation of oxygen homeostasis, and is a transcription factor that targets angiogenesis, vascular remodeling, glucose utilization, iron transport and apoptosis. HIF is synthesized consititutively, and hydroxylation of at least one of two critical proline residues mediates their interaction with the von Hippel Lindau E3 ubiquitin ligase complex, which targets them for rapid degradation. This reaction is calalysed by prolyl 4-hydroxylases. Fumarate and succinate have been identified as potent inhibitors of prolyl hydroxylases, thus leading to the stabilisation of HIF.

Major metabolic pathways converging on the TCA cycle

Several catabolic pathways converge on the TCA cycle. Reactions that form intermediates of the TCA cycle in order to replenish them (especially during the scarcity of the intermediates) are called anaplerotic reactions.

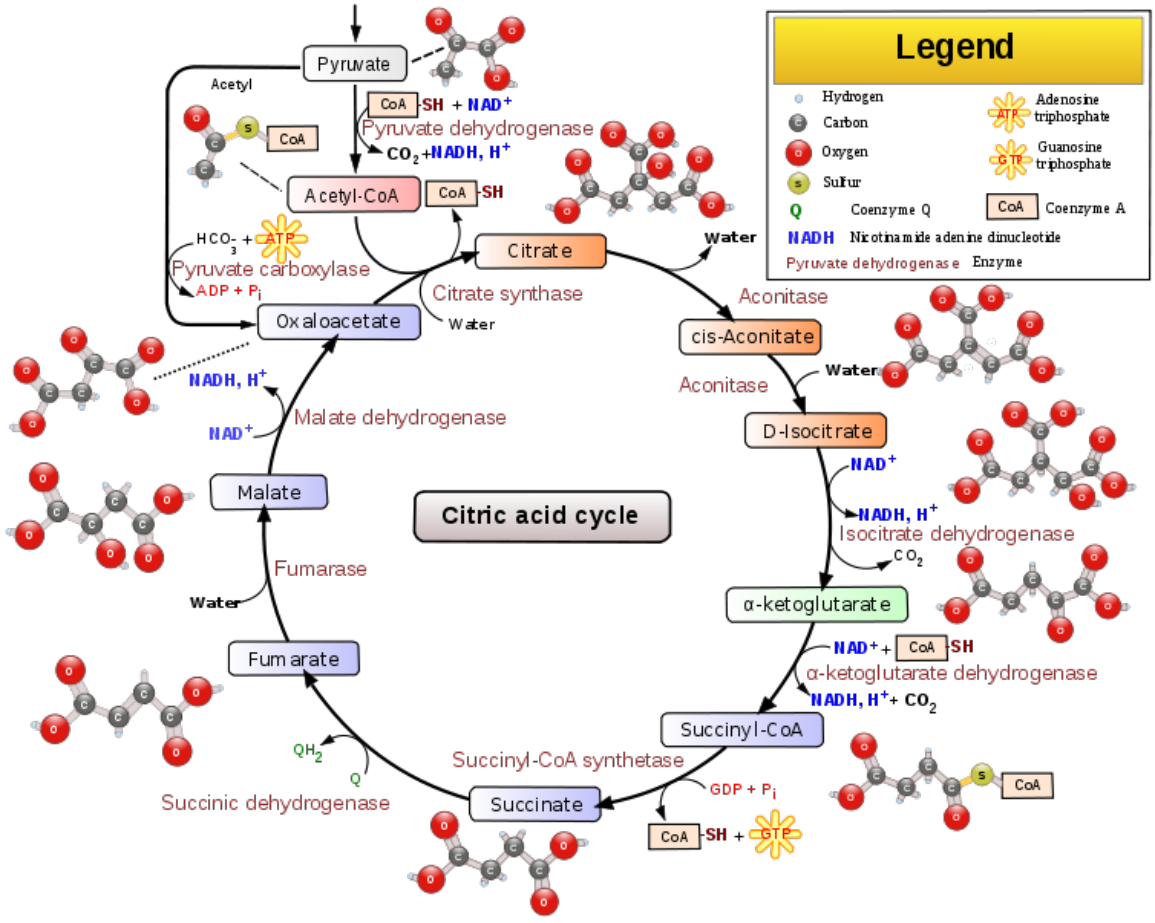
The citric acid cycle is the third step in carbohydrate catabolism (the breakdown of sugars). Glycolysis breaks glucose (a six-carbon-molecule) down into pyruvate (a three-carbon molecule). In eukaryotes, pyruvate moves into the mitochondria. It is converted into acetyl-CoA by decarboxylation and enters the citric acid cycle.

In protein catabolism, proteins are broken down by proteases into their constituent amino acids. The carbon backbone of these amino acids can become a source of energy by being converted to acetyl-CoA and entering into the citric acid cycle.

In fat catabolism, triglycerides are hydrolyzed to break them into fatty acids and glycerol. In the liver the glycerol can be converted into glucose via dihydroxyacetone phosphate and glyceraldehyde-3-phosphate by way of gluconeogenesis. In many tissues, especially heart tissue, fatty acids are broken down through a process known as beta oxidation, which results in acetyl-CoA, which can be used in the citric acid cycle. Beta oxidation of fatty acids with an odd number of methylene groups produces propionyl CoA, which is then converted into succinyl-CoA and fed into the citric acid cycle.

The total energy gained from the complete breakdown of one molecule of glucose by glycolysis, the citric acid cycle, and oxidative phosphorylation equals about 30 ATP molecules, in eukaryotes. The citric acid cycle is called an amphibolic pathway because it participates in both catabolism and anabolism.

Interactive pathway map



Citric acid cycle edit

Chapter- 4

Ethanol Fermentation

A laboratory vessel being used for the fermentation of straw.

Ethanol fermentation, also referred to as **alcoholic fermentation**, is a biological process in which sugars such as glucose, fructose, and sucrose are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products. Because yeasts perform this process in the absence of oxygen, ethanol fermentation is classified as anaerobic. Ethanol fermentation occurs in the production of alcoholic beverages and ethanol fuel, and in the rising of bread dough.

The chemical process of fermentation of glucose

The chemical equation below summarizes the fermentation of glucose, whose chemical formula is $C_6H_{12}O_6$. One mole of glucose is converted into two moles of ethanol and two moles of carbon dioxide:

$$C_{12}H_{22}O_{11} + H_2O + \text{invertase} \rightarrow C_6H_{12}O_6$$
$$C_6H_{12}O_6 + \text{Zymase} \rightarrow 2C_2H_5OH + 2CO_2$$

C_2H_5OH is the chemical formula for ethanol.

Before fermentation takes place, one glucose molecule is broken down into two pyruvic molecules. This is known as glycolysis. Glycolysis is summarized by the chemical equation:

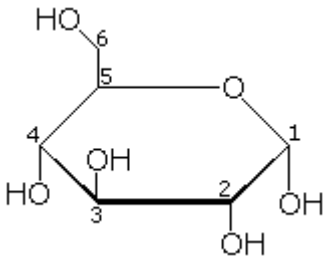
$$C_6H_{12}O_6 + 2\ ADP + 2\ P_i + 2\ NAD^+ \rightarrow 2\ CH_3COCOO^- + 2\ ATP + 2\ NADH + 2\ H_2O + 2H^+$$

The chemical formula of pyruvate is CH_3COCOO^-. P_i stands for the inorganic phosphate. As shown by the reaction equation, glycolysis causes the reduction of two molecules of NAD^+ to NADH. Two ADP molecules are also converted to two ATP and two water molecules via substrate-level phosphorylation.

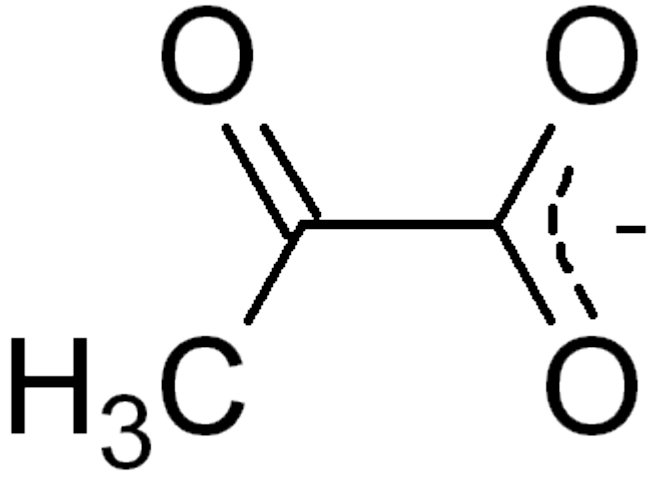
Effect of oxygen

The fermentation process does not require oxygen. If oxygen is present, some species of yeast (*Kluyveromyces lactis*, *Kluyveromyces lipolytica*) oxidize pyruvate completely to carbon dioxide and water. This process is called respiration. Thus these yeasts produce ethanol only in an anaerobic environment.

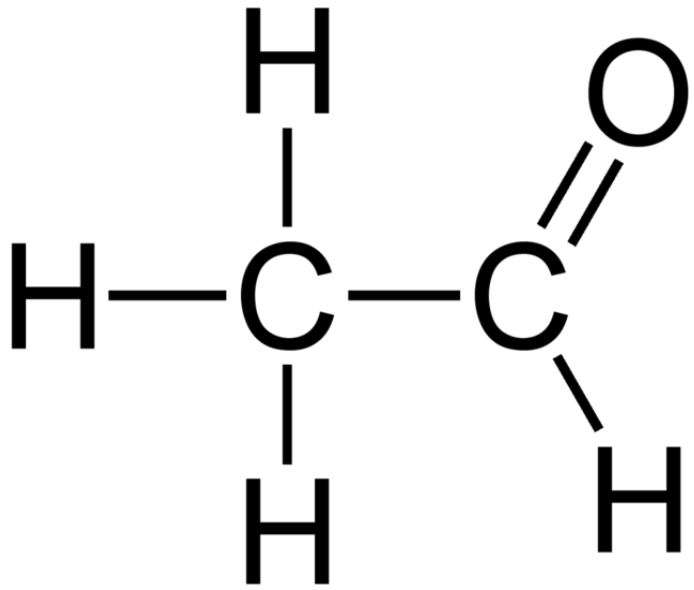
However, many yeasts such as the commonly used baker's yeast *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe*, prefer fermentation to respiration. These yeasts will produce ethanol even under aerobic conditions given the right sources of nutrition.



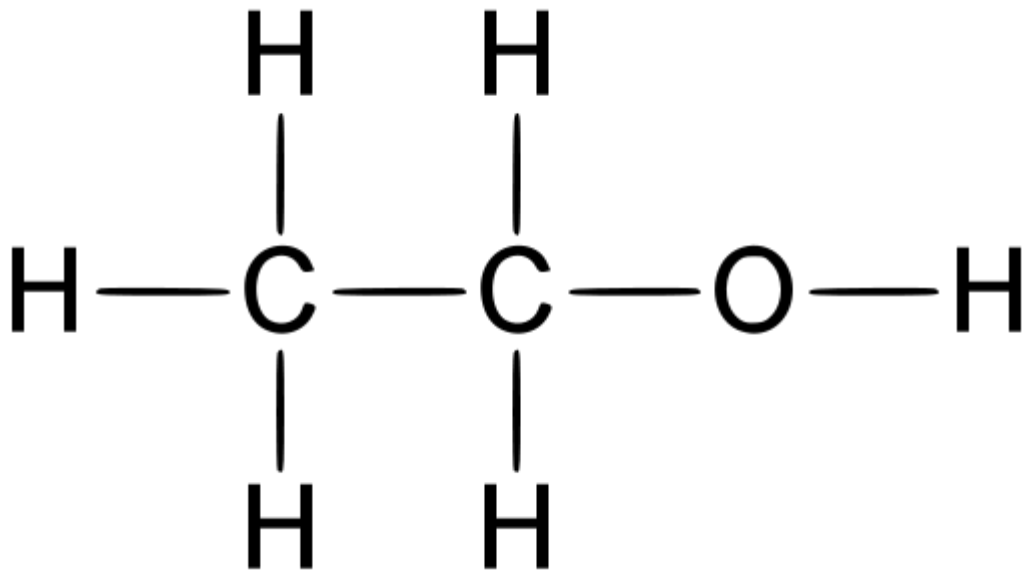
Glucose depicted in Haworth projection



Pyruvate



Acetaldehyde



Ethanol

Uses

The formation of carbon dioxide — a byproduct of yeast's respiration — causes bread to rise.

Ethanol fermentation is responsible for the rising of bread dough. Yeast organisms consume sugars in the dough and produce ethanol and carbon dioxide as waste products. The carbon dioxide forms bubbles in the dough, expanding it into something of a foam. Nearly all the ethanol evaporates from the dough when the bread is baked.

All alcoholic beverages, except those produced by carbonic maceration, are produced by ethanol fermentation by yeast. Wine and brandy are produced by fermentation of the natural sugars present in fruits, especially grapes. Beer and whiskey are produced by fermentation of grain starches that have been converted to sugar by the enzyme amylase, which is present in grain kernels that have been germinated. Amylase-treated grain or amylase-treated potatoes are fermented for the production of vodka. Rum is produced by fermentation of cane sugar. In all cases, the fermentation must take place in a vessel that allows carbon dioxide to escape, but prevents outside air from coming in, as exposure to oxygen would prevent the formation of ethanol.

Similar yeast fermentation of various carbohydrate products is used to produce much of the ethanol used for fuel.

Feedstocks for fuel production

The dominant ethanol feedstock in warmer regions is sugarcane. In temperate regions, sugar beet is sometimes used instead.

In the United States, the main feedstock for the production of ethanol is currently corn. Approximately 2.8 gallons of ethanol are produced from one bushel of corn (0.42 liter per kilogram). While much of the corn turns into ethanol, some of the corn also yields by-products such as DDGS (distillers dried grains with solubles) that can be used as feed for livestock. A bushel of corn produces about 18 pounds of DDGS (320 kilograms of DDGS per metric ton of maize). Although most of the fermentation plants have been built in corn-producing regions, sorghum is also an important feedstock for ethanol production in the Plains states. Pearl millet is showing promise as an ethanol feedstock for the southeastern U.S. and the potential of duckweed is being studied.

In some parts of Europe, particularly France and Italy, grapes have become a *de facto* feedstock for fuel ethanol by the distillation of surplus wine. In Japan, it has been proposed to use rice normally made into sake as an ethanol source.

Cassava as ethanol feedstock

Ethanol can be made from mineral oil or from sugars or starches. Starches are cheapest. The starchy crop with highest energy content per acre is cassava, which grows in tropical countries.

Thailand already had a large cassava industry in the 1990s, for use as cattle feed and as a cheap admixture to wheat flour. Nigeria and Ghana are already establishing cassava-to-ethanol plants. Brazil is doing that too (sugarcane and cassava grow on very different

types of soil). So are many other countries. Production of ethanol from cassava is currently economically feasible when crude oil prices are above US$120 per barrel.

New varieties of cassava are being developed, so the future situation remains uncertain. Currently, cassava can yield between 25-40 tonnes per hectare (with irrigation and fertilizer), and from a tonne of cassava roots, circa 200 liters of ethanol can be produced (assuming cassava with 22% starch content). A liter of ethanol contains circa 21.46 MJ of energy. The overall energy efficiency of cassava-root to ethanol conversion is circa 32%.

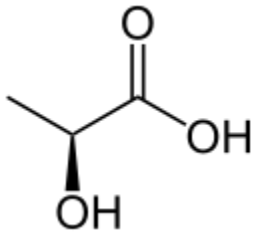
The yeast used for processing cassava is *Endomycopsis fibuligera*, sometimes used together with bacterium *Zymomonas mobilis*.

Microbes used in ethanol fermentation

- Yeast
- Zymomonas mobilis

Chapter- 5

Lactic Acid Fermentation



One isomer of lactic acid

Lactic acid fermentation is a biological process by which sugars such as glucose, fructose, and sucrose, are converted into cellular energy and the metabolic byproduct lactate. It is an anaerobic fermentation reaction that occurs in some bacteria and animal cells, such as muscle cells, in the absence of oxygen. If oxygen is present in the cell, many organisms will bypass fermentation and undergo cellular respiration; however, facultative anaerobic organisms will both ferment and undergo respiration in the presence of oxygen.

In *homofermentative fermentation*, one molecule of glucose is ultimately converted to two molecules of lactic acid. *Heterofermentative fermentation*, in contrast, yields carbon dioxide and ethanol in addition to lactic acid, in a process called the phosphoketolase pathway.

Chemical process

The process of lactic acid fermentation using glucose is summarized below. In homolactic fermentation, one molecule of glucose is converted to two molecules of lactic acid:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH$$

In heterolactic fermentation, the reaction proceeds as follows, with one molecule of glucose converted to one molecule of lactic acid, one molecule of ethanol, and one molecule of carbon dioxide:

$$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$$

Before lactic acid fermentation can occur, the molecule of glucose must be split into two molecules of pyruvate. This process is called glycolysis.

Glycolysis

To extract chemical energy from glucose, the glucose molecule must be split up into two molecules of pyruvate. This process also generates two molecules of adenosine triphosphate as an immediate energy yield and two molecules of NADH.

$$C_6H_{12}O_6 + 2\ ADP + 2\ P_i + 2\ NAD^+ \rightarrow 2\ CH_3COCOO^- + 2\ ATP + 2\ NADH + 2\ H_2O + 2H^+$$

In aerobic respiration, the pyruvate is further oxidized completely, generating additional ATP and NADH in the citric acid cycle and by oxidative phosphorylation. However, this can occur only in the presence of oxygen. Oxygen is toxic to organisms that are obligate anaerobes, and is not required by facultative anaerobic organisms. In the absence of oxygen, one of the fermentation pathways occurs in order to regenerate NAD^+; lactic acid fermentation is one of these pathways.

Fermentation

Lactic acid fermentation is the simplest type of fermentation. In essence, it is a redox reaction. In anaerobic conditions, the cell's primary mechanism of ATP production is glycolysis. Glycolysis reduces – that is, transfers electrons to – NAD^+, forming NADH. However, there is only a limited supply of NAD^+ available in a cell. For glycolysis to continue, NADH must be oxidized – that is, have electrons taken away – to regenerate the NAD^+. This is usually done through an electron transport chain in a process called oxidative phosphorylation; however, this mechanism is not available without oxygen.

Instead, the NADH donates its extra electrons to the pyruvate molecules formed during glycolysis. Since the NADH has lost electrons, NAD^+ regenerates and is again available for glycolysis. Lactic acid, for which this process is named, is formed by the reduction of pyruvate.

In homolactic acid fermentation, both molecules of pyruvate are converted to lactate. In heterolactic acid fermentation, one molecule of pyruvate is converted to lactate; the other is converted to ethanol and carbon dioxide. Homolactic acid fermentation is unique in that it is one of the only respiration processes that do not produce a gas as a byproduct.

Purpose

Some bacteria and yeasts organisms are unable to cope with the presence of oxygen. These organisms use fermentation as a method of obtaining energy in the form of ATP.

Because the production of lactic acid frees up NAD^+, the process of glycolysis can continue.

Lactic acid fermentation also occurs in animal muscle cells under conditions when oxygen is low. Extreme exercise would be an example of this. In this situation, the lactate is carried away by the circulatory system to the liver, where it is converted back to pyruvate through the Cori cycle.

Fermentation, however, is far less effective than cellular respiration, producing only two ATP molecules per glucose molecule consumed. The typical yield from cellular respiration is anywhere from 34-38 molecules of ATP. Thus, it is typically seen only in small organisms, such as bacteria and yeast, that can survive on this low energy yield.

Applications

Lactic acid fermentation is used in many areas of the world to produce foods that cannot be produced through other methods. The most commercially important genus of lactic acid-fermenting bacteria is *Lactobacillus*, though other bacteria and even yeast are sometimes used. Two of the most common applications of lactic acid fermentation are in the production of yogurt and sauerkraut.

Yogurt production

The main method of producing yogurt is through the lactic acid fermentation of milk with harmless bacteria. The primary bacteria used are typically *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and US law requires all yogurts to contain these two cultures (though others may be added as probiotic cultures). These bacteria produce lactic acid in the milk culture, decreasing its pH and causing it to congeal. The bacteria also produce compounds that give yogurt its distinctive flavor. An additional effect of the lowered pH is the incompatibility of the acidic environment with many other types of harmful bacteria.

For a probiotic yogurt, additional types of bacteria such as *Lactobacillus acidophilus* are also added to the culture.

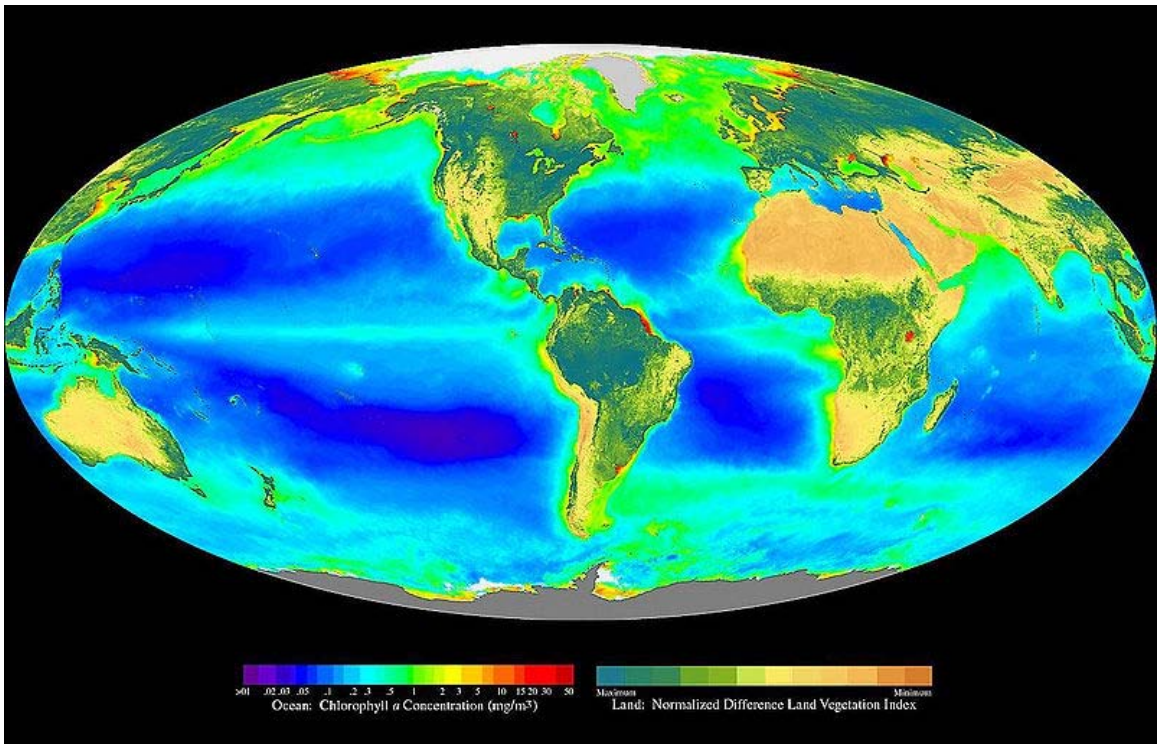
Sauerkraut

Lactic acid fermentation is also used in the production of sauerkraut. The main type of bacteria used in the production of sauerkraut is of the genus *Leuconostoc*.

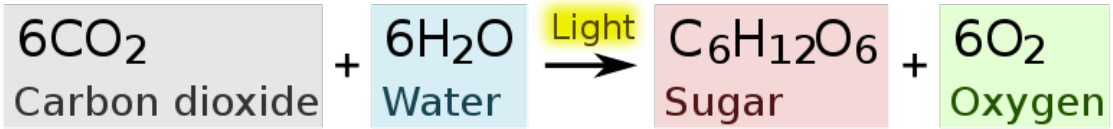
As in yogurt, when the acidity rises due to lactic acid-fermenting organisms, many other pathogenic microorganisms are killed. The bacteria produce lactic acid, as well as simple alcohols and other hydrocarbons. These may then combine to form esters, contributing to the unique flavor of sauerkraut.

Chapter- 6

Photosynthesis



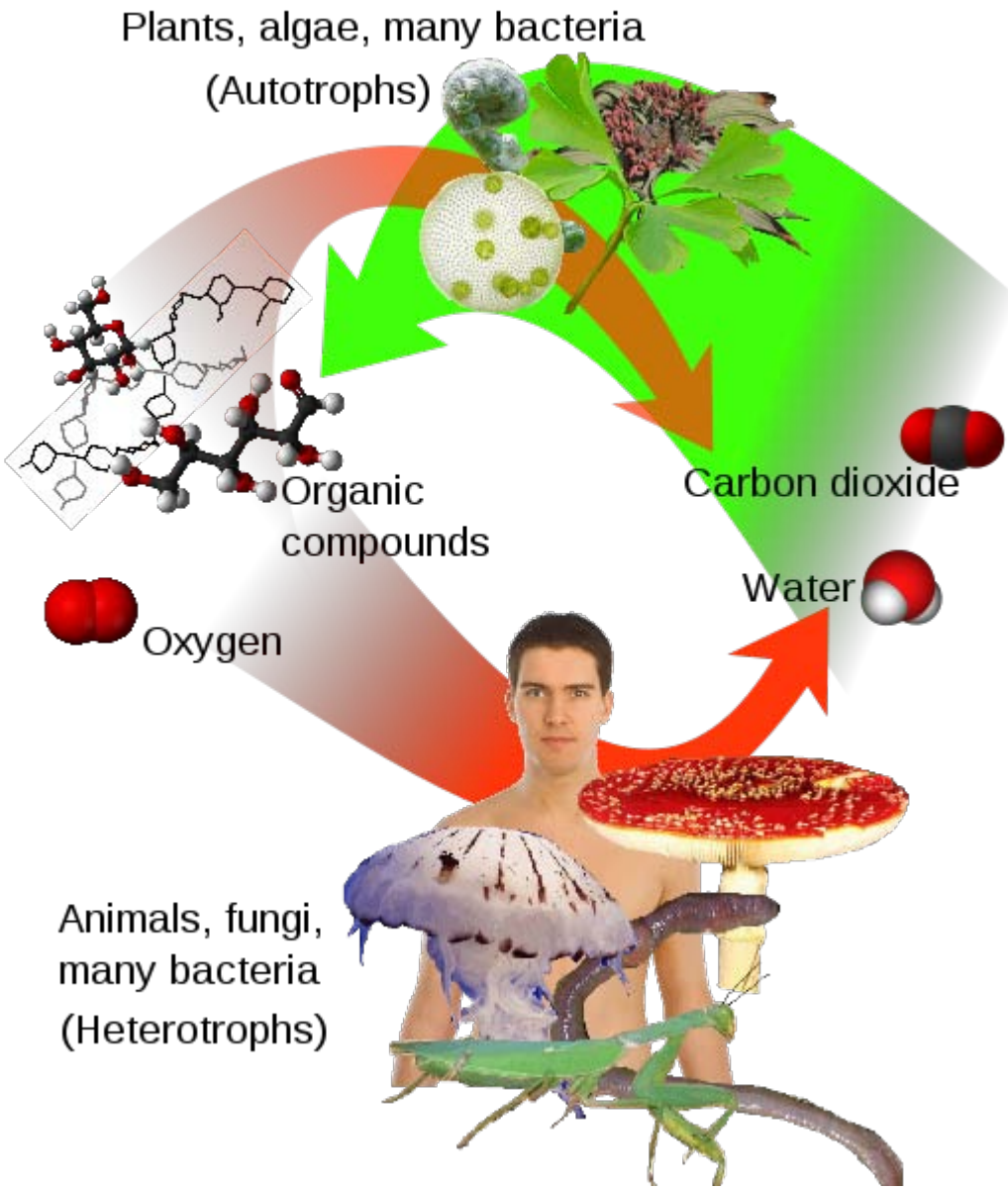
Composite image showing the global distribution of photosynthesis, including both oceanic phytoplankton and vegetation



Overall equation for the type of photosynthesis that occurs in plants

Photosynthesis is a process that converts carbon dioxide into organic compounds, especially sugars, using the energy from sunlight. Photosynthesis occurs in plants, algae, and many species of bacteria, but not in archaea. Photosynthetic organisms are called *photoautotrophs*, since they can create their own food. In plants, algae, and cyanobacteria, photosynthesis uses carbon dioxide and water, releasing oxygen as a waste product. Photosynthesis is vital for all aerobic life on Earth. As well as maintaining the normal level of oxygen in the atmosphere, nearly all life either depends on it directly as a source of energy, or indirectly as the ultimate source of the energy in their food (the exceptions are chemoautotrophs that live in rocks or around deep sea hydrothermal vents). The rate of energy capture by photosynthesis is immense, approximately 100 terawatts, which is about six times larger than the power consumption of human civilization. As well as energy, photosynthesis is also the source of the carbon in all the organic compounds within organisms' bodies. In all, photosynthetic organisms convert around 100–115 teragrams of carbon into biomass per year.

Although photosynthesis can happen in different ways in different species, some features are always the same. For example, the process always begins when energy from light is absorbed by proteins called photosynthetic reaction centers that contain chlorophylls. In plants, these proteins are held inside organelles called chloroplasts, while in bacteria they are embedded in the plasma membrane. Some of the light energy gathered by chlorophylls is stored in the form of adenosine triphosphate (ATP). The rest of the energy is used to remove electrons from a substance such as water. These electrons are then used in the reactions that turn carbon dioxide into organic compounds. In plants, algae and cyanobacteria, this is done by a sequence of reactions called the Calvin cycle, but different sets of reactions are found in some bacteria, such as the reverse Krebs cycle in *Chlorobium*. Many photosynthetic organisms have adaptations that concentrate or store carbon dioxide. This helps reduce a wasteful process called photorespiration that can consume part of the sugar produced during photosynthesis.

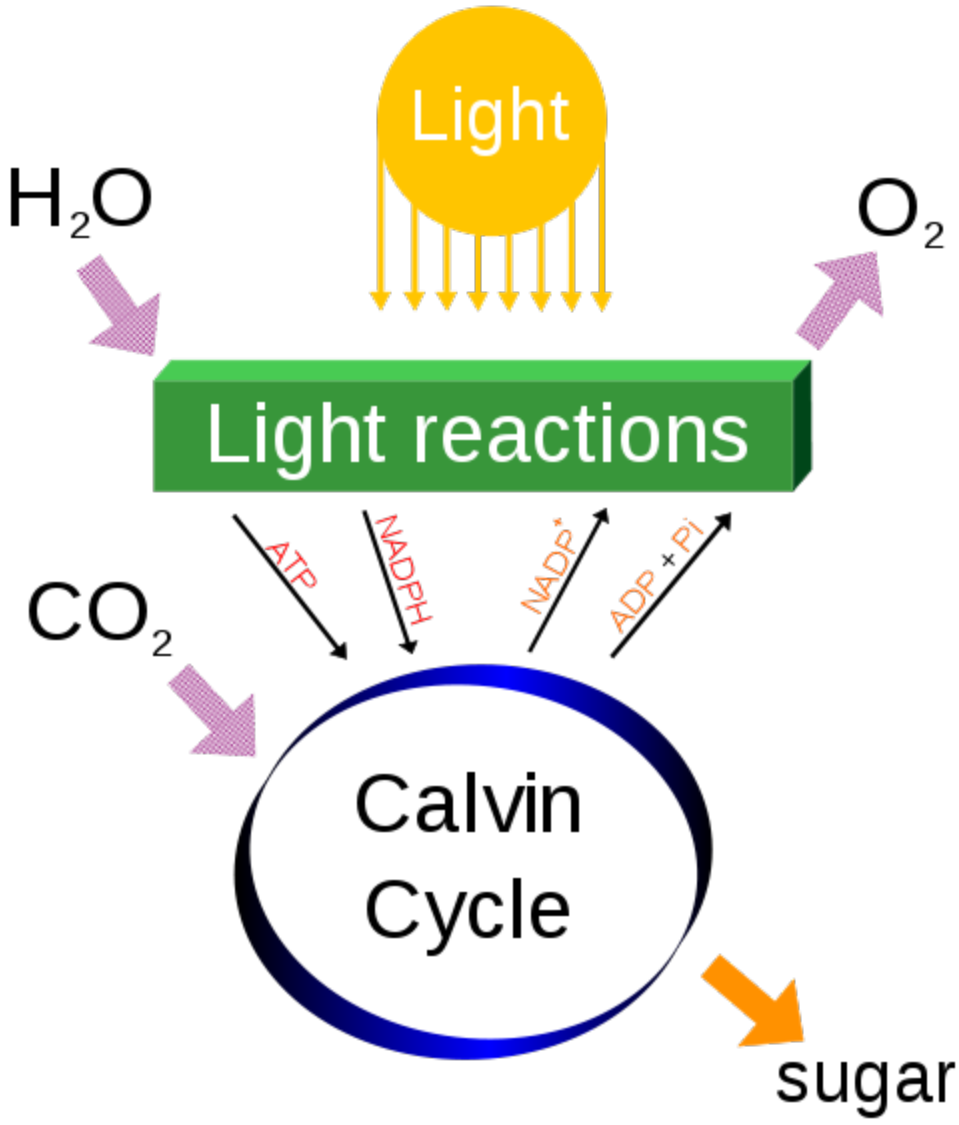


Overview of cycle between autotrophs and heterotrophs. Photosynthesis is the main means by which plants, algae and many bacteria produce organic compounds and oxygen from carbon dioxide and water (green arrow).

The first photosynthetic organisms probably evolved about 3,500 million years ago, early in the evolutionary history of life, when all forms of life on Earth were microorganisms and the atmosphere had much more carbon dioxide. They most likely used hydrogen or hydrogen sulfide as sources of electrons, rather than water. Cyanobacteria appeared later, around 3,000 million years ago, and drastically changed the Earth when they began to oxygenate the atmosphere, beginning about 2,400 million years ago. This new atmosphere allowed the evolution of complex life such as protists. Eventually, no later than a billion years ago, one of these protists formed a symbiotic relationship with a

cyanobacterium, producing the ancestor of many plants and algae. The chloroplasts in modern plants are the descendants of these ancient symbiotic cyanobacteria.

Overview



Photosynthesis changes the energy from the sun into chemical energy, splits water to liberate O_2, and fixes CO_2 into sugar.

Photosynthetic organisms are photoautotrophs, which means that they are able to synthesize food directly from carbon dioxide using energy from light. However, not all organisms that use light as a source of energy carry out photosynthesis, since *photoheterotrophs* use organic compounds, rather than carbon dioxide, as a source of carbon. In plants, algae and cyanobacteria, photosynthesis releases oxygen. This is called *oxygenic photosynthesis*. Although there are some differences between oxygenic

photosynthesis in plants, algae and cyanobacteria, the overall process is quite similar in these organisms. However, there are some types of bacteria that carry out anoxygenic photosynthesis, which consumes carbon dioxide but does not release oxygen.

Carbon dioxide is converted into sugars in a process called carbon fixation. Carbon fixation is a redox reaction, so photosynthesis needs to supply both a source of energy to drive this process, and the electrons needed to convert carbon dioxide into carbohydrate, which is a reduction reaction. In general outline, photosynthesis is the opposite of cellular respiration, where glucose and other compounds are oxidized to produce carbon dioxide, water, and release chemical energy. However, the two processes take place through a different sequence of chemical reactions and in different cellular compartments.

The general equation for photosynthesis is therefore:

$$2n\ CO_2 + 2n\ H_2O + \text{photons} \rightarrow 2(CH_2O)_n + n\ O_2 + 2n\ A$$

Carbon dioxide + electron donor + light energy → carbohydrate + oxygen + oxidized electron donor

Since water is used as the electron donor in oxygenic photosynthesis, the equation for this process is:

$$2n\ CO_2 + 2n\ H_2O + \text{photons} \rightarrow 2(CH_2O)_n + 2n\ O_2$$

carbon dioxide + water + light energy → carbohydrate + oxygen

Other processes substitute other compounds (such as arsenite) for water in the electron-supply role; the microbes use sunlight to oxidize arsenite to arsenate: The equation for this reaction is:

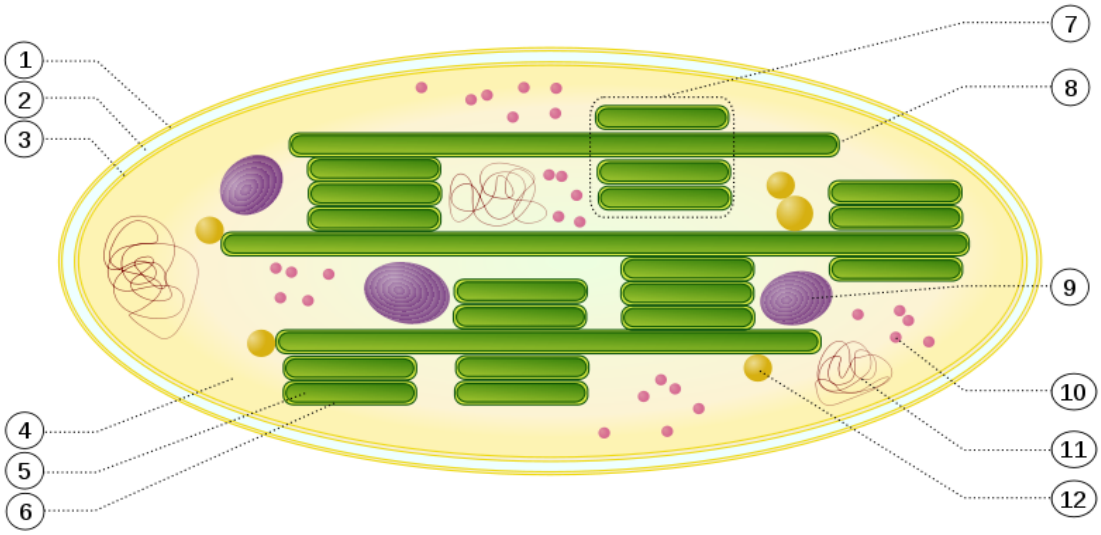
$$(AsO_3^{3-}) + CO_2 + \text{photons} \rightarrow CO + (AsO_4^{3-})$$

carbon dioxide + arsenite + light energy → arsenate + carbon monoxide (used to build other compounds in subsequent reactions)

Photosynthesis occurs in two stages. In the first stage, *light-dependent reactions* or *light reactions* capture the energy of light and use it to make the energy-storage molecules ATP and NADPH. During the second stage, the *light-independent reactions* use these products to capture and reduce carbon dioxide.

Most organisms that utilize photosynthesis to produce oxygen use visible light to do so, although at least three use infrared radiation.

Photosynthetic membranes and organelles



Chloroplast ultrastructure:
1. outer membrane
2. intermembrane space
3. inner membrane (1+2+3: envelope)
4. stroma (aqueous fluid)
5. thylakoid lumen (inside of thylakoid)
6. thylakoid membrane
7. granum (stack of thylakoids)
8. thylakoid (lamella)
9. starch
10. ribosome
11. plastidial DNA
12. plastoglobule (drop of lipids)

The proteins that gather light for photosynthesis are embedded within cell membranes. The simplest way these are arranged is in photosynthetic bacteria, where these proteins are held within the plasma membrane. However, this membrane may be tightly folded into cylindrical sheets called thylakoids, or bunched up into round vesicles called *intracytoplasmic membranes*. These structures can fill most of the interior of a cell, giving the membrane a very large surface area and therefore increasing the amount of light that the bacteria can absorb.

In plants and algae, photosynthesis takes place in organelles called chloroplasts. A typical plant cell contains about 10 to 100 chloroplasts. The chloroplast is enclosed by a membrane. This membrane is composed of a phospholipid inner membrane, a phospholipid outer membrane, and an intermembrane space between them. Within the membrane is an aqueous fluid called the stroma. The stroma contains stacks (grana) of thylakoids, which are the site of photosynthesis. The thylakoids are flattened disks, bounded by a membrane with a lumen or thylakoid space within it. The site of

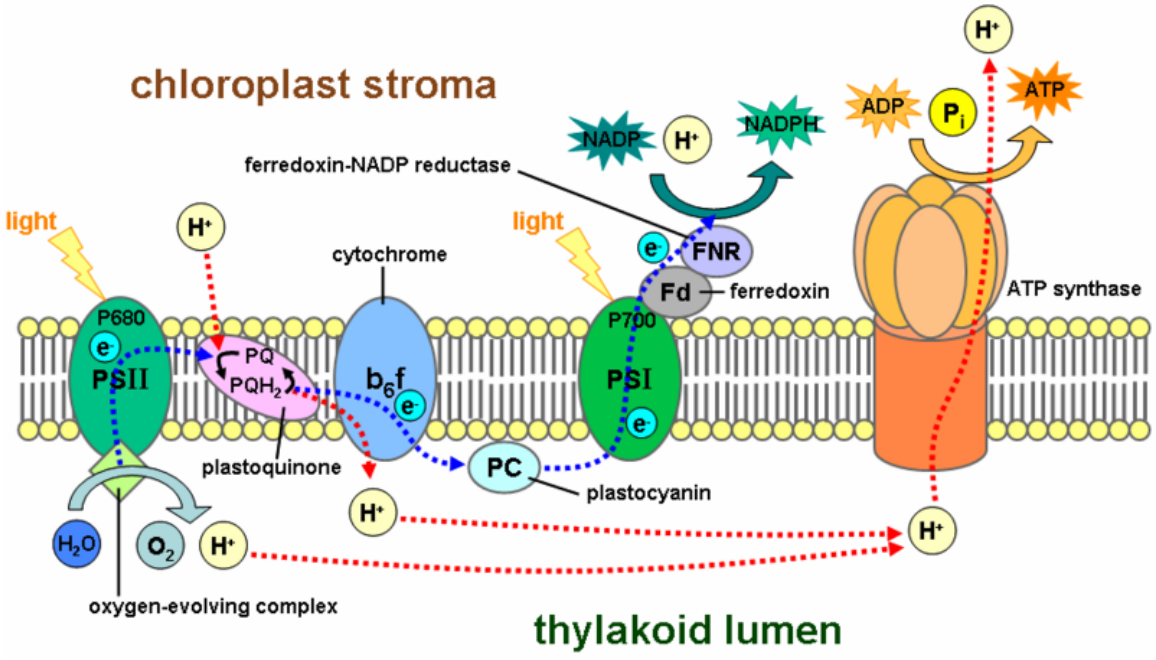
photosynthesis is the thylakoid membrane, which contains integral and peripheral membrane protein complexes, including the pigments that absorb light energy, which form the photosystems.

Plants absorb light primarily using the pigment chlorophyll, which is the reason that most plants have a green color. Besides chlorophyll, plants also use pigments such as carotenes and xanthophylls. Algae also use chlorophyll, but various other pigments are present as phycocyanin, carotenes, and xanthophylls in green algae, phycoerythrin in red algae (rhodophytes) and fucoxanthin in brown algae and diatoms resulting in a wide variety of colors.

These pigments are embedded in plants and algae in special antenna-proteins. In such proteins all the pigments are ordered to work well together. Such a protein is also called a light-harvesting complex.

Although all cells in the green parts of a plant have chloroplasts, most of the energy is captured in the leaves. The cells in the interior tissues of a leaf, called the mesophyll, can contain between 450,000 and 800,000 chloroplasts for every square millimeter of leaf. The surface of the leaf is uniformly coated with a water-resistant waxy cuticle that protects the leaf from excessive evaporation of water and decreases the absorption of ultraviolet or blue light to reduce heating. The transparent epidermis layer allows light to pass through to the palisade mesophyll cells where most of the photosynthesis takes place.

Light reactions



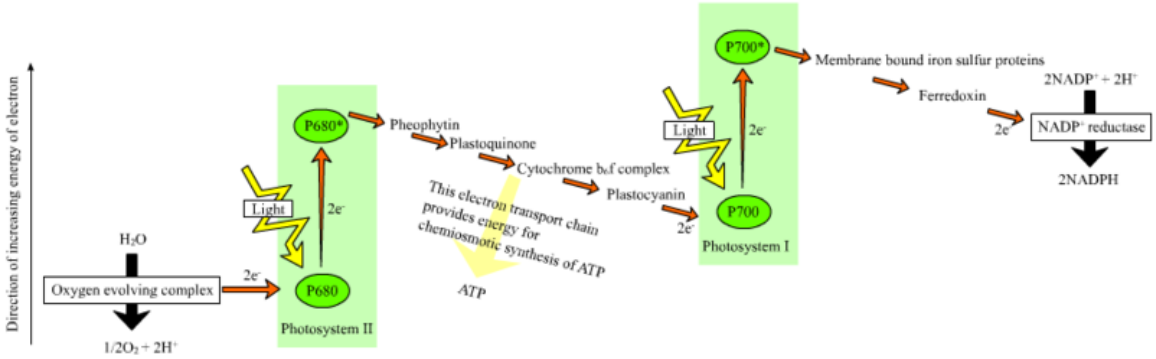
Light-dependent reactions of photosynthesis at the thylakoid membrane

In the light reactions, one molecule of the pigment chlorophyll absorbs one photon and loses one electron. This electron is passed to a modified form of chlorophyll called pheophytin, which passes the electron to a quinone molecule, allowing the start of a flow of electrons down an electron transport chain that leads to the ultimate reduction of NADP to NADPH. In addition, this creates a proton gradient across the chloroplast membrane; its dissipation is used by ATP synthase for the concomitant synthesis of ATP. The chlorophyll molecule regains the lost electron from a water molecule through a process called photolysis, which releases a dioxygen (O_2) molecule. The overall equation for the light-dependent reactions under the conditions of non-cyclic electron flow in green plants is:

$$2\ H_2O + 2\ NADP^+ + 3\ ADP + 3\ P_i + light \rightarrow 2\ NADPH + 2\ H^+ + 3\ ATP + O_2$$

Not all wavelengths of light can support photosynthesis. The photosynthetic action spectrum depends on the type of accessory pigments present. For example, in green plants, the action spectrum resembles the absorption spectrum for chlorophylls and carotenoids with peaks for violet-blue and red light. In red algae, the action spectrum overlaps with the absorption spectrum of phycobilins for blue-green light, which allows these algae to grow in deeper waters that filter out the longer wavelengths used by green plants. The non-absorbed part of the light spectrum is what gives photosynthetic organisms their color (e.g., green plants, red algae, purple bacteria) and is the least effective for photosynthesis in the respective organisms.

Z scheme



The "Z scheme"

In plants, light-dependent reactions occur in the thylakoid membranes of the chloroplasts and use light energy to synthesize ATP and NADPH. The light-dependent reaction has two forms: cyclic and non-cyclic. In the non-cyclic reaction, the photons are captured in the light-harvesting antenna complexes of photosystem II by chlorophyll and other accessory pigments. When a chlorophyll molecule at the core of the photosystem II reaction center obtains sufficient excitation energy from the adjacent antenna pigments, an electron is transferred to the primary electron-acceptor molecule, pheophytin, through a process called photoinduced charge separation. These electrons are shuttled through an electron transport chain, the so called ***Z-scheme*** shown in the diagram, that initially

functions to generate a chemiosmotic potential across the membrane. An ATP synthase enzyme uses the chemiosmotic potential to make ATP during photophosphorylation, whereas NADPH is a product of the terminal redox reaction in the *Z-scheme*. The electron enters a chlorophyll molecule in Photosystem I. The electron is excited due to the light absorbed by the photosystem. A second electron carrier accepts the electron, which again is passed down lowering energies of electron acceptors. The energy created by the electron acceptors is used to move hydrogen ions across the thylakoid membrane into the lumen. The electron is used to reduce the co-enzyme NADP, which has functions in the light-independent reaction. The cyclic reaction is similar to that of the non-cyclic, but differs in the form that it generates only ATP, and no reduced NADP (NADPH) is created. The cyclic reaction takes place only at photosystem I. Once the electron is displaced from the photosystem, the electron is passed down the electron acceptor molecules and returns back to photosystem I, from where it was emitted, hence the name *cyclic reaction*.

Water photolysis

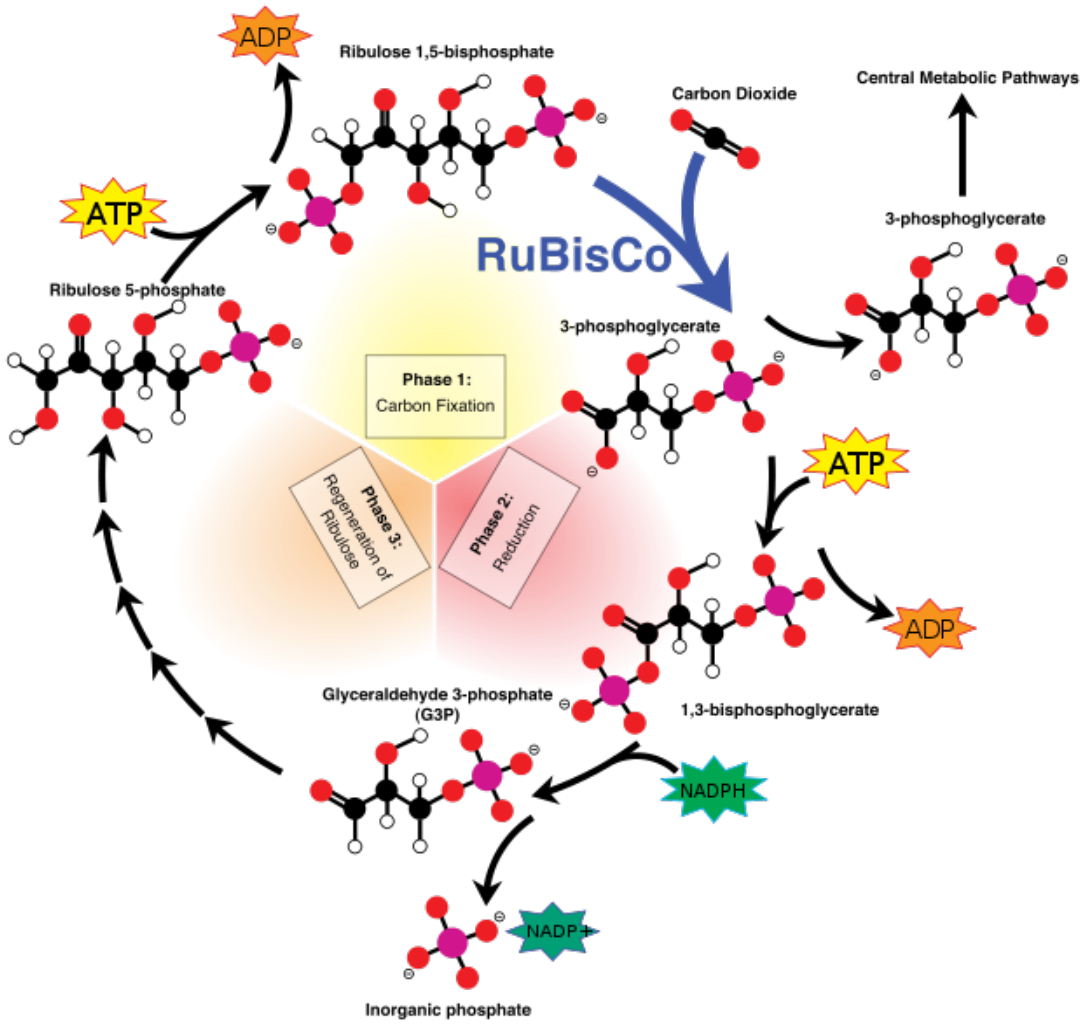
The NADPH is the main reducing agent in chloroplasts, providing a source of energetic electrons to other reactions. Its production leaves chlorophyll with a deficit of electrons (oxidized), which must be obtained from some other reducing agent. The excited electrons lost from chlorophyll in photosystem I are replaced from the electron transport chain by plastocyanin. However, since photosystem II includes the first steps of the *Z-scheme*, an external source of electrons is required to reduce its oxidized **chlorophyll *a*** molecules. The source of electrons in green-plant and cyanobacterial photosynthesis is water. Two water molecules are oxidized by four successive charge-separation reactions by photosystem II to yield a molecule of diatomic oxygen and four hydrogen ions; the electron yielded in each step is transferred to a redox-active tyrosine residue that then reduces the photoxidized paired-chlorophyll *a* species called P680 that serves as the primary (light-driven) electron donor in the photosystem II reaction center. The oxidation of water is catalyzed in photosystem II by a redox-active structure that contains four manganese ions and a calcium ion; this oxygen-evolving complex binds two water molecules and stores the four oxidizing equivalents that are required to drive the water-oxidizing reaction. Photosystem II is the only known biological enzyme that carries out this oxidation of water. The hydrogen ions contribute to the transmembrane chemiosmotic potential that leads to ATP synthesis. Oxygen is a waste product of light-dependent reactions, but the majority of organisms on Earth use oxygen for cellular respiration, including photosynthetic organisms.

Light-independent reactions

The Calvin Cycle

In the Light-independent or dark reactions the enzyme RuBisCO captures CO_2 from the atmosphere and in a process that requires the newly formed NADPH, called the Calvin-Benson Cycle, releases three-carbon sugars, which are later combined to form sucrose and starch. The overall equation for the light-independent reactions in green plants is:

$3\ CO_2 + 9\ ATP + 6\ NADPH + 6\ H^+ \rightarrow C_3H_6O_3\text{-phosphate} + 9\ ADP + 8\ P_i + 6\ NADP^+ + 3\ H_2O$



Overview of the Calvin cycle and carbon fixation

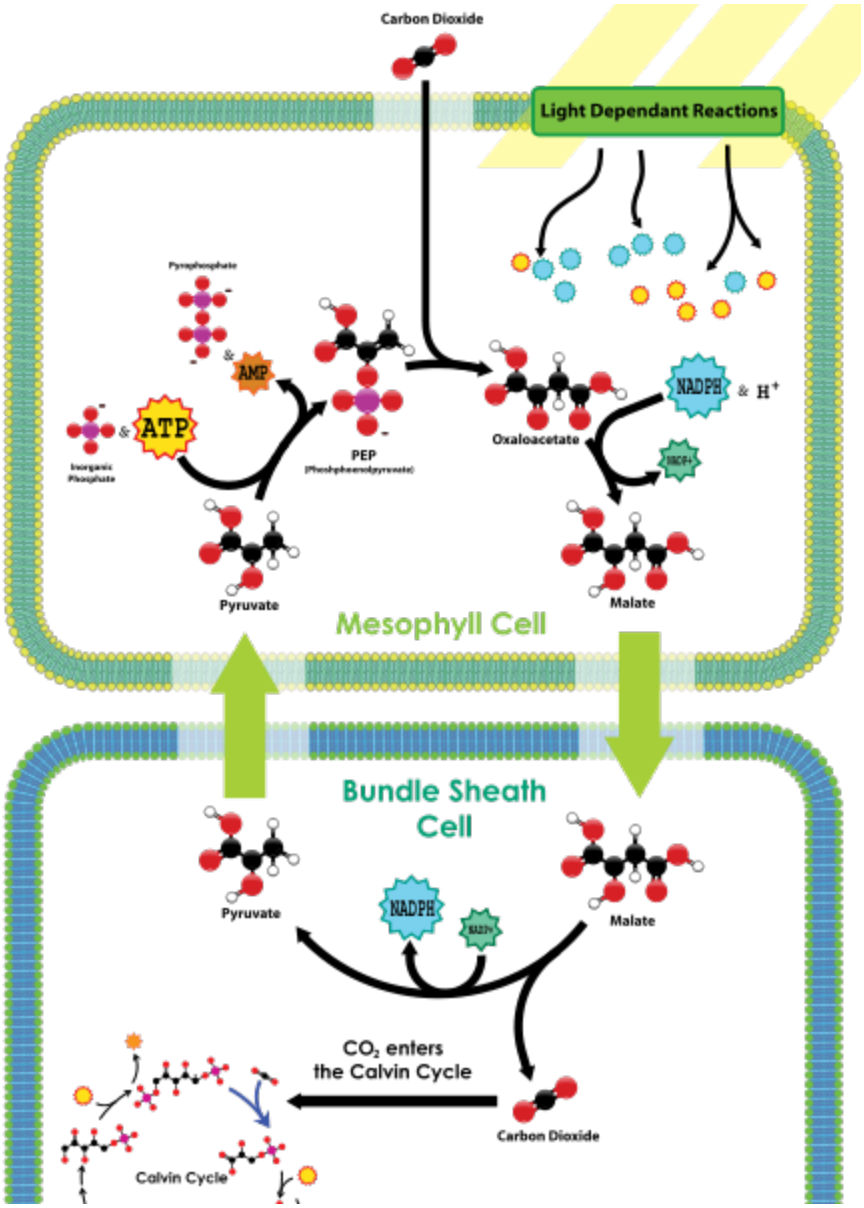
To be more specific, carbon fixation produces an intermediate product, which is then converted to the final carbohydrate products. The carbon skeletons produced by photosynthesis are then variously used to form other organic compounds, such as the building material cellulose, as precursors for lipid and amino acid biosynthesis, or as a

fuel in cellular respiration. The latter occurs not only in plants but also in animals when the energy from plants gets passed through a food chain.

The fixation or reduction of carbon dioxide is a process in which carbon dioxide combines with a five-carbon sugar, ribulose 1,5-bisphosphate (RuBP), to yield two molecules of a three-carbon compound, glycerate 3-phosphate (GP), also known as 3-phosphoglycerate (PGA). GP, in the presence of ATP and NADPH from the light-dependent stages, is reduced to glyceraldehyde 3-phosphate (G3P). This product is also referred to as 3-phosphoglyceraldehyde (PGAL) or even as triose phosphate. Triose is a 3-carbon sugar. Most (5 out of 6 molecules) of the G3P produced is used to regenerate RuBP so the process can continue. The 1 out of 6 molecules of the triose phosphates not "recycled" often condense to form hexose phosphates, which ultimately yield sucrose, starch and cellulose. The sugars produced during carbon metabolism yield carbon skeletons that can be used for other metabolic reactions like the production of amino acids and lipids.

Carbon concentrating mechanisms

On land



Overview of C4 carbon fixation

In hot and dry conditions, plants close their stomata to prevent the loss of water. Under these conditions, CO_2 will decrease, and oxygen gas, produced by the light reactions of photosynthesis, will decrease in the stem, not leaves, causing an increase of photorespiration by the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase and decrease in carbon fixation. Some plants have evolved mechanisms to increase the CO_2 concentration in the leaves under these conditions.

C_4 plants chemically fix carbon dioxide in the cells of the mesophyll by adding it to the three-carbon molecule phosphoenolpyruvate (PEP), a reaction catalyzed by an enzyme called PEP carboxylase and which creates the four-carbon organic acid, oxaloacetic acid. Oxaloacetic acid or malate synthesized by this process is then translocated to specialized bundle sheath cells where the enzyme, rubisco, and other Calvin cycle enzymes are located, and where CO_2 released by decarboxylation of the four-carbon acids is then fixed by rubisco activity to the three-carbon sugar 3-phosphoglyceric acids. The physical separation of rubisco from the oxygen-generating light reactions reduces photorespiration and increases CO_2 fixation and thus photosynthetic capacity of the leaf. C_4 plants can produce more sugar than C_3 plants in conditions of high light and temperature. Many important crop plants are C_4 plants, including maize, sorghum, sugarcane, and millet. Plants that do not use PEP-carboxylase in carbon fixation are called C_3 plants because the primary carboxylation reaction, catalyzed by rubisco, produces the three-carbon sugar 3-phosphoglyceric acids directly in the Calvin-Benson cycle. Over 90% of plants use C_3 carbon fixation, compared to 3% that use C_4 carbon fixation.

Xerophytes, such as cacti and most succulents, also use PEP carboxylase to capture carbon dioxide in a process called Crassulacean acid metabolism (CAM). In contrast to C_4 metabolism, which *physically* separates the CO_2 fixation to PEP from the Calvin cycle, CAM *temporally* separates these two processes. CAM plants have a different leaf anatomy from C_3 plants, and fix the CO_2 at night, when their stomata are open. CAM plants store the CO_2 mostly in the form of malic acid via carboxylation of phosphoenolpyruvate to oxaloacetate, which is then reduced to malate. Decarboxylation of malate during the day releases CO_2 inside the leaves, thus allowing carbon fixation to 3-phosphoglycerate by rubisco. 16,000 species of plants use CAM.

In water

Cyanobacteria possess carboxysomes which increase the concentration of CO_2 around rubisco to increase the rate of photosynthesis. This operates by carbonic anhydrase producing hydrocarbonate ions (HCO_3^-) which are then pumped into the carboxysome, before being processed by a different carbonic anhydrase to produce CO_2. Pyrenoids in algae and hornworts also act to concentrate CO_2 around rubisco.

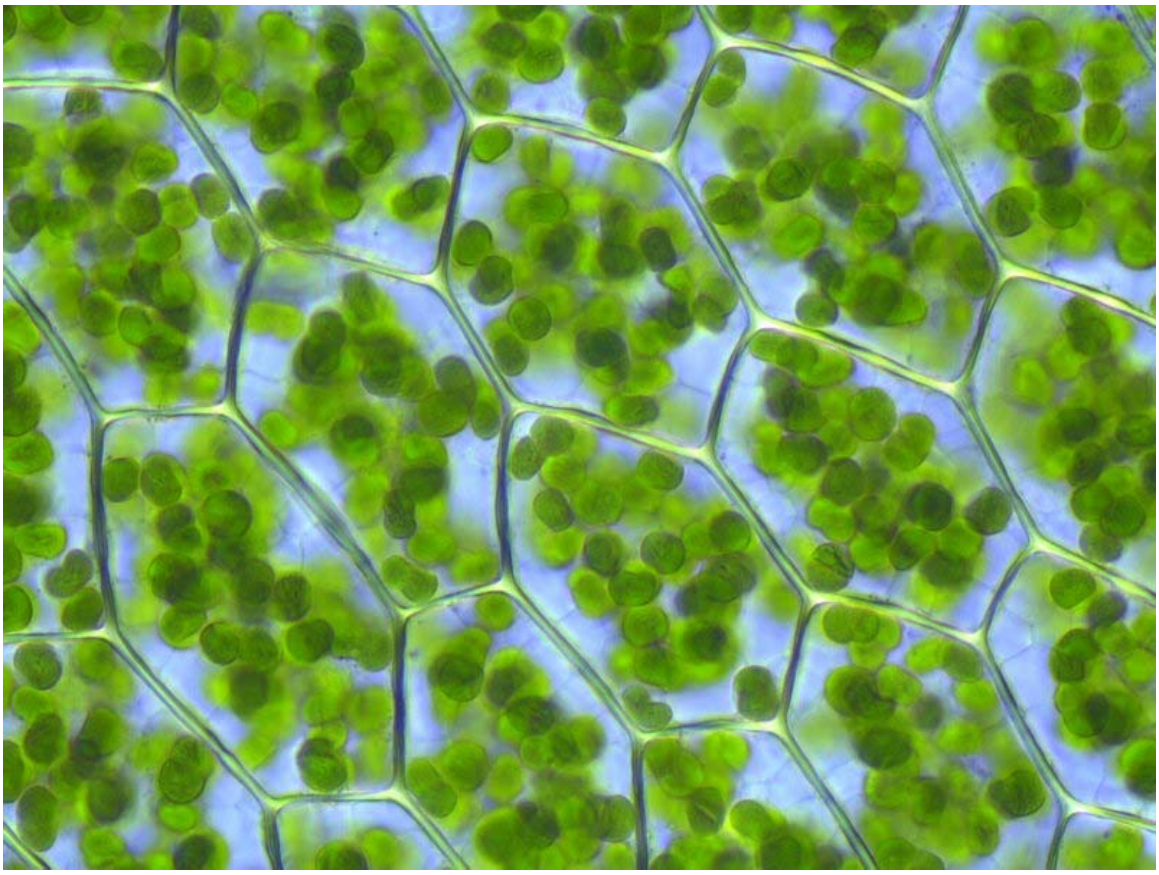
Order and kinetics

The overall process of photosynthesis takes place in four stages. The first, energy transfer in antenna chlorophyll takes place in the femtosecond (1 femtosecond (fs) = 10^{-15} s) to picosecond (1 picosecond (ps) = 10^{-12} s) time scale. The next phase, the transfer of electrons in photochemical reactions, takes place in the picosecond to nanosecond time scale (1 nanosecond (ns) = 10^{-9} s). The third phase, the electron transport chain and ATP synthesis, takes place on the microsecond (1 microsecond (μs) = 10^{-6} s) to millisecond (1 millisecond (ms) = 10^{-3} s) time scale. The final phase is carbon fixation and export of stable products and takes place in the millisecond to second time scale. The first three stages occur in the thylakoid membranes.

Efficiency

Plants usually convert light into chemical energy with a photosynthetic efficiency of 3–6%. Actual plants' photosynthetic efficiency varies with the frequency of the light being converted, light intensity, temperature and proportion of carbon dioxide in the atmosphere, and can vary from 0.1% to 8%. By comparison, solar panels convert light into electric energy at an efficiency of approximately 6–20% for mass-produced panels, and up to 41% in a research laboratory.

Evolution



Plant cells with visible chloroplasts (from a moss, *Plagiomnium affine*)

Early photosynthetic systems, such as those from green and purple sulfur and green and purple nonsulfur bacteria, are thought to have been anoxygenic, using various molecules as electron donors. Green and purple sulfur bacteria are thought to have used hydrogen and sulfur as an electron donor. Green nonsulfur bacteria used various amino and other organic acids. Purple nonsulfur bacteria used a variety of nonspecific organic molecules. The use of these molecules is consistent with the geological evidence that the atmosphere was highly reduced at that time.

Fossils of what are thought to be filamentous photosynthetic organisms have been dated at 3.4 billion years old.

The main source of oxygen in the atmosphere is oxygenic photosynthesis, and its first appearance is sometimes referred to as the oxygen catastrophe. Geological evidence suggests that oxygenic photosynthesis, such as that in cyanobacteria, became important during the Paleoproterozoic era around 2 billion years ago. Modern photosynthesis in plants and most photosynthetic prokaryotes is oxygenic. Oxygenic photosynthesis uses water as an electron donor, which is oxidized to molecular oxygen (O_2) in the photosynthetic reaction center.

Symbiosis and the origin of chloroplasts

Several groups of animals have formed symbiotic relationships with photosynthetic algae. These are most common in corals, sponges and sea anemones, possibly due to these animals having particularly simple body plans and large surface areas compared to their volumes. In addition, a few marine mollusks *Elysia viridis* and *Elysia chlorotica* also maintain a symbiotic relationship with chloroplasts they capture from the algae in their diet and then store in their bodies. This allows the molluscs to survive solely by photosynthesis for several months at a time. Some of the genes from the plant cell nucleus have even been transferred to the slugs, so that the chloroplasts can be supplied with proteins that they need to survive.

An even closer form of symbiosis may explain the origin of chloroplasts. Chloroplasts have many similarities with photosynthetic bacteria, including a circular chromosome, prokaryotic-type ribosomes, and similar proteins in the photosynthetic reaction center. The endosymbiotic theory suggests that photosynthetic bacteria were acquired (by endocytosis) by early eukaryotic cells to form the first plant cells. Therefore, chloroplasts may be photosynthetic bacteria that adapted to life inside plant cells. Like mitochondria, chloroplasts still possess their own DNA, separate from the nuclear DNA of their plant host cells and the genes in this chloroplast DNA resemble those in cyanobacteria. DNA in chloroplasts codes for redox proteins such as photosynthetic reaction centers. The CoRR Hypothesis proposes that this **Co**-location is required for **R**edox **R**egulation.

Cyanobacteria and the evolution of photosynthesis

The biochemical capacity to use water as the source for electrons in photosynthesis evolved once, in a common ancestor of extant cyanobacteria. The geological record indicates that this transforming event took place early in Earth's history, at least 2450–2320 million years ago (Ma), and possibly much earlier. Available evidence from geobiological studies of Archean (>2500 Ma) sedimentary rocks indicates that life existed 3500 Ma, but the question of when oxygenic photosynthesis evolved is still unanswered. A clear paleontological window on cyanobacterial evolution opened about 2000 Ma, revealing an already-diverse biota of blue-greens. Cyanobacteria remained principal primary producers throughout the Proterozoic Eon (2500–543 Ma), in part because the redox structure of the oceans favored photoautotrophs capable of nitrogen

fixation. Green algae joined blue-greens as major primary producers on continental shelves near the end of the Proterozoic, but only with the Mesozoic (251–65 Ma) radiations of dinoflagellates, coccolithophorids, and diatoms did primary production in marine shelf waters take modern form. Cyanobacteria remain critical to marine ecosystems as primary producers in oceanic gyres, as agents of biological nitrogen fixation, and, in modified form, as the plastids of marine algae.

A 2010 study by researchers at Tel Aviv University discovered that the Oriental hornet (*Vespa orientalis*) converts sunlight into electric power using a pigment called xanthopterin. This is the first scientific evidence of a member of the animal kingdom engaging in photosynthesis.

Discovery

Although some of the steps in photosynthesis are still not completely understood, the overall photosynthetic equation has been known since the 19th century.

Jan van Helmont began the research of the process in the mid-17th century when he carefully measured the mass of the soil used by a plant and the mass of the plant as it grew. After noticing that the soil mass changed very little, he hypothesized that the mass of the growing plant must come from the water, the only substance he added to the potted plant. His hypothesis was partially accurate—much of the gained mass also comes from carbon dioxide as well as water. However, this was a signaling point to the idea that the bulk of a plant's biomass comes from the inputs of photosynthesis, not the soil itself.

Joseph Priestley, a chemist and minister, discovered that when he isolated a volume of air under an inverted jar, and burned a candle in it, the candle would burn out very quickly, much before it ran out of wax. He further discovered that a mouse could similarly "injure" air. He then showed that the air that had been "injured" by the candle and the mouse could be restored by a plant.

In 1778, Jan Ingenhousz, court physician to the Austrian Empress, repeated Priestley's experiments. He discovered that it was the influence of sunlight on the plant that could cause it to revive a mouse in a matter of hours.

In 1796, Jean Senebier, a Swiss pastor, botanist, and naturalist, demonstrated that green plants consume carbon dioxide and release oxygen under the influence of light. Soon afterwards, Nicolas-Théodore de Saussure showed that the increase in mass of the plant as it grows could not be due only to uptake of CO_2, but also to the incorporation of water. Thus the basic reaction by which photosynthesis is used to produce food (such as glucose) was outlined.

Cornelis Van Niel made key discoveries explaining the chemistry of photosynthesis. By studying purple sulfur bacteria and green bacteria he was the first scientist to demonstrate that photosynthesis is a light-dependent redox reaction, in which hydrogen reduces carbon dioxide.

Robert Emerson discovered two light reactions by testing plant productivity using different wavelengths of light. With the red alone, the light reactions were suppressed. When blue and red were combined, the output was much more substantial. Thus, there were two photosystems, one aborbing up to 600 nm wavelengths, the other up to 700. The former is known as PSII, the latter is PSI. PSI contains only chlorophyll a, PSII contains primarily chlorophyll a with most of the available chlorophyll b, among other pigments.

Further experiments to prove that the oxygen developed during the photosynthesis of green plants came from water, were performed by Robert Hill in 1937 and 1939. He showed that isolated chloroplasts give off oxygen in the presence of unnatural reducing agents like iron oxalate, ferricyanide or benzoquinone after exposure to light. The Hill reaction is as follows:

$$2\ H_2O + 2\ A + (\text{light, chloroplasts}) \rightarrow 2\ AH_2 + O_2$$

where A is the electron acceptor. Therefore, in light the electron acceptor is reduced and oxygen is evolved. Cyt b_6, now known as a plastoquinone, is one electron acceptor.

Samuel Ruben and Martin Kamen used radioactive isotopes to determine that the oxygen liberated in photosynthesis came from the water.

Melvin Calvin and Andrew Benson, along with James Bassham, elucidated the path of carbon assimilation (the photosynthetic carbon reduction cycle) in plants. The carbon reduction cycle is known as the Calvin cycle, which inappropriately ignores the contribution of Bassham and Benson. Many scientists refer to the cycle as the Calvin-Benson Cycle, Benson-Calvin, and some even call it the Calvin-Benson-Bassham (or CBB) Cycle.

A Nobel Prize winning scientist, Rudolph A. Marcus, was able to discover the function and significance of the electron transport chain.

Otto Heinrich Warburg and Dean Burk discovered the I-quantum photosynthesis reaction that splits the CO2, activated by the respiration.

Factors

The leaf is the primary site of photosynthesis in plants.

There are three main factors affecting photosynthesis and several corollary factors. The three main are:

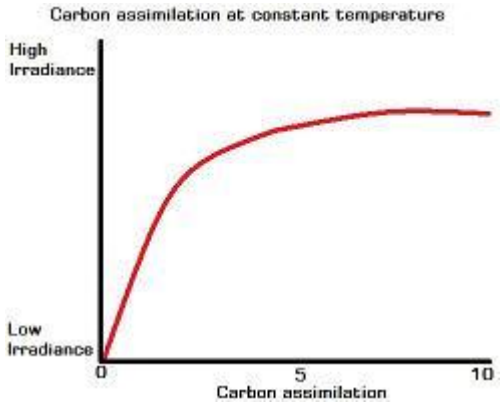
- Light irradiance and wavelength
- Carbon dioxide concentration
- Temperature.

Light intensity (irradiance), wavelength and temperature

In the early 20th century Frederick Frost Blackman along with Albert Einstein investigated the effects of light intensity (irradiance) and temperature on the rate of carbon assimilation.

- At constant temperature, the rate of carbon assimilation varies with irradiance, initially increasing as the irradiance increases. However at higher irradiance this relationship no longer holds and the rate of carbon assimilation reaches a plateau.
- At constant irradiance, the rate of carbon assimilation increases as the temperature is increased over a limited range. This effect is only seen at high irradiance levels.

At low irradiance, increasing the temperature has little influence on the rate of carbon assimilation.



Carbon assimilation at a constant temperature.

These two experiments illustrate vital points: firstly, from research it is known that photochemical reactions are not generally affected by temperature. However, these experiments clearly show that temperature affects the rate of carbon assimilation, so there must be two sets of reactions in the full process of carbon assimilation. These are of course the light-dependent 'photochemical' stage and the light-independent, temperature-dependent stage. Second, Blackman's experiments illustrate the concept of limiting factors. Another limiting factor is the wavelength of light. Cyanobacteria, which reside several meters underwater, cannot receive the correct wavelengths required to cause photoinduced charge separation in conventional photosynthetic pigments. To combat this problem, a series of proteins with different pigments surround the reaction center.This unit is called a phycobilisome.

Carbon dioxide levels and photorespiration

As carbon dioxide concentrations rise, the rate at which sugars are made by the light-independent reactions increases until limited by other factors. RuBisCO, the enzyme that captures carbon dioxide in the light-independent reactions, has a binding affinity for both carbon dioxide and oxygen. When the concentration of carbon dioxide is high, RuBisCO will fix carbon dioxide. However, if the carbon dioxide concentration is low, RuBisCO will bind oxygen instead of carbon dioxide. This process, called photorespiration, uses energy, but does not produce sugars.

RuBisCO oxygenase activity is disadvantageous to plants for several reasons:

1. One product of oxygenase activity is phosphoglycolate (2 carbon) instead of 3-phosphoglycerate (3 carbon). Phosphoglycolate cannot be metabolized by the Calvin-Benson cycle and represents carbon lost from the cycle. A high oxygenase activity, therefore, drains the sugars that are required to recycle ribulose 5-bisphosphate and for the continuation of the Calvin-Benson cycle.

2. Phosphoglycolate is quickly metabolized to glycolate that is toxic to a plant at a high concentration; it inhibits photosynthesis.
3. Salvaging glycolate is an energetically expensive process that uses the glycolate pathway and only 75% of the carbon is returned to the Calvin-Benson cycle as 3-phosphoglycerate. The reactions also produce ammonia (NH_3) which is able to diffuse out of the plant leading to a loss of nitrogen.

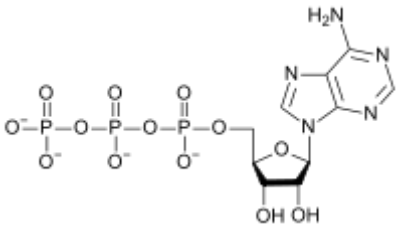
   A highly simplified summary is:
   2 glycolate + ATP → 3-phosphoglycerate + carbon dioxide + ADP +NH_3

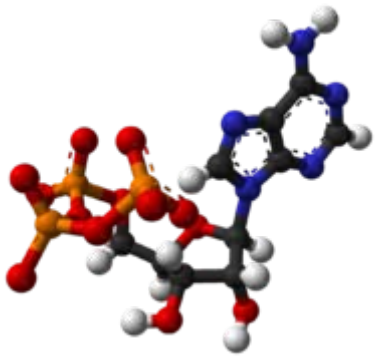
The salvaging pathway for the products of RuBisCO oxygenase activity is more commonly known as photorespiration, since it is characterized by light-dependent oxygen consumption and the release of carbon dioxide.

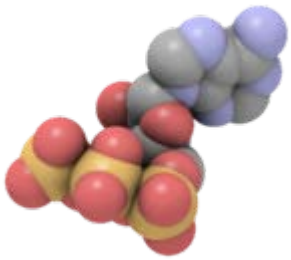
Chapter- 7

Adenosine Triphosphate

Adenosine triphosphate







IUPAC name
[(2*R*,3*S*,4*R*,5*R*)-5-(6-aminopurin-9-yl)-3,4-dihydroxyoxolan-2-yl]methyl(hydroxyphosphonooxyphosphoryl)hydrogen phosphate

Other names
adenosine 5'-(tetrahydrogen triphosphate)

Identifiers	
CAS number	56-65-5 ✓
PubChem	5957
ChemSpider	5742 ✓
UNII	8L70Q75FXE ✓
ChEMBL	CHEMBL14249 ✓
IUPHAR ligand	1713
Properties	
Molecular formula	$C_{10}H_{16}N_5O_{13}P_3$
Molar mass	507.18 g mol^{-1}
Density	1.04 g/cm^3 (disodium salt)
Melting point	187 °C (disodium salt) *decomposes*
Acidity (pK_a)	6.5

Adenosine-5'-triphosphate (**ATP**) is a multifunctional nucleotide used in cells as a coenzyme. It is often called the "molecular unit of currency" of intracellular energy transfer. ATP transports chemical energy within cells for metabolism. It is produced by photophosphorylation and cellular respiration and used by enzymes and structural proteins in many cellular processes, including biosynthetic reactions, motility, and cell division. One molecule of ATP contains three phosphate groups, and it is produced by ATP synthase from inorganic phosphate and adenosine diphosphate (ADP) or adenosine monophosphate (AMP).

Metabolic processes that use ATP as an energy source convert it back into its precursors. ATP is therefore continuously recycled in organisms: the human body, which on average contains 250 grams (8.8 oz) of ATP turns over its own weight in ATP each day.

ATP is used as a substrate in signal transduction pathways by kinases that phosphorylate proteins and lipids, as well as by adenylate cyclase, which uses ATP to produce the second messenger molecule cyclic AMP. The ratio between ATP and AMP is used as a way for a cell to sense how much energy is available and control the metabolic pathways that produce and consume ATP. Apart from its roles in energy metabolism and signaling, ATP is also incorporated into nucleic acids by polymerases in the processes of DNA replication and transcription.

The structure of this molecule consists of a purine base (adenine) attached to the 1' carbon atom of a pentose sugar (ribose). Three phosphate groups are attached at the 5' carbon atom of the pentose sugar. It is the addition and removal of these phosphate groups that inter-convert ATP, ADP and AMP. When ATP is used in DNA synthesis, the ribose sugar is first converted to deoxyribose by ribonucleotide reductase.

ATP was discovered in 1929 by Karl Lohmann, but its correct structure was not determined until some years later. It was proposed to be the main energy-transfer

molecule in the cell by Fritz Albert Lipmann in 1941. It was first artificially synthesized by Alexander Todd in 1948.

Physical and chemical properties

ATP consists of adenosine — composed of an adenine ring and a ribose sugar — and three phosphate groups (triphosphate). The phosphoryl groups, starting with the group closest to the ribose, are referred to as the alpha (α), beta (β), and gamma (γ) phosphates. Consequently, as a nucleotide, it (and its relatives ADP and AMP) is basically a monomer of RNA. ATP is highly soluble in water and is quite stable in solutions between pH 6.8–7.4, but is rapidly hydrolysed at extreme pH. Consequently, ATP is best stored as an anhydrous salt.

ATP is an unstable molecule in unbuffered water, in which it hydrolyses to ADP and phosphate. This is because the strength of the bonds between the phosphate groups in ATP are less than the strength of the hydrogen bonds (hydration bonds), between its products (ADP + phosphate), and water. Thus, if ATP and ADP are in chemical equilibrium in water, almost all of the ATP will eventually be converted to ADP. A system that is far from equilibrium contains Gibbs free energy, and is capable of doing work. Living cells maintain the ratio of ATP to ADP at a point ten orders of magnitude from equilibrium, with ATP concentrations a thousandfold higher than the concentration of ADP. This displacement from equilibrium means that the hydrolysis of ATP in the cell releases a large amount of free energy.

Two high-energy phosphate bonds (phosphoanhydride bonds) (those that connect adjacent phosphates) in an ATP molecule are responsible for the high energy content of this molecule. In the context of biochemical reactions, these anhydride bonds are frequently—and sometimes controversially—referred to as *high-energy bonds*. Energy stored in ATP may be released upon hydrolysis of the anhydride bonds. The bonds formed after hydrolysis—or the phosphorylation of a residue by ATP—are lower in energy than the phosphoanhydride bonds of ATP. During enzyme-catalyzed hydrolysis of ATP or phosphorylation by ATP, the available free energy can be harnessed by a living system to do work.

Any unstable system of potentially reactive molecules could potentially serve as a way of storing free energy, if the cell maintained their concentration far from the equilibrium point of the reaction. However, as is the case with most polymeric biomolecules, the breakdown of RNA, DNA, and ATP into simpler monomers is driven by both energy-release and entropy-increase considerations, in both standard concentrations, and also those concentrations encountered within the cell.

The standard amount of energy released from hydrolysis of ATP can be calculated from the changes in energy under non-natural (standard) conditions, then correcting to biological concentrations. The net change in heat energy (enthalpy) at standard temperature and pressure of the decomposition of ATP into hydrated ADP and hydrated inorganic phosphate is −20.5 kJ/mol, with a change in free energy of 3.4 kJ/mol. The

energy released by cleaving either a phosphate (P_i) or pyrophosphate (PP_i) unit from ATP at standard state of 1 M are:

$$ATP + H_2O \rightarrow ADP + P_i \quad \Delta G^\circ = -30.5 \text{ kJ/mol } (-7.3 \text{ kcal/mol})$$
$$ATP + H_2O \rightarrow AMP + PP_i \quad \Delta G^\circ = -45.6 \text{ kJ/mol } (-10.9 \text{ kcal/mol})$$

These values can be used to calculate the change in energy under physiological conditions and the cellular ATP/ADP ratio. However, a more representative value (which takes AMP into consideration) called the Energy charge is increasingly being employed. The values given for the Gibbs free energy for this reaction are dependent on a number of factors, including overall ionic strength and the presence of alkaline earth metal ions such as Mg^{2+} and Ca^{2+}. Under typical cellular conditions, ΔG is approximately −57 kJ/mol (−14 kcal/mol).

Ionization in biological systems

ATP has multiple ionizable groups with different acid dissociation constants. In neutral solution, ATP is ionized and exists mostly as ATP^{4-}, with a small proportion of ATP^{3-}. As ATP has several negatively charged groups in neutral solution, it can chelate metals with very high affinity. The binding constant for various metal ions are (given as per mole) as Mg^{2+} (9 554), Na^+ (13), Ca^{2+} (3 722), K^+ (8), Sr^{2+} (1 381) and Li^+ (25). Due to the strength of these interactions, ATP exists in the cell mostly in a complex with Mg^{2+}.

Biosynthesis

The ATP concentration inside the cell is typically 1–10 *mM*. ATP can be produced by redox reactions using simple and complex sugars (carbohydrates) or lipids as an energy source. For ATP to be synthesized from complex fuels, they first need to be broken down into their basic components. Carbohydrates are hydrolysed into simple sugars, such as glucose and fructose. Fats (triglycerides) are metabolised to give fatty acids and glycerol.

The overall process of oxidizing glucose to carbon dioxide is known as cellular respiration and can produce about 30 molecules of ATP from a single molecule of glucose. ATP can be produced by a number of distinct cellular processes; the three main pathways used to generate energy in eukaryotic organisms are glycolysis and the citric acid cycle/oxidative phosphorylation, both components of cellular respiration; and beta-oxidation. The majority of this ATP production by a non-photosynthetic aerobic eukaryote takes place in the mitochondria, which can make up nearly 25% of the total volume of a typical cell.

Glycolysis

In glycolysis, glucose and glycerol are metabolized to pyruvate via the glycolytic pathway. In most organisms, this process occurs in the cytosol, but in some protozoa such as the kinetoplastids, this is carried out in a specialized organelle called the glycosome. Glycolysis generates a net two molecules of ATP through substrate phosphorylation

catalyzed by two enzymes: PGK and pyruvate kinase. Two molecules of NADH are also produced, which can be oxidized via the electron transport chain and result in the generation of additional ATP by ATP synthase. The pyruvate generated as an end-product of glycolysis is a substrate for the Krebs Cycle.

Glucose

In the mitochondrion, pyruvate is oxidized by the pyruvate dehydrogenase complex to Acetyl group, which is fully oxidized to carbon dioxide by the citric acid cycle (also known as the Krebs Cycle). Every "turn" of the citric acid cycle produces two molecules of carbon dioxide, one molecule of the ATP equivalent guanosine triphosphate (GTP) through substrate-level phosphorylation catalyzed by succinyl-CoA synthetase, three molecules of the reduced coenzyme NADH, and one molecule of the reduced coenzyme $FADH_2$. Both of these latter molecules are recycled to their oxidized states (NAD^+ and FAD, respectively) via the electron transport chain, which generates additional ATP by oxidative phosphorylation. The oxidation of an NADH molecule results in the synthesis of between 2-3 ATP molecules, and the oxidation of one $FADH_2$ yields between 1-2 ATP molecules. The majority of cellular ATP is generated by this process. Although the citric acid cycle itself does not involve molecular oxygen, it is an obligately aerobic process because O_2 is needed to recycle the reduced NADH and $FADH_2$ to their oxidized states. In the absence of oxygen the citric acid cycle will cease to function due to the lack of available NAD^+ and FAD.

The generation of ATP by the mitochondrion from cytosolic NADH relies on the malate-aspartate shuttle (and to a lesser extent, the glycerol-phosphate shuttle) because the inner mitochondrial membrane is impermeable to NADH and NAD^+. Instead of transferring the generated NADH, a malate dehydrogenase enzyme converts oxaloacetate to malate, which is translocated to the mitochondrial matrix. Another malate dehydrogenase-catalyzed reaction occurs in the opposite direction, producing oxaloacetate and NADH from the newly transported malate and the mitochondrion's interior store of NAD^+. A transaminase converts the oxaloacetate to aspartate for transport back across the membrane and into the intermembrane space.

In oxidative phosphorylation, the passage of electrons from NADH and $FADH_2$ through the electron transport chain powers the pumping of protons out of the mitochondrial matrix and into the intermembrane space. This creates a proton motive force that is the net effect of a pH gradient and an electric potential gradient across the inner mitochondrial membrane. Flow of protons down this potential gradient — that is, from the intermembrane space to the matrix — provides the driving force for ATP synthesis by ATP synthase. This enzyme contains a rotor subunit that physically rotates relative to the static portions of the protein during ATP synthesis.

Most of the ATP synthesized in the mitochondria will be used for cellular processes in the cytosol; thus it must be exported from its site of synthesis in the mitochondrial matrix. The inner membrane contains an antiporter, the ADP/ATP translocase, which is an integral membrane protein used to exchange newly-synthesized ATP in the matrix for

ADP in the intermembrane space. This translocase is driven by the membrane potential, as it results in the movement of about 4 negative charges out of the mitochondrial membrane in exchange for 3 negative charges moved inside. However, it is also necessary to transport phosphate into the mitochondrion; the phosphate carrier moves a proton in with each phosphate, partially dissipating the proton gradient.

Beta oxidation

Fatty acids can also be broken down to acetyl-CoA by beta-oxidation. Each round of this cycle reduces the length of the acyl chain by two carbon atoms and produces one NADH and one $FADH_2$ molecule, which are used to generate ATP by oxidative phosphorylation. Because NADH and $FADH_2$ are energy-rich molecules, dozens of ATP molecules can be generated by the beta-oxidation of a single long acyl chain. The high energy yield of this process and the compact storage of fat explain why it is the most dense source of dietary calories.

Anaerobic respiration

Anaerobic respiration or fermentation entails the generation of energy via the process of oxidation in the absence of O_2 as an electron acceptor. In most eukaryotes, glucose is used as both an energy store and an electron donor. The equation for the oxidation of glucose to lactic acid is:

$$C_6H_{12}O_6 \longrightarrow 2CH_3CH(OH)COOH + 2\ ATP$$

In prokaryotes, multiple electron acceptors can be used in anaerobic respiration. These include nitrate, sulfate or carbon dioxide. These processes lead to the ecologically-important processes of denitrification, sulfate reduction and acetogenesis, respectively.

ATP replenishment by nucleoside diphosphate kinases

ATP can also be synthesized through several so-called "replenishment" reactions catalyzed by the enzyme families of nucleoside diphosphate kinases (NDKs), which use other nucleoside triphosphates as a high-energy phosphate donor, and the ATP:guanido-phosphotransferase family,

ATP production during photosynthesis

In plants, ATP is synthesized in thylakoid membrane of the chloroplast during the light-dependent reactions of photosynthesis in a process called photophosphorylation. Here, light energy is used to pump protons across the chloroplast membrane. This produces a proton-motive force and this drives the ATP synthase, exactly as in oxidative phosphorylation. Some of the ATP produced in the chloroplasts is consumed in the Calvin cycle, which produces triose sugars.

ATP recycling

The total quantity of ATP in the human body is about 0.1 mole. The majority of ATP is not usually synthesised *de novo*, but is generated from ADP by the aforementioned processes. Thus, at any given time, the total amount of ATP + ADP remains fairly constant.

The energy used by human cells requires the hydrolysis of 100 to 150 moles of ATP daily which is around 50 to 75 kg. Typically, a human will use up their body weight of ATP over the course of the day. This means that each ATP molecule is recycled 1000 to 1500 times during a single day (100 / 0.1 = 1000). ATP cannot be stored, hence its consumption closely follows its synthesis.

Regulation of biosynthesis

ATP production in an aerobic eukaryotic cell is tightly regulated by allosteric mechanisms, by feedback effects, and by the substrate concentration dependence of individual enzymes within the glycolysis and oxidative phosphorylation pathways. Key control points occur in enzymatic reactions that are so energetically favorable that they are effectively irreversible under physiological conditions.

In glycolysis, hexokinase is directly inhibited by its product, glucose-6-phosphate, and pyruvate kinase is inhibited by ATP itself. The main control point for the glycolytic pathway is phosphofructokinase (PFK), which is allosterically inhibited by high concentrations of ATP and activated by high concentrations of AMP. The inhibition of PFK by ATP is unusual, since ATP is also a substrate in the reaction catalyzed by PFK; the biologically active form of the enzyme is a tetramer that exists in two possible conformations, only one of which binds the second substrate fructose-6-phosphate (F6P). The protein has two binding sites for ATP - the active site is accessible in either protein conformation, but ATP binding to the inhibitor site stabilizes the conformation that binds F6P poorly. A number of other small molecules can compensate for the ATP-induced shift in equilibrium conformation and reactivate PFK, including cyclic AMP, ammonium ions, inorganic phosphate, and fructose 1,6 and 2,6 biphosphate.

The citric acid cycle is regulated mainly by the availability of key substrates, particularly the ratio of NAD^+ to NADH and the concentrations of calcium, inorganic phosphate, ATP, ADP, and AMP. Citrate - the molecule that gives its name to the cycle - is a feedback inhibitor of citrate synthase and also inhibits PFK, providing a direct link between the regulation of the citric acid cycle and glycolysis.

In oxidative phosphorylation, the key control point is the reaction catalyzed by cytochrome c oxidase, which is regulated by the availability of its substrate—the reduced form of cytochrome c. The amount of reduced cytochrome c available is directly related to the amounts of other substrates:

$$\frac{1}{2}NADH + cyt\ c_{ox} + ADP + P_i \iff \frac{1}{2}NAD^+ + cyt\ c_{red} + ATP$$

which directly implies this equation:

$$\frac{cyt\ c_{red}}{cyt\ c_{ox}} = \left(\frac{[NADH]}{[NAD]^+}\right)^{\frac{1}{2}} \left(\frac{[ADP][Pi]}{[ATP]}\right) K_{eq}$$

Thus, a high ratio of [NADH] to [NAD^+] or a low ratio of [ADP] [P_i] to [ATP] imply a high amount of reduced cytochrome c and a high level of cytochrome c oxidase activity. An additional level of regulation is introduced by the transport rates of ATP and NADH between the mitochondrial matrix and the cytoplasm.

Functions in cells

Metabolism, synthesis, and active transport

ATP is consumed in the cell by energy-requiring (endothermic) processes and can be generated by energy-releasing (exothermic) processes. In this way ATP transfers energy between spatially-separate metabolic reactions. ATP is the main energy source for the majority of cellular functions. This includes the synthesis of macromolecules, including DNA and RNA, and proteins. ATP also plays a critical role in the transport of macromolecules across cell membranes, e.g. exocytosis and endocytosis.

Roles in cell structure and locomotion

ATP is critically involved in maintaining cell structure by facilitating assembly and disassembly of elements of the cytoskeleton. In a related process, ATP is required for the shortening of actin and myosin filament crossbridges required for muscle contraction. This latter process is one of the main energy requirements of animals and is essential for locomotion and respiration.

Cell signalling

Extracellular signalling

ATP is also a signalling molecule. ATP, ADP, or adenosine are recognised by purinergic receptors. Purinoreceptors might be the most abundant receptors in mammalian tissues (Abbracchio M.P. et al., 2008).

In humans, this signalling role is important in both the central and peripheral nervous system. Activity-dependent release of ATP from synapses, axons and glia activates purinergic membrane receptors known as P2. The *P2Y* receptors are *metabotropic*, i.e. G protein-coupled and modulate mainly intracellular calcium and sometimes cyclic AMP

levels. Though named between $P2Y_1$ and $P2Y_{15}$, only nine members of the P2Y family have been cloned, and some are only related through weak homology and several ($P2Y_5$, $P2Y_7$, $P2Y_9$, $P2Y_{10}$) do not function as receptors that raise cytosolic calcium. The *P2X ionotropic* receptor subgroup comprises seven members ($P2X_1$–$P2X_7$) which are ligand-gated Ca^{2+}-permeable ion channels that open when bound to an extracellular purine nucleotide. In contrast to P2 receptors (agonist order ATP > ADP > AMP > ADO), purinergic nucleotides like ATP are not strong agonists of P1 receptors which are strongly activated by adenosine and other nucleosides (ADO > AMP > ADP > ATP). P1 receptors have A1, A2a, A2b, and A3 subtypes ("A" as a remnant of old nomenclature of *adenosine receptor*), all of which are G protein-coupled receptors, A1 and A3 being coupled to Gi, and A2a and A2b being coupled to Gs. All adenosine receptors were shown to activate at least one subfamily of mitogen-activated protein kinases. The actions of adenosine are often antagonistic or synergistic to the actions of ATP. In the CNS, adenosine has multiple functions, such as modulation of neural development, neuron and glial signalling and the control of innate and adaptive immune systems (Abbracchio M.P. et al., 2008).

Intracellular signalling

ATP is critical in signal transduction processes. It is used by kinases as the source of phosphate groups in their phosphate transfer reactions. Kinase activity on substrates such as proteins or membrane lipids are a common form of signal transduction. Phosphorylation of a protein by a kinase can activate this cascade such as the mitogen-activated protein kinase cascade.

ATP is also used by adenylate cyclase and is transformed to the second messenger molecule cyclic AMP, which is involved in triggering calcium signals by the release of calcium from intracellular stores. This form of signal transduction is particularly important in brain function, although it is involved in the regulation of a multitude of other cellular processes.

DNA and RNA synthesis

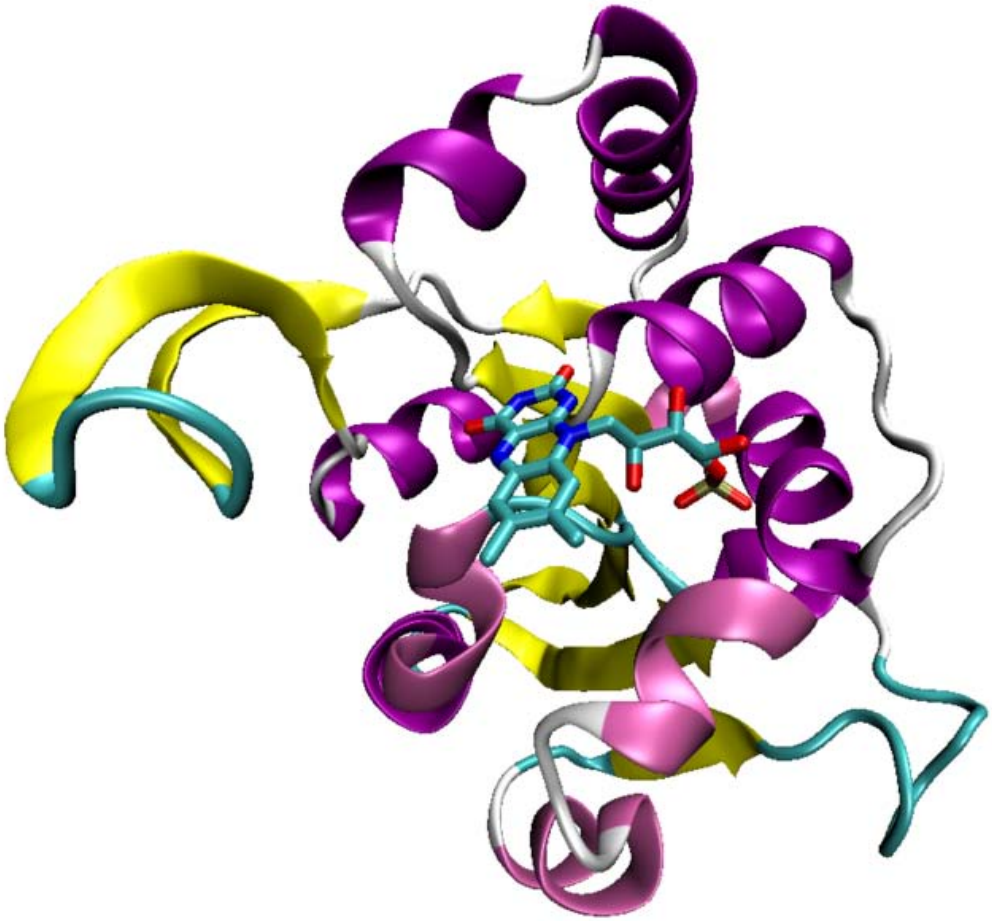
In all known organisms, the deoxyribonucleotides that make up DNA are synthesized by the action of ribonucleotide reductase (RNR) enzymes on their corresponding ribonucleotides. These enzymes reduce the sugar residue from ribose to deoxyribose by removing oxygen from the 2' hydroxyl group; the substrates are ribonucleoside diphosphates and the products deoxyribonucleoside diphosphates (the latter are denoted dADP, dCDP, dGDP, and dUDP respectively.) All ribonucleotide reductase enzymes use a common sulfhydryl radical mechanism reliant on reactive cysteine residues that oxidize to form disulfide bonds in the course of the reaction. RNR enzymes are recycled by reaction with thioredoxin or glutaredoxin.

The regulation of RNR and related enzymes maintains a balance of dNTPs relative to each other and relative to NTPs in the cell. Very low dNTP concentration inhibits DNA synthesis and DNA repair and is lethal to the cell, while an abnormal ratio of dNTPs is

mutagenic due to the increased likelihood of the DNA polymerase incorporating the wrong dNTP during DNA synthesis. Regulation of or differential specificity of RNR has been proposed as a mechanism for alterations in the relative sizes of intracellular dNTP pools under cellular stress such as hypoxia.

In the synthesis of the nucleic acid RNA, ATP is one of the four nucleotides incorporated directly into RNA molecules by RNA polymerases. The energy driving this polymerization comes from cleaving off a pyrophosphate (two phosphate groups). The process is similar in DNA biosynthesis, except that ATP is reduced to the deoxyribonucleotide dATP, before incorporation into DNA.

Binding to proteins



An example of the Rossmann fold, a structural domain of a decarboxylase enzyme from the bacterium *Staphylococcus epidermidis* (PDB ID 1G5Q) with a bound flavin mononucleotide cofactor.

Some proteins that bind ATP do so in a characteristic protein fold known as the Rossmann fold, which is a general nucleotide-binding structural domain that can also bind the cofactor NAD. The most common ATP-binding proteins, known as kinases,

share a small number of common folds; the protein kinases, the largest kinase superfamily, all share common structural features specialized for ATP binding and phosphate transfer.

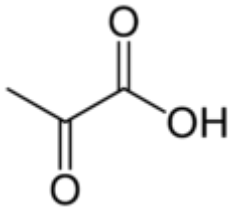
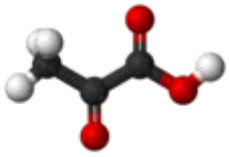
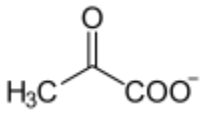
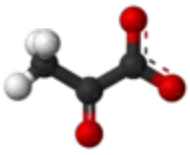
ATP in complexes with proteins generally requires the presence of a divalent cation, almost always magnesium, which binds to the ATP phosphate groups. The presence of magnesium greatly decreases the dissociation constant of ATP from its protein binding partner without affecting the ability of the enzyme to catalyze its reaction once the ATP has bound. The presence of magnesium ions can serve as a mechanism for kinase regulation.

ATP analogues

Biochemistry laboratories often use *in vitro* studies to explore ATP-dependent molecular processes. Enzyme inhibitors of ATP-dependent enzymes such as kinases are needed to examine the binding sites and transition states involved in ATP-dependent reactions. ATP analogs are also used in X-ray crystallography to determine a protein structure in complex with ATP, often together with other substrates. Most useful ATP analogs cannot be hydrolyzed as ATP would be; instead they trap the enzyme in a structure closely related to the ATP-bound state. Adenosine 5'-(gamma-thiotriphosphate) is an extremely common ATP analog in which one of the gamma-phosphate oxygens is replaced by a sulfur atom; this molecule is hydrolyzed at a dramatically slower rate than ATP itself and functions as an inhibitor of ATP-dependent processes. In crystallographic studies, hydrolysis transition states are modeled by the bound vanadate ion. However, caution is warranted in interpreting the results of experiments using ATP analogs, since some enzymes can hydrolyze them at appreciable rates at high concentration.

Chapter- 8

Pyruvic Acid

Pyruvic acid	
 	
IUPAC name 2-oxopropanoic acid	
Other names α-ketopropionic acid; acetylformic acid; pyroracemic acid; Pyr	
Identifiers	
CAS number	127-17-3 ✓
PubChem	1060
ChemSpider	1031 ✓
UNII	8558G7RUTR ✓
ChEMBL	CHEMBL1162144 ✓
Properties	
Molecular formula	$C_3H_4O_3$
Molar mass	88.06 g/mol
Density	1.250 g/cm³
Melting point	11.8 °C, 285 K, 53 °F
Boiling point	165 °C, 438 K, 329 °F
Acidity (pK_a)	2.50
Related compounds	
Other anions	pyruvate ion  
Related keto-acids, carboxylic acids	acetic acid glyoxylic acid oxalic acid

Related compounds	propionic acid acetoacetic acid propionaldehyde glyceraldehyde methylglyoxal sodium pyruvate

Pyruvic acid ($CH_3COCOOH$) is an organic acid, a ketone, as well the simplest of the alpha-keto acids. The carboxylate (COOH) ion (anion) of pyruvic acid, CH_3COCOO^-, is known as **pyruvate**, and is a key intersection in several metabolic pathways. It can be made from glucose through glycolysis, supplies energy to living cells in the citric acid cycle (also known as the Krebs cycle), and can also be converted to carbohydrates via gluconeogenesis, to fatty acids or energy through acetyl-CoA, to the amino acid alanine and to ethanol.

Chemistry

In 1834, Théophile-Jules Pelouze distilled both tartaric acid (L-tartaric acid) and racemic acid (a mix of D- and L-tartaric acid) and isolated pyrotartaric acid (methyl succinic acid) and another acid which Jöns Jacob Berzelius characterized the following year and named pyruvic acid. Pyruvic acid is a colorless liquid with a smell similar to that of acetic acid and is miscible with water. In the laboratory, pyruvic acid may be prepared by heating a mixture of tartaric acid and potassium hydrogen sulfate, by the oxidation of propylene glycol by a strong oxidizer (eg. potassium permanganate or bleach), or by the hydrolysis of acetyl cyanide, formed by reaction of acetyl chloride with potassium cyanide:

$$CH_3COCl + KCN \rightarrow CH_3COCN + KCl$$
$$CH_3COCN \rightarrow CH_3COCOOH$$

Biochemistry

Pyruvate is an important chemical compound in biochemistry. It is the output of the anaerobic metabolism of glucose known as glycolysis. One molecule of glucose breaks down into two molecules of pyruvate, which are then used to provide further energy, in one of two ways. Pyruvate is converted into acetyl-coenzyme A, which is the main input for a series of reactions known as the Krebs cycle. Pyruvate is also converted to oxaloacetate by an anaplerotic reaction which replenishes Krebs cycle intermediates; alternatively, the oxaloacetate is used for gluconeogenesis. These reactions are named after Hans Adolf Krebs, the biochemist awarded the 1953 Nobel Prize for physiology, jointly with Fritz Lipmann, for research into metabolic processes. The cycle is also called the citric acid cycle, because citric acid is one of the intermediate compounds formed during the reactions.

If insufficient oxygen is available, the acid is broken down anaerobically, creating lactate in animals and ethanol in plants and microorganisms. Pyruvate from glycolysis is converted by anaerobic respiration to lactate using the enzyme lactate dehydrogenase and

the coenzyme NADH in lactate fermentation, or to acetaldehyde and then to ethanol in alcoholic fermentation.

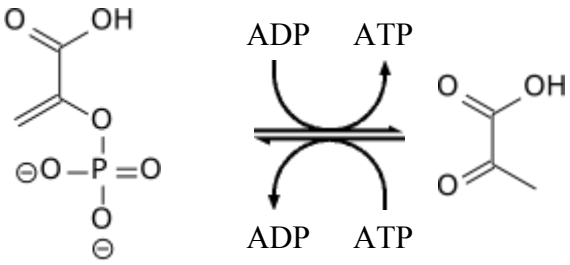
Pyruvate is a key intersection in the network of metabolic pathways. Pyruvate can be converted into carbohydrates via gluconeogenesis, to fatty acids or energy through acetyl-CoA, to the amino acid alanine and to ethanol. Therefore it unites several key metabolic processes.

The pyruvic acid derivative bromopyruvic acid is being studied for potential cancer treatment applications by researchers at Johns Hopkins University in ways that would support the Warburg hypothesis on the cause(s) of cancer.

Pyruvate production by glycolysis

In glycolysis, phosphoenolpyruvate (PEP) is converted to pyruvate by pyruvate kinase. This reaction is strongly exergonic and irreversible; in gluconeogenesis it takes two enzymes, pyruvate carboxylase and PEP carboxykinase, to catalyze the reverse transformation of pyruvate to PEP.

phosphoenolpyruvate | pyruvate kinase | pyruvate



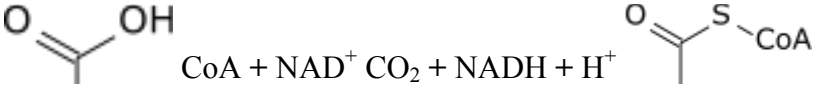
pyruvate kinase

Compound C00074 at KEGG Pathway Database. Enzyme 2.7.1.40 at KEGG Pathway Database. Compound C00022 at KEGG Pathway Database.

Pyruvate decarboxylation to acetyl CoA

Pyruvate decarboxylation by the pyruvate dehydrogenase complex produces acetyl-CoA.

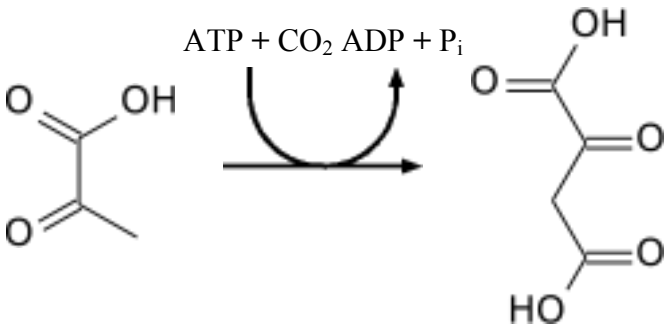
pyruvate | pyruvate dehydrogenase complex | acetyl-CoA



Pyruvate carboxylation to oxaloacetate

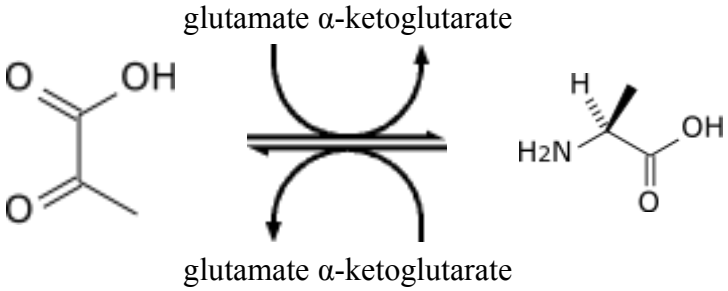
Carboxylation by the pyruvate carboxylase produces oxaloacetate.

pyruvate	pyruvate carboxylase	oxaloacetate



Transamination by the alanine aminotransferase

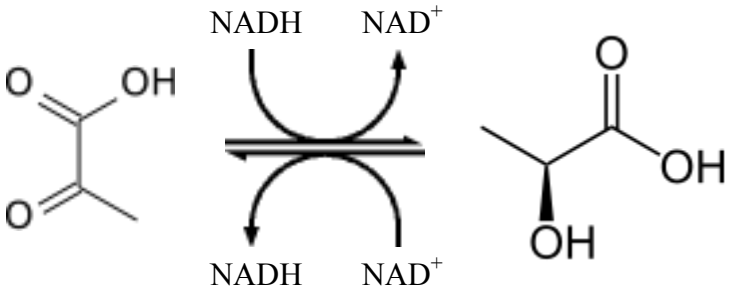
pyruvate	alanine transaminase	alanine



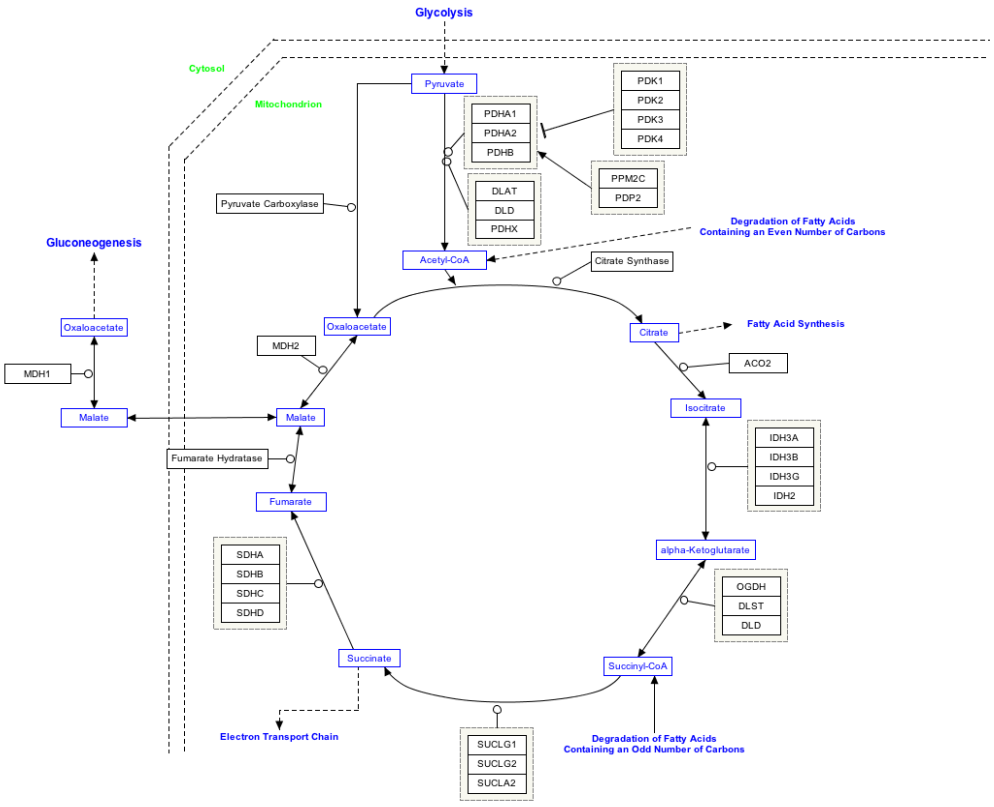
Reduction to lactic acid

Reduction by the lactate dehydrogenase produces lactic acid.

pyruvate	lactate dehydrogenase	lactic acid



Interactive pathway map



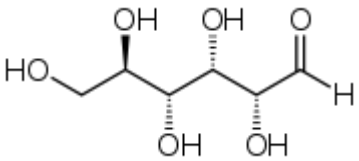
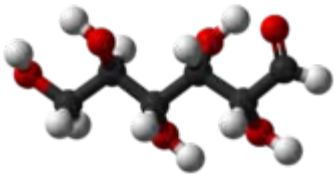
Citric acid cycle edit

Origin of life

Current evolutionary theory on the origin of life posits that the first organisms were anaerobic because the atmosphere of prebiotic Earth was, in theory, almost barren of diatomic oxygen. As such, requisite biochemical materials must have preceded life. In vitro, iron sulfide at sufficient pressure and temperature catalyzes the formation of pyruvate. Thus, argues Günter Wächtershäuser, the mixing of iron-rich crust with hydrothermal vent fluid is suspected of providing the fertile basis for the formation of life.

Chapter- 9

Glucose

D-glucose


Preferred IUPAC name Dextrose Systematic name (2*R*,3*S*,4*R*,5*R*)-2,3,5,4,6-Pentahydroxyhexanal **Other names** Blood sugar Dextrose Corn sugar D-Glucose Grape sugar

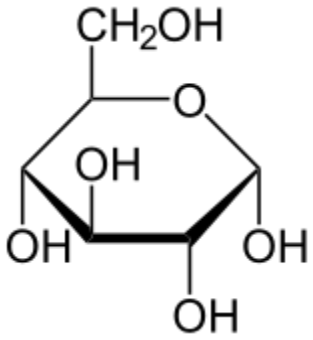
Identifiers	
Abbreviations	Glc
CAS number	50-99-7 ✓
PubChem	5793
ChemSpider	5589 ✓
UNII	5SL0G7R0OK ✓
EC number	200-075-1
KEGG	C00031 ✓
MeSH	Glucose
ChEBI	CHEBI:4167
ChEMBL	CHEMBL1216 ✗
RTECS number	LZ6600000

ATC code	B05CX01,V04CA02, V06DC01
Beilstein Reference	1281604
Gmelin Reference	83256
3DMet	B04623
Properties	
Molecular formula	$C_6H_{12}O_6$
Molar mass	180.16 g/mol
Exact mass	180.063388
Density	1.54 g/cm^3
Melting point	α-D-glucose: 146 °C β-D-glucose: 150 °C
Solubility in water	91 g/100 ml (25 °C)
Solubility in methanol	0.037 M
Solubility in ethanol	0.006 M
Solubility in tetrahydrofuran	0.016 M
Thermochemistry	
Std enthalpy of formation $\Delta_f H^{\ominus}_{298}$	−1271 kJ/mol
Std enthalpy of combustion $\Delta_c H^{\ominus}_{298}$	−2805 kJ/mol
Standard molar entropy $S^{\ominus}_{298}$	209.2 J K^{-1} mol^{-1}
Hazards	
MSDS	ICSC 0865
EU Index	not listed

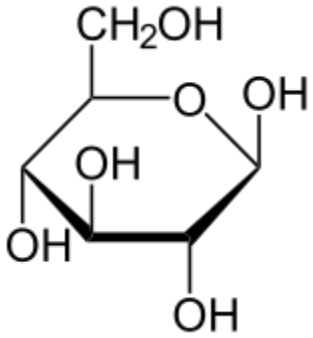
Glucose is a monosaccharide (simple sugar) with formula $C_6H_{12}O_6$ or H-(C=O)-$(CHOH)_5$-H, whose five hydroxyl (OH) groups are arranged in a specific way along its six-carbon backbone.

The name may refer to any of two stereoisomers, which are mirror images of each other. It usually means the isomer that is most common in nature, properly called **D-glucose**, **dextrose**, or **grape sugar**. This isomer is an important carbohydrate in biology, used by most cells as a source of energy and a metabolic intermediate. D-Glucose is one of the main products of photosynthesis and the starting material of cellular respiration. Starch and cellulose are polymers derived from the dehydration of D-glucose. The other stereoisomer, called L-glucose, is hardly found in nature.

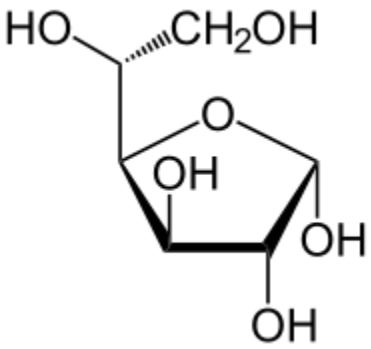
The open chain form of D-glucose is actually unstable. In solution, it exists in equilibrium with four cyclic isomers, called **α-D-glucopyranose**, **β-D-glucopyranose**, **α-D-glucofuranose** and **β-D-glucofuranose**. The terms "glucose" and "D-glucose" are generally used for these cyclic forms as well.



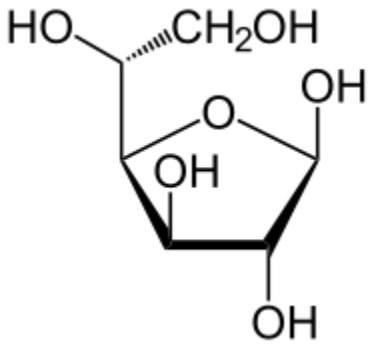
α-D-
Glucopyranose



β-D-
Glucopyranose



α-D-
Glucofuranose



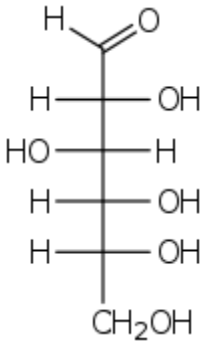
β-D-
Glucofuranose

The glucopyranose forms predominate in solution, and are the only forms observed in the solid state. They are crystalline colorless solids, highly soluble in water and acetic acid, poorly soluble in methanol and ethanol. They melt at 146°C (α) and 150°C (β), and decompose at higher temperatures into carbon and water.

The name "glucose" comes from the Greek word *glukus* (γλυκύς), meaning "sweet". The suffix "-ose" denotes a sugar. The name "dextrose" and the 'D-' prefix come from Latin *dexter* ("right"), referring to the handedness of the molecules.

Structure and nomenclature

Open-chain form



D-glucose in Fischer projection

In its fleeting open-chain form, the glucose molecule has an open (as opposed to cyclic) and unbranched backbone of six carbon atoms, C-1 through C-6; where C-1 is part of an aldehyde group H(C=O)-, and each of the other five carbons bears one hydroxyl group -OH. The remaining bonds of the backbone carbons are satisfied by hydrogen atoms -H. Therefore glucose is an hexose and an aldose, or an aldohexose.

Each of the four carbons C-2 through C-5 is chiral, meaning that its four bonds connect to four distinct parts of the molecule. (Carbon C-2, for example, connects to -(C=O)H, -OH, -H, and $-(CHOH)_4H$.) In D-glucose, these four parts must be in a specific three-dimensional arrangement. Namely, when the molecule is drawn in the Fischer projection, the hydroxyls on C-2, C-4, and C-5 must be on the right side, while that on C-3 must be on the left side.

The positions of those four hydroxyls are exacly reversed in the Fischer diagram of L-Glucose. D- and L-glucose are two of the 16 possible aldohexoses; the other 14 are allose, altrose, mannose, gulose, idose, galactose, and talose, each with two isomers, 'D-' and 'L-'.

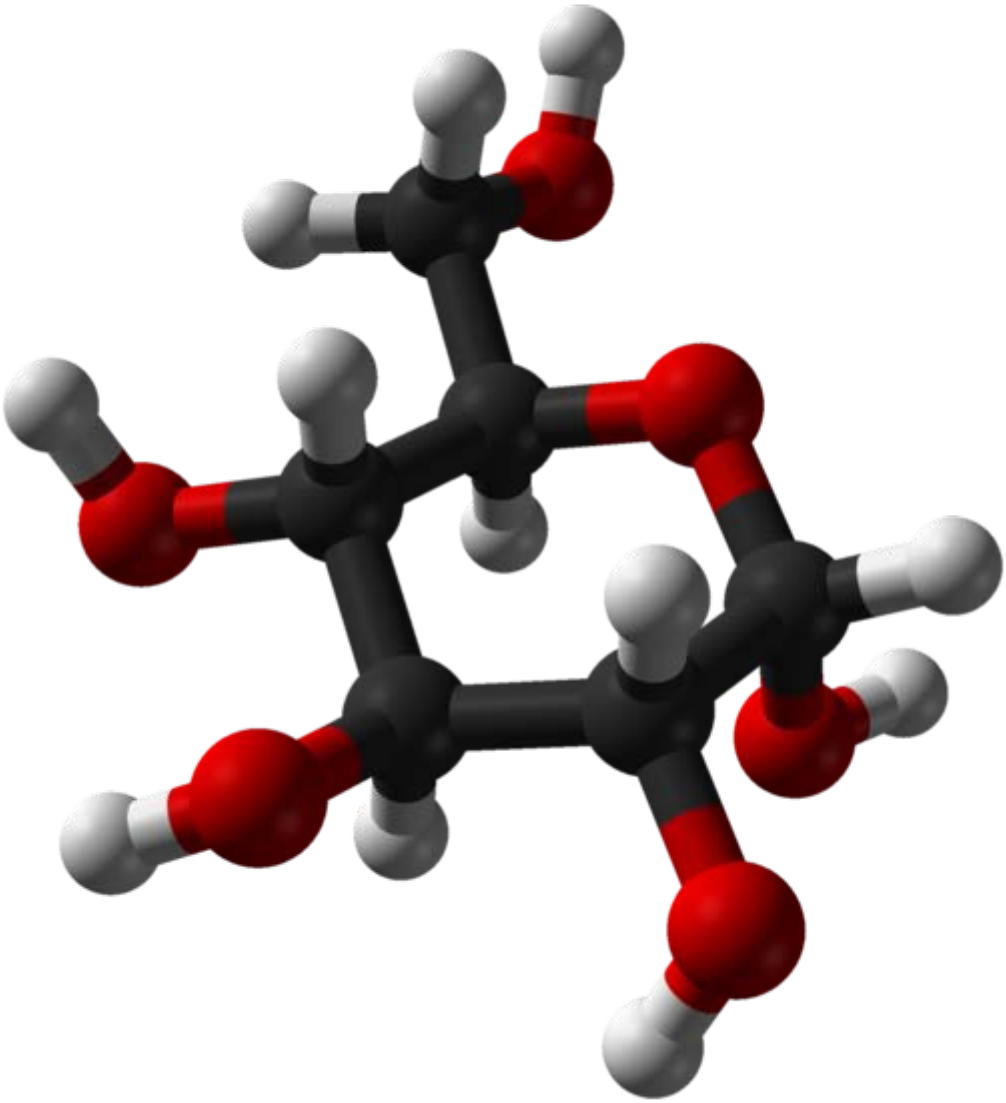
Cyclic forms

In solutions, the open-chain form of glucose (either 'D-' or 'L-') exists in equilibrium with several cyclic isomers, each containing a ring of carbons closed by one oxygen atom. The ring arises from the open-chain form by a nucleophilic addition reaction between the aldehyde group -(C=O)H at C-1 and the hydroxyl group -OH at C-4 or C-5, yielding a hemiacetal group -C(OH)H-O-.

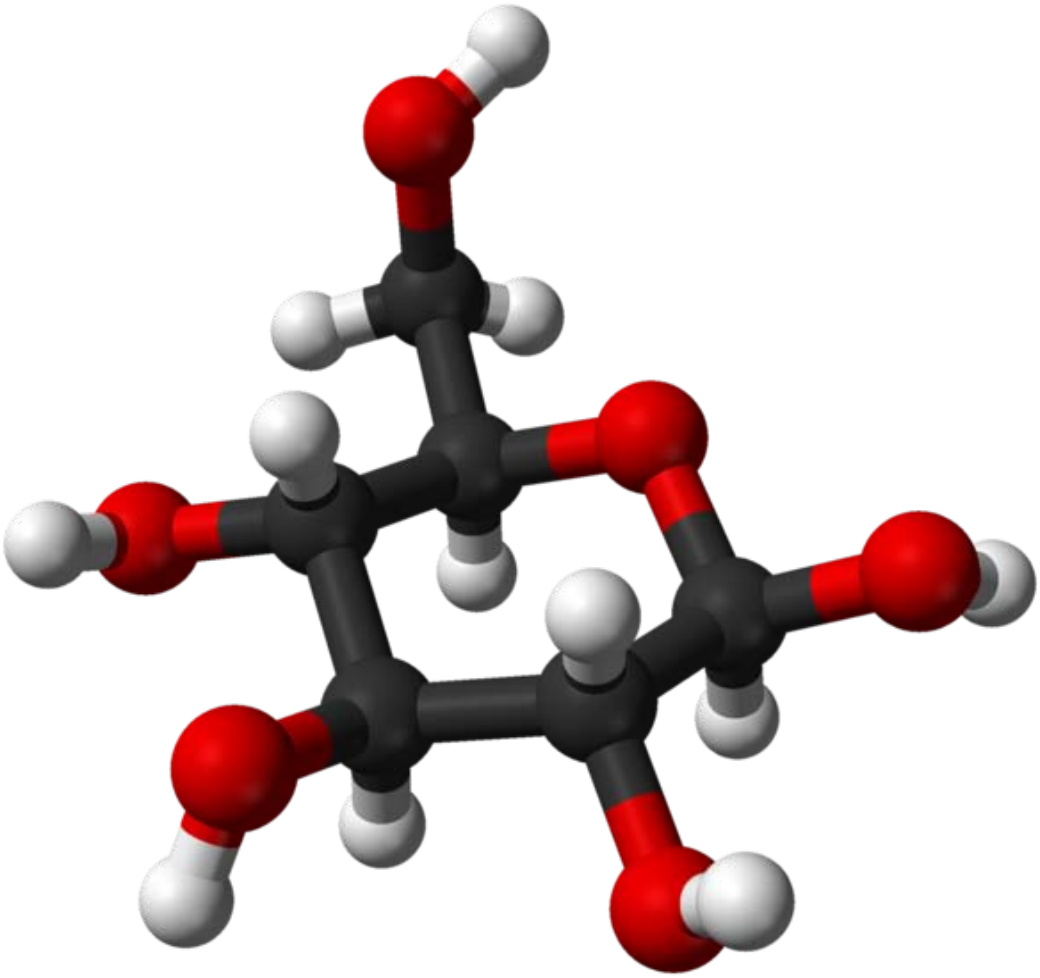
The reaction between C-1 and C-5 creates a molecule with six-membered ring, called glucopyranose — after the cyclic ether pyran, the simplest molecule with the same carbon-oxygen ring. The (much rarer) reaction between C-1 and C-4 creates a molecule with a five-membered ring, called glucofuranose, after the cyclic ether furan. In either case, each carbon in the ring has one hydrogen and one hydroxyl attached, except for the last carbon (C-4 or C-5) where the hydroxyl is replaced by the remainder of the open molecule (which is $-(CHOH)_2-H$ or -(CHOH)-H, respectively).

The ring-closing reaction makes carbon C-1 chiral, too, since its four bonds lead to -H, to -OH, to carbon C-2, and to the ring oxygen. These four parts of the molecule may be arranged around C-1 (the anomeric carbon) in two distinct ways, designated by the prefixes 'α-' and 'β-'. When a glucopyranose molecule is drawn in the Haworth projection, the designation 'α-' means that the hydroxyl group attached to C-1 and the $-CH_2OH$ group at C-5 lies on opposite sides of the ring's plane (a *trans* arrangement), while 'β-' means that they are on the same side of the plane (a *cis* arrangement).

Therefore, the open isomer D-glucose gives rise to four distinct cyclic isomers: α-D-glucopyranose, β-D-glucopyranose, α-D-glucofuranose, and β-D-glucofuranose; which are all chiral.



α-D-
Glucopyranose



β-D-
Glucopyranose

The other open-chain isomer L-glucose similarly gives rise to four distinct cyclic forms of L-glucose, each the mirror image of the corresponding D-glucose.

The rings are not planar but twisted in three dimensions. The glucopyranose ring (α or β) can assume several non-planar shapes, analogous to the 'chair' and 'boat' conformations of cyclohexane. Similarly, the glucofuranose ring may assume several shapes, analogous to the 'envelope' conformations of cyclopentane.

Rotational isomers

Each glucose isomer is subject to rotational isomerism. Within the cyclic form of glucose, rotation may occur around the O6-C6-C5-O5 torsion angle, termed the ω-angle, to form three staggered rotamer conformations called *gauche-gauche* (gg), *gauche-trans* (gt) and *trans-gauche* (tg). For methyl α-D-glucopyranose at equilibrium the ratio of

molecules in each rotamer conformation is reported as 57:38:5 gg:gt:tg. This tendency for the ω-angle to prefer to adopt a *gauche* conformation is attributed to the gauche effect.

Physical properties

Solutions

All forms of glucose are colorless and easily soluble in water, acetic acid, and several other solvents. They are only sparingly soluble in methanol and ethanol.

The open-chain form is thermodynamically unstable, and it spontaneously tautomerizes to the cyclic forms. (Although the ring closure reaction could in theory create four- or three-atom rings, these would be highly strained and are not observed.) In solutions at room temperature, the four cyclic isomers interconvert over a timescale of hours, in a process called mutarotation. Starting from any proportions, the mixture converges stable ratio of α:β 36:64. The ratio would be α:β 11:89 if it were not for the influence of the anomeric effect. Mutarotation is considerably slower at temperatures close to 0°C.

Mutarotation consists of a temporary reversal of the ring-forming reaction, resulting in the open-chain form, followed by a re-forming of the ring. The ring closure step may use a different -OH group than the one recreated by the opening step (thus switching between pyranose and furanose forms), and/or the new hemiacetal group created on C-1 may have the same or opposite handedness as the original one (thus switching between the α and β forms). Thus, even though the open-chain form is barely detectable in solution, it is an essential component of the equilibrium.

Solid state

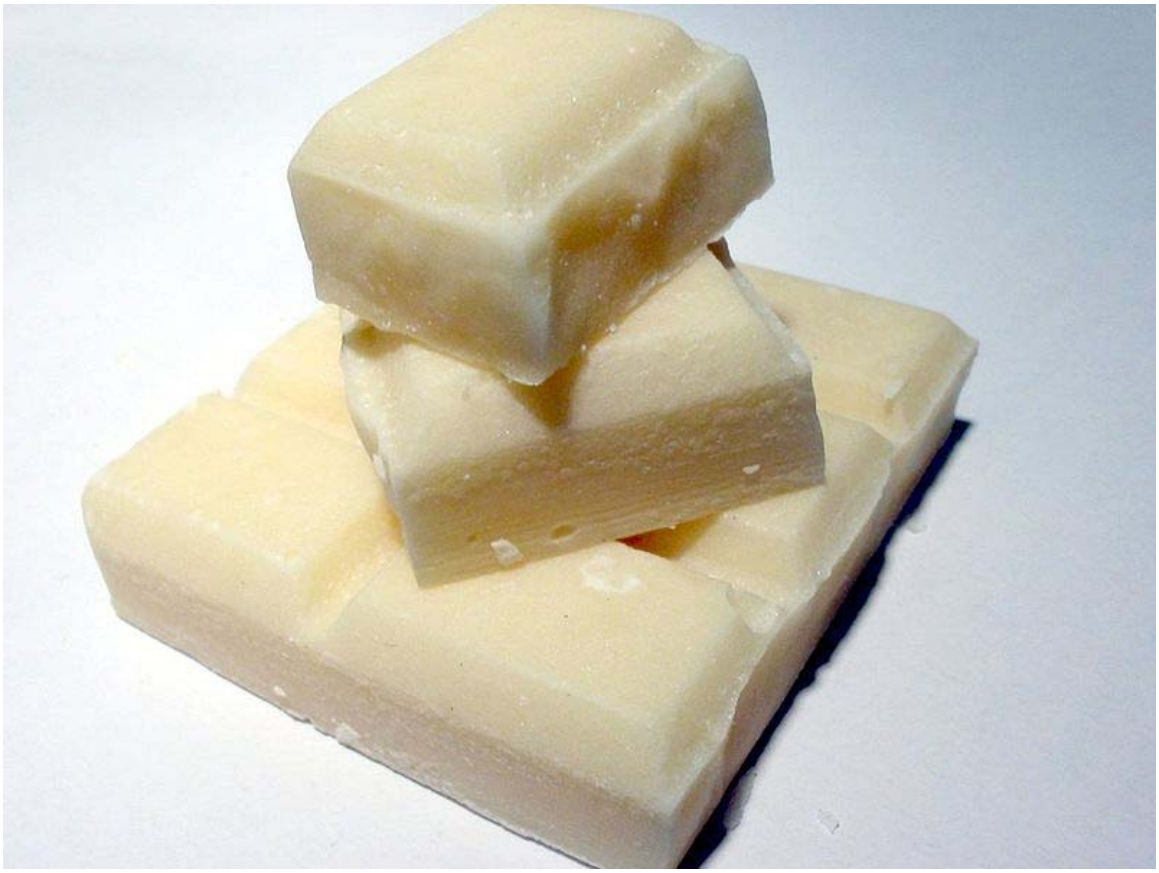
Depending on conditions, three major solid forms of glucose can be crystallised from water solutions: α-glucopyranose, β-glucopyranose, and β-glucopyranose hydrate.

Optical activity

Whether in water or in the solid form, D-glucose is dextrorotatory, meaning that it will rotate the direction of polarized light clockwise. The effect is due to the chirality of the molecules, and indeed the mirror-image isomer, L-glucose, is levorotatory (rotates polarized light counterclockwise) by the same amount. The strength of the effect is different for each of the five tautomers.

Note that the D- prefix does not refer directly to the optical properties of the compound. It indicates that the C-2 chiral center has the same handedness as that of D-glutaraldehyde (which was so labeled because *it* is dextrorotatory). The fact that D-glucose is dextrorotatory is a combined effect of its four chiral centers, not just of C-2; and indeed some of the other D-aldohexoses are levorotatory.

Production



Glucose tablets

Biosynthesis

In plants and some prokaryotes, glucose is a product of photosynthesis. In animals and fungi, glucose results from the breakdown of glycogen, a process known as glycogenolysis. In plants the breakdown substrate is starch.

In animals, glucose is synthesized in the liver and kidneys from non-carbohydrate intermediates, such as pyruvate and glycerol, by a process known as gluconeogenesis.

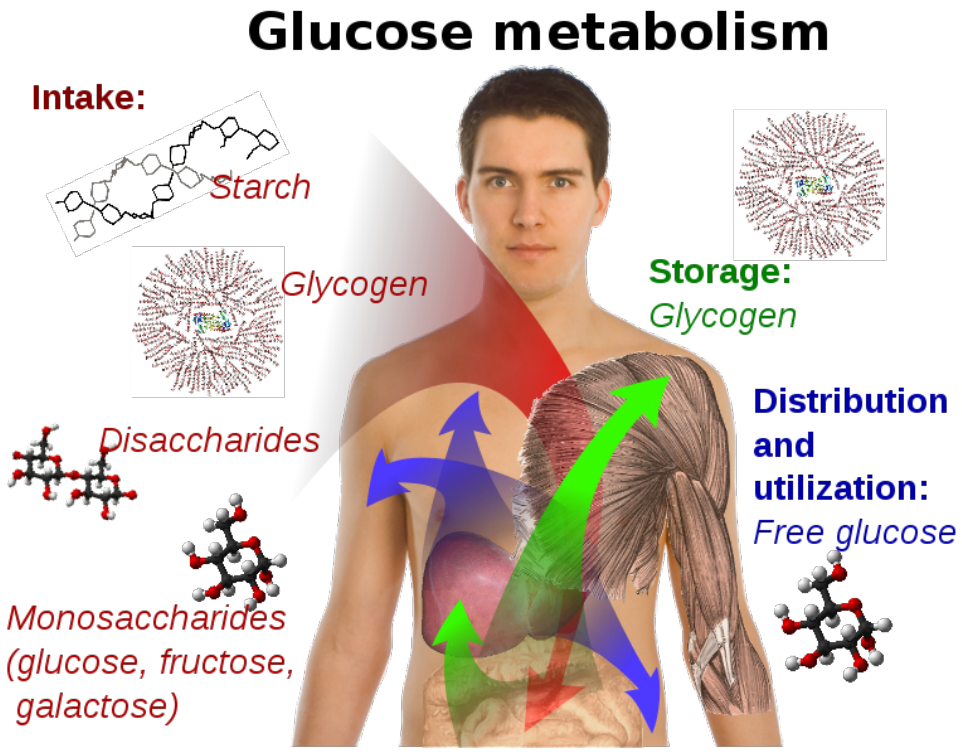
In some deep-sea bacteria glucose is produced by chemosynthesis.

Commercial

Glucose is produced commercially via the enzymatic hydrolysis of starch. Many crops can be used as the source of starch. Maize, rice, wheat, cassava, corn husk and sago are all used in various parts of the world. In the United States, cornstarch (from maize) is used almost exclusively. Most commercial glucose occurs as a component of invert

sugar, an approximately 1:1 mixture of glucose and fructose. In principle, cellulose could be hydrolysed to glucose, but this process is not yet commercially practical.

Function



Glucose metabolism and various forms of it in the process.
-Glucose-containing compounds and isomeric forms are digested and taken up by the body in the intestines, including starch, glycogen, disaccharides and monosaccharides.
-Glucose is stored in mainly the liver and muscles as glycogen.
-It is distributed and utilized in tissues as free glucose.

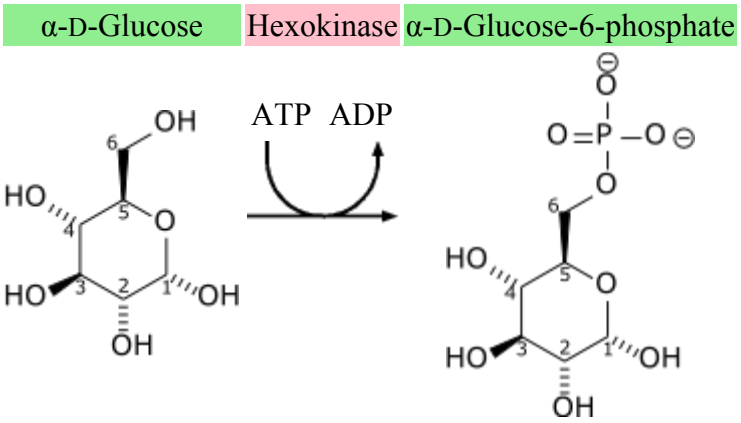
Scientists can speculate on the reasons why glucose, and not another monosaccharide such as fructose, is so widely used in organisms. One reason might be that glucose has a lower tendency, relative to other hexose sugars, to react non-specifically with the amino groups of proteins. This reaction (glycation) reduces or destroys the function of many enzymes. The low rate of glycation is due to glucose's preference for the less reactive cyclic isomer. Nevertheless, many of the long-term complications of diabetes (e.g., blindness, renal failure, and peripheral neuropathy) are probably due to the glycation of proteins or lipids. In contrast, enzyme-regulated addition of glucose to proteins by glycosylation is often essential to their function.

As an energy source

Glucose is a ubiquitous fuel in biology. It is used as an energy source in most organisms, from bacteria to humans. Use of glucose may be by either aerobic respiration, anaerobic

respiration, or fermentation. Carbohydrates are the human body's key source of energy, through aerobic respiration, providing approximately 3.75 kilocalories (16 kilojoules) of food energy per gram. Breakdown of carbohydrates (e.g. starch) yields mono- and disaccharides, most of which is glucose. Through glycolysis and later in the reactions of the citric acid cycle (TCAC), glucose is oxidized to eventually form CO_2 and water, yielding energy sources, mostly in the form of ATP. The insulin reaction, and other mechanisms, regulate the concentration of glucose in the blood. A high fasting blood sugar level is an indication of prediabetic and diabetic conditions.

Glucose is a primary source of energy for the brain, and hence its availability influences psychological processes. When glucose is low, psychological processes requiring mental effort (e.g., self-control, effortful decision-making) are impaired.



Glucose in glycolysis

Use of glucose as an energy source in cells is via aerobic or anaerobic respiration. Both of these start with the early steps of the glycolysis metabolic pathway. The first step of this is the phosphorylation of glucose by hexokinase to prepare it for later breakdown to provide energy. The major reason for the immediate phosphorylation of glucose by a hexokinase is to prevent diffusion out of the cell. The phosphorylation adds a charged phosphate group so the glucose 6-phosphate cannot easily cross the cell membrane. Irreversible first steps of a metabolic pathway are common for regulatory purposes.

In anaerobic respiration one glucose molecule produces a net gain of 2 ATP molecules (4 ATP molecules are produced during glycolysis but 2 are required by enzymes used during the process). In aerobic respiration a molecule of glucose is much more profitable in that a net worth of 32 ATP molecules are generated (34 gross with 2 being required in the process).

As a precursor

Glucose is critical in the production of proteins and in lipid metabolism. In plants and most animals, it is also a precursor for vitamin C (ascorbic acid) production. It is modified for use in these processes by the glycolysis pathway.

Glucose is used as a precursor for the synthesis of several important substances. Starch, cellulose, and glycogen ("animal starch") are common glucose polymers (polysaccharides). Lactose, the predominant sugar in milk, is a glucose-galactose disaccharide. In sucrose, another important disaccharide, glucose is joined to fructose. These synthesis processes also rely on the phosphorylation of glucose through the first step of glycolysis.

Industrial use

In industry, glucose is used as a precursor to make vitamin C in the Reichstein process, to make citric acid, gluconic acid, bio-ethanol, polylactic acid, sorbitol.

Sources and absorption

Most dietary carbohydrates contain glucose, either as their only building block, as in starch and glycogen, or together with another monosaccharide, as in sucrose and lactose.

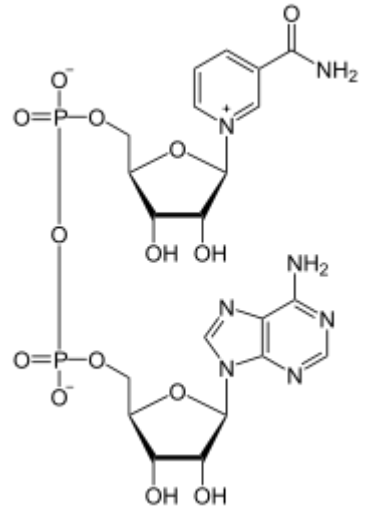
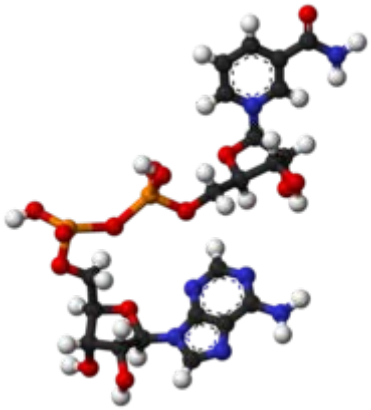
In the lumen of the duodenum and small intestine, the glucose oligo- and polysaccharides are broken down to monosaccharides by the pancreatic and intestinal glycosidases. Other polysaccharides cannot be processed by the human intestine and require assistance by intestinal flora if they are to be broken down; the most notable exceptions are sucrose (fructose-glucose) and lactose (galactose-glucose). Glucose is then transported across the apical membrane of the enterocytes by SLC5A1, and later across their basal membrane by SLC2A2. Some of the glucose is directly utilized as an energy source by brain cells, intestinal cells and red blood cells, while the rest reaches the liver, adipose tissue and muscle cells, where it is absorbed and stored as glycogen (under the influence of insulin). Liver cell glycogen can be converted to glucose and returned to the blood when insulin is low or absent; muscle cell glycogen is not returned to the blood because of a lack of enzymes. In fat cells, glucose is used to power reactions that synthesize some fat types and have other purposes. Glycogen is the body's 'glucose energy storage' mechanism because it is much more 'space efficient' and less reactive than glucose itself.

History

Because glucose is a basic necessity of many organisms, a correct understanding of its chemical makeup and structure contributed greatly to a general advancement in organic chemistry. This understanding occurred largely as a result of the investigations of Emil Fischer, a German chemist who received the 1902 Nobel Prize in Chemistry as a result of his findings. The synthesis of glucose established the structure of organic material and consequently formed the first definitive validation of Jacobus Henricus van't Hoff's theories of chemical kinetics and the arrangements of chemical bonds in carbon-bearing molecules. Between 1891 and 1894, Fischer established the stereochemical configuration of all the known sugars and correctly predicted the possible isomers, applying van't Hoff's theory of asymmetrical carbon atoms.

Chapter- 10

Nicotinamide Adenine Dinucleotide

Nicotinamide adenine dinucleotide


Other names
Diphosphopyridine nucleotide (DPN^+), Coenzyme I
Identifiers
CAS number: 53-84-9 ✓, 58-68-4 (NADH)
PubChem: 925
ChemSpider: 5681 ✓

UNII	0U46U6E8UK ✓
KEGG	C00003 ✓
ChEBI	CHEBI:13389
IUPHAR ligand	2451
RTECS number	UU3450000
Properties	
Molecular formula	$C_{21}H_{27}N_7O_{14}P_2$
Molar mass	663.43 g/mol
Appearance	White powder
Melting point	160 °C
Hazards	
Main hazards	Not hazardous

Nicotinamide adenine dinucleotide, abbreviated **NAD**$^+$, is a coenzyme found in all living cells. The compound is a dinucleotide, since it consists of two nucleotides joined through their phosphate groups, with one nucleotide containing an adenine base and the other containing nicotinamide.

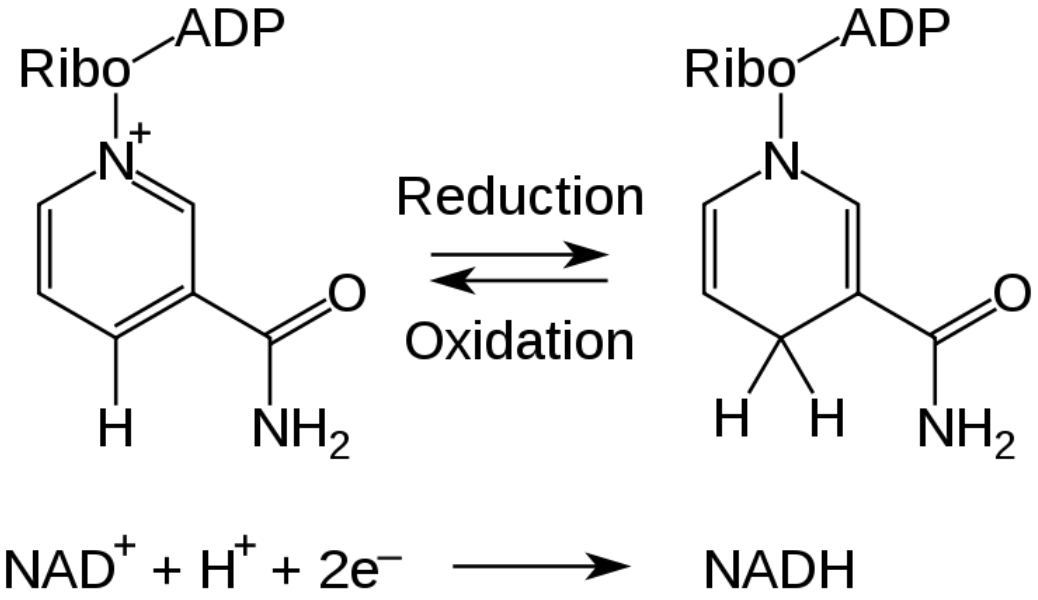
In metabolism, NAD^+ is involved in redox reactions, carrying electrons from one reaction to another. The coenzyme is, therefore, found in two forms in cells: NAD^+ is an oxidizing agent – it accepts electrons from other molecules and becomes reduced. This reaction forms **NADH**, which can then be used as a reducing agent to donate electrons. These electron transfer reactions are the main function of NAD^+. However, it is also used in other cellular processes, the most notable one being a substrate of enzymes that add or remove chemical groups from proteins, in posttranslational modifications. Because of the importance of these functions, the enzymes involved in NAD^+ metabolism are targets for drug discovery.

In organisms, NAD^+ can be synthesized from simple building-blocks (*de novo*) from the amino acids tryptophan or aspartic acid. In an alternative fashion, more complex components of the coenzymes are taken up from food as the vitamin called niacin. Similar compounds are released by reactions that break down the structure of NAD^+. These preformed components then pass through a salvage pathway that recycles them back into the active form. Some NAD^+ is also converted into nicotinamide adenine dinucleotide phosphate ($NADP^+$); the chemistry of this related coenzyme is similar to that of NAD^+, but it has different roles in metabolism.

Physical and chemical properties

Nicotinamide adenine dinucleotide, like all *dinucleotide*s, consists of two nucleotides joined by a pair of bridging phosphate groups. The nucleotides consist of ribose rings, one with adenine attached to the first carbon atom (the 1' position) and the other with nicotinamide at this position. The nicotinamide moiety can be attached in two orientations to this anomeric carbon atom. Because of these two possible structures, the compound exists as two diastereomers. It is the β-nicotinamide diastereomer of NAD^+

that is found in organisms. These nucleotides are joined together by a bridge of two phosphate groups through the 5' carbons.



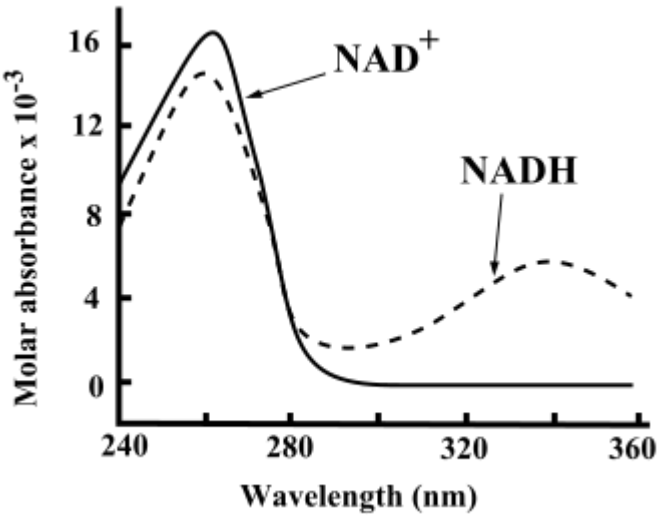
The redox reactions of nicotinamide adenine dinucleotide.

In metabolism, the compound accepts or donates electrons in redox reactions. Such reactions (summarized in formula below) involve the removal of two hydrogen atoms from the reactant (R), in the form of a hydride ion (H^-), and a proton (H^+). The proton is released into solution, while the reductant RH_2 is oxidized and NAD^+ reduced to NADH by transfer of the hydride to the nicotinamide ring.

$$RH_2 + NAD^+ \rightarrow NADH + H^+ + R$$

From the hydride electron pair, one electron is transferred to the positively charged nitrogen of the nicotinamide ring of NAD^+, and the second hydrogen atom transferred to the C4 carbon atom opposite this nitrogen. The midpoint potential of the NAD^+/NADH redox pair is −0.32 volts, which makes NADH a strong reducing agent. The reaction is easily reversible, when NADH reduces another molecule and is re-oxidized to NAD^+. This means the coenzyme can continuously cycle between the NAD^+ and NADH forms without being consumed.

In appearance, all forms of this coenzyme are white amorphous powders that are hygroscopic and highly water-soluble. The solids are stable if stored dry and in the dark. Solutions of NAD^+ are colorless and stable for about a week at 4 °C and neutral pH, but decompose rapidly in acids or alkalis. Upon decomposition, they form products that are enzyme inhibitors.



UV absorption spectra of NAD^+ and NADH.

Both NAD^+ and NADH absorb strongly in the ultraviolet due to the adenine base. For example, peak absorption of NAD^+ is at a wavelength of 259 nanometers (nm), with an extinction coefficient of 16,900 $M^{-1}cm^{-1}$. NADH also absorbs at higher wavelengths, with a second peak in UV absorption at 339 nm with an extinction coefficient of 6,220 $M^{-1}cm^{-1}$. This difference in the ultraviolet absorption spectra between the oxidized and reduced forms of the coenzymes at higher wavelengths makes it simple to measure the conversion of one to another in enzyme assays – by measuring the amount of UV absorption at 340 nm using a spectrophotometer.

NAD^+ and NADH also differ in their fluorescence. NADH in solution has an emission peak at 460 nm and a fluorescence lifetime of 0.4 nanoseconds, while the oxidized form of the coenzyme does not fluoresce. The properties of the fluorescence signal changes when NADH binds to proteins, so these changes can be used to measure dissociation constants, which are useful in the study of enzyme kinetics. These changes in fluorescence are also used to measure changes in the redox state of living cells, through fluorescence microscopy.

Concentration and state in cells

In rat liver, the total amount of NAD^+ and NADH is approximately 1 μmole per gram of wet weight, about 10 times the concentration of $NADP^+$ and NADPH in the same cells. The actual concentration of NAD^+ in cell cytosol is harder to measure, with recent estimates in animal cells, ranging around 0.3 mM, and approximately 1.0 to 2.0 mM in yeast. However, over 80% is bound to proteins, so the concentration in solution is much lower.

Data for other compartments in the cell are limited, although, in the mitochondrion the concentration of NAD^+ is similar to that in the cytosol. This NAD^+ is carried into the

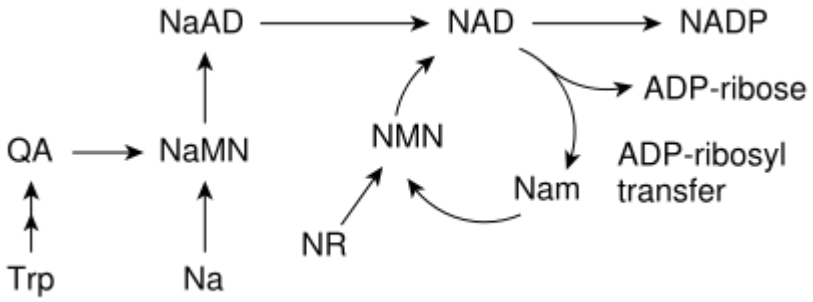
mitochondrion by a specific membrane transport protein, since the coenzyme cannot diffuse across membranes.

The balance between the oxidized and reduced forms of nicotinamide adenine dinucleotide is called the NAD^+/NADH ratio. This ratio is an important component of what is called the *redox state* of a cell, a measurement that reflects both the metabolic activities and the health of cells. The effects of the NAD^+/NADH ratio are complex, controlling the activity of several key enzymes, including glyceraldehyde 3-phosphate dehydrogenase and pyruvate dehydrogenase. In healthy mammalian tissues, estimates of the ratio between free NAD^+ and NADH in the cytoplasm typically lie around 700; the ratio is thus favourable for oxidative reactions. The ratio of total NAD^+/NADH is much lower, with estimates ranging from 0.05 to 4. In contrast, the $NADP^+$/NADPH ratio is normally about 0.005, so NADPH is the dominant form of this coenzyme. These different ratios are key to the different metabolic roles of NADH and NADPH.

Biosynthesis

NAD^+ is synthesized through two metabolic pathways. It is produced either in a *de novo* pathway from amino acids or in salvage pathways by recycling preformed components such as nicotinamide back to NAD^+.

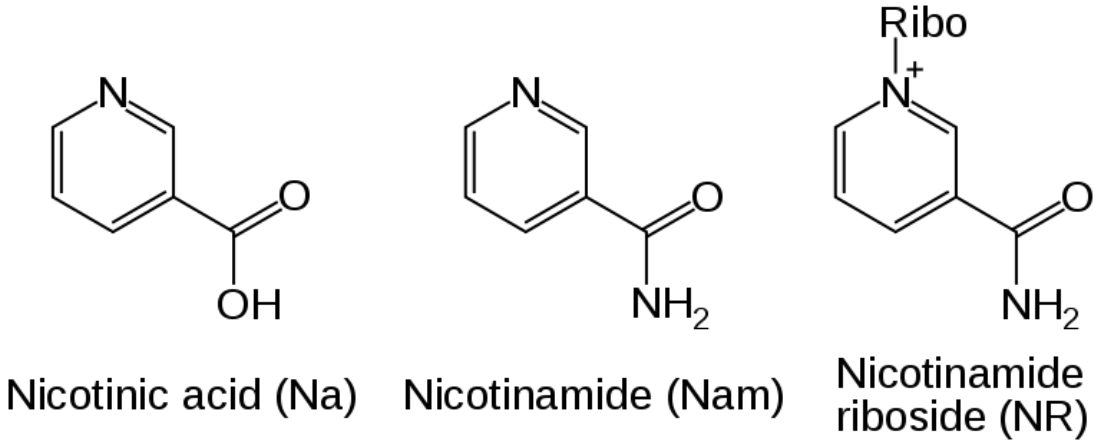
De novo production



Some metabolic pathways that synthesize and consume NAD^+ in vertebrates. The abbreviations are defined in the text.

Most organisms synthesize NAD^+ from simple components. The specific set of reactions differs among organisms, but a common feature is the generation of quinolinic acid (QA) from an amino acid – either tryptophan (Trp) in animals and some bacteria, or aspartic acid in some bacteria and plants. The quinolinic acid is converted to nicotinic acid mononucleotide (NaMN) by transfer of a phosphoribose moiety. An adenylate moiety is then transferred to form nicotinic acid adenine dinucleotide (NaAD). Finally, the nicotinic acid moiety in NaAD is amidated to a nicotinamide (Nam) moiety, forming nicotinamide adenine dinucleotide.

In a further step, some NAD^+ is converted into $NADP^+$ by NAD+ kinase, which phosphorylates NAD^+. In most organisms, this enzyme uses ATP as the source of the phosphate group, although several bacteria such as *Mycobacterium tuberculosis* and a hyperthermophilic archaeon *Pyrococcus horikoshii*, use inorganic polyphosphate as an alternative phosphoryl donor.



Salvage pathways use three precursors for NAD^+.

Salvage pathways

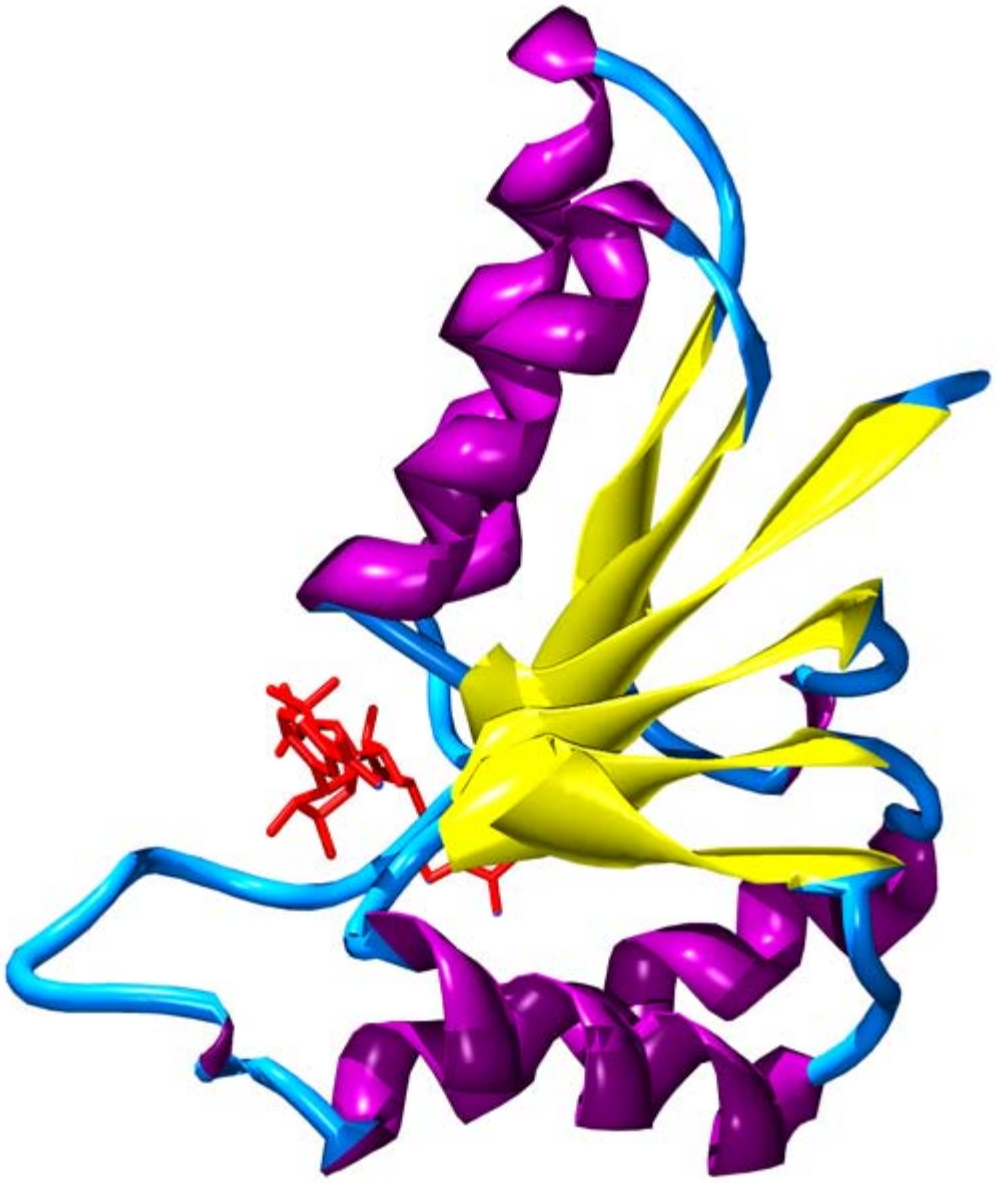
Besides assembling NAD^+ *de novo* from simple amino acid precursors, cells also salvage preformed compounds containing nicotinamide. Although other precursors are known, the three natural compounds containing the nicotinamide ring and used in these salvage metabolic pathways are nicotinic acid (Na), nicotinamide (Nam) and nicotinamide riboside (NR). The precursors are fed into the $NAD(P)^+$ biosynthetic pathway, shown above, through adenylation and phosphoribosylation reactions. These compounds can be taken up from the diet, where the mixture of nicotinic acid and nicotinamide are called vitamin B_3 or *niacin*. However, these compounds are also produced within cells, when the nicotinamide moiety is released from NAD^+ in ADP-ribose transfer reactions. Indeed, the enzymes involved in these salvage pathways appear to be concentrated in the cell nucleus, which may compensate for the high level of reactions that consume NAD^+ in this organelle. Cells can also take up extracellular NAD^+ from their surroundings.

Despite the presence of the *de novo* pathway, the salvage reactions are essential in humans; a lack of niacin in the diet causes the vitamin deficiency disease pellagra. This high requirement for NAD^+ results from the constant consumption of the coenzyme in reactions such as posttranslational modifications, since the cycling of NAD^+ between oxidized and reduced forms in redox reactions does not change the overall levels of the coenzyme.

The salvage pathways used in microorganisms differ from those of mammals. Some pathogens, such as the yeast *Candida glabrata* and the bacterium *Haemophilus influenzae*

are NAD^+ auxotrophs – they cannot synthesize NAD^+ – but possess salvage pathways and thus are dependent on external sources of NAD^+ or its precursors. Even more surprising is the intracellular pathogen *Chlamydia trachomatis*, which lacks recognizable candidates for any genes involved in the biosynthesis or salvage of both NAD^+ and $NADP^+$, and must acquire these coenzymes from its host.

Functions



Rossmann fold in part of the lactate dehydrogenase of *Cryptosporidium parvum*, showing NAD^+ in red, beta sheets in yellow, and alpha helices in purple.

Nicotinamide adenine dinucleotide has several essential roles in metabolism. It acts as a coenzyme in redox reactions, as a donor of ADP-ribose moieties in ADP-ribosylation

reactions, as a precursor of the second messenger molecule cyclic ADP-ribose, as well as acting as a substrate for bacterial DNA ligases and a group of enzymes called sirtuins that use NAD^+ to remove acetyl groups from proteins.

Oxidoreductases

The main role of NAD^+ in metabolism is the transfer of electrons from one molecule to another. Reactions of this type are catalyzed by a large group of enzymes called oxidoreductases. The correct names for these enzymes contain the names of both their substrates: for example NADH-ubiquinone oxidoreductase catalyzes the oxidation of NADH by coenzyme Q. However, these enzymes are also referred to as *dehydrogenases* or *reductases*, with NADH-ubiquinone oxidoreductase commonly being called *NADH dehydrogenase* or sometimes *coenzyme Q reductase*.

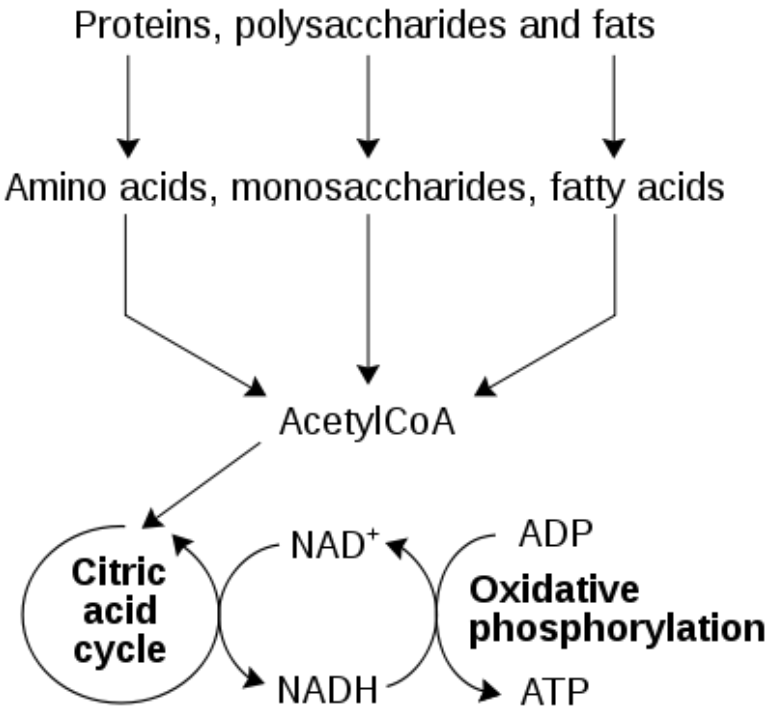
When bound to a protein, NAD^+ and NADH are usually held within a structural motif known as the Rossmann fold. The motif is named after Michael Rossmann who was the first scientist to notice how common this structure is within nucleotide-binding proteins. This fold contains three or more parallel beta strands linked by two alpha helices in the order beta-alpha-beta-alpha-beta. This forms a beta sheet flanked by a layer of alpha helices on each side. Because each Rossmann fold binds one nucleotide, binding domains for the dinucleotide NAD^+ consist of two paired Rossmann folds, with each fold binding one nucleotide within the cofactor. However, this fold is not universal among NAD-dependent enzymes, since a class of bacterial enzymes involved in amino acid metabolism have recently been discovered that bind the coenzyme, but lack this motif.

3-D conformation of NAD^+.

When bound in the active site of an oxidoreductase, the nicotinamide ring of the coenzyme is positioned so that it can accept a hydride from the other substrate. Since the C4 carbon that accepts the hydrogen is prochiral, this can be exploited in enzyme kinetics to give information about the enzyme's mechanism. This is done by mixing an enzyme with a substrate that has deuterium atoms substituted for the hydrogens, so the enzyme will reduce NAD^+ by transferring deuterium rather than hydrogen. In this case, an enzyme can produce one of two stereoisomers of NADH. In some enzymes the hydrogen is transferred from above the plane of the nicotinamide ring; these are called *class A* oxidoreductases, whereas class B enzymes transfer the atom from below.

Despite the similarity in how proteins bind the two coenzymes, enzymes almost always show a high level of specificity for either NAD^+ or $NADP^+$. This specificity reflects the distinct metabolic roles of the respective coenzymes, and is the result of distinct sets of amino acid residues in the two types of coenzyme-binding pocket. For instance, in the active site of NADP-dependent enzymes, an ionic bond is formed between a basic amino acid side-chain and the acidic phosphate group of $NADP^+$. On the converse, in NAD-dependent enzymes the charge in this pocket is reversed, preventing $NADP^+$ from binding. However, there are a few exceptions to this general rule, and enzymes such as aldose reductase, glucose-6-phosphate dehydrogenase, and methylenetetrahydrofolate reductase can use both coenzymes in some species.

Role in redox metabolism



A simplified outline of redox metabolism, showing how NAD^+ and NADH link the citric acid cycle and oxidative phosphorylation.

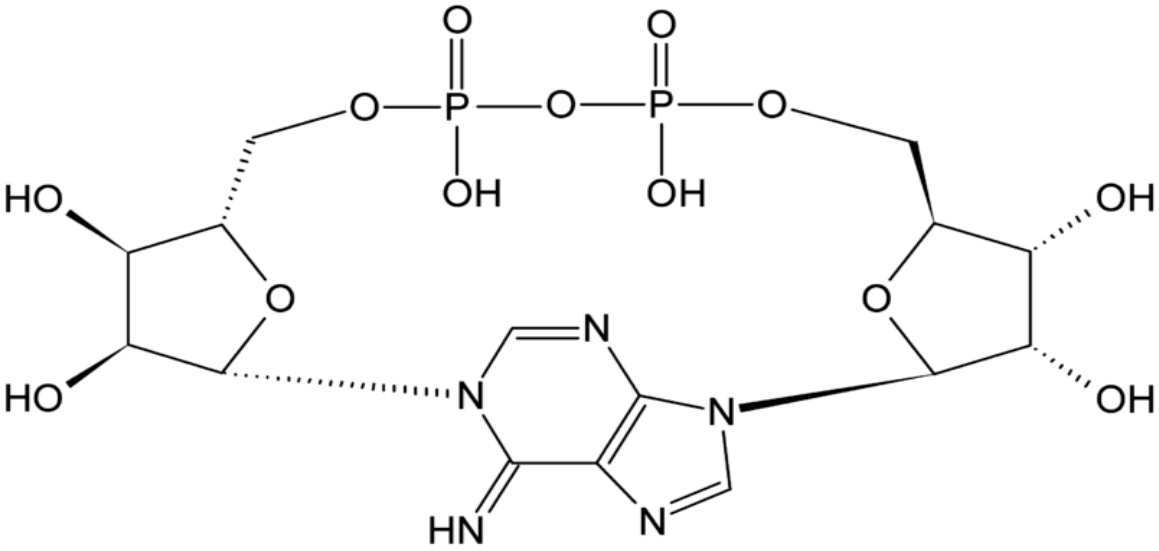
The redox reactions catalyzed by oxidoreductases are vital in all parts of metabolism, but one particularly important area where these reactions occur is in the release of energy from nutrients. Here, reduced compounds such as glucose are oxidized, thereby releasing energy. This energy is transferred to NAD^+ by reduction to NADH, as part of glycolysis and the citric acid cycle. In eukaryotes the electrons carried by the NADH that is produced in the cytoplasm by glycolysis are transferred into the mitochondrion (to reduce mitochondrial NAD^+) by mitochondrial shuttles, such as the malate-aspartate shuttle. The mitochondrial NADH is then oxidized in turn by the electron transport chain, which pumps protons across a membrane and generates ATP through oxidative phosphorylation. These shuttle systems also have the same transport function in chloroplasts.

Since both the oxidized and reduced forms of nicotinamide adenine dinucleotide are used in these linked sets of reactions, the cell maintains significant concentrations of both NAD^+ and NADH, with the high NAD^+/NADH ratio allowing this coenzyme to act as both an oxidizing and a reducing agent. In contrast, the main function of NADPH is as a reducing agent in anabolism, with this coenzyme being involved in pathways such as fatty acid synthesis and photosynthesis. Since NADPH is needed to drive redox reactions as a strong reducing agent, the $NADP^+$/NADPH ratio is kept very low.

Although it is important in catabolism, NADH is also used in anabolic reactions, such as gluconeogenesis. This need for NADH in anabolism poses a problem for prokaryotes growing on nutrients that release only a small amount of energy. For example, nitrifying bacteria such as *Nitrobacter* oxidize nitrite to nitrate, which releases sufficient energy to pump protons and generate ATP, but not enough to produce NADH directly. As NADH is still needed for anabolic reactions, these bacteria use a nitrite oxidoreductase to produce enough proton-motive force to run part of the electron transport chain in reverse, generating NADH.

Non-redox roles

The coenzyme NAD^+ is also consumed in ADP-ribose transfer reactions. For example, enzymes called ADP-ribosyltransferases add the ADP-ribose moiety of this molecule to proteins, in a posttranslational modification called ADP-ribosylation. NAD^+ may also be added onto cellular RNA as a base modification. ADP-ribosylation involves either the addition of a single ADP-ribose moiety, in *mono-ADP-ribosylation*, or the transferral of ADP-ribose to proteins in long branched chains, which is called *poly(ADP-ribosyl)ation*. Mono-ADP-ribosylation was first identified as the mechanism of a group of bacterial toxins, notably cholera toxin, but it is also involved in normal cell signaling. Poly(ADP-ribosyl)ation is carried out by the poly(ADP-ribose) polymerases. The poly(ADP-ribose) structure is involved in the regulation of several cellular events and is most important in the cell nucleus, in processes such as DNA repair and telomere maintenance. In addition to these functions within the cell, a group of extracellular ADP-ribosyltransferases has recently been discovered, but their functions remain obscure.



The structure of cyclic ADP-ribose.

Another function of this coenzyme in cell signaling is as a precursor of cyclic ADP-ribose, which is produced from NAD^+ by ADP-ribosyl cyclases, as part of a second messenger system. This molecule acts in calcium signaling by releasing calcium from intracellular stores. It does this by binding to and opening a class of calcium channels called ryanodine receptors, which are located in the membranes of organelles, such as the endoplasmic reticulum.

NAD^+ is also consumed by sirtuins, which are NAD-dependent deacetylases, such as Sir2. These enzymes act by transferring an acetyl group from their substrate protein to the ADP-ribose moiety of NAD^+; this cleaves the coenzyme and releases nicotinamide and O-acetyl-ADP-ribose. The sirtuins mainly seem to be involved in regulating transcription through deacetylating histones and altering nucleosome structure. However, non-histone proteins can be deacetylated by sirtuins as well. These activities of sirtuins are particularly interesting because of their importance in the regulation of aging.

Other NAD-dependent enzymes include bacterial DNA ligases, which join two DNA ends by using NAD^+ as a substrate to donate an adenosine monophosphate (AMP) moiety to the 5' phosphate of one DNA end. This intermediate is then attacked by the 3' hydroxyl group of the other DNA end, forming a new phosphodiester bond. This contrasts with eukaryotic DNA ligases, which use ATP to form the DNA-AMP intermediate.

Pharmacology and medical uses

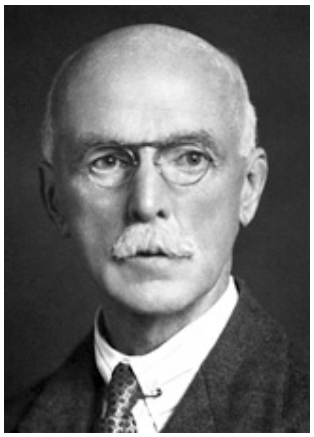
The enzymes that make and use NAD^+ and NADH are important in both current pharmacology and the research into future treatments for disease. Drug design and drug development exploits NAD^+ in three ways: as a direct target of drugs, by designing enzyme inhibitors or activators based on its structure that change the activity of NAD-dependent enzymes, and by trying to inhibit NAD^+ biosynthesis.

The coenzyme NAD^+ is not itself currently used as a treatment for any disease. However, it is potentially useful in the therapy of neurodegenerative diseases such as Alzheimer's and Parkinson disease. Evidence on the use of NAD^+ in neurodegeneration is mixed; studies in mice are promising, whereas a placebo-controlled clinical trial failed to show any effect. NAD^+ is also a direct target of the drug isoniazid, which is used in the treatment of tuberculosis, an infection caused by *Mycobacterium tuberculosis*. Isoniazid is a prodrug and once it has entered the bacteria, it is activated by a peroxidase, which oxidizes the compound into a free radical form. This radical then reacts with NADH, to produce adducts that are very potent inhibitors of the enzymes enoyl-acyl carrier protein reductase, and dihydrofolate reductase.

Since a large number of oxidoreductases use NAD^+ and NADH as substrates, and bind them using a highly conserved structural motif, the idea that inhibitors based on NAD^+ could be specific to one enzyme is surprising. However, this can be possible: for example, inhibitors based on the compounds mycophenolic acid and tiazofurin inhibit IMP dehydrogenase at the NAD^+ binding site. Because of the importance of this enzyme in purine metabolism, these compounds may be useful as anti-cancer, anti-viral, or immunosuppressive drugs. Other drugs are not enzyme inhibitors, but instead activate enzymes involved in NAD^+ metabolism. Sirtuins are a particularly interesting target for such drugs, since activation of these NAD-dependent deacetylases extends lifespan. Compounds such as resveratrol increase the activity of these enzymes, which may be important in their ability to delay aging in both vertebrate, and invertebrate model organisms.

Because of the differences in the metabolic pathways of NAD^+ biosynthesis between organisms, such as between bacteria and humans, this area of metabolism is a promising area for the development of new antibiotics. For example, the enzyme nicotinamidase, which converts nicotinamide to nicotinic acid, is a target for drug design, as this enzyme is absent in humans but present in yeast and bacteria.

History



Arthur Harden, co-discoverer of NAD.

The coenzyme NAD^+ was first discovered by the British biochemists Arthur Harden and William Youndin in 1906. They noticed that adding boiled and filtered yeast extract greatly accelerated alcoholic fermentation in unboiled yeast extracts. They called the unidentified factor responsible for this effect a *coferment*. Through a long and difficult purification from yeast extracts, this heat-stable factor was identified as a nucleotide sugar phosphate by Hans von Euler-Chelpin. In 1936, the German scientist Otto Heinrich Warburg showed the function of the nucleotide coenzyme in hydride transfer and identified the nicotinamide portion as the site of redox reactions.

A source of nicotinamide was identified in 1938, when Conrad Elvehjem purified niacin from liver and showed this vitamin contained nicotinic acid and nicotinamide. Then, in 1939, he provided the first strong evidence that niacin was used to synthesize NAD^+. In the early 1940s, Arthur Kornberg made another important contribution towards understanding NAD^+ metabolism, by being the first to detect an enzyme in the biosynthetic pathway. Subsequently, in 1949, the American biochemists Morris Friedkin and Albert L. Lehninger proved that NADH linked metabolic pathways such as the citric acid cycle with the synthesis of ATP in oxidative phosphorylation. Finally, in 1959, Jack Preiss and Philip Handler discovered the intermediates and enzymes involved in the biosynthesis of NAD^+; consequently, *de novo* synthesis is often called the Preiss-Handler pathway in their honor.

The non-redox roles of NAD(P) are a recent discovery. The first of these functions to be identified was the use of NAD^+ as the ADP-ribose donor in ADP-ribosylation reactions, observed in the early 1960s. Later studies in the 1980s and 1990s revealed the activities of NAD^+ and $NADP^+$ metabolites in cell signaling – such as the action of cyclic ADP-ribose, which was discovered in 1987. The metabolism of NAD^+ has remained an area of intense research into the 21st century, with interest being heightened after the discovery of the NAD^+-dependent protein deacetylases called sirtuins in 2000, by Shinichiro Imai and coworkers at the Massachusetts Institute of Technology.