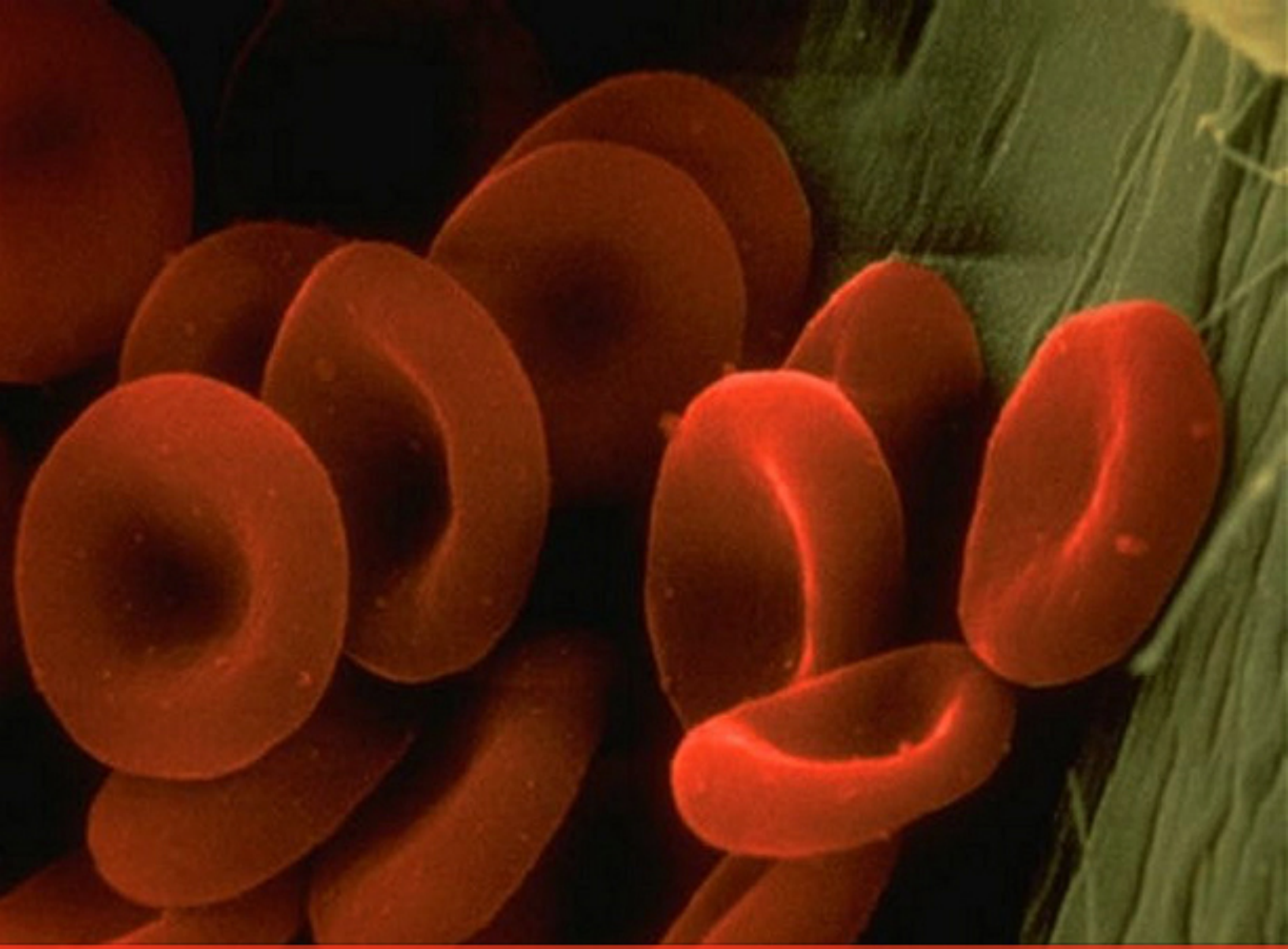

Blood Proteins

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Table of Contents

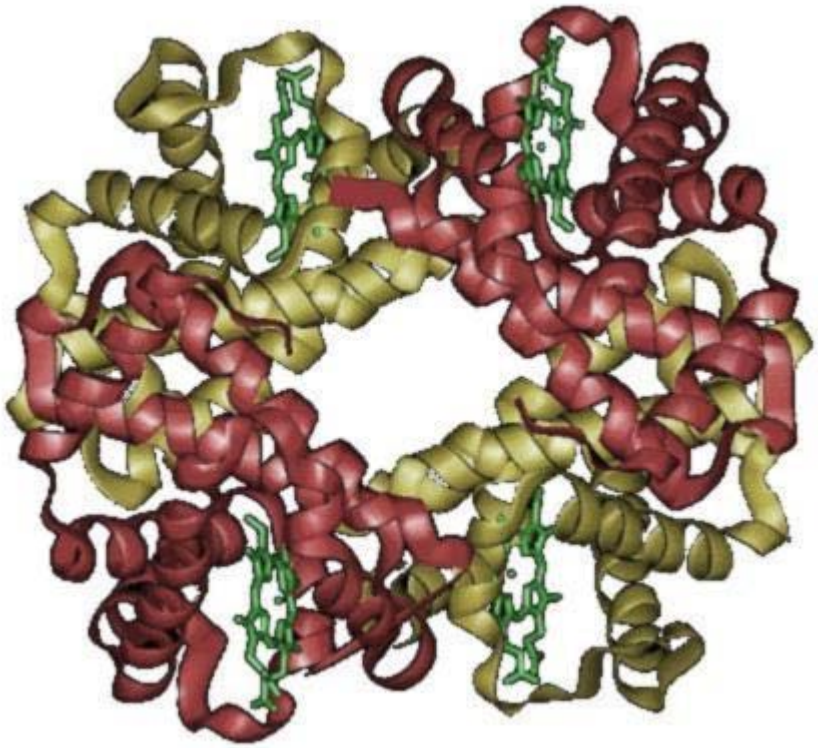
Introduction

Blood proteins, also called *serum proteins*, are proteins found in blood plasma. Serum total protein in blood is 7g/dl. They serve many different functions, including

- circulatory transport molecules for lipids, hormones, vitamins and metals
- enzymes, complement components, protease inhibitors, and kinin precursors
- regulation of acellular activity and functioning and in the immune system.

Separating serum proteins by electrophoresis is a valuable diagnostic tool as well as a way to monitor clinical progress.

Often mentioned blood proteins:



Structure of hemoglobin

Blood protein	Normal level	%	Function
Albumins	3.5-5.0 g/dl	60%	create oncotic pressure and transports other molecules
immunoglobulins	1.0-1.5 g/dl	18%	participate in immune system
Fibrinogens	0.2-0.45 g/dl	4%	blood coagulation
alpha 1-antitrypsin			neutralize trypsin that has leaked from the digestive system
Regulatory proteins		<1%	Regulation of gene expression

Other types of blood proteins include: Prealbumin Alpha 1 antitrypsin Alpha 1 acid glycoprotein Alpha 1 fetoprotein Haptoglobin Alpha 2 macroglobulin Ceruloplasmin Transferring C3/C4 Beta 2 microglobulin Beta lipoprotein Gamma globulin proteins C-reactive protein (CRP)

- alpha2-macroglobulin
- Other globulins, which are of three types- alpha, beta and gamma.
- Lipoproteins (chylomicrons, VLDL, LDL, HDL)
- Transferrin
- Prothrombin
- MBL or MBP

All the plasma proteins are synthesized in liver except gamma globulins.

60% of plasma proteins are made up of the protein albumin, which are major contributors to osmotic pressure of plasma which assists in the transport of lipids and steroid hormones. Globulins make up 35% of plasma proteins and are used in the transport of ions, hormones and lipids assisting in immune function. 4% is fibrinogen which is essential in the clotting of blood and can be converted into insoluble fibrin. Regulatory proteins which make up less than 1% of plasma proteins are proteins such as enzymes, proenzymes and hormones. Current research regarding blood plasma proteins is centered on performing proteomics analyses of serum/plasma in the search for biomarkers. These efforts started with two-dimensional gel electrophoresis efforts in the 1970s and in more recent times this research has been performed using LC-tandem MS based proteomics.

Chapter 1

Albumin and Cholesterylester Transfer Protein

Albumin

Serum albumin family

Structure of human serum albumin.

Identifiers	
Symbol	Serum_albumin
Pfam	PF00273
Pfam clan	CL0282
InterPro	IPR014760
SMART	SM00103
PROSITE	PS51438
SCOP	1ao6

Albumin (Latin: albus, white) refers generally to any protein that is water soluble, which is moderately soluble in concentrated salt solutions, and experiences heat denaturation.

They are commonly found in blood plasma, and are unique to other plasma proteins in that they are not glycosylated. Substances containing albumin, such as egg white, are called *albuminoids*.

A number of serum transport proteins are known to be evolutionarily related, including serum albumin, alpha-fetoprotein, vitamin D-binding protein and afamin.

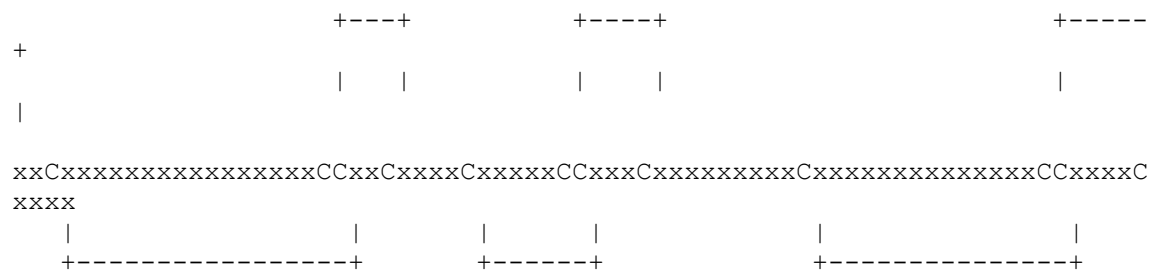
Function

Albumin is the main protein of plasma; it binds water, cations (such as Ca^{2+}, Na^{+} and K^{+}), fatty acids, hormones, bilirubin and drugs - its main function is to regulate the colloidal osmotic pressure of blood. Alpha-fetoprotein (alpha-fetoglobulin) is a fetal plasma protein that binds various cations, fatty acids and bilirubin. Vitamin D-binding protein binds to vitamin D and its metabolites, as well as to fatty acids. The biological role of afamin (alpha-albumin) has not yet been characterised.

Structure

The 3D structure of human serum albumin has been determined by X-ray crystallography to a resolution of 2.8 Å.

Albumin comprises three homologous domains that assemble to form a heart-shaped molecule. Each domain is a product of two subdomains that possess common structural motifs. The principal regions of ligand binding to human serum albumin are located in hydrophobic cavities in subdomains IIA and IIIA, which exhibit similar chemistry. Structurally, the serum albumins are similar, each domain containing five or six internal disulphide bonds, as shown schematically below:



Types

Serum albumin

Serum albumin is the most abundant blood plasma protein and is produced in the liver and forms a large proportion of all plasma protein. The human version is human serum albumin, and it normally constitutes about 60% of human plasma protein.

Serum albumins are important in regulating blood volume by maintaining the oncotic pressure (also known as colloid osmotic pressure) of the blood compartment. They also serve as carriers for molecules of low water solubility this way isolating their hydrophobic nature, including lipid soluble hormones, bile salts, unconjugated bilirubin, free fatty acids (apoprotein), calcium, ions (transferrin), and some drugs like warfarin, phenobutazone, clofibrate & phenytoin. For this reason, it's sometimes referred as a molecular "taxi". Competition between drugs for albumin binding sites may cause drug interaction by increasing the free fraction of one of the drugs, thereby affecting potency.

Specific types include:

- human serum albumin
- bovine serum albumin (cattle serum albumin) or BSA, often used in medical and molecular biology labs.

Low albumin (hypoalbuminemia) may be caused by liver disease, nephrotic syndrome, burns, protein-losing enteropathy, malabsorption, malnutrition, late pregnancy, artefact, genetic variations and malignancy.

High albumin (hyperalbuminemia) is almost always caused by dehydration. In some cases of retinol (Vitamin A) deficiency the albumin level can be elevated to high-normal values (e.g., 4.9 g/dL). This is because retinol causes cells to swell with water (this is also the reason too much Vitamin A is toxic). In lab experiments it has been shown that All-trans retinoic acid down regulates human albumin production

Normal range of human serum albumin in adults (> 3 y.o.) is 3.5 to 5 g/dL. For children less than three years of age, the normal range is broader, 2.9-5.5 g/dL.

Albumin binds to the cell surface receptor Albondin.

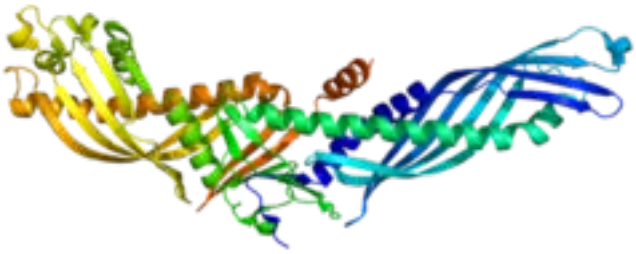
Other types

Other types include the storage protein ovalbumin in egg white, and different storage albumins in the seeds of some plants.

- Note that the protein 'albumin' is spelled with an "i", while "albumen" with an "e", is the white of an egg which contains (among other things) several dozen types of albumin (with an 'i'), mostly ovalbumin.

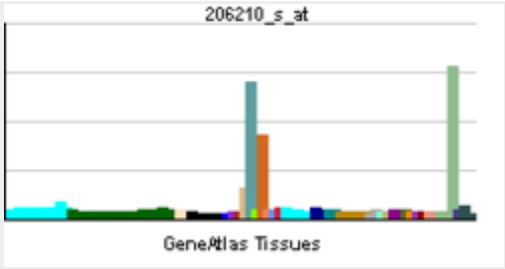
Cholesterylester transfer protein

Cholesteryl ester transfer protein, plasma



PDB rendering based on 2obd.

Available structures		
Identifiers		
Symbols	CETP;	
External IDs	OMIM: 118470 HomoloGene: 47904 GeneCards: CETP Gene	
RNA expression pattern		



Orthologs		
Species	**Human**	**Mouse**
Entrez	1071	n/a
Ensembl	ENSG00000087237	n/a
UniProt	P11597	n/a
RefSeq (mRNA)	NM_000078	n/a
RefSeq (protein)	NP_000069	n/a

Location (UCSC)	Chr 16: 55.55 - 55.58 Mb	n/a

Cholesteryl ester transfer protein (CETP), also called **plasma lipid transfer protein**, is a plasma protein that facilitates the transport of cholesteryl esters and triglycerides between the lipoproteins. It collects triglycerides from very-low-density (VLDL) or low-density lipoproteins (LDL) and exchanges them for cholesteryl esters from high-density lipoproteins (HDL), and vice versa. Most of the time, however, CETP does a homoexchange, trading a triglyceride for a triglyceride or a cholesteryl ester for a cholesteryl ester.

Genetics

The *CETP* gene is located on the sixteenth chromosome (16q21).

Role in disease

Rare mutations leading to increased function of CETP have been linked to accelerated atherosclerosis. In contrast, a polymorphism (I405V) of the *CETP* gene leading to lower serum levels has also been linked to exceptional longevity and to metabolic response to nutritional intervention. However, this mutation also increases the prevalence of coronary heart disease in patients with hypertriglyceridemia. The D442G mutation, which lowers CETP levels and increases HDL levels, also increases coronary heart disease.

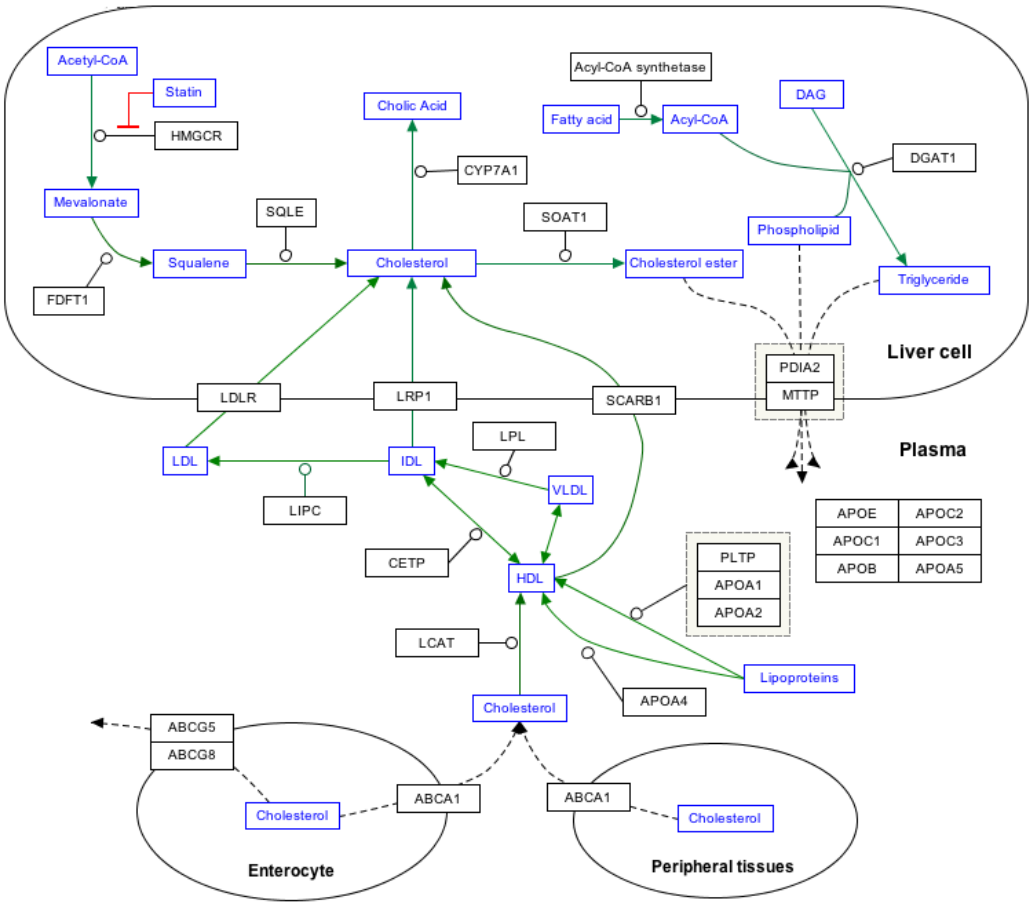
Elaidic acid, a major component of trans fat, increases CETP activity.

Pharmacology

As HDL can alleviate atherosclerosis and other cardiovascular diseases, and certain disease states such as the metabolic syndrome feature low HDL, pharmacological inhibition of CETP is being studied as a method of improving HDL levels. To be specific, in a 2004 study, the small molecular agent torcetrapib was shown to increase HDL levels, alone and with a statin, and lower LDL when co-administered with a statin. Studies into cardiovascular endpoints, however, were largely disappointing. While they confirmed the change in lipid levels, most reported an increase in blood pressure, no change in atherosclerosis, and, in a trial of a combination of torcetrapib and atorvastatin, an increase in cardiovascular events and mortality.

A compound related to torcetrapib, with the investigative name JTT-705/R1658, is also being studied. It increases HDL levels by 30%, as compared to 60% by torcetrapib. Another CETP inhibitor under development is Merck's MK-0859 anacetrapib, which in initial studies is not shown to increase blood pressure.

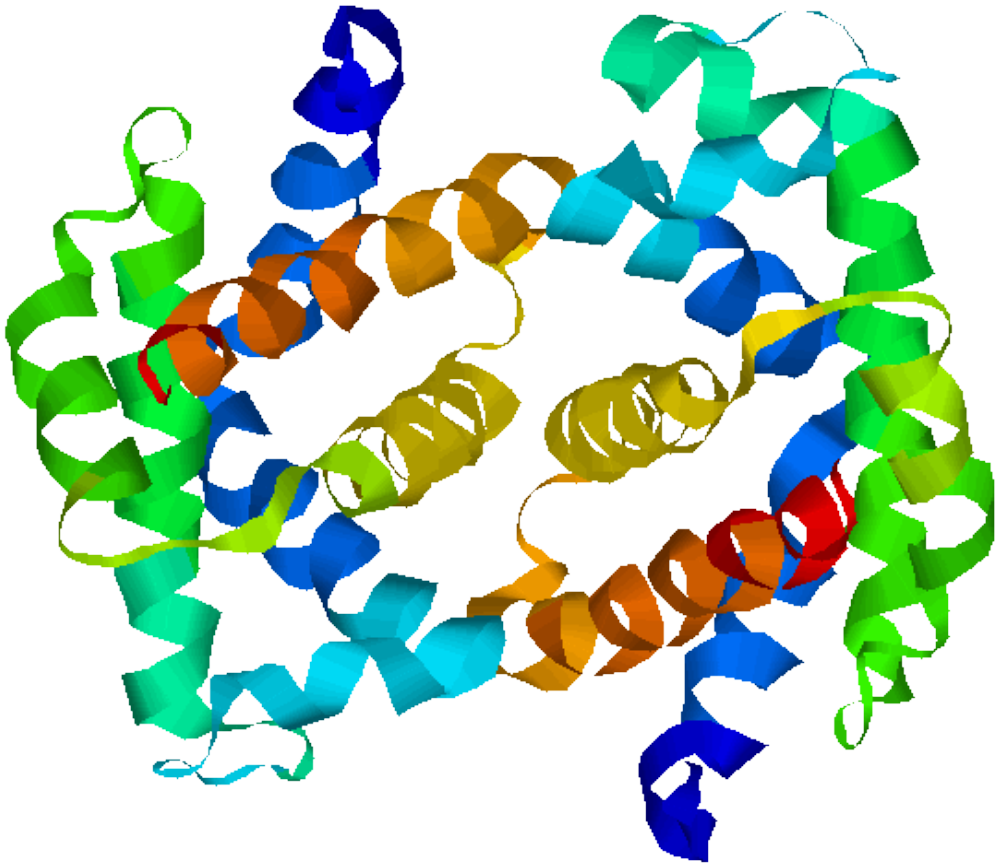
Interactive pathway map



Statin Pathway

Chapter 2

Fetal Hemoglobin



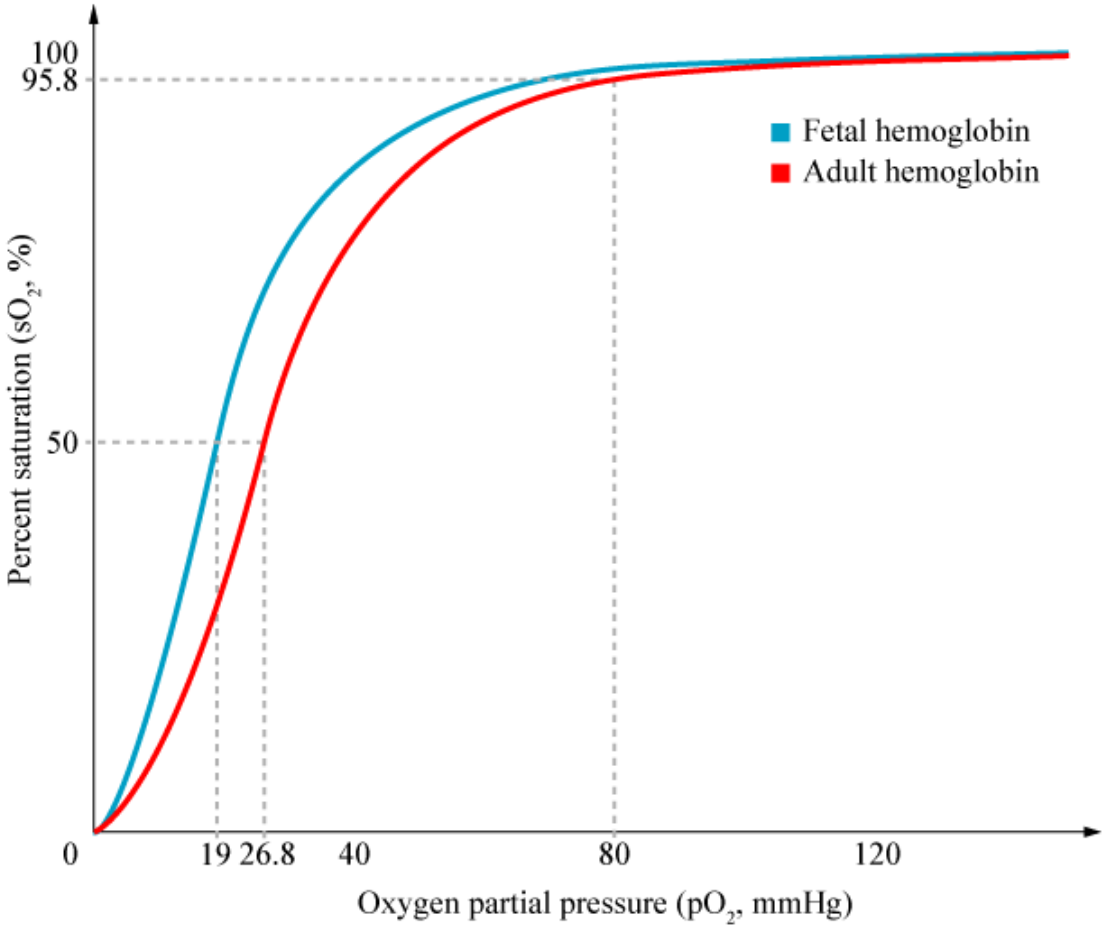
Fetal hemoglobin protein structure

Fetal hemoglobin, or **foetal haemoglobin**, (also **hemoglobin F** or **HbF**) is the main oxygen transport protein in the fetus during the last seven months of development in the uterus and in the newborn until roughly 6 months old. Functionally, fetal hemoglobin differs most from adult hemoglobin in that it is able to bind oxygen with greater affinity

than the adult form, giving the developing fetus better access to oxygen from the mother's bloodstream.

In newborns, fetal hemoglobin is nearly completely replaced by adult hemoglobin by approximately the twelfth week of postnatal life. In adults, fetal hemoglobin production can be reactivated pharmacologically, which is useful in the treatment of such diseases as sickle-cell disease.

Overview



The oxygen saturation curve for fetal hemoglobin (blue) appears left-shifted when compared to adult hemoglobin (red) since fetal hemoglobin has a greater affinity for oxygen.

Oxygenated blood is delivered to the fetus via the umbilical vein from the placenta, which is anchored to the wall of the mother's uterus. As blood courses through the mother, oxygen is delivered to capillary beds for gas exchange, and by the time blood reaches the capillaries of the placenta, its oxygen saturation has decreased considerably. In order to recover enough oxygen to sustain itself, the fetus must be able to bind oxygen with a greater affinity than the mother.

Fetal hemoglobin's affinity for oxygen is substantially greater than that of adult hemoglobin. Notably, the P50 value for fetal hemoglobin (i.e., the partial pressure of oxygen at which the protein is 50% saturated; lower values indicate greater affinity) is roughly 19 mmHg, whereas adult hemoglobin has a value of approximately 26.8 mmHg. As a result, the so-called "oxygen saturation curve", which plots percent saturation *vs.* pO_2, is left-shifted for fetal hemoglobin in comparison to the same curve in adult hemoglobin.

This greater affinity for oxygen is explained by fetal hemoglobin's interaction with 2,3-bisphosphoglycerate (2,3-BPG or 2,3-DPG). In adult red blood cells, this substance decreases the affinity of hemoglobin for oxygen. It is also present in fetal red blood cells, but does not interact with fetal hemoglobin, leaving its affinity for oxygen unchanged. Adult hemoglobin alone actually has a higher affinity for oxygen than its fetal equivalent, but the levels of 2,3-BPG reduce it.

For mothers to deliver oxygen to a fetus, it is necessary for the fetal hemoglobin to extract oxygen from the maternal oxygenated hemoglobin across the placenta. This requires the fetal hemoglobin to have a higher oxygen affinity than that of the maternal carrier. This is achieved by a fetal hemoglobin subunit γ (gamma), instead of the b (beta) subunit. The γ subunit has less positive charges than the adult hemoglobin b subunit. This means that 2,3-bisphosphoglycerate (2,3-BPG) is less electrostatically bound to fetal hemoglobin as compared to adult hemoglobin. This means that 2,3-BPG is less effective in lowering the oxygen affinity of the fetal hemoglobin. This lowered affinity allows for adult hemoglobin (the maternal hemoglobin of the mother) to readily transfer its oxygen to the fetal hemoglobin.

Distribution

After the first 10 to 12 weeks of development, the fetus' primary form of hemoglobin switches from embryonic hemoglobin to fetal hemoglobin. At birth, fetal hemoglobin comprises 50-95% of the child's hemoglobin. These levels decline after six months as adult hemoglobin synthesis is activated while fetal hemoglobin synthesis is deactivated. Soon after, adult hemoglobin (hemoglobin A in particular) takes over as the predominant form of hemoglobin in normal children. Certain genetic abnormalities can cause the switch to adult hemoglobin synthesis to fail, resulting in a condition known as hereditary persistence of fetal hemoglobin (HPFH).

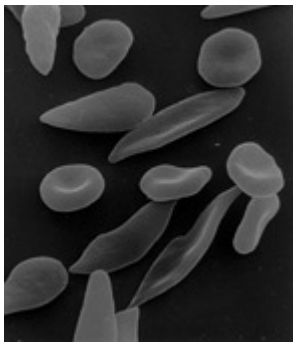
Structure and genetics

Most types of normal hemoglobin, including hemoglobin A, hemoglobin A2, and hemoglobin F, are tetramers composed of four protein subunits and four heme prosthetic groups. Whereas adult hemoglobin is composed of two alpha and two beta subunits, fetal hemoglobin is composed of two alpha and two gamma subunits, commonly denoted as $\alpha_2\gamma_2$. Because of its presence in fetal hemoglobin, the gamma subunit is commonly called the "fetal" hemoglobin subunit.

In humans, each chromosome 11 contains two similar copies of the gene that encodes the gamma subunit, γG (glycine as residue 136) and γA (alanine as residue 136). (The beta subunit is also on Chromosome 11) The gene that codes for the alpha subunit is located on chromosome 16 and is also present in duplicate.

Clinical significance

Treatment of sickle-cell disease



Increasing the body's production of fetal hemoglobin is used as a strategy to treat sickle-cell disease.

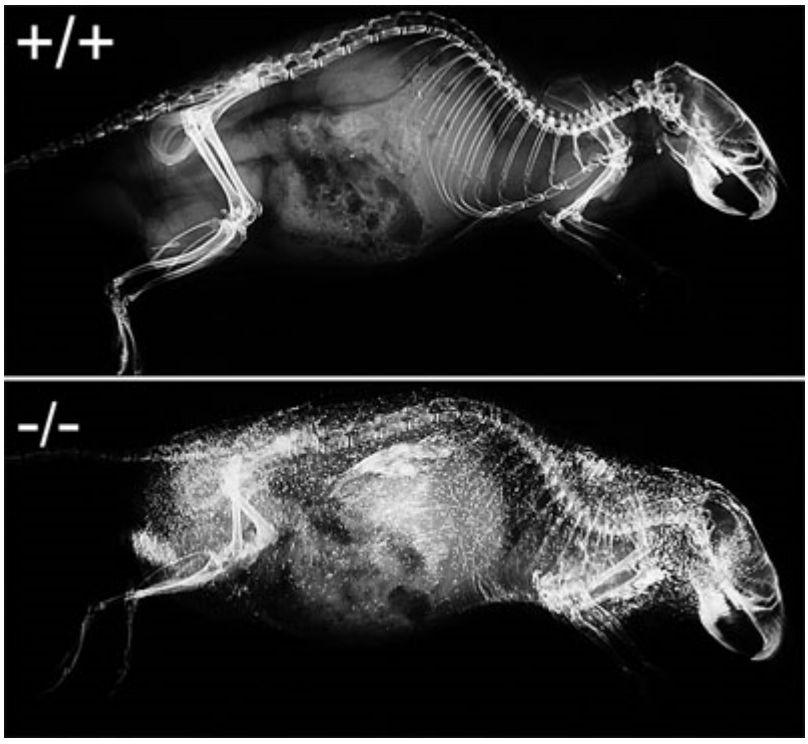
When fetal hemoglobin production is switched off after birth, normal children begin producing adult hemoglobin (HbA). Children with sickle-cell disease instead begin producing a defective form of hemoglobin called hemoglobin S. This variety of hemoglobin aggregates, forming filaments that cause red blood cells to change their shape from round to sickle-shaped, which have a greater tendency to stack on top of one another and block blood vessels. These invariably lead to so-called *painful vaso-occlusive episodes*, which are a hallmark of the disease.

If fetal hemoglobin remains the predominant form of hemoglobin after birth, the number of painful episodes decreases in patients with sickle-cell disease. Hydroxyurea promotes the production of fetal hemoglobin and can be used to treat individuals with sickle-cell disease. Combination therapy with hydroxyurea and recombinant erythropoietin—rather than treatment with hydroxyurea alone—has been shown to further elevate hemoglobin F levels and to promote the development of HbF-containing F-cells.

Chapter 3

Fetuin and Fibrin

Fetuin



X-ray picture of a Fetuin-A knockout mouse (-/-) compared to a wildtype mouse (+/+). The bright dots in the fetuin-A deficient mouse indicate calcified lesions throughout the body.

Fetuins are blood proteins that are made in the liver and secreted into the blood stream. They belong to a large group of binding proteins mediating the transport and availability of a wide variety of cargo substances in the blood stream. The best known representative of these carrier proteins is serum albumin, the most abundant protein in the blood plasma

of adult animals. Fetuin is more abundant in fetal blood, hence the name "fetuin" (from Latin, *fetus*). Fetal calf serum contains more fetuin than albumin, while adult serum contains more albumin than fetuin.

Family members

Human fetuin is synonymous with α2-HS-glycoprotein (genetic symbol AHSG), α2-HS, A2HS, AHS, HSGA, and fetuin-A. Fetuin-A exists as a single-copy gene in the human and mouse genomes. A closely related gene, fetuin-B, also exists in the human, rat, and mouse genomes. Like fetuin-A, fetuin-B is made predominantly by the liver and to a lesser extent by a number of secretory tissues. Fetuins exist in all vertebrate genomes including fish and reptiles. Fetuins are members of a family of proteins that evolved from the protein cystatin by gene duplication and exchange of gene segments. Fetuins thus belong to the cystatin superfamily of proteins. Fetuin relatives within this superfamiliy are the histidine-rich glycoprotein (HRG) and kininogen (KNG).

A2-HS-glycoprotein	
Identifiers	
Symbol	AHSG
Alt. symbols	FETUA, A2HS, HSGA
Entrez	197
HUGO	349
OMIM	138680
RefSeq	NM_001622
UniProt	P02765
Other data	
Locus	Chr. 3 *q27.3*

fetuin-B	
Identifiers	
Symbol	FETUB
Alt. symbols	16G2, Gugu
Entrez	26998
HUGO	3658
OMIM	605954
RefSeq	NM_014375
UniProt	Q9UGM5
Other data	
Locus	Chr. 3 *q27.3*

Animal studies

The function of Fetuin-A in the body was determined by gene knockout technology in mice. Knocking out the gene for fetuin-A rendered the mice completely fetuin-A deficient. Feeding a mineral-rich diet to fetuin-A-deficient mice resulted in widespread calcification (ectopic mineralization) of lung, heart, and kidneys in these mice. The calcification became drastically exacerbated when the fetuin-A knockout was combined with the genetic background DBA/2. The mouse strain DBA/2 is known for its proneness to calcify damaged tissues, a process called "dystrophic calcification". Fetuin-A deficiency dramatically increased the calcification proneness of these mice in that all mice sponteneously calcified throughout their body even without a mineral-rich diet or

surgical tissue trauma. Fetuin-A is therefore regarded as a potent inhibitor of systemic calcification.

Fibrin

Fibrin (also called **Factor Ia**) is a fibrous protein involved in the clotting of blood, and is non-globular. It is a fibrillar protein that is polymerised to form a "mesh" that forms a hemostatic plug or clot (in conjunction with platelets) over a wound site.

Fibrin is involved in the following biological processes: signal transduction, blood coagulation, platelet activation, and protein polymerization.

Role in disease

Excessive generation of fibrin due to activation of the coagulation cascade leads to thrombosis, more commonly known as a clot, while ineffective generation or premature lysis of fibrin predisposes to hemorrhage.

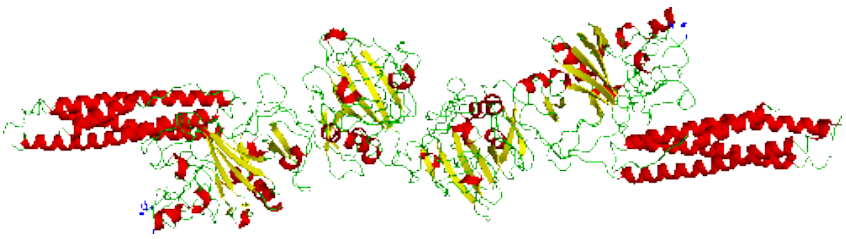
Dysfunction or disease of the liver can lead to a decrease in fibrinogen production or the production of abnormal fibrinogen molecules with reduced activity (dysfibrinogenaemia). Hereditary abnormalities of fibrinogen (the gene is carried on chromosome 4) are of both quantitative and qualitative in nature and include; afibrinogenaemia, hypofibrinogenaemia, dysfibrinogenaemia, and hypodysfibrinogenaemia.

Consequences of reduced, absent, or dysfunctional fibrin is likely to render patients as hemophiliacs.

Physiology

Fibrin from different animal sources is generally glycosylated with complex type diantennary asparagine linked glycans. Variety is just found in the degree of core fucosylation and in the type of sialic acid and galactose linkage.

Structure



Crystal structure of the double-d fragment from human fibrin

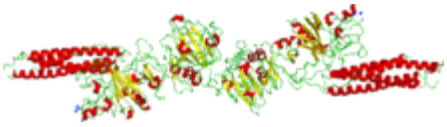
The image at the left is a crystal structure of the double-d fragment from human fibrin with two bound ligands. The experimental method used to obtain the image was X-ray diffraction, and it has a resolution of 2.30 Å. The structure is mainly made up of single alpha helices shown in red and beta sheets shown in yellow. The two blue structures are the bound ligands. The chemical structures of the ligands are Ca^{+2} ion, alpha-D-mannose ($C_6H_{12}O_6$), and D-glucosamine ($C_8H_{15}NO_6$).

Chapter 4

Fibrinogen and Fibulin

Fibrinogen

fibrinogen alpha chain



Crystallographic structure of a fragment of human fibrin.

Identifiers	
Symbol	FGA
Entrez	2243
HUGO	3661
OMIM	134820
RefSeq	NM_000508
UniProt	P02671
Other data	
Locus	Chr. 4 *q28*

fibrinogen beta chain

Identifiers	
Symbol	FGB
Entrez	2244
HUGO	3662
OMIM	134830

RefSeq	NM_005141
UniProt	P02675
Other data	
Locus	Chr. 4 *q28*

fibrinogen gamma chain

Identifiers	
Symbol	FGG
Entrez	2266
HUGO	3694
OMIM	134850
RefSeq	NM_021870
UniProt	P02679
Other data	
Locus	Chr. 4 *q28*

Fibrinogen (*factor I*) is a soluble plasma glycoprotein, synthesised by the liver, that is converted by thrombin into fibrin during blood coagulation. This is achieved through processes in the coagulation cascade that activate the zymogen prothrombin to the serine protease thrombin, which is responsible for converting fibrinogen into fibrin. Fibrin is then cross linked by factor XIII to form a clot. FXIIIa stabilizes fibrin further by incorporation of the fibrinolysis inhibitors alpha-2-antiplasmin and TAFI (thrombin activatable fibrinolysis inhibitor, procarboxypeptidase B), and binding to several adhesive proteins of various cells. Both the activation of Factor XIII by thrombin and plasminogen activator (t-PA) are catalyzed by fibrin. Fibrin specifically binds the activated coagulation factors factor Xa and thrombin and entraps them in the network of fibers, thus functioning as a temporary inhibitor of these enzymes which stay active and can be released during fibrinolysis. Recent research has shown that fibrin plays a key role in the inflammatory response and development of rheumatoid arthritis.

Fibrinogen deficiency

Congenital deficiency (afibrinogenemia) or disturbed function of fibrinogen has been described in a few cases.

It can lead to either bleeding, thromboembolic complications or is clinically without pathological findings. More common are acquired deficiency stages which can be detected by laboratory tests in blood plasma or in whole blood by means of thrombelastometry. Acquired deficiency is found after hemodilution, blood losses and/or consumption such as in trauma patients, during some phases of disseminated

intravascular coagulation (DIC), and also in sepsis. In patients with fibrinogen deficiency, the correction of bleeding is possible by infusion of fresh frozen plasma (FFP), cryoprecipitate (a fibrinogen rich plasma fraction) or by fibrinogen concentrates. There is increasing evidence that correction of fibrinogen deficiency or fibrinogen polymerization disorders is very important in patients with bleeding.

Diagnostic use

Fibrinogen levels can be measured in venous blood. Normal levels are about 1.5-2.77 g/L, depending on the method which is used. Typically fibrinogen is measured in citrated plasma samples in the laboratory, however the analysis of whole blood samples by use of thrombelastometry (platelet function is inhibited with cytochalasin D) is also possible. Higher levels are, amongst others, associated with cardiovascular disease (>3.43 g/L). It may be elevated in any form of inflammation, as it is an acute phase protein; for example, it is especially apparent in human gingival tissue during the initial phase of periodontal disease.

It is used in veterinary medicine as an inflammatory marker: in horses a level above the normal range of 1.0-4.0 g/L suggests some degree of systemic inflammatory response.

Low levels of fibrinogen can indicate a systemic activation of the clotting system, with consumption of clotting factors faster than synthesis. This excessive clotting factor consumption condition is known as disseminated intravascular coagulation or "DIC." DIC can be difficult to diagnose, but a strong clue is low fibrinogen levels in the setting of prolonged clotting times (PT or aPTT), in the context of acute critical illness such as sepsis or trauma. Besides low fibrinogen level, fibrin polymerization disorders which can be induced by several factors, including plasma expanders, can also lead to severe bleeding problems. Fibrin polymerization disorders can be detected by viscoelastic methods such as thrombelastometry.

Physiology

Fibrinogen is a 340 KDa glycoprotein synthesised in the liver by hepatocytes. The concentration in blood plasma is 1.5-4.0 g/L (normally measured using the Clauss method) or about 7 μM. In its natural form, fibrinogen can form bridges between platelets, by binding to their GpIIb/IIIa surface membrane proteins; however its major function is as the precursor to fibrin.

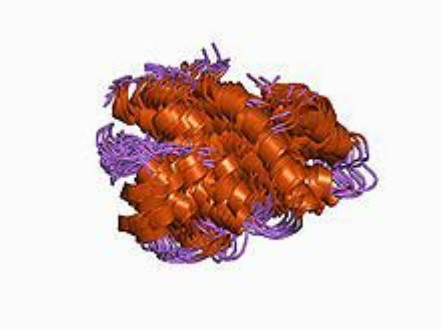
Fibrinogen, the principal protein of vertebrate blood clotting is a hexamer containing two sets of three different chains (α, β, and γ), linked to each other by disulfide bonds. The N-terminal sections of these three chains contain the cysteines that participate in the cross-linking of the chains. The C-terminal parts of the α, β and γ chains contain a domain of about 225 amino-acid residues, which can function as a molecular recognition unit. In fibrinogen as well as in angiopoietin this domain is implicated in protein-protein interactions. In lectins, such as mammalian ficolins and invertebrate tachylectin 5A, the fibrinogen C-terminal domain binds carbohydrates. On the fibrinogen α and β chains,

there is a small peptide sequence (called a fibrinopeptide). These small peptides are what prevent fibrinogen from spontaneously forming polymers with itself.

The conversion of fibrinogen to fibrin occurs in several steps. First, thrombin cleaves the N-terminus of the fibrinogen alpha and beta chains to fibrinopeptide A and B respectively. The resulting fibrin monomers polymerize end to end to from protofibrils which in turn associate laterally to form fibrin fibers. In a final step, the fibrin fibers associate to form the fibrin gel.

Fibulin

Anaphylotoxin-like domain



Structure of porcine C5adesArg.

Identifiers	
Symbol	ANATO
Pfam	PF01821
InterPro	IPR000020
SMART	ANATO
PROSITE	PDOC00906
SCOP	1c5a

Fibulin (FY-beau-lin) (now known as Fibulin-1 FBLN1) is the prototypic member of a multigene family, currently with seven members. Fibulin-1 is a calcium-bindng glycoprotein. In vertebrates, fibulin-1 is found in blood and extracellular matrices. In the extracellular matrix, fibulin-1 associates with basement membranes and elastic fibers. The association with these matrix structures is mediated by its ability to interact with numerous extracellular matrix constituents including fibronectin, proteoglycans, laminins and tropoelastin. In blood, fibulin-1 binds to fibrinogen and incorporates into clots.

Fibulins are secreted glycoproteins that become incorporated into a fibrillar extracellular matrix when expressed by cultured cells or added exogenously to cell monolayers. The five known members of the family share an elongated structure and many calcium-binding sites, owing to the presence of tandem arrays of epidermal growth factor-like domains. They have overlapping binding sites for several basement-membrane proteins, tropoelastin, fibrillin, fibronectin and proteoglycans, and they participate in diverse supramolecular structures. The amino-terminal domain I of fibulin consists of three anaphylatoxin-like (AT) modules, each approximately 40 residues long and containing four or six cysteines. The structure of an AT module was determined for the complement-derived anaphylatoxin C3a, and was found to be a compact alpha-helical fold that is stabilized by three disulphide bridges in the pattern Cys14, Cys25 and Cys36 (where Cys is cysteine). The bulk of the remaining portion of the fibulin molecule is a series of nine EGF-like repeats.

Chapter 5

Glycated Hemoglobin

Glycated hemoglobin (**glycosylated hemoglobin**, *hemoglobin A1c, HbA_{1c}, A1C*, or *Hb_{1c}*; sometimes also **HbA1c**) is a form of hemoglobin which is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous months prior to the measurement.

The 2010 American Diabetes Association Standards of Medical Care in Diabetes added the A1c ≥ 48 mmol/mol (≥6.5%) as another criterion for the diagnosis of diabetes, but this is controversial and has not been universally adopted.

In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, and retinopathy. Monitoring the HbA_{1c} in type-1 diabetic patients may improve treatment.

History

Hemoglobin A1c was first separated from other forms of hemoglobin by Huisman and Meyering in 1958 using a chromatographic column. It was first characterized as a glycoprotein by Bookchin and Gallop in 1968. Its increase in diabetes was first described in 1969 by Samuel Rahbar et. al. The reactions leading to its formation were characterized by Bunn and his co-workers in 1975. The use of hemoglobin A1c for monitoring the degree of control of glucose metabolism in diabetic patients was proposed in 1976 by Anthony Cerami, Ronald Koenig and coworkers.

Underlying principle

In the normal 120-day lifespan of the red blood cell, glucose molecules react with hemoglobin, forming glycated hemoglobin. In individuals with poorly controlled diabetes, the quantities of these glycated hemoglobins are much higher than in healthy people.

Once a hemoglobin molecule is glycated, it remains that way. A buildup of glycated hemoglobin within the red cell, therefore, reflects the average level of glucose to which the cell has been exposed during its life-cycle. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation. The HbA_{1c} level is proportional to average blood glucose concentration over the previous four weeks to three months. Some researchers state that the major proportion of its value is related to a rather shorter period of two to four weeks.

The 2010 American Diabetes Association Standards of Medical Care in Diabetes added the A1c ≥ 48 mmol/mol (≥6.5%) as another criterion for the diagnosis of diabetes, but this is controversial and has not been universally adopted.

Measuring A1C

There are a number of techniques used to measure A1C.

Laboratories use:

- high-performance liquid chromatography (HPLC): The HbA1c result is calculated as a ratio to total haemoglobin by using a chromatogram.
- immunoassay

Point of care (e.g. doctor's surgery) devices use:

- immunoassay
- boronate affinity chromatography

In the United States, POC A1C tests are certified by the National Glycohemoglobin Standardization Program (NGSP) to standardise them against the results of the 1993 Diabetes Control and Complications Trial (DCCT). There is an additional percentage scale, Mono S, that is in use by Japan and Sweden.

Switch to IFCC units

The American Diabetes Association (ADA), European Association for the Study of Diabetes (EASD) and International Diabetes Federation (IDF) have agreed that, in the future, HbA_{1c} is to be reported in the International Federation of Clinical Chemistry (IFCC) units. IFCC reporting was introduced in Europe except for the UK in 2003, and the UK has as of 1 June 2009 introduced dual reporting until 1 June 2011.

Conversion between DCCT and IFCC is by the following equation: IFCC-HbA_{1c} (mmol/mol) = [DCCT-HbA_{1c} (%) - 2.15] × 10.929

IFCC-HbA_{1c} (mmol/mol)	**DCCT- HbA_{1c}** (%)	**Mono S- HbA_{1c}** (%)
10	3.1	2.0
20	4.0	2.9
30	4.9	3.9
40	5.8	4.8
45	6.3	5.3
50	6.7	5.8
55	7.2	6.3
60	7.6	6.8
65	8.1	7.2
70	8.6	7.7
80	9.5	8.7
90	10.4	9.6
100	11.3	10.6

Interpretation of results

Laboratory results may differ depending on the analytical technique, the age of the subject, and biological variation among individuals. Two individuals with the same average blood sugar can have A1C values that differ by as much as 3 percentage points. Results can be unreliable in many circumstances, such as after blood loss, for example, after surgery, blood transfusions, anemia, or high erythrocyte turnover; in the presence of chronic renal or liver disease; after administration of high-dose vitamin C; or erythropoetin treatment. In general, the reference range (that found in healthy persons), is about 20–40 mmol/mol (4%–5.9%).

Higher levels of HbA_{1c} are found in people with persistently elevated blood sugar, as in diabetes mellitus. While diabetic patient treatment goals vary, many include a target range of HbA_{1c} values. A diabetic person with good glucose control has a HbA_{1c} level that is close to or within the reference range. **The International Diabetes Federation and American College of Endocrinology recommend HbA_{1c} values below 48 mmol/mol (6.5%), while American Diabetes Association recommends that the HbA_{1c} be below 53 mmol/mol (7.0%) for most patients**. Recent results from large trials suggest that a target below 53 mmol/mol (7%) may be excessive: Below 53 mmol/mol (7%) the health benefits of reduced A1C become smaller, and the intensive glycemic control required to reach this level leads to an increased rate of dangerous hypoglycemic episodes. A retrospective study of 47,970 diabetes patients found that patients with an A1C greater than 48 mmol/mol (6.5%) had an increased mortality rate. Practitioners must consider an individual patient's health, his/her risk of hypoglycemia, and his/her specific

health risks when setting a target A1C level. For example, patients at high risk of microvascular complications may gain further benefits from reducing A1C below 53 mmol/mol (7%). Because patients are responsible for averting or responding to their own hypoglycemic episodes, the patient's input and the doctor's assessment of the patient's self-care skills are also important.

A high HbA_{1c} represents poor glucose control. However, a 'good' HbA_{1c} in a patient with diabetes can still be riddled with a history of recent hypoglycemia, or even spikes of hyperglycemia. Regular blood glucose monitoring is still the best method for the analysis of overall vascular health with respect to blood sugar control. Often, patients with diabetes mellitus are scolded by their doctors for having an HbA_{1c} which is too low, because a lower A1C would indicate a likelihood of frequent hypoglycemia in the recent past. This is often assessed with blood sugar data, and receptions are typically mixed. A balance of long-term health (hyperglycemia prevention) versus short-term health (hypoglycemia prevention) is always a constant concern for both patients and their doctors. Doctors are especially sensitive about lower level HbA_{1c}'s with patients who regularly drive, this being a prime example of a short-term motivation for preventing hypoglycemia. Many diabetics have died behind the wheel as a result of a low blood sugar, especially for the reason that frequent hypoglycemia results in a higher tolerance (sometimes, the patient is seized with a feeling of panic, an increased heart rate, profuse sweating, etc.) for the condition, and some patients may not even consciously realize their blood sugar has dropped to dangerous levels. In addition to acquired tolerance, the use of alcohol and certain drugs (marijuana, for example) can create moderately similar symptoms to those of hypoglycemia (especially when used in combination) and for this reason the patient may not realize he/she has developed hypoglycemia.

Persistent elevations in blood sugar (and, therefore, HbA_{1c}) increase the risk for the long-term vascular complications of diabetes such as coronary disease, heart attack, stroke, heart failure, kidney failure, blindness, erectile dysfunction, neuropathy (loss of sensation, especially in the feet), gangrene, and gastroparesis (slowed emptying of the stomach). Poor blood glucose control also increases the risk of short-term complications of surgery such as poor wound healing.

Lower-than-expected levels of HbA_{1c} can be seen in people with shortened red blood cell lifespan, such as with glucose-6-phosphate dehydrogenase deficiency, sickle-cell disease, or any other condition causing premature red blood cell death. On the converse, higher-than-expected levels can be seen in people with a longer red blood cell lifespan, such as with Vitamin B_{12} or folate deficiency.

The approximate mapping between HbA_{1c} values given in percentage (%) and eAG (estimated average glucose) measurements is given by the following equation:

$$eAG(mg/dl) = 28.7 \times A1C - 46.7$$
$$eAG(mmol/l) = 1.59 \times A1C - 2.59$$

Data in parentheses are 95% confidence intervals

HbA_{1c}		eAG (estimated average glucose)	
(%)	(mmol/mol)	(mmol/L)	(mg/dL)
5	31	5.4 (4.2–6.7)	97 (76–120)
6	42	7.0 (5.5–8.5)	126 (100–152)
7	53	8.6 (6.8–10.3)	154 (123–185)
8	64	10.2 (8.1–12.1)	183 (147–217)
9	75	11.8 (9.4–13.9)	212 (170–249)
10	86	13.4 (10.7–15.7)	240 (193–282)
11	97	14.9 (12.0–17.5)	269 (217–314)
12	108	16.5 (13.3–19.3)	298 (240–347)

Indications and use

Glycated hemoglobin testing is recommended for both (a) checking blood sugar control in people who might be pre-diabetic and (b) monitoring blood sugar control in patients with more elevated levels, termed diabetes mellitus. There is a significant proportion of people who are unaware of their elevated HbA_{1c} level before they have blood lab work. For a single blood sample, it provides far more revealing information on glycemic behavior than a fasting blood sugar value: Fasting blood sugar tests are crucial in making treatment decisions. The American Diabetes Association guidelines are similar to others in advising that the glycosylated hemoglobin test be performed at least two times a year in patients with diabetes that are meeting treatment goals (and that have stable glycemic control) and quarterly in patients with diabetes whose therapy has changed or that are not meeting glycemic goals.

Glycated hemoglobin measurement is not appropriate where there has been a change in diet or treatment within 6 weeks. Likewise, the test assumes a normal red blood cell aging process and mix of hemoglobin subtypes (predominantly HbA in normal adults). Hence, people with recent blood loss, hemolytic anemia, or genetic differences in the hemoglobin molecule (hemoglobinopathy) such as sickle-cell disease and other conditions, as well as those that have donated blood recently, are not suitable for this test.

Due to glycated hemoglobin's variability (as shown in the table above), additional measures should be checked in patients at or near recommended goals. People with hemoglobin A1C values at 64 mmol/mol (8.0%) or less should be provided additional testing to determine whether the HbA_{1c} values are due to averaging out high blood glucose (hyperglycemia) with low blood glucose (hypoglycemia) or the HbA_{1c} is more reflective of an elevated blood glucose that does not vary much throughout the day.

Devices such as continuous blood glucose monitoring allow people with diabetes to determine their blood glucose levels on a continuous basis, testing every few minutes. Continuous use of blood glucose monitors is becoming more common, and the devices are covered by many health insurance plans but not by Medicare. The supplies tend to be expensive, since the sensors must be changed at least weekly. Another test that is useful in determining if HbA_{1c} values are due to wide variations of blood glucose throughout the day is 1,5 Anhydroglucitol, also known as GlycoMark. GlycoMark reflects only the times that the person experiences hyperglycemia above 180 mg/dL over a two-week period.

Concentrations of hemoglobin A1 (HbA1) are increased, both in diabetic patients and in patients with renal failure, when measured by ion-exchange chromatography. The thiobarbituric acid method (a chemical method specific for the detection of glycation) shows that patients with renal failure have values for glycated hemoglobin similar to those observed in normal subjects, suggesting that the high values in these patients are a result of binding of something other than glucose to hemoglobin.

In autoimmune hemolytic anemia, concentrations of hemoglobin A1 (HbA1) is undetectable. Administration of prednisolone (PSL) will allow the HbA1 to be detected. The alternative fructosamine test may be used in these circumstances and it also reflects an average of blood glucose levels over the preceding 2 to 3 weeks.

All the major institutions like International Expert Committee Report, drawn from the International Diabetes Federation (IDF), the European Association for the Study of diabetes (EASD), and the American Diabetes Association (ADA), suggests the A1C level of 48 mmol/mol (6.5%) as a diagnostic level. The Committee Report further states that, when A1C testing cannot be done, the fasting and glucose tolerance tests be done.

Diagnosis of diabetes during pregnancy continues to require fasting and glucose tolerance measurements for gestational diabetes, and not the glycated hemoglobin.

Modification by exercise training

A meta-analysis of research done to identify the effect of two different kinds of training programs (combined aerobic and eccentric resistance exercise program and aerobic exercise only) on the glycosylated hemoglobin levels of individuals with T2DM found that the effect of combining resistance exercise with aerobic exercise improved the glucose control more than just the aerobics alone. The average effect of the training programs included reductions of glycosylated hemoglobin of 9 mmol/mol (0.8 %), which was a result similar to that of long-term diet and drug or insulin therapy (which result in a reduction of 6,5 - 9 mmol/mol [or 0.6–0.8 %]).

Standardization & traceability

HbA1c is now standardized & traceable to IFCC methods HPLC-CE & HPLC-MS. A new unit (mmol/mol) is used as part of this standardization.

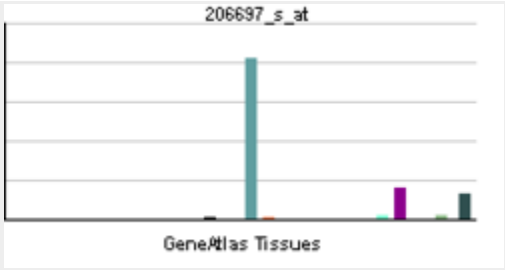
Chapter 6

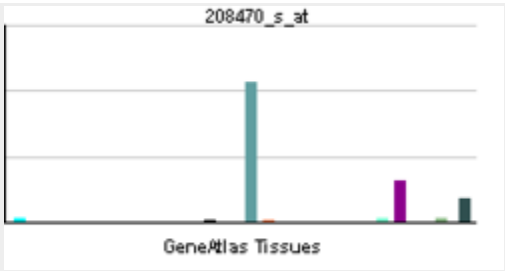
Haptoglobin

Haptoglobin

Identifiers	
Symbols	HP; MGC111141; hp2-alpha
External IDs	OMIM: 140100 MGI: 96211 HomoloGene: 121756 GeneCards: HP Gene

RNA expression pattern





Orthologs

Species	Human	Mouse
Entrez	3240	15439
Ensembl	ENSG00000197711	ENSMUSG00000031722

UniProt	P00738	Q61646
RefSeq (mRNA)	NM_005143	NM_017370
RefSeq (protein)	NP_005134	NP_059066
Location (UCSC)	Chr 16: 70.65 - 70.65 Mb	Chr 8: 112.1 - 112.1 Mb

Haptoglobin (abbreviated as **Hp**) is a protein that in humans is encoded by the *HP* gene. In blood plasma, haptoglobin binds free hemoglobin (Hb) released from erythrocytes with high affinity and thereby inhibits its oxidative activity. The haptoglobin-hemoglobin complex will then be removed by the reticuloendothelial system (mostly the spleen). In clinical settings, the haptoglobulin assay is used to screen for and monitor intravascular hemolytic anemia. In intravascular hemolysis free hemoglobin will be released into circulation and hence haptoglobin will bind the Hb. This causes a decline in Hp levels. Conversely, in extravascular hemolysis the reticuloendothelial system, especially splenic monocytes, phagocytose the erythrocytes and hemoglobin is not released into circulation and hence haptoglobin levels are normal.

Function

This gene encodes a preproprotein that is processed to yield both alpha and beta chains, which subsequently combine as a tetramer to produce haptoglobin. Haptoglobin functions to bind free plasma hemoglobin, which allows degradative enzymes to gain access to the hemoglobin while at the same time preventing loss of iron through the kidneys and protecting the kidneys from damage by hemoglobin. For this reason it is often referred to as the suicide protein.

Synthesis

Haptoglobin is produced mostly by hepatocytes but also by other tissues: e.g., skin, lung, and kidney. In addition, the haptoglobin gene is expressed in murine and human adipose tissue.

Haptoglobin had been shown to be expressed in adipose tissue of cattle as well (Saremi et al, 2010).

Structure

Haptoglobin, in its simplest form, consists of two α- and two β-chains, connected by disulfide bridges. The chains originate from a common precursor protein, which is proteolytically cleaved during protein synthesis.

Hp exists in two allelic forms in the human population, so-called *Hp1* and *Hp2*, the latter one having arisen due to the partial duplication of *Hp1* gene. Three genotypes of Hp, therefore, are found in humans: Hp1-1, Hp2-1, and Hp2-2. Hp of different genotypes have been shown to bind hemoglobin with different affinities, with Hp2-2 being the weakest binder.

In other species

Hp has been found in all mammals studied so far, some birds, e.g., cormorant and ostrich but also, in its simpler form, in bony fish, e.g., zebrafish. It is interesting to note that Hp is absent in at least some amphibians (*Xenopus*) and neognathous birds (chicken and goose).

Clinical significance

Mutations in this gene and/or its regulatory regions cause ahaptoglobinemia or hypohaptoglobinemia. This gene has also been linked to diabetic nephropathy, the incidence of coronary artery disease in type 1 diabetes, Crohn's disease, inflammatory disease behavior, primary sclerosing cholangitis, susceptibility to idiopathic Parkinson's disease, and a reduced incidence of Plasmodium falciparum malaria.

Since the reticuloendothelial system will remove the haptoglobin-hemoglobin complex from the body, haptoglobin levels will be decreased in hemolytic anemias. In the process of binding hemoglobin, haptoglobin sequesters the iron within hemoglobin, preventing iron-utilizing bacteria from benefiting from hemolysis. It is theorized that, because of this, haptoglobin has evolved into an acute-phase protein. HP has a protective influence on the hemolytic kidney.

Some studies associate the HP with the risk of developing schizophrenia.

Test protocol

Haptoglobin is ordered whenever a patient exhibits symptoms of anemia, such as pallor, fatigue, or shortness of breath, along with physical signs of hemolysis, such as jaundice or dark-colored urine. The test is also commonly ordered as a hemolytic anemia battery, which also includes a reticulocyte count and a peripheral blood smear. It can also be ordered along with a Direct Antiglobulin Test when a patient is suspected of having a transfusion reaction or symptoms of autoimmune hemolytic anemia. Also, it may be ordered in conjunction with a bilirubin.

Interpretation

A decrease in haptoglobin can support a diagnosis of hemolytic anemia, especially when correlated with a decreased RBC count, Hemoglobin, and Hematocrit, and also an increased reticulocyte count.

If the reticulocyte count is increased, but the haptoglobin level is normal, this may indicate that cellular destruction is occurring in the spleen and liver, which may indicate a drug-induced hemolysis, or a red cell dysplasia. The spleen and liver recognize an error in the red cells (either Drug coating the red cell membrane or a dysfunctional red cell membrane), and destroy the cell. This type of destruction does not release hemoglobin into the peripheral blood, so the haptoglobin cannot bind to it. Thus, the haptoglobin will stay normal.

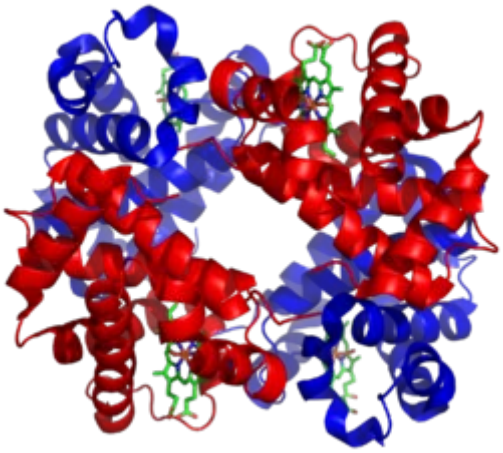
If there are symptoms of anemia but both the reticulocyte count and the haptoglobin level are normal, the anemia is most likely not due to hemolysis, but instead some other error in cellular production, such as aplastic anemia

Haptoglobin levels that are decreased but do not accompany signs of anemia may indicate liver damage, as the liver is not producing enough haptoglobin to begin with.

As haptoglobin is indeed an acute-phase protein, any inflammatory process (infection, extreme stress, burns, major crush injury, allergy, etc.) may increase the levels of plasma haptoglobin.

Chapter 7

Hemoglobin

Hemoglobin, human, adult (heterotetramer, $(\alpha\beta)_2$)	
	
Structure of human hemoglobin. The protein's α and β subunits are in red and blue, and the iron-containing heme groups in green. From PDB 1GZX *Proteopedia Hemoglobin*	
-	
Protein type	metalloprotein, globulin
Function	oxygen-transport

Cofactor(s)		heme (4)
Subunit Name	Gene	Chromosomal Locus
Hb-α1	HBA1	Chr. 16 p13.3
Hb-α2	HBA2	Chr. 16 p13.3
Hb-β	HBB	Chr. 11 p15.5

Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (except the fish family Channichthyidae) and the tissues of some invertebrates. Hemoglobin in the blood is what transports oxygen from the lungs or gills to the rest of the body (i.e. the tissues) where it releases the oxygen for cell use, and collects carbon dioxide to bring it back to the lungs.

In mammals the protein makes up about 97% of the red blood cells' dry content, and around 35% of the total content (including water). Hemoglobin has an oxygen binding capacity of 1.34 ml O_2 per gram of hemoglobin, which increases the total blood oxygen capacity seventyfold compared to dissolved oxygen in blood. The mammalian hemoglobin molecule can bind (carry) up to four oxygen molecules.

Hemoglobin is involved in the transport of other gases: it carries some of the body's respiratory carbon dioxide (about 10% of the total) as carbaminohemoglobin, in which CO_2 is bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen.

Hemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain hemoglobin include the A9 dopaminergic neurons in the substantia nigra, macrophages, alveolar cells, and mesangial cells in the kidney. In these tissues, hemoglobin has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism.

Hemoglobin and hemoglobin-like molecules are also found in many invertebrates, fungi, and plants. In these organisms, hemoglobins may carry oxygen, or they may act to transport and regulate other things such as carbon dioxide, nitric oxide, hydrogen sulfide and sulfide. A variant of the molecule, called leghemoglobin, is used to scavenge oxygen, to keep it from poisoning anaerobic systems, such as nitrogen-fixing nodules of leguminous plants.

Research history

The oxygen-carrying protein hemoglobin was discovered by Hünefeld in 1840. In 1851, Otto Funke published a series of articles in which he described growing hemoglobin

crystals by successively diluting red blood cells with a solvent such as pure water, alcohol or ether, followed by slow evaporation of the solvent from the resulting protein solution. Hemoglobin's reversible oxygenation was described a few years later by Felix Hoppe-Seyler.

In 1959 Max Perutz determined the molecular structure of hemoglobin by X-ray crystallography. This work resulted in his sharing with John Kendrew the 1962 Nobel Prize in Chemistry.

The role of hemoglobin in the blood was elucidated by physiologist Claude Bernard. The name *hemoglobin* is derived from the words *heme* and *globin*, reflecting the fact that each subunit of hemoglobin is a globular protein with an embedded heme (or haem) group. Each heme group contains one iron atom, that can bind one oxygen molecule through ion-induced dipole forces. The most common type of hemoglobin in mammals contains four such subunits.

Genetics

Hemoglobin consists mostly of protein (the "globin" chains), and these proteins, in turn, are composed of sequences of amino acids. These sequences are linear, in the manner of letters in a written sentence or beads on a string. In all proteins, it is the variation in the type of amino acids in the protein sequence of amino acids, which determine the protein's chemical properties and function. This is true of hemoglobin, where the sequence of amino acids may affect crucial functions such as the protein's affinity for oxygen.

There is more than one hemoglobin gene. The amino acid sequences of the globin proteins in hemoglobins usually differ between species, although the differences grow with the evolutionary distance between species. For example, the most common hemoglobin sequences in humans and chimpanzees are nearly identical, differing by only one amino acid in both the alpha and the beta globin protein chains. These differences grow larger between less closely related species.

Even within a species, different variants of hemoglobin always exist, although one sequence is usually a "most common" one in each species. Mutations in the genes for the hemoglobin protein in a species result in hemoglobin variants. Many of these mutant forms of hemoglobin cause no disease. Some of these mutant forms of hemoglobin, however, cause a group of hereditary diseases termed the *hemoglobinopathies*. The best known hemoglobinopathy is sickle-cell disease, which was the first human disease whose mechanism was understood at the molecular level. A (mostly) separate set of diseases called thalassemias involves underproduction of normal and sometimes abnormal hemoglobins, through problems and mutations in globin gene regulation. All these diseases produce anemia.

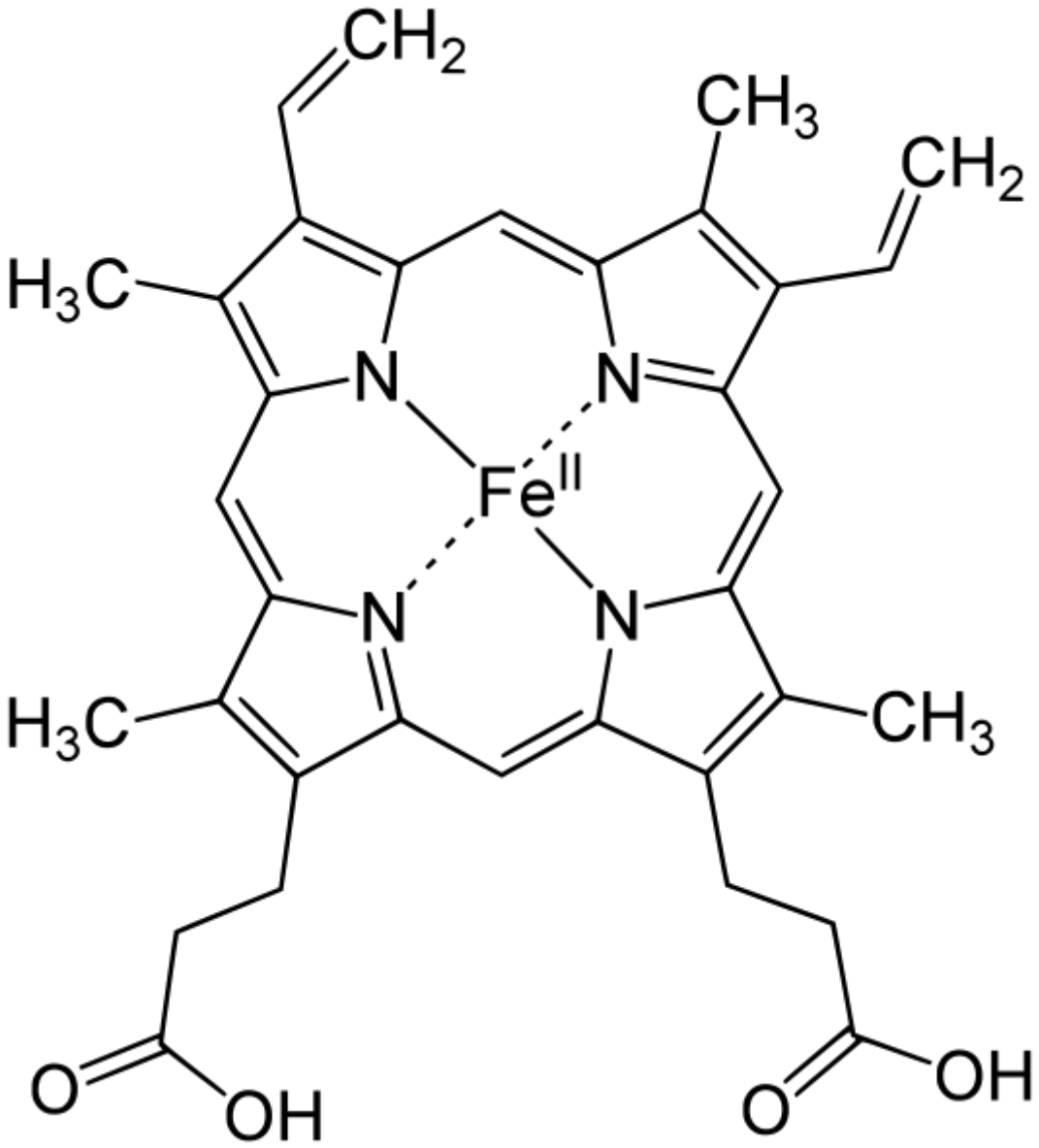
Variations in hemoglobin amino acid sequences, as with other proteins, may be adaptive. For example, recent studies have suggested genetic variants in deer mice that help explain how deer mice that live in the mountains are able to survive in the thin air that

accompanies high altitudes. A researcher from the University of Nebraska-Lincoln found mutations in four different genes that can account for differences between deer mice that live in lowland prairies versus the mountains. After examining wild mice captured from both highlands and lowlands, it was found that: the genes of the two breeds are "virtually identical–except for those that govern the oxygen-carrying capacity of their hemoglobin". "The genetic difference enables highland mice to make more efficient use of their oxygen", since less is available at higher altitudes, such as those in the mountains. Mammoth hemoglobin featured mutations that allowed for oxygen delivery at lower temperatures, thus enabling mammoths to migrate to higher latitudes during the Pleistocene.

Synthesis

Hemoglobin (Hb) is synthesized in a complex series of steps. The heme part is synthesized in a series of steps in the mitochondria and the cytosol of immature red blood cells, while the globin protein parts are synthesized by ribosomes in the cytosol. Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. At this point, the nucleus is lost in mammalian red blood cells, but not in birds and many other species. Even after the loss of the nucleus in mammals, residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loses its RNA soon after entering the vasculature (this hemoglobin-synthetic RNA in fact gives the reticulocyte its reticulated appearance and name).

Structure



Heme b group

Hemoglobin has a quaternary structure characteristic of many multi-subunit globular proteins. Most of the amino acids in hemoglobin form alpha helices, connected by short non-helical segments. Hydrogen bonds stabilize the helical sections inside this protein, causing attractions within the molecule, folding each polypeptide chain into a specific shape. Hemoglobin's quaternary structure comes from its four subunits in roughly a tetrahedral arrangement.

In most vertebrates, the hemoglobin molecule is an assembly of four globular protein subunits. Each subunit is composed of a protein chain tightly associated with a non-

protein heme group. Each protein chain arranges into a set of alpha-helix structural segments connected together in a globin fold arrangement, so called because this arrangement is the same folding motif used in other heme/globin proteins such as myoglobin. This folding pattern contains a pocket that strongly binds the heme group.

A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together (by methene bridges) with the iron ion bound in the center. The iron ion, which is the site of oxygen binding, coordinates with the four nitrogens in the center of the ring, which all lie in one plane. The iron is bound strongly (covalently) to the globular protein via the imidazole ring of the F8 histidine residue (also known as the proximal histidine) below the porphyrin ring. A sixth position can reversibly bind oxygen by a coordinate covalent bond, completing the octahedral group of six ligands. Oxygen binds in an "end-on bent" geometry where one oxygen atom binds Fe and the other protrudes at an angle. When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron.

Even though carbon dioxide is carried by hemoglobin, it does not compete with oxygen for the iron-binding positions, but is actually bound to the protein chains of the structure.

The iron ion may be either in the Fe^{2+} or in the Fe^{3+} state, but ferrihemoglobin (methemoglobin) (Fe^{3+}) cannot bind oxygen. In binding, oxygen temporarily and reversibly oxidizes (Fe^{2+}) to (Fe^{3+}) while oxygen temporally turns into superoxide, thus iron must exist in the +2 oxidation state to bind oxygen. If superoxide ion associated to Fe^{3+} is protonated the hemoglobin iron will remain oxidized and incapable to bind oxygen. In such cases, the enzyme methemoglobin reductase will be able to eventually reactivate methemoglobin by reducing the iron center.

In adult humans, the most common hemoglobin type is a tetramer (which contains 4 subunit proteins) called **hemoglobin A**, consisting of two α and two β subunits non-covalently bound, each made of 141 and 146 amino acid residues, respectively. This is denoted as $\alpha_2\beta_2$. The subunits are structurally similar and about the same size. Each subunit has a molecular weight of about 17,000 daltons, for a total molecular weight of the tetramer of about 68,000 daltons (64,458 g/mol). Thus, 1 g/dL = 0.01551 mmol/L. Hemoglobin A is the most intensively studied of the hemoglobin molecules.

In human infants, the hemoglobin molecule is made up of 2 α chains and 2 gamma chains. The gamma chains are gradually replaced by β chains as the infant grows.

The four polypeptide chains are bound to each other by salt bridges, hydrogen bonds, and the hydrophobic effect. There are two kinds of contacts between the α and β chains: $\alpha_1\beta_1$ and $\alpha_1\beta_2$.

Oxygen Saturation

In general, hemoglobin can be saturated with oxygen molecules (oxyhemoglobin), or desaturated with oxygen molecules (deoxyhemoglobin).

Oxyhemoglobin

Oxyhemoglobin is formed during physiological respiration when oxygen binds to the heme component of the protein hemoglobin in red blood cells. This process occurs in the pulmonary capillaries adjacent to the alveoli of the lungs. The oxygen then travels through the blood stream to be dropped off at cells where it is utilized in aerobic glycolysis and in the production of ATP by the process of oxidative phosphorylation. It does not, however, help to counteract a decrease in blood pH. Ventilation, or breathing, may reverse this condition by removal of carbon dioxide, thus causing a shift up in pH.

Hemoglobin exists in two forms, a **taut form** (T) and a **relaxed form** (R). Various factors such as low pH, high CO_2 and high 2,3 BPG at the level of the tissues favor the taut form, which has low oxygen affinity and releases oxygen in the tissues. The opposite of these aforementioned factors at the level of the lung capillaries favors the relaxed form which can better bind oxygen.

Deoxyhemoglobin

Deoxyhemoglobin is the form of hemoglobin without the bound oxygen. The absorption spectra of oxyhemoglobin and deoxyhemoglobin differ. The oxyhemoglobin has significantly lower absorption of the 660 nm wavelength than deoxyhemoglobin, while at 940 nm its absorption is slightly higher. This difference is used for measurement of the amount of oxygen in patient's blood by an instrument called pulse oximeter. This difference also accounts for the presentation of cyanosis, the blue to purplish color that tissues develop during hypoxia.

Iron's oxidation state in oxyhemoglobin

Assigning oxygenated hemoglobin's oxidation state is difficult because oxyhemoglobin ($Hb\text{-}O_2$), by experimental measurement, is diamagnetic (no net unpaired electrons), yet the low-energy electron configurations in both oxygen and iron are paramagnetic (suggesting at least one unpaired electron in the complex). The lowest-energy form of oxygen, and the lowest energy forms of the relevant oxidation states of iron, are these:

- Triplet oxygen, the lowest energy molecular oxygen species, has two unpaired electrons in antibonding π^* molecular orbitals.
- Iron(II) tends to exist in a high-spin configuration where unpaired electrons exist in E_g antibonding orbitals.
- Iron(III) has an odd number of electrons, and thus must have one or more unpaired electrons, in any energy state.

All of these structures are paramagnetic (have unpaired electrons), not diamagnetic. Thus, a non-intuitive (e.g., a higher-energy for at least one species) distribution of electrons in the combination of iron and oxygen must exist, in order to explain the observed diamagnetism and no unpaired electrons.

The three logical possibilities to produce diamagnetic (no net spin) $Hb\text{-}O_2$ are:

1. Low-spin Fe^{2+} binds to singlet oxygen. Both low-spin iron and singlet oxygen are diamagnetic. However, the singlet form of oxygen is the higher-energy form of the molecule.
2. Low-spin Fe^{3+} binds to $\mathbf{.}O_2^-$ (the superoxide ion) and the two unpaired electrons couple antiferromagnetically, giving diamagnetic properties.
3. Low-spin Fe^{4+} binds to peroxide, O_2^{2-}. Both are diamagnetic.

Direct experimental data:

- X-ray photoelectron spectroscopy suggests iron has an oxidation state of approximately 3.2
- infrared stretching frequencies of the O-O bond suggests a bond length fitting with superoxide (a bond order of about 1.6, with superoxide being 1.5).

Thus, the nearest formal oxidation state of iron in Hb-O_2 is the +3 state, with oxygen in the -1 state (as superoxide $\mathbf{.}O_2^-$). The diamagnetism in this configuration arises from the single unpaired electron on superoxide aligning antiferromagnetically from the single unpaired electron on iron, to give no net spin to the entire configuration, in accordance with diamagnetic oxyhemoglobin from experiment.

The second choice of the three logical possibilities above for diamagnetic oxyhemoglobin being found correct by experiment, is not surprising: singlet oxygen (possibility #1) and large separations of charge (possibility #3) are both unfavorably high-energy states. Iron's shift to a higher oxidation state in Hb-O_2 decreases the atom's size, and allows it into the plane of the porphyrin ring, pulling on the coordinated histidine residue and initiating the allosteric changes seen in the globulins.

Early postulates by bio-inorganic chemists claimed that possibility #1 (above) was correct and that iron should exist in oxidation state II. This seemed particularly likely since the iron oxidation state III as methemoglobin, when **not** accompanied by superoxide $\mathbf{.}O_2^-$ to "hold" the oxidation electron, was known to render hemoglobin incapable of binding normal triplet O_2 as it occurs in the air. It was thus assumed that iron remained as Fe(II) when oxygen gas was bound in the lungs. The iron chemistry in this previous classical model was elegant, but the required presence of the required diamagnetic high-energy singlet oxygen was never explained. It was classically argued that the binding of an oxygen molecule placed high-spin iron(II) in an octahedral field of strong-field ligands; this change in field would increase the crystal field splitting energy, causing iron's electrons to pair into the low-spin configuration, which would be diamagnetic in Fe(II). This forced low-spin pairing is indeed thought to happen in iron when oxygen binds, but is not enough to explain iron's change in size. Extraction of an additional electron from iron by oxygen is required to explain both iron's smaller size and observed increased oxidation state, and oxygen's weaker bond.

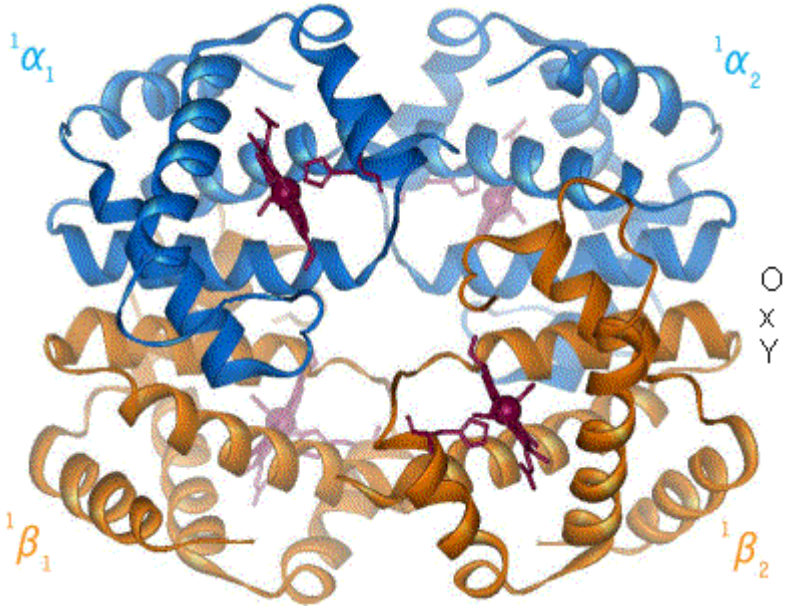
It should be noted that the assignment of a whole-number oxidation state is a formalism, as the covalent bonds are not required to have perfect bond orders involving whole electron-transfer. Thus, all three models for paramagnetic Hb-O_2 may contribute to some

small degree (by resonance) to the actual electronic configuration of Hb-O_2. However, the model of iron in Hb-O_2 being Fe(III) is more correct than the classical idea that it remains Fe(II).

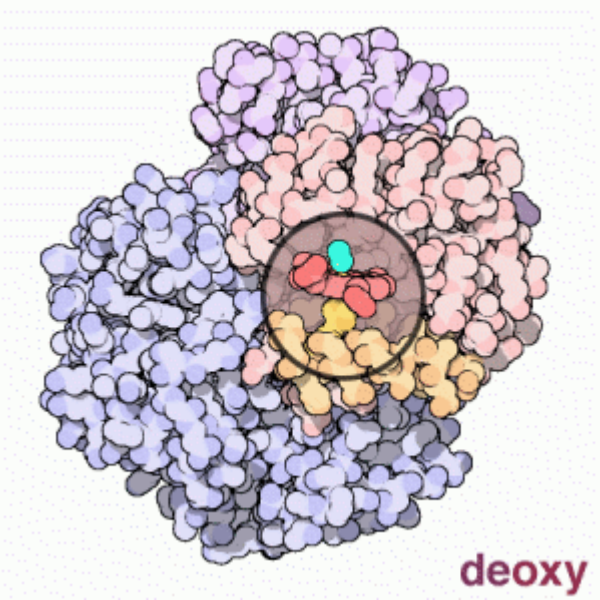
Binding for ligands other than oxygen

Besides the oxygen ligand, which binds to hemoglobin in a cooperative manner, hemoglobin ligands also include competitive inhibitors such as carbon monoxide (CO) and allosteric ligands such as carbon dioxide (CO_2) and nitric oxide (NO). The carbon dioxide is bound to amino groups of the globin proteins as carbaminohemoglobin, and is thought to account for about 10% of carbon dioxide transport in mammals. Nitric oxide is bound to specific thiol groups in the globin protein to form an S-nitrosothiol which dissociates into free nitric oxide and thiol again, as the hemoglobin releases oxygen from its heme site. This nitric oxide transport to peripheral tissues is hypothesised to assist oxygen transport in tissues, by releasing vasodilatory nitric oxide to tissues in which oxygen levels are low.

Cooperative



A schematic visual model of oxygen-binding process, showing all four monomers and hemes, and protein chains only as diagramatic coils, to facilitate visualization into the molecule. Oxygen is not shown in this model, but, for each of the iron atoms, it binds to the iron (red sphere) in the flat heme. For example, in the upper left of the four hemes shown, oxygen binds at the left of the iron atom shown in the upper left of diagram. This causes the iron atom to move backward into the heme which holds it (the iron moves upward as it binds oxygen, in this illustration), tugging the histidine residue (modeled as a red pentagon on the right of the iron) closer, as it does. This, in turn, pulls on the protein chain holding the histidine.



Another view of how binding and release of ligands induces a conformational (structural) change in hemoglobin. Only one of the four heme groups is shown, but more of the electron cloud of the protein chain is included in this diagram, as compared with above. The binding and release of oxygen (shown now in green) illustrates the structural differences between oxy- and deoxyhemoglobin, respectively. The histidine, which is pulled by motion of the iron atom, is shown here in yellow.

When oxygen binds to the iron complex, it causes the iron atom to move back toward the center of the plane of the porphyrin ring. At the same time, the imidazole side-chain of the histidine residue interacting at the other pole of the iron is pulled toward the porphyrin ring. This interaction forces the plane of the ring sideways toward the outside of the tetramer, and also induces a strain in the protein helix containing the histidine as it moves nearer to the iron atom. This strain is transmitted to the remaining three monomers in the tetramer, where it induces a similar conformational change in the other heme sites such that binding of oxygen to these sites becomes easier.

In the tetrameric form of normal adult hemoglobin, the binding of oxygen is, thus, a cooperative process. The binding affinity of hemoglobin for oxygen is increased by the oxygen saturation of the molecule, with the first oxygens bound influencing the shape of the binding sites for the next oxygens, in a way favorable for binding. This positive cooperative binding is achieved through steric conformational changes of the hemoglobin protein complex as discussed above; i.e., when one subunit protein in hemoglobin becomes oxygenated, a conformational or structural change in the whole complex is initiated, causing the other subunits to gain an increased affinity for oxygen. As a consequence, the oxygen binding curve of hemoglobin is sigmoidal, or *S*-shaped, as opposed to the normal hyperbolic curve associated with noncooperative binding.

The dynamic mechanism of the cooperativity in hemoglobin and its relation with the low-frequency resonance has been discussed.

Competitive

Hemoglobin's oxygen-binding capacity is decreased in the presence of carbon monoxide because both gases compete for the same binding sites on hemoglobin, carbon monoxide binding preferentially in place of oxygen.

The binding of oxygen is affected by molecules such as carbon monoxide (CO) (for example, from tobacco smoking, car exhaust, and incomplete combustion in furnaces). CO competes with oxygen at the heme binding site. Hemoglobin binding affinity for CO is 250 times greater than its affinity for oxygen, meaning that small amounts of CO dramatically reduce hemoglobin's ability to transport oxygen. When hemoglobin combines with CO, it forms a very bright red compound called carboxyhemoglobin, which may cause the skin of CO poisoning victims to appear pink in death, instead of white or blue. When inspired air contains CO levels as low as 0.02%, headache and nausea occur; if the CO concentration is increased to 0.1%, unconsciousness will follow. In heavy smokers, up to 20% of the oxygen-active sites can be blocked by CO.

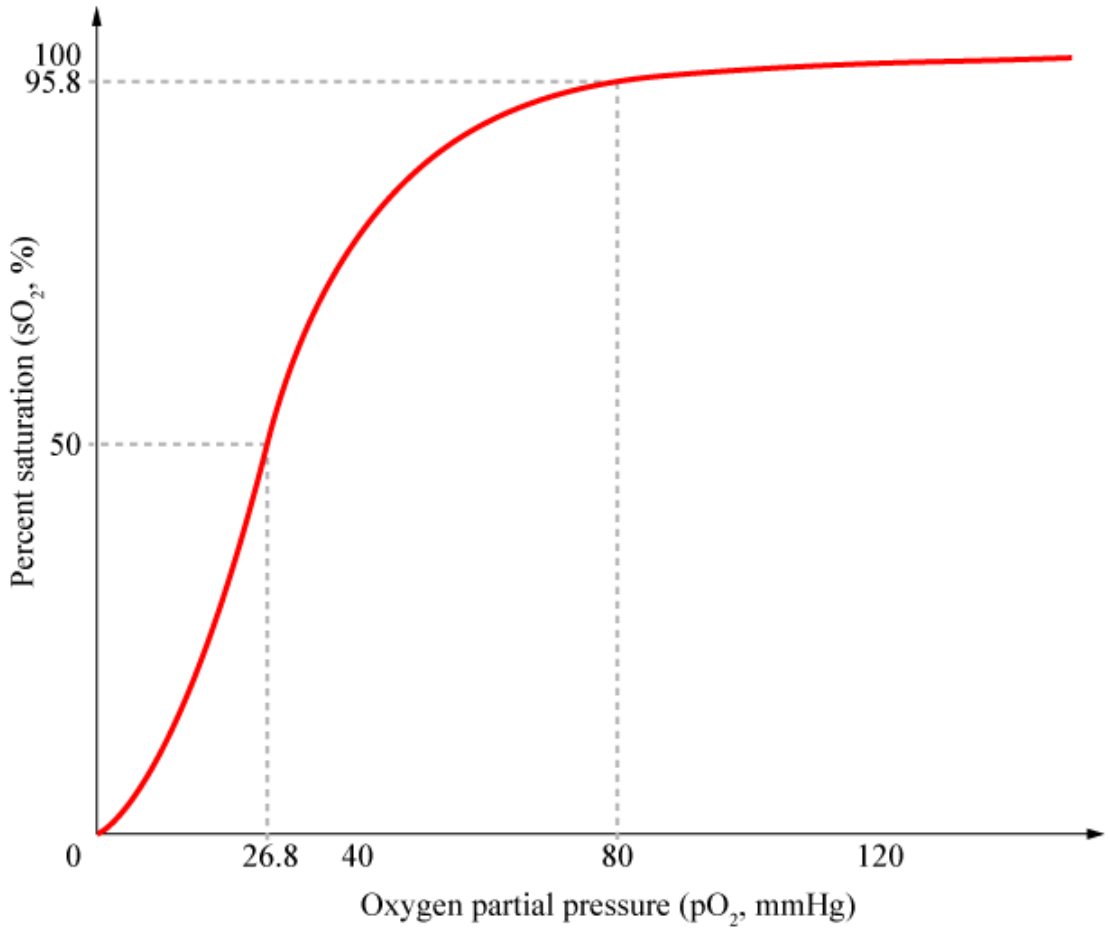
In similar fashion, hemoglobin also has competitive binding affinity for cyanide (CN^-), sulfur monoxide (SO), nitric oxide (NO), and sulfide(S^{2-}), including hydrogen sulfide (H_2S). All of these bind to iron in heme without changing its oxidation state, but they nevertheless inhibit oxygen-binding, causing grave toxicity.

The iron atom in the heme group must initially be in the ferrous (Fe^{2+}) oxidation state to support oxygen and other gases' binding and transport (it temporarily switches to ferric during the time oxygen is bound, as explained above). Initial oxidation to the ferric (Fe^{3+}) state without oxygen converts hemoglobin into "hemiglobin" or methemoglobin (pronounced "MET-hemoglobin"), which cannot bind oxygen. Hemoglobin in normal red blood cells is protected by a reduction system to keep this from happening. Nitric oxide is capable of converting a small fraction of hemoglobin to methemoglobin in red blood cells. The latter reaction is a remnant activity of the more ancient nitric oxide dioxygenase function of globins.

Allosteric

Carbon *di*oxide occupies a different binding site on the hemoglobin. Carbon dioxide is more readily dissolved in deoxygenated blood, facilitating its removal from the body after the oxygen has been released to tissues undergoing metabolism. This increased affinity for carbon dioxide by the venous blood is known as the Haldane effect. Through the enzyme carbonic anhydrase, carbon dioxide reacts with water to give carbonic acid, which decomposes into bicarbonate and protons:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$$



The sigmoidal shape of hemoglobin's oxygen-dissociation curve results from cooperative binding of oxygen to hemoglobin.

Hence blood with high carbon dioxide levels is also lower in pH (more acidic). Hemoglobin can bind protons and carbon dioxide, which causes a conformational change in the protein and facilitates the release of oxygen. Protons bind at various places on the protein, while carbon dioxide binds at the α-amino group. Carbon dioxide binds to hemoglobin and forms carbaminohemoglobin. This decrease in hemoglobin's affinity for oxygen by the binding of carbon dioxide and acid is known as the Bohr effect (shifts the O_2-saturation curve to the *right*). Conversely, when the carbon dioxide levels in the blood decrease (i.e., in the lung capillaries), carbon dioxide and protons are released from hemoglobin, increasing the oxygen affinity of the protein. A reduction in the total binding capacity of hemoglobin to oxygen (i.e. shifting the curve down, not just to the right) due to reduced pH is called the root effect. This is seen in bony fish.

It is necessary for hemoglobin to release the oxygen that it binds; if not, there is no point in binding it. The sigmoidal curve of hemoglobin makes it efficient in binding (taking up O_2 in lungs), and efficient in unloading (unloading O_2 in tissues).

In people acclimated to high altitudes, the concentration of 2,3-Bisphosphoglycerate (2,3-BPG) in the blood is increased, which allows these individuals to deliver a larger amount of oxygen to tissues under conditions of lower oxygen tension. This phenomenon, where molecule Y affects the binding of molecule X to a transport molecule Z, is called a *heterotropic* allosteric effect.

A variant hemoglobin, called fetal hemoglobin (HbF, $\alpha_2\gamma_2$), is found in the developing fetus, and binds oxygen with greater affinity than adult hemoglobin. This means that the oxygen binding curve for fetal hemoglobin is left-shifted (i.e., a higher percentage of hemoglobin has oxygen bound to it at lower oxygen tension), in comparison to that of adult hemoglobin. As a result, fetal blood in the placenta is able to take oxygen from maternal blood.

Hemoglobin also carries nitric oxide in the globin part of the molecule. This improves oxygen delivery in the periphery and contributes to the control of respiration. NO binds reversibly to a specific cysteine residue in globin; the binding depends on the state (R or T) of the hemoglobin. The resulting S-nitrosylated hemoglobin influences various NO-related activities such as the control of vascular resistance, blood pressure and respiration. NO is not released in the cytoplasm of erythrocytes but transported by an anion exchanger called AE1 out of them.

A study was performed to examine the influence of the form of hemoglobin (Hb) on the partitioning of inhaled volatile organic compounds (VOCs) into [human and animal] blood. Benzene was the prototypic VOC used in the investigations for this research due to the similar properties it shares with many other VOCs. To be specific, this study analyses the influence of the water solubility of Hb on the partitioning coefficient (PC) of a VOC as compared to the influence of the "species" or form of Hb. The different forms of blood used include: human hemoglobin (HbA), rat Hb, and sickle-cell hemoglobin (HbS). Rat Hb contains little water and is in a quasi-crystalline form, found inside the red blood cells (RBC), meaning they are more hydrophobic than human Hb, which are water-soluble. Sickle-cell hemoglobin (HbS) is water-soluble, however it can become water-insoluble, forming hydrophobic polymers, when deoxygenated. The findings state that the benzene PC for rat Hb was much higher than human that for Hb; however, the tests that measured the PCs of the oxygenated and deoxygenated forms of HbA and HbS did not differ, indicating that the affinity of benzene was not affected by the water solubility of Hb.

Types in humans

Hemoglobin variants are a part of the normal embryonic and fetal development, but may also be pathologic mutant forms of hemoglobin in a population, caused by variations in genetics. Some well-known hemoglobin variants such as sickle-cell anemia are responsible for diseases, and are considered hemoglobinopathies. Other variants cause no detectable pathology, and are thus considered non-pathological variants.

In the embryo:

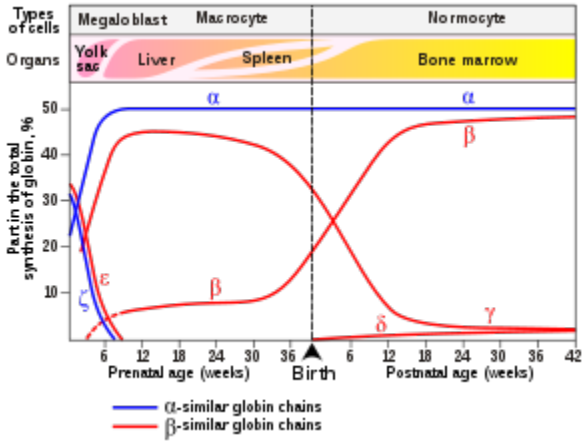
- Gower 1 ($\zeta_2\varepsilon_2$)
- Gower 2 ($\alpha_2\varepsilon_2$) (PDB 1A9W)
- Hemoglobin Portland ($\zeta_2\gamma_2$)

In the fetus:

- Hemoglobin F ($\alpha_2\gamma_2$) (PDB 1FDH)

In adults:

- Hemoglobin A ($\alpha_2\beta_2$) (PDB 1BZ0) - The most common with a normal amount over 95%
- Hemoglobin A_2 ($\alpha_2\delta_2$) - δ chain synthesis begins late in the third trimester and in adults, it has a normal range of 1.5-3.5%
- Hemoglobin F ($\alpha_2\gamma_2$) - In adults Hemoglobin F is restricted to a limited population of red cells called F-cells. However, the level of Hb F can be elevated in persons with sickle-cell disease and beta-thalassemia.



Gene expression of hemoglobin before and after birth. Also identifies the types of cells and organs in which the gene expression (data on *Wood W.G.*, (1976). **Br. Med. Bull. 32, 282.**)

Variant forms that cause disease:

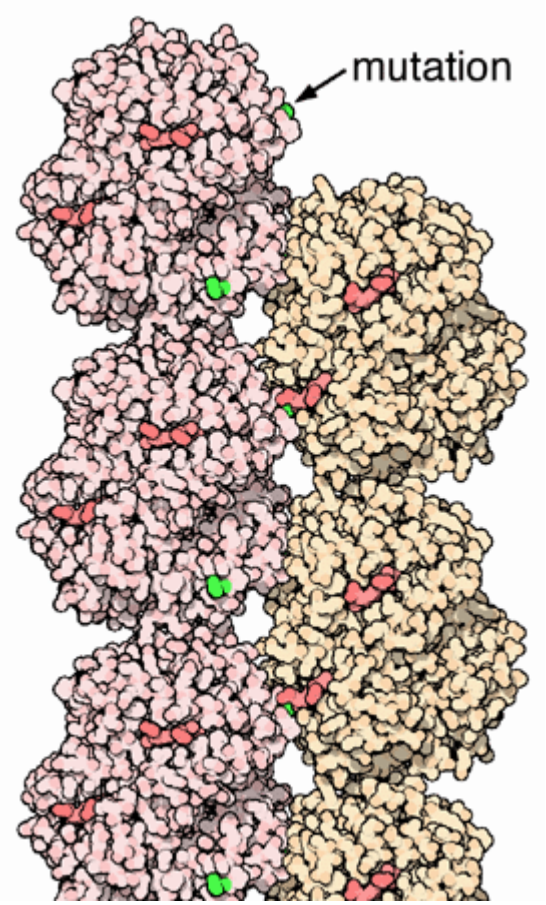
- Hemoglobin H (β_4) - A variant form of hemoglobin, formed by a tetramer of β chains, which may be present in variants of α thalassemia.
- Hemoglobin Barts (γ_4) - A variant form of hemoglobin, formed by a tetramer of γ chains, which may be present in variants of α thalassemia.
- Hemoglobin S ($\alpha_2\beta^S{}_2$) - A variant form of hemoglobin found in people with sickle cell disease. There is a variation in the β-chain gene, causing a change in the properties of hemoglobin, which results in sickling of red blood cells.

- Hemoglobin C ($\alpha_2\beta^C_2$) - Another variant due to a variation in the β-chain gene. This variant causes a mild chronic hemolytic anemia.
- Hemoglobin E ($\alpha_2\beta^E_2$) - Another variant due to a variation in the β-chain gene. This variant causes a mild chronic hemolytic anemia.
- Hemoglobin AS - A heterozygous form causing Sickle cell trait with one adult gene and one sickle cell disease gene
- Hemoglobin SC disease - Another heterozygous form with one sickle gene and another encoding Hemoglobin C.

Degradation in vertebrate animals

When red cells reach the end of their life due to aging or defects, they are broken down, the hemoglobin molecule is broken up and the iron gets recycled. When the porphyrin ring is broken up, the fragments are normally secreted in the bile by the liver. This process also produces one molecule of carbon monoxide for every molecule of heme degraded. This is one of the few natural sources of carbon monoxide production in the human body, and is responsible for the normal blood levels of carbon monoxide even in people breathing pure air. The other major final product of heme degradation is bilirubin. Increased levels of this chemical are detected in the blood if red cells are being destroyed more rapidly than usual. Improperly degraded hemoglobin protein or hemoglobin that has been released from the blood cells too rapidly can clog small blood vessels, especially the delicate blood filtering vessels of the kidneys, causing kidney damage.

Role in disease



In sickle cell hemoglobin (HbS) glutamic acid in position 6 (in beta chain) is mutated to valine. This change allows the deoxygenated form of the hemoglobin to stick to each other.

Hemoglobin deficiency can be caused either by decreased amount of hemoglobin molecules, as in anemia, or by decreased ability of each molecule to bind oxygen at the same partial pressure of oxygen. Hemoglobinopathies (genetic defects resulting in abnormal structure of the hemoglobin molecule) may cause both. In any case, hemoglobin deficiency decreases blood oxygen-carrying capacity. Hemoglobin deficiency is, in general, strictly distinguished from hypoxemia, defined as decreased partial pressure of oxygen in blood, although both are causes of hypoxia (insufficient oxygen supply to tissues).

Other common causes of low hemoglobin include loss of blood, nutritional deficiency, bone marrow problems, chemotherapy, kidney failure, or abnormal hemoglobin (such as that of sickle-cell disease).

High hemoglobin levels may be caused by exposure to high altitudes, smoking, dehydration, or tumors.

The ability of each hemoglobin molecule to carry oxygen is normally modified by altered blood pH or CO_2, causing an altered oxygen-hemoglobin dissociation curve. However, it can also be pathologically altered in, e.g., carbon monoxide poisoning.

Decrease of hemoglobin, with or without an absolute decrease of red blood cells, leads to symptoms of anemia. Anemia has many different causes, although iron deficiency and its resultant iron deficiency anemia are the most common causes in the Western world. As absence of iron decreases heme synthesis, red blood cells in iron deficiency anemia are *hypochromic* (lacking the red hemoglobin pigment) and *microcytic* (smaller than normal). Other anemias are rarer. In hemolysis (accelerated breakdown of red blood cells), associated jaundice is caused by the hemoglobin metabolite bilirubin, and the circulating hemoglobin can cause renal failure.

Some mutations in the globin chain are associated with the hemoglobinopathies, such as sickle-cell disease and thalassemia.

There is a group of genetic disorders, known as the *porphyrias* that are characterized by errors in metabolic pathways of heme synthesis. King George III of the United Kingdom was probably the most famous porphyria sufferer.

To a small extent, hemoglobin A slowly combines with glucose at the terminal valine (an alpha aminoacid) of each β chain. The resulting molecule is often referred to as Hb A_{1c}. As the concentration of glucose in the blood increases, the percentage of Hb A that turns into Hb A_{1c} increases. In diabetics whose glucose usually runs high, the percent Hb A_{1c} also runs high. Because of the slow rate of Hb A combination with glucose, the Hb A_{1c} percentage is representative of glucose level in the blood averaged over a longer time (the half-life of red blood cells, which is typically 50–55 days).

Glycosylated hemoglobin is the form of hemoglobin to which glucose is bound. The binding of glucose to amino acids in the hemoglobin takes place spontaneously (without the help of an enzyme) in many proteins, and is not known to serve a useful purpose. However, the binding to hemoglobin does serve as a record for average blood glucose levels over the lifetime of red cells, which is approximately 120 days. The levels of glycosylated hemoglobin are therefore measured in order to monitor the long-term control of the chronic disease of type 2 diabetes mellitus (T2DM). Poor control of T2DM results in high levels of glycosylated hemoglobin in the red blood cells. The normal reference range is approximately 4–5.9 %. Though difficult to obtain, values less than 7 % are recommended for people with T2DM. Levels greater than 9 % are associated with poor control of the glycosylated hemoglobin, and levels greater than 12 % are associated with very poor control. Diabetics who keep their glycosylated hemoglobin levels close to 7 % have a much better chance of avoiding the complications that may accompany diabetes (than those whose levels are 8 % or higher).

Elevated levels of hemoglobin are associated with increased numbers or sizes of red blood cells, called polycythemia. This elevation may be caused by congenital heart

disease, cor pulmonale, pulmonary fibrosis, too much erythropoietin, or polycythemia vera.

Elevation in levels of hemoglobin were found in one study of the yogic practice of Yoga Nidra (yogic sleep) for half an hour daily.

Diagnostic uses

Hemoglobin concentration measurement is among the most commonly performed blood tests, usually as part of a complete blood count. For example it is typically tested before or after blood donation. Results are reported in g/L, g/dL or mol/L. 1 g/dL equals about 0.6206 mmol/L. Normal levels are:

- Men: 13.8 to 18.0 g/dL (138 to 182 g/L, or 8.56 to 11.3 mmol/L)
- Women: 12.1 to 15.1 g/dL (121 to 151 g/L, or 7.51 to 9.37 mmol/L)
- Children: 11 to 16 g/dL (111 to 160 g/L, or 6.83 to 9.93 mmol/L)
- Pregnant women: 11 to 12 g/dL (110 to 120 g/L, or 6.83 to 7.45 mmol/L)

Normal values of hemoglobin in the 1st and 3rd trimesters of pregnant women must be at least 11 g/dL and at least 10.5 g/dL during the 2nd trimester.

If the concentration is below normal, this is called anemia. Anemias are classified by the size of red blood cells, the cells that contain hemoglobin in vertebrates. The anemia is called "microcytic" if red cells are small, "macrocytic" if they are large, and "normocytic" otherwise.

Hematocrit, the proportion of blood volume occupied by red blood cells, is typically about three times the hemoglobin level. For example, if the hemoglobin is measured at 17, that compares with a hematocrit of 51.

Long-term control of blood sugar concentration can be measured by the concentration of Hb A_{1c}. Measuring it directly would require many samples because blood sugar levels vary widely through the day. Hb A_{1c} is the product of the irreversible reaction of hemoglobin A with glucose. A higher glucose concentration results in more Hb A_{1c}. Because the reaction is slow, the Hb A_{1c} proportion represents glucose level in blood averaged over the half-life of red blood cells, is typically 50–55 days. An Hb A_{1c} proportion of 6.0% or less show good long-term glucose control, while values above 7.0% are elevated. This test is especially useful for diabetics.

The functional magnetic resonance imaging (fMRI) machine uses the signal from deoxyhemoglobin, which is sensitive to magnetic fields since it is paramagnetic.

Analogues in non-vertebrate organisms

A variety of oxygen-transport and -binding proteins exist in organisms throughout the animal and plant kingdoms. Organisms including bacteria, protozoans, and fungi all have

hemoglobin-like proteins whose known and predicted roles include the reversible binding of gaseous ligands. Since many of these proteins contain globins and the heme moiety (iron in a flat porphyrin support), they are often called hemoglobins, even if their overall tertiary structure is very different from that of vertebrate hemoglobin. In particular, the distinction of "myoglobin" and hemoglobin in lower animals is often impossible, because some of these organisms do not contain muscles. Or, they may have a recognizable separate circulatory system but not one that deals with oxygen transport (for example, many insects and other arthropods). In all these groups, heme/globin-containing molecules (even monomeric globin ones) that deal with gas-binding are referred to as oxyhemoglobins. In addition to dealing with transport and sensing of oxygen, they may also deal with NO, CO_2, sulfide compounds, and even O_2 scavenging in environments that must be anaerobic. They may even deal with detoxification of chlorinated materials in a way analogous to heme-containing P450 enzymes and peroxidases.

The giant tube worm *Riftia pachyptila* showing red hemoglobin-containing plumes

The structure of hemoglobins varies across species. Hemoglobin occurs in all kingdoms of organisms, but not in all organisms. Primitive species such as bacteria, protozoa, algae, and plants often have single-globin hemoglobins. Many nematode worms, molluscs, and crustaceans contain very large multisubunit molecules, much larger than those in vertebrates. In particular, chimeric hemoglobins found in fungi and giant annelids may contain both globin and other types of proteins.

One of the most striking occurrences and uses of hemoglobin in organisms is in the giant tube worm (*Riftia pachyptila*, also called Vestimentifera), which can reach 2.4 meters

length and populates ocean volcanic vents. Instead of a digestive tract, these worms contain a population of bacteria constituting half the organism's weight. The bacteria react with H_2S from the vent and O_2 from the water to produce energy to make food from H_2O and CO_2. The worms end with a deep red fan-like structure ("plume"), which extends into the water and absorbs H_2S and O_2 for the bacteria, and CO_2 for use as synthetic raw material similar to photosynthetic plants. The structures are bright-red due to their containing several extraordinarily complex hemoglobins that have up to 144 globin chains, each including associated heme structures. These hemoglobins are remarkable for being able to carry oxygen in the presence of sulfide, and even to carry sulfide, without being completely "poisoned" or inhibited by it as hemoglobins in most other species are.

Other oxygen-binding proteins

Myoglobin: Found in the muscle tissue of many vertebrates, including humans, it gives muscle tissue a distinct red or dark gray color. It is very similar to hemoglobin in structure and sequence, but is not a tetramer; instead, it is a monomer that lacks cooperative binding. It is used to store oxygen rather than transport it.

Hemocyanin: The second most common oxygen-transporting protein found in nature, it is found in the blood of many arthropods and molluscs. Uses copper prosthetic groups instead of iron heme groups and is blue in color when oxygenated.

Hemerythrin: Some marine invertebrates and a few species of annelid use this iron-containing non-heme protein to carry oxygen in their blood. Appears pink/violet when oxygenated, clear when not.

Chlorocruorin: Found in many annelids, it is very similar to erythrocruorin, but the heme group is significantly different in structure. Appears green when deoxygenated and red when oxygenated.

Vanabins: Also known as **vanadium chromagens**, they are found in the blood of sea squirts. There were once hypothesized to use the rare metal vanadium as an oxygen binding prosthetic group. However, although they do contain vandadium by preference, they apparently bind little oxygen, and thus have some other function, which has not been elucidated (sea squirts also contain some hemoglobin). They may act as toxins.

Erythrocruorin: Found in many annelids, including earthworms, it is a giant free-floating blood protein containing many dozens—possibly hundreds—of iron- and heme-bearing protein subunits bound together into a single protein complex with a molecular mass greater than 3.5 million daltons.

Pinnaglobin: Only seen in the mollusc *Pinna squamosa*. Brown manganese-based porphyrin protein.

Leghemoglobin: In leguminous plants, such as alfalfa or soybeans, the nitrogen fixing bacteria in the roots are protected from oxygen by this iron heme containing oxygen-binding protein. The specific enzyme protected is nitrogenase, which is unable to reduce nitrogen gas in the presence of free oxygen.

Coboglobin: A synthetic cobalt-based porphyrin. Coboprotein would appear colorless when oxygenated, but yellow when in veins.

Presence in nonerythroid cells

Some nonerythroid cells (i.e., cells other than the red blood cell line) contain hemoglobin. In the brain, these include the A9 dopaminergic neurons in the substantia nigra, astrocytes in the cerebral cortex and hippocampus, and in all mature oligodendrocytes. It has been suggested that brain hemoglobin in these cell may enable the "storage of oxygen to provide a homeostatic mechanism in anoxic conditions, which is especially important for A9 DA neurons that have an elevated metabolism with a high requirement for energy production". It has been noted further that "A9 dopaminergic neurons may be at particular risk since in addition to their high mitochondrial activity they are under intense oxidative stress caused by the production of hydrogen peroxide via autoxidation and/or monoamine oxidase (MAO)-mediated deamination of dopamine and the subsequent reaction of accessible ferrous iron to generate highly toxic hydroxyl radicals". This may explain the risk of these cells for degeneration in Parkinson's disease. The presence of iron from hemoglobin in these cells also results in the post-mortem darkness of these cells, which is the origin of the Latin name, substantia *nigra*.

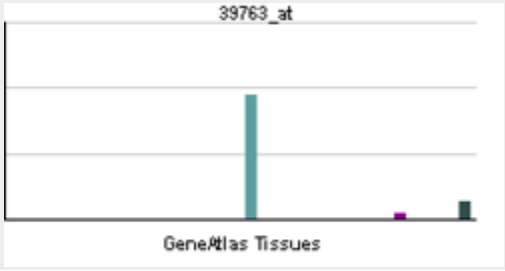
Outside the brain, hemoglobin has non-oxygen-carrying functions as an antioxidant and a regulator of iron metabolism in macrophages, alveolar cells, and mesangial cells in the kidney.

Chapter 8

Hemopexin and Mannan-Binding Lectin

Hemopexin

Hemopexin	
Identifiers	
Symbols	HPX;
External IDs	OMIM: 142290 MGI: 105112 HomoloGene: 511 GeneCards: HPX Gene
EC number	3.2.1.35
RNA expression pattern	



Orthologs		
Species	**Human**	**Mouse**
Entrez	3263	15458
Ensembl	ENSG00000110169	ENSMUSG00000030895
UniProt	P02790	Q3UKP2
RefSeq (mRNA)	NM_000613	NM_017371
RefSeq (protein)	NP_000604	NP_059067
Location (UCSC)	Chr 11: 6.41 - 6.42 Mb	Chr 7: 105.47 - 105.47 Mb

Hemopexin (or **haemopexin**; HPX), also known as **beta-1B-glycoprotein**, is a protein that in humans is encoded by the *HPX* gene and belongs to hemopexin family of proteins.

Function

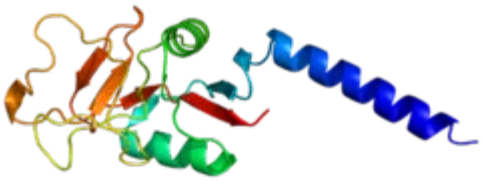
Hemopexin binds heme with the highest affinity of any known protein. Its function of scavenging the heme released or lost by the turnover of heme proteins such as hemoglobin and thus protects the body from the oxidative damage that free heme can cause. In addition, hemopexin releases its bound ligand for internalisation upon interacting with a specific receptor situated on the surface of liver cells. This function of hemopexin is to preserve the body's iron.

Clinical significance

Its levels in serum reflect how much heme is present in the blood. Therefore, low hemopexin levels indicates that there has been significant degradation of heme containing compounds and hemopexin is made to scavenge any heme it can. Low hemopexin levels are one of the diagnostic features of a hemolytic anemia.

Mannan-binding lectin

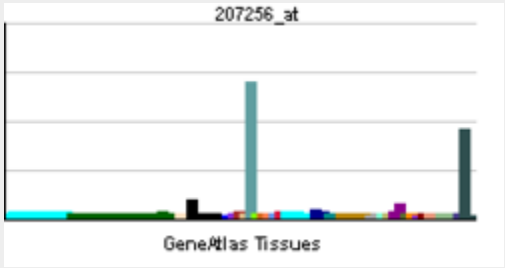
Mannose-binding lectin (protein C) 2, soluble (opsonic defect)



PDB rendering based on 1hup.

Available structures	
Identifiers	
Symbols	MBL2; COLEC1; HSMBPC; MBL; MBP; MBP1; MGC116832; MGC116833
External IDs	OMIM: 154545 MGI: 96924 HomoloGene: 88328 GeneCards: MBL2 Gene

RNA expression pattern



Orthologs

Species	Human	Mouse
Entrez	4153	17195
Ensembl	ENSG00000165471	ENSMUSG00000024863
UniProt	P11226	Q3UEK1
RefSeq (mRNA)	NM_000242	NM_010776
RefSeq (protein)	NP_000233	NP_034906

Location (UCSC)	Chr 10: 54.2 - 54.2 Mb	Chr 19: 30.3 - 30.31 Mb

Mannose binding lectin (MBL), also named mannose- or mannan-binding protein (MBP), is an important factor in innate immunity.

Function

MBL belongs to the class of collectins in the C-type lectin superfamily, whose function appears to be pattern recognition in the first line of defense in the pre-immune host.

MBL recognizes carbohydrate patterns, found on the surface of a large number of pathogenic micro-organisms, including bacteria, viruses, protozoa and fungi.

Binding of MBL to a micro-organism results in activation of the lectin pathway of the complement system.

Structure

MBL has an oligomeric structure (400-700 kDa), built of subunits that contain three identical peptide chains of 32 kDa each.

Although MBL can form several oligomeric forms, there are indications that dimers and trimers are not biologically active and at least a tetramer form is needed for activation of complement.

Activation

The complement system can be activated through three pathways the classical pathway, the alternative pathway, and the mannose-binding (MB) lectin pathway. The most-recently discovered mannose-binding lectin pathway activates complement through the mannose-binding lectin protein. MBL binds to carbohydrates (specifically Mannose and Fucose residues) found on the surface of many pathogens.

For example, MBL has been show to bind to:

- yeasts such as Candida albicans
- viruses such as HIV and influenza A
- many bacteria including Salmonella and Streptococci
- parasites like Leishmania

Complexes

MBL in the blood is complexed with (bound to) another protein, a serine protease called MASP-2 (MBL-associated serine protease).

In order to activate the complement system when MBL binds to its target (for example, mannose on the surface of a bacterium), the MASP protein functions to cleave the blood protein C4 into C4a and C4b. The C4b fragments can then bind to the surface of the bacterium, and initiate the formation of a C3 convertase.

The subsequent complement cascade catalyzed by C3 convertase results in creating a membrane attack complex, which causes lysis of the pathogen that MBL bound to.

Clinical significance

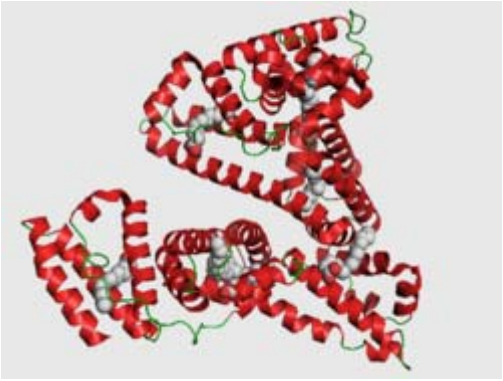
It is produced in the liver as a response to infection, and is part of many other factors termed acute phase proteins.

Chapter 9

Serum Albumin and Thyroxine-Binding Globulin

Serum albumin

Albumin



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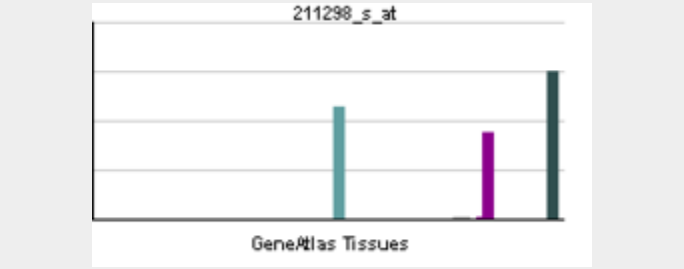
Available structures	
Identifiers	
Symbols	ALB; DKFZp779N1935; PRO0883; PRO0903; PRO1341
External IDs	OMIM: 103600 MGI: 87991 HomoloGene: 405 GeneCards: ALB Gene
RNA expression pattern	



Orthologs		
Species	**Human**	**Mouse**
Entrez	213	11657
Ensembl	ENSG00000163631	ENSMUSG00000029368
UniProt	P02768	Q3TV03
RefSeq (mRNA)	NM_000477	NM_009654
RefSeq (protein)	NP_000468	NP_033784
Location (UCSC)	Chr 4: 74.49 - 74.51 Mb	Chr 5: 91.54 - 91.55 Mb

Serum albumin, often referred to simply as **albumin** is a protein that in humans is encoded by the *ALB* gene.

Serum albumin is the most abundant plasma protein in mammals. Albumin is essential for maintaining the osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues. It also acts as a plasma carrier by non-specifically binding several hydrophobic steroid hormones and as a transport protein for hemin and fatty acids. Too much serum albumin in the body can be harmful.

Function

Major contributors to oncotic pressure (known also as colloid osmotic pressure) of plasma; carriers for various substances.

Albumin is a soluble, monomeric protein which comprises about one-half of the blood serum protein. Albumin functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones and plays a role in stabilizing extracellular fluid volume. Albumin is a globular un-glycosylated serum protein of molecular weight 65,000. Albumin is synthesized in the liver as preproalbumin which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum.

The product, proalbumin, is in turn cleaved in the Golgi vesicles to produce the secreted albumin.

Types

Serum albumin is widely distributed in mammals. Examples include:

- The human version is human serum albumin.
- Bovine serum albumin, or BSA, is commonly used in immunodiagnostic procedures, clinical chemistry reagents, cell culture media, protein chemistry research and molecular biology laboratories (usually to leverage its non-specific protein binding properties).

Physical properties

Albumin (when ionized in water at pH 7.4, as found in the body) is negatively charged. The glomerular basement membrane is also negatively charged in the body; some studies suggest that this prevents the filtration of albumin in the urine. According to this theory, that charge plays a major role in the selective exclusion of albumin from the glomerular filtrate. A defect in this property results in nephrotic syndrome leading to albumin loss in the urine. Nephrotic syndrome patients are sometimes given albumin to replace the lost albumin.

Because smaller animals (for example rats) function at a lower blood pressure, they need less oncotic pressure to balance this, and thus need less albumin to maintain proper fluid distribution.

Structure

The general structure of Albumin is characterized by several long α (alpha) helices, this allows it to maintain a relatively static shape, something essential for regulating blood pressure.

Serum albumin contains eleven distinct binding domains for hydrophobic compounds. One hemin and six long-chain fatty acids can bind to serum albumin at the same time.

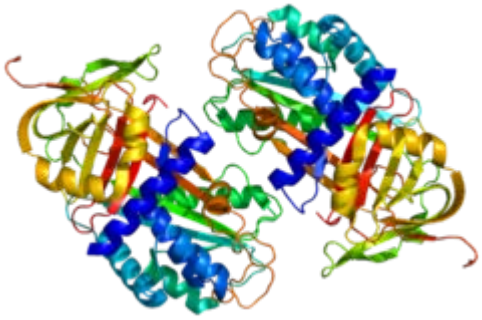
Serum albumin family

Structure of human serum albumin.

Identifiers	
Symbol	Serum_albumin
Pfam	PF00273
Pfam clan	CL0282
InterPro	IPR014760
SMART	SM00103
PROSITE	PS51438
SCOP	1ao6

Thyroxine-binding globulin

Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7



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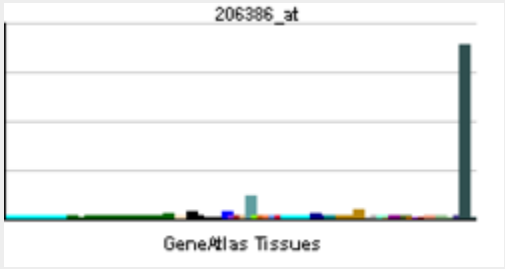
Available structures	
Identifiers	
Symbols	SERPINA7; TBG
External IDs	OMIM: 314200 MGI: 3041197 HomoloGene: 20113 GeneCards: SERPINA7 Gene
RNA expression pattern	



Orthologs		
Species	**Human**	**Mouse**
Entrez	6906	331535
Ensembl	ENSG00000123561	ENSMUSG00000031271
UniProt	P05543	Q3UEL9
RefSeq (mRNA)	NM_000354	NM_177920

RefSeq (protein)	NP_000345	NP_808588
Location (UCSC)	Chr X: 105.16 - 105.17 Mb	Chr X: 134.43 - 134.43 Mb

Thyroxine-binding globulin (TBG) binds thyroid hormone in circulation. It is one of three proteins (along with transthyretin and albumin) responsible for carrying the thyroid hormones thyroxine (T4) and 3,5,3'-triiodothyronine (T3) in the bloodstream. Of these three proteins, TBG has the highest affinity for T4 and T3, but is present in the lowest concentration. Despite its low concentration, TBG carries the majority of T4 in the blood. Due to the very low concentration of T4 & T3 in the blood, TBG is rarely more than 25% saturated with its ligand. Unlike transthyretin and albumin, TBG has a single binding site for T4/T3. TBG is synthesized primarily in the liver as a 54 kDa protein. In terms of genomics, TBG is a serpin; however, it has no inhibitory function like many other members of this class of proteins.

Role in Diagnosis

TBG tests are sometimes used in finding the reason for elevated or diminished levels of thyroid hormone. This is done by measuring resin binding to labeled thyroid hormone, which happens only when the labeled thyroid hormone is free.

The patient's serum is mixed with the labeled thyroid hormone; then, the resin is added to the whole mixture to measure the amount of free labeled thyroid hormone. So, for instance, if the patient is truly hypothyroid, and TBG levels are normal, then there are many sites open for binding on the TBG, since the total thyroid hormone level is low. Therefore, when the labeled hormone is added, it will bind mostly to the TBG, leaving little of it left for binding to the resin. In contrast, however, if the patient is truly hyperthyroid, and TBG levels are normal, the patient's endogenous hormone will saturate the TBG binding sites more, leaving less room for the labeled hormone, which allows greater binding to the resin.

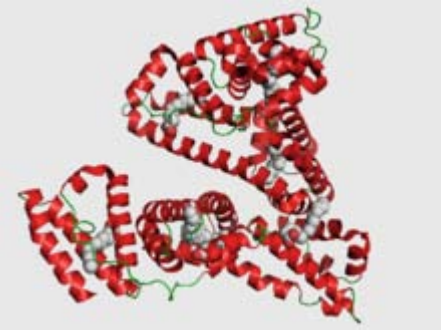
In the situations described above, TBG testing is not very useful. However, if total thyroid hormone levels point to hypothyroidism or hyperthyroidism in the absence of accompanying symptoms, the utility of TBG testing becomes more evident, since TBG production can be modified by other factors such as estrogen levels, corticosteroid levels, or liver failure. If, for example, the TBG level is high, which can occur when estrogen levels are high, the TBG will bind more thyroid hormone, decreasing the free hormone available in the blood, which leads to stimulation of TSH, and the production of more thyroid hormone. In this case, the total thyroid hormone level will be high. However, when labeled hormone is added, since TBG is so high, it will bind to the TBG, leaving little free labeled hormone for uptake into the resin. On the converse, in the presence of corticosteroids, which lower TBG levels, the total thyroid hormone (bound and free) in the blood will be low. However, when the labeled hormone is added, since so little TBG

is available in the blood, only a small portion of it will bind, leaving plenty available for uptake by the resin.

Chapter 10

Human Serum Albumin

Human serum albumin



PDB rendering based on 1e7h.

Identifiers	
Symbol	ALB
Entrez	213
HUGO	399
OMIM	103600
PDB	1E7H
RefSeq	NM_000477
UniProt	P02768
Other data	
Locus	Chr. 4 *q13.3*

Human serum albumin is the most abundant protein in human blood plasma. It is produced in the liver. Albumin comprises about half of the blood serum protein. It is soluble and monomeric.

The gene for albumin is located on chromosome 4 and mutations in this gene can result in various anomalous proteins. The human albumin gene is 16,961 nucleotides long from the putative 'cap' site to the first poly(A) addition site. It is split into 15 exons that are symmetrically placed within the 3 domains thought to have arisen by triplication of a single primordial domain.

Albumin is synthesized in the liver as preproalbumin, which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum. The product, proalbumin, is in turn cleaved in the Golgi vesicles to produce the secreted albumin.

The reference range for albumin concentrations in blood is 3.4 to 5.4 g/dL. It has a serum half-life of approximately 20 days. It has a molecular mass of 67 kDa.

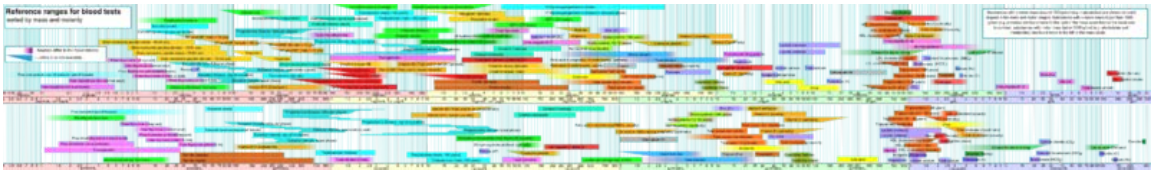
Function

- Maintains oncotic pressure
- Transports thyroid hormones
- Transports other hormones, in particular, ones that are fat-soluble
- Transports fatty acids ("free" fatty acids) to the liver
- Transports unconjugated bilirubin
- Transports many drugs; serum albumin levels can affect the half-life of drugs
- Competitively binds calcium ions (Ca^{2+})
- Buffers pH
- Serum albumin, as a negative acute-phase protein, is down-regulated in inflammatory states. As such, it is not a valid marker of nutritional status; rather, it is a marker in inflammatory states
- Prevents photodegradation of folic acid

Measurement

Plasma albumin is a component of liver function tests (LFTs), but may be ordered separately. Albumin can be measured in serum (yellow-top tube), plain tube with no additives (red-top tube), or heparin plasma (green-top tube). The reference interval is 36 - 52 g/L. (upper limit increased from 47 g/L on the 15th June 2007). One of the methods used is bromocresol green on a Roche Modular or Olympus AU2700 analyser.

Reference ranges



Reference ranges for blood tests, comparing blood content of albumin (shown in purple at right) with other constituents.

Pathology

Hypoalbuminemia

Low blood albumin levels (hypoalbuminemia) can be caused by:

- Liver disease; cirrhosis of the liver is most common
- Excess excretion by the kidneys (as in nephrotic syndrome)
- Excess loss in bowel (protein-losing enteropathy, e.g., Menetrier's)
- Burns (plasma loss in the absence of skin barrier)
- Redistribution (hemodilution [as in pregnancy], increased vascular permeability or decreased lymphatic clearance)
- Acute disease states (referred to as a negative acute-phase protein)
- Mutation causing analbuminemia (very rare)

Hyperalbuminemia

Typically, this condition is a sign of severe or chronic dehydration. Chronic dehydration needs to be treated with zinc as well as with water. Zinc reduces cell swelling caused by increased intake of water (hypotonicity) and also increases retention of salt. In the dehydrated state, the body has too high an osmolarity and, it appears, discards zinc to prevent this. Zinc also regulates transport of the cellular osmolyte taurine, and albumin is known to increase cellular taurine absorption. Zinc has been shown to increase retinol (vitamin A) production from beta-carotene, and in lab experiments retinol reduced human albumin production. It is possible that a retinol (vitamin A) deficiency alone could cause albumin levels to become raised. Patients recovering from chronic dehydration may develop dry eyes as the body uses up its vitamin A store. It is interesting to note that retinol causes cells to swell with water (this is likely one reason that too much vitamin A is toxic). Hyperalbuminemia is also associated with high protein diets.

Glycosylation

It has been known for a long time that human blood proteins like hemoglobin and serum albumin may undergo a slow non-enzymatic glycation, mainly by formation of a Schiff base between ε-amino groups of lysine (and sometimes arginine) residues and glucose molecules in blood (Maillard reaction). This reaction can be inhibited in the presence of

antioxidant agents. Although this reaction may happen normally, elevated glycoalbumin is observed in diabetes mellitus.

Glycation has the potential to alter the biological structure and function of the serum albumin protein.

Moreover, the glycation can result in the formation of Advanced Glycosylation End-Products (AGE), which result in abnormal biological effects. Accumulation of AGEs leads to tissue damage via alteration of the structures and functions of tissue proteins, stimulation of cellular responses, through receptors specific for AGE-proteins, and generation of reactive oxygen intermediates. AGEs also react with DNA, thus causing mutations and DNA transposition. Thermal processing of proteins and carbohydrates brings major changes in allergenicity. AGEs are antigenic and represent many of the important neoantigens found in cooked or stored foods. They also interfere with the normal product of nitric oxide in cells.

Although there are several lysine and arginine residues in the serum albumin structure, very few of them can take part in the glycation reaction. It is not clear exactly why only these residues are glycated in serum albumin, but it is suggested that non-covalent binding of glucose to serum albumin prior to the covalent bond formation might be the reason.

Loss via kidneys

In the healthy kidney, albumin's size and negative electric charge exclude it from excretion in the glomerulus. This is not always the case, as in some diseases including diabetic nephropathy, a major complication of uncontrolled diabetes in which proteins can cross the glomerulus. The lost albumin can be detected by a simple urine test. Depending on the amount of albumin lost, a patient may have normal renal function, microalbuminuria, or albuminuria.

Amino acid sequence

The approximate sequence of human serum albumin is:

*MKWVTFISLL FLFSSAYSRG VFRR*DAHKSE VAHRFKDLGE ENFKALVLIA FAQYLQQCPF
EDHVKLVNEV TEFAKTCVAD ESAENCDKSL HTLFGDKLCT VATLRETYGE MADCCAKQEP
ERNECFLQHK DDNPNLPRLV RPEVDVMCTA FHDNEETFLK KYLYEIARRH PYFYAPELLF
FAKRYKAAFT ECCQAADKAA CLLPKLDELR DEGKASSAKQ RLKCASLQKF GERAFKAWAV
ARLSQRFPKA EFAEVSKLVT DLTKVHTECC HGDLLECADD RADLAKYICE NQDSISSKLK
ECCEKPLLEK SHCIAEVEND EMPADLPSLA ADFVESKDVC KNYAEAKDVF LGMFLYEYAR
RHPDYSVVLL LRLAKTYETT LEKCCAAADP HECYAKVFDE FKPLVEEPQN LIKQNCELFE
QLGEYKFQNA LLVRYTKKVP QVSTPTLVEV SRNLGKVGSK CCKHPEAKRM PCAEDYLSVV
LNQLCVLHEK TPVSDRVTKC CTESLVNRRP CFSALEVDET YVPKEFNAET FTFHADICTL
SEKERQIKKQ TALVELVKHK PKATKEQLKA VMDDFAAFVE KCCKADDKET CFAEEGKKLV
AASQAALGL

The italicized first 24 amino acids are signal and propeptide portions not observed in the transcribed, translated, and transported protein but present in the gene. There are 609 amino acids in this sequence with only 585 amino acids in the final product observed in the blood.

Interactions

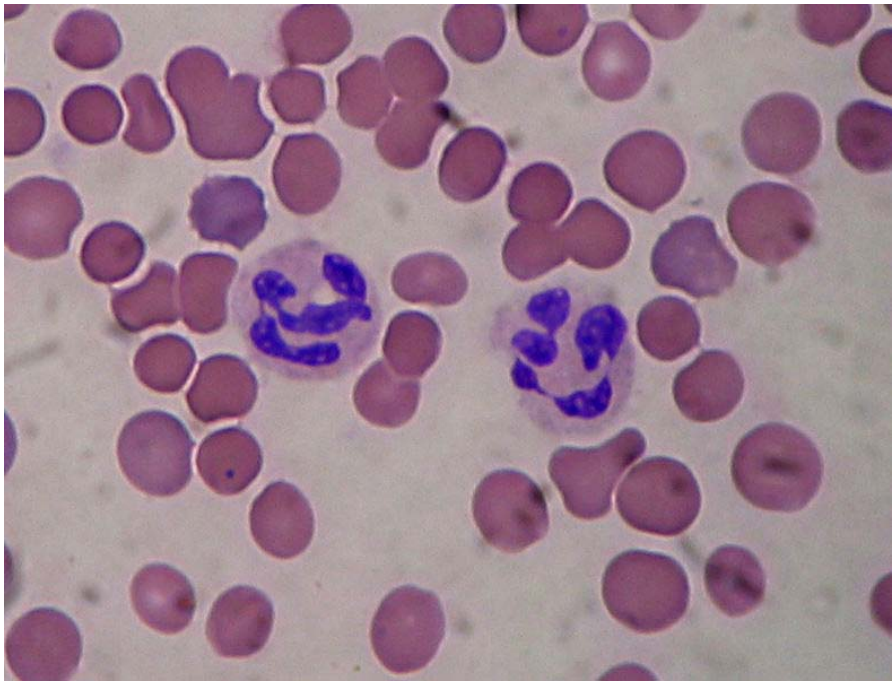
Human serum albumin has been shown to interact with FCGRT.

Chapter 11

Acute-Phase Protein and Apolipoprotein

Acute-phase protein

Acute-phase proteins are a class of proteins whose plasma concentrations increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) in response to inflammation. This response is called the *acute-phase reaction* (also called acute-phase response).



Inflammatory cells and red blood cells

In response to injury, local inflammatory cells (neutrophil granulocytes and macrophages) secrete a number of cytokines into the bloodstream, most notable of which are the interleukins IL-1, IL-6 and IL-8, and TNF-α.

The liver responds by producing a large number of **acute-phase reactants**. At the same time, the production of a number of other proteins is reduced; these are, therefore, referred to as "negative" acute-phase reactants.

Positive

Positive acute-phase proteins serve different physiological functions for the immune system. Some act to destroy or inhibit growth of microbes, e.g., C-reactive protein, Mannose-binding protein, complement factors, ferritin, ceruloplasmin, Serum amyloid A and haptoglobin. Others give negative feedback on the inflammatory response, e.g. serpins. Alpha 2-macroglobulin and coagulation factors affect coagulation, mainly stimulating it. This pro-coagulant effect may limit infection by trapping pathogens in local blood clots. Also, some products of the coagulation system can contribute to the innate immune system by their ability to increase vascular permeability and act as chemotactic agents for phagocytic cells.

"Positive" acute-phase proteins:

Protein	Immune system function
C-reactive protein	Opsonin on microbes
Serum amyloid P component	Opsonin
Serum amyloid A	• Recruitment of immune cells to inflammatory sites • Induction of enzymes that degrade extracellular matrix
Complement factors	Opsonization, lysis and clumping of target cells. Chemotaxis
Mannan-binding lectin	Mannan-binding lectin pathway of complement activation
Fibrinogen, prothrombin, factor VIII, von Willebrand factor	Coagulation factors, trapping invading microbes in blood clots. Some cause chemotaxis
Plasminogen	Degradation of blood clots
Alpha 2-macroglobulin	• Inhibitor of coagulation by inhibiting thrombin. • Inhibitor of fibrinolysis by inhibiting plasmin
Ferritin	Binding iron, inhibiting microbe iron uptake
Hepcidin	Stimulates the internalization of ferroportin, preventing release of iron bound by ferritin within intestinal enterocytes and macrophages
Ceruloplasmin	Oxidizes iron, facilitating for ferritin, inhibiting microbe iron uptake
Haptoglobin	Binds hemoglobin, inhibiting microbe iron uptake
Orosomucoid (Alpha-1-acid	Steroid carrier

glycoprotein, AGP)	
Alpha 1-antitrypsin	Serpin, downregulates inflammation
Alpha 1-antichymotrypsin	Serpin, downregulates inflammation

Negative

"Negative" acute-phase proteins decrease in inflammation. Examples include albumin, transferrin, transthyretin, retinol-binding protein, antithrombin, transcortin. The decrease of such proteins may be used as markers of inflammation. The physiological role of decreased synthesis of such proteins is generally to provide amino acids in order to produce "positive" acute-phase proteins more efficiently. Theoretically, a decrease in transferrin could additionally be decreased by an upregulation of transferrin receptors, but the latter do not appear to change with inflammation.

Clinical significance

Measurement of acute-phase proteins, especially C-reactive protein, is a useful marker of inflammation in both medical and veterinary clinical pathology. It correlates with the erythrocyte sedimentation rate (ESR).

Apolipoprotein

Apolipoproteins are proteins that bind to lipids (oil-soluble substances such as fat and cholesterol) to form lipoproteins, which transport the lipids through the lymphatic and circulatory systems.

The lipid components of lipoproteins are not soluble in water; however, because of their detergent-like (amphipathic) properties, apolipoproteins and other amphipathic molecules (such as phospholipids) can surround the lipids, creating the lipoprotein particle that is itself water-soluble, and can thus be carried through water-based circulation (i.e., blood, lymph).

Apolipoproteins also serve as enzyme cofactors, receptor ligands, and lipid transfer carriers that regulate the metabolism of lipoproteins and their uptake in tissues.

Functions

- They are enzyme coenzymes (C-II for lipoprotein lipase and A-I for lecithin-cholesterol acyltransferase)
- Lipid transport proteins
- Ligands for interaction with lipoprotein receptors in tissues (apoB100 and apoE for LDL-receptors, apoA-I for HDL receptors)

Classes

There are six major classes of apolipoproteins and several sub-classes:

- A (apo A-I, apo A-II, apo A-IV, and apo A-V)
- B (apo B48 and apo B100)
- C (apo C-I, apo C-II, apo C-III, and apo C-IV)
- D
- E
- H

Hundreds of genetic polymorphisms of the apolipoproteins have been described, and many of them alter their structure and function.

Synthesis and regulation

Apolipoprotein synthesis in the intestine is regulated principally by the fat content of the diet.

Apolipoprotein synthesis in the liver is controlled by a host of factors, including dietary composition, hormones (insulin, glucagon, thyroxin, estrogens, androgens), alcohol intake, and various drugs (statins, niacin,and fibric acids). Apo B is an integral apoprotein whereas the others are peripheral apoproteins.

Chapter 12

Coagulation

Coagulation is a complex process by which blood forms clots. It is an important part of hemostasis (the cessation of blood loss from a damaged vessel), wherein a damaged blood vessel wall is covered by a platelet and fibrin-containing clot to stop bleeding and begin repair of the damaged vessel. Disorders of coagulation can lead to an increased risk of bleeding (hemorrhage) or obstructive clotting (thrombosis).

Coagulation is highly conserved throughout biology; in all mammals, coagulation involves both a cellular (platelet) and a protein (coagulation factor) component. The system in humans has been the most extensively researched and is therefore the best understood.

Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium (lining of the vessel). Exposure of the blood to proteins such as tissue factor initiates changes to blood platelets and the plasma protein fibrinogen, a clotting factor. Platelets immediately form a plug at the site of injury; this is called *primary hemostasis*. *Secondary hemostasis* occurs simultaneously: Proteins in the blood plasma, called *coagulation factors* or *clotting factors*, respond in a complex cascade to form fibrin strands, which strengthen the platelet plug.

Physiology

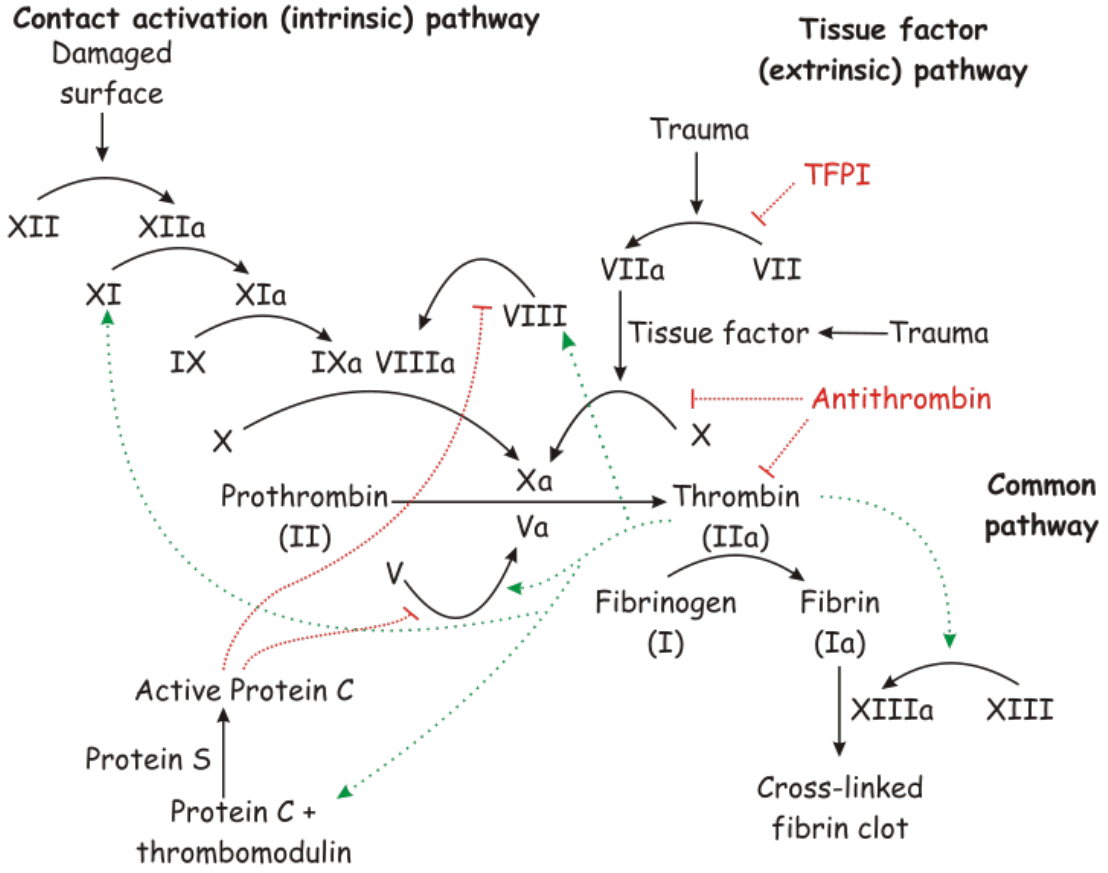
Platelet activation

Damage to blood vessel walls exposes subendothelium proteins, most notably von Willebrand factor (vWF), present under the endothelium. vWF is a protein secreted by healthy endothelium, forming a layer between the endothelium and underlying basement membrane. When the endothelium is damaged, the normally-isolated, underlying vWF is exposed to white blood cells and recruits Factor VIII, collagen, and other clotting factors. Circulating platelets bind to collagen with surface collagen-specific glycoprotein Ia/IIa receptors. This adhesion is strengthened further by additional circulating proteins vWF,

which forms additional links between the platelets glycoprotein Ib/IX/V and the collagen fibrils. These adhesions activate the platelets.

Activated platelets release the contents of stored granules into the blood plasma. The granules include ADP, serotonin, platelet-activating factor (PAF), vWF, platelet factor 4, and thromboxane A_2 (TXA_2), which, in turn, activate additional platelets. The granules' contents activate a G_q-linked protein receptor cascade, resulting in increased calcium concentration in the platelets' cytosol. The calcium activates protein kinase C, which, in turn, activates phospholipase A_2 (PLA_2). PLA_2 then modifies the integrin membrane glycoprotein IIb/IIIa, increasing its affinity to bind fibrinogen. The activated platelets change shape from spherical to stellate, and the fibrinogen cross-links with glycoprotein IIb/IIIa aid in aggregation of adjacent platelets (completing primary hemostasis).

The coagulation cascade



The coagulation cascade of secondary hemostasis.

The coagulation cascade of secondary hemostasis has two pathways which lead to *fibrin* formation. These are the *contact activation pathway* (also known as the intrinsic pathway), and the *tissue factor pathway* (also known as the extrinsic pathway). It was previously thought that the coagulation cascade consisted of two pathways of equal

importance joined to a common pathway. It is now known that the primary pathway for the initiation of blood coagulation is the *tissue factor* pathway. The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lowercase *a* appended to indicate an active form.

The coagulation factors are generally serine proteases (enzymes). There are some exceptions. For example, FVIII and FV are glycoproteins, and Factor XIII is a transglutaminase. Serine proteases act by cleaving other proteins at specific sites. The coagulation factors circulate as inactive zymogens. The coagulation cascade is classically divided into three pathways. The *tissue factor* and *contact activation* pathways both activate the "final common pathway" of factor X, thrombin and fibrin.

Tissue factor pathway (extrinsic)

The main role of the tissue factor pathway is to generate a "thrombin burst," a process by which thrombin, the most important constituent of the coagulation cascade in terms of its feedback activation roles, is released instantaneously. FVIIa circulates in a higher amount than any other activated coagulation factor.

- Following damage to the blood vessel, FVII leaves the circulation and comes into contact with tissue factor (TF) expressed on tissue-factor-bearing cells (stromal fibroblasts and leukocytes), forming an activated complex (TF-FVIIa).
- TF-FVIIa activates FIX and FX.
- FVII is itself activated by thrombin, FXIa, FXII and FXa.
- The activation of FXa by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI).
- FXa and its co-factor FVa form the prothrombinase complex, which activates prothrombin to thrombin.
- Thrombin then activates other components of the coagulation cascade, including FV and FVIII (which activates FXI, which, in turn, activates FIX), and activates and releases FVIII from being bound to vWF.
- FVIIIa is the co-factor of FIXa, and together they form the "tenase" complex, which activates FX; and so the cycle continues. ("Tenase" is a contraction of "ten" and the suffix "-ase" used for enzymes.)

Contact activation pathway (intrinsic)

The contact activation pathway begins with formation of the primary complex on collagen by high-molecular-weight kininogen (HMWK), prekallikrein, and FXII (Hageman factor). Prekallikrein is converted to kallikrein and FXII becomes FXIIa. FXIIa converts FXI into FXIa. Factor XIa activates FIX, which with its co-factor FVIIIa form the tenase complex, which activates FX to FXa. The minor role that the contact activation pathway has in initiating clot formation can be illustrated by the fact that

patients with severe deficiencies of FXII, HMWK, and prekallikrein do not have a bleeding disorder. Instead, contact activation system seems to be more involved in inflammation. Patients without FXII (Hageman factor) suffer from constant infections.

Final common pathway

Thrombin has a large array of functions. Its primary role is the conversion of fibrinogen to fibrin, the building block of a hemostatic plug. In addition, it activates Factors VIII and V and their inhibitor protein C (in the presence of thrombomodulin), and it activates Factor XIII, which forms covalent bonds that crosslink the fibrin polymers that form from activated monomers.

Following activation by the contact factor or tissue factor pathways, the coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX to form the tenase complex, until it is down-regulated by the anticoagulant pathways.

Cofactors

Various substances are required for the proper functioning of the coagulation cascade:

- Calcium and phospholipid (a platelet membrane constituent) are required for the tenase and prothrombinase complexes to function. Calcium mediates the binding of the complexes via the terminal gamma-carboxy residues on FXa and FIXa to the phospholipid surfaces expressed by platelets, as well as procoagulant microparticles or microvesicles shed from them. Calcium is also required at other points in the coagulation cascade.
- Vitamin K is an essential factor to a hepatic gamma-glutamyl carboxylase that adds a carboxyl group to glutamic acid residues on factors II, VII, IX and X, as well as Protein S, Protein C and Protein Z. In adding the gamma-carboxyl group to glutamate residues on the immature clotting factors Vitamin K is itself oxidized. Another enzyme, *Vitamin K epoxide reductase*, (VKORC) reduces vitamin K back to its active form. Vitamin K epoxide reductase is pharmacologically important as a target for anticoagulant drugs warfarin and related coumarins such as acenocoumarol, phenprocoumon, and dicumarol. These drugs create a deficiency of reduced vitamin K by blocking VKORC, thereby inhibiting maturation of clotting factors. Other deficiencies of vitamin K (e.g., in malabsorption), or disease (hepatocellular carcinoma) impairs the function of the enzyme and leads to the formation of PIVKAs (proteins formed in vitamin K absence); this causes partial or non-gamma carboxylation, and affects the coagulation factors' ability to bind to expressed phospholipid.

Regulators

Five mechanisms keep platelet activation and the coagulation cascade in check. Abnormalities can lead to an increased tendency toward thrombosis:

- Protein C is a major physiological anticoagulant. It is a vitamin K-dependent serine protease enzyme that is activated by thrombin into activated protein C (APC). Protein C is activated in a sequence that starts with Protein C and thrombin binding to a cell surface protein thrombomodulin. Thrombomodulin binds these proteins in such a way that it activates Protein C. The activated form, along with protein S and a phospholipid as cofactors, degrades FVa and FVIIIa. Quantitative or qualitative deficiency of either may lead to thrombophilia (a tendency to develop thrombosis). Impaired action of Protein C (activated Protein C resistance), for example by having the "Leiden" variant of Factor V or high levels of FVIII also may lead to a thrombotic tendency.
- Antithrombin is a serine protease inhibitor (serpin) that degrades the serine proteases: thrombin, FIXa, FXa, FXIa, and FXIIa. It is constantly active, but its adhesion to these factors is increased by the presence of heparan sulfate (a glycosaminoglycan) or the administration of heparins (different heparinoids increase affinity to FXa, thrombin, or both). Quantitative or qualitative deficiency of antithrombin (inborn or acquired, e.g., in proteinuria) leads to thrombophilia.
- Tissue factor pathway inhibitor (TFPI) limits the action of tissue factor (TF). It also inhibits excessive TF-mediated activation of FIX and FX.
- Plasmin is generated by proteolytic cleavage of plasminogen, a plasma protein synthesized in the liver. This cleavage is catalyzed by tissue plasminogen activator (t-PA), which is synthesized and secreted by endothelium. Plasmin proteolytically cleaves fibrin into fibrin degradation products that inhibit excessive fibrin formation.
- Prostacyclin (PGI_2) is released by endothelium and activates platelet G_s protein-linked receptors. This, in turn, activates adenylyl cyclase, which synthesizes cAMP. cAMP inhibits platelet activation by decreasing cytosolic levels of calcium and, by doing so, inhibits the release of granules that would lead to activation of additional platelets and the coagulation cascade.

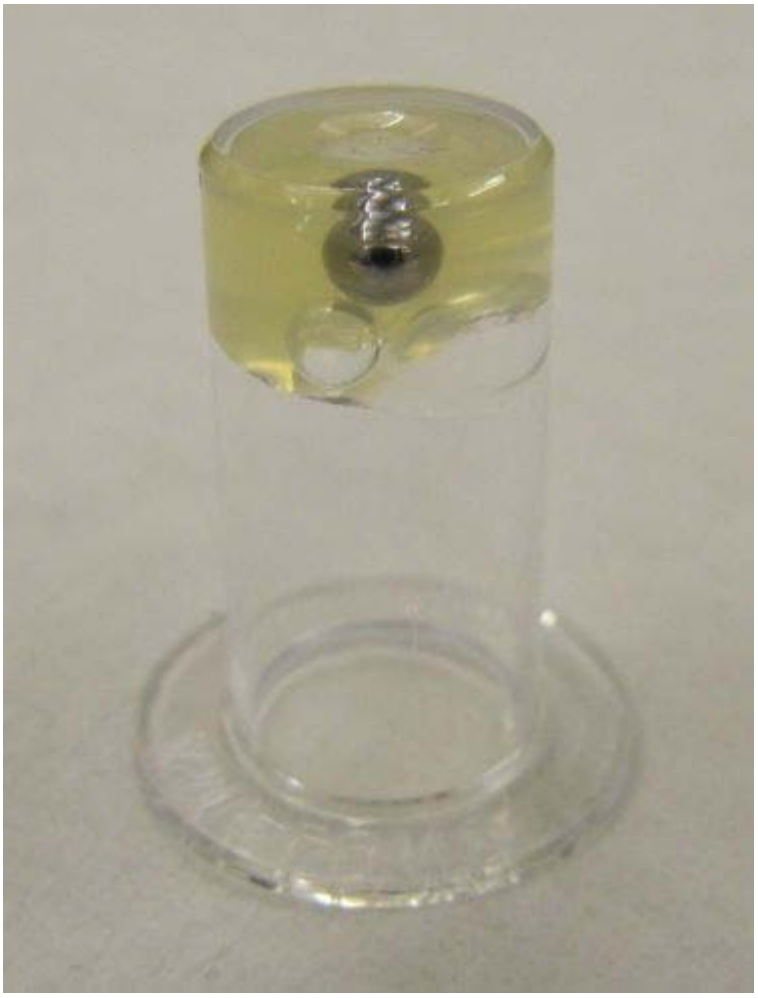
Fibrinolysis

Eventually, blood clots are reorganised and resorbed by a process termed *fibrinolysis*. The main enzyme responsible for this process (plasmin) is regulated by various activators and inhibitors.

Role in immune system

The coagulation system overlaps with the immune system. Coagulation can physically trap invading microbes in blood clots. Also, some products of the coagulation system can contribute to the innate immune system by their ability to increase vascular permeability and act as chemotactic agents for phagocytic cells. In addition, some of the products of the coagulation system are directly antimicrobial. For example, beta-lysine, a protein produced by platelets during coagulation, can cause lysis of many Gram-positive bacteria by acting as a cationic detergent. Many acute-phase proteins of inflammation are involved in the coagulation system. In addition, pathogenic bacteria may secrete agents that alter the coagulation system, e.g. coagulase and streptokinase.

Testing of coagulation



Blood plasma after the addition of Tissue Factor forms a gel-like structure (Test for prothrombin time).

Numerous tests are used to assess the function of the coagulation system:

- Common: aPTT, PT (also used to determine INR), fibrinogen testing (often by the Clauss method), platelet count, platelet function testing (often by PFA-100).
- Other: TCT, bleeding time, mixing test (whether an abnormality corrects if the patient's plasma is mixed with normal plasma), coagulation factor assays, antiphosholipid antibodies, D-dimer, genetic tests (e.g. factor V Leiden, prothrombin mutation G20210A), dilute Russell's viper venom time (dRVVT), miscellaneous platelet function tests, thromboelastography (TEG or Sonoclot), euglobulin lysis time (ELT).

The contact activation (intrinsic) pathway is initiated by activation of the "contact factors" of plasma, and can be measured by the activated partial thromboplastin time (aPTT) test.

The tissue factor (extrinsic) pathway is initiated by release of tissue factor (a specific cellular lipoprotein), and can be measured by the prothrombin time (PT) test. PT results are often reported as ratio (INR value) to monitor dosing of oral anticoagulants such as warfarin.

The quantitative and qualitative screening of fibrinogen is measured by the thrombin clotting time (TCT). Measurement of the exact amount of fibrinogen present in the blood is generally done using the Clauss method for fibrinogen testing. Many analysers are capable of measuring a "derived fibrinogen" level from the graph of the Prothrombin time clot.

If a coagulation factor is part of the contact activation or tissue factor pathway, a deficiency of that factor will affect only one of the tests: Thus hemophilia A, a deficiency of factor VIII, which is part of the contact activation pathway, results in an abnormally prolonged aPTT test but a normal PT test. The exceptions are prothrombin, fibrinogen, and some variants of FX that can be detected only by either aPTT or PT. If an abnormal PT or aPTT is present, additional testing will occur to determine which (if any) factor is present as aberrant concentrations.

Deficiencies of fibrinogen (quantitative or qualitative) will affect all screening tests.

Condition	Prothrombin time	Partial thromboplastin time	Bleeding time	Platelet count
Vitamin K deficiency or warfarin	prolonged	prolonged	unaffected	unaffected
Disseminated intravascular coagulation	prolonged	prolonged	prolonged	decreased
Von Willebrand disease	unaffected	prolonged	prolonged	unaffected
Haemophilia	unaffected	prolonged	unaffected	unaffected
Aspirin	unaffected	unaffected	prolonged	unaffected
Thrombocytopenia	unaffected	unaffected	prolonged	decreased
Early Liver failure	prolonged	unaffected	unaffected	unaffected
End-stage Liver failure	prolonged	prolonged	prolonged	decreased
Uremia	unaffected	unaffected	prolonged	unaffected
Congenital afibrinogenemia	prolonged	prolonged	prolonged	unaffected
Factor V deficiency	prolonged	prolonged	unaffected	unaffected
Factor X deficiency as seen in amyloid purpura	prolonged	prolonged	unaffected	unaffected
Glanzmann's thrombasthenia	unaffected	unaffected	prolonged	unaffected
Bernard-Soulier	unaffected	unaffected	prolonged	decreased

syndrome

Role in disease

Problems with coagulation may dispose to hemorrhage, thrombosis, and occasionally both, depending on the nature of the pathology.

Platelet disorders

Platelet conditions may be inborn or acquired. Some inborn platelet pathologies are Glanzmann's thrombasthenia, Bernard-Soulier syndrome (abnormal glycoprotein Ib-IX-V complex), gray platelet syndrome (deficient alpha granules), and delta storage pool deficiency (deficient dense granules). Most are rare conditions. Most inborn platelet pathologies predispose to hemorrhage. Von Willebrand disease is due to deficiency or abnormal function of von Willebrand factor, and leads to a similar bleeding pattern; its milder forms are relatively common.

Decreased platelet numbers may be due to various causes, including insufficient production (e.g., in myelodysplastic syndrome or other bone marrow disorders), destruction by the immune system (immune thrombocytopenic purpura/ITP), and consumption due to various causes (thrombotic thrombocytopenic purpura/TTP, hemolytic-uremic syndrome/HUS, paroxysmal nocturnal hemoglobinuria/PNH, disseminated intravascular coagulation/DIC, heparin-induced thrombocytopenia/HIT). Most consumptive conditions lead to platelet activation, and some are associated with thrombosis.

Disease and clinical significance of thrombosis

The best-known coagulation factor disorders are the hemophilias. The three main forms are hemophilia A (factor VIII deficiency), hemophilia B (factor IX deficiency or "Christmas disease") and hemophilia C (factor XI deficiency, mild bleeding tendency). Hemophilia A and B are X-linked recessive disorders, whereas Hemophilia C is much more rare autosomal recessive disorder most commonly seen in Ashkenazi Jews.

Von Willebrand disease (which behaves more like a platelet disorder except in severe cases), is the most common hereditary bleeding disorder and is characterized as being inherited autosomal recessive or dominant. In this disease, there is a defect in von Willebrand factor (vWF), which mediates the binding of glycoprotein Ib (GPIb) to collagen. This binding helps mediate the activation of platelets and formation of primary hemostasis.

Bernard-Soulier syndrome is a defect or deficiency in GPIb. GPIb, the receptor for vWF, can be defective and lead to lack of primary clot formation (primary hemostasis) and increased bleeding tendency. This is an autosomal recessive inherited disorder.

Thrombasthenia of Glanzman and Naegeli (Glanzmann thrombasthenia) is extremely rare. It is characterized by a defect in GPIIb/IIIa fibrinogen receptor complex. When GPIIb/IIIa receptor is dysfunctional, fibrinogen cannot cross-link platelets, which inhibits primary hemostasis. This is an autosomal recessive inherited disorder.

In liver failure (acute and chronic forms), there is insufficient production of coagulation factors by the liver; this may increase bleeding risk.

Deficiency of Vitamin K may also contribute to bleeding disorders because clotting factor maturation depends on Vitamin K.

Thrombosis is the pathological development of blood clots. These clots may break free and become mobile, forming an embolus or grow to such a size that occludes the vessel in which it developed. An embolism is said to occur when the thrombus (blood clot) becomes a mobile embolus and migrates to another part of the body, interfering with blood circulation and hence impairing organ function downstream of the occlusion. This causes ischemia and often leads to ischemic necrosis of tissue. Most cases of thrombosis are due to acquired extrinsic problems (surgery, cancer, immobility, obesity, economy class syndrome), but a small proportion of people harbor predisposing conditions known collectively as thrombophilia (e.g., antiphospholipid syndrome, factor V Leiden, and various other rarer genetic disorders).

Mutations in factor XII have been associated with an asymptomatic prolongation in the clotting time and possibly a tendency toward thrombophlebitis. Other mutations have been linked with a rare form of hereditary angioedema (type III).

Pharmacology

Procoagulants

The use of adsorbent chemicals, such as zeolites, and other hemostatic agents are also used for use in sealing severe injuries quickly (such as in traumatic bleeding secondary to gunshot wounds). Thrombin and fibrin glue are used surgically to treat bleeding and to thrombose aneurysms.

Desmopressin is used to improve platelet function by activating arginine vasopressin receptor 1A.

Coagulation factor concentrates are used to treat hemophilia, to reverse the effects of anticoagulants, and to treat bleeding in patients with impaired coagulation factor synthesis or increased consumption. Prothrombin complex concentrate, cryoprecipitate and fresh frozen plasma are commonly-used coagulation factor products. Recombinant activated human factor VII is increasingly popular in the treatment of major bleeding.

Tranexamic acid and aminocaproic acid inhibit fibrinolysis, and lead to a *de facto* reduced bleeding rate. Before its withdrawal, aprotinin was used in some forms of major surgery to decrease bleeding risk and need for blood products.

Anticoagulants

Anticoagulants and anti-platelet agents are amongst the most commonly used medications. Anti-platelet agents include aspirin, clopidogrel, dipyridamole and ticlopidine; the parenteral glycoprotein IIb/IIIa inhibitors are used during angioplasty. Of the anticoagulants, warfarin (and related coumarins) and heparin are the most commonly used. Warfarin affects the vitamin K-dependent clotting factors (II, VII, IX,X) , whereas heparin and related compounds increase the action of antithrombin on thrombin and factor Xa. A newer class of drugs, the direct thrombin inhibitors, is under development; some members are already in clinical use (such as lepirudin). Also under development are other small molecular compounds that interfere directly with the enzymatic action of particular coagulation factors (e.g., rivaroxaban, dabigatran, apixaban).

Coagulation factors

Coagulation factors and related substances

Number and/or name	Function
I (fibrinogen)	Forms clot (fibrin)
II (prothrombin)	Its active form (IIa) activates I, V, VII, VIII, XI, XIII, protein C, platelets
Tissue factor	Co-factor of VIIa (formerly known as factor III)
Calcium	Required for coagulation factors to bind to phospholipid (formerly known as factor IV)
V (proaccelerin, labile factor)	Co-factor of X with which it forms the prothrombinase complex
VI	*Unassigned* – old name of Factor Va
VII (stable factor, proconvertin)	Activates IX, X
VIII (Antihemophilic factor A)	Co-factor of IX with which it forms the tenase complex
IX (Antihemophilic factor B or Christmas factor)	Activates X: forms tenase complex with factor VIII
X (Stuart-Prower factor)	Activates II: forms prothrombinase complex with factor V
XI (plasma thromboplastin antecedent)	Activates IX
XII (Hageman factor)	Activates factor XI, VII and prekallikrein
XIII (fibrin-stabilizing factor)	Crosslinks fibrin
von Willebrand factor	Binds to VIII, mediates platelet adhesion
prekallikrein (Fletcher factor)	Activates XII and prekallikrein; cleaves HMWK

high-molecular-weight kininogen (HMWK) (Fitzgerald factor)	Supports reciprocal activation of XII, XI, and prekallikrein
fibronectin	Mediates cell adhesion
antithrombin III	Inhibits IIa, Xa, and other proteases;
heparin cofactor II	Inhibits IIa, cofactor for heparin and dermatan sulfate ("minor antithrombin")
protein C	Inactivates Va and VIIIa
protein S	Cofactor for activated protein C (APC, inactive when bound to C4b-binding protein)
protein Z	Mediates thrombin adhesion to phospholipids and stimulates degradation of factor X by ZPI
Protein Z-related protease inhibitor (ZPI)	Degrades factors X (in presence of protein Z) and XI (independently)
plasminogen	Converts to plasmin, lyses fibrin and other proteins
alpha 2-antiplasmin	Inhibits plasmin
tissue plasminogen activator (tPA)	Activates plasminogen
urokinase	Activates plasminogen
plasminogen activator inhibitor-1 (PAI1)	Inactivates tPA & urokinase (endothelial PAI)
plasminogen activator inhibitor-2 (PAI2)	Inactivates tPA & urokinase (placental PAI)
cancer procoagulant	Pathological factor X activator linked to thrombosis in cancer

History

Initial discoveries

Theories on the coagulation of blood have existed since antiquity. Physiologist Johannes Müller (1801-1858) described fibrin, the substance of a thrombus. Its soluble precursor, fibrinogen, was thus named by Rudolf Virchow (1821-1902), and isolated chemically by Prosper Sylvain Denis (1799-1863). Alexander Schmidt suggested that the conversion from fibrinogen to fibrin is the result of an enzymatic process, and labeled the hypothetical enzyme "thrombin" and its precursor "prothrombin". Arthus discovered in 1890 that calcium was essential in coagulation. Platelets were identified in 1865, and their function was elucidated by Giulio Bizzozero in 1882.

The theory that thrombin is generated by the presence of tissue factor was consolidated by Paul Morawitz in 1905. At this stage, it was known that *thrombokinase/thromboplastin* (factor III) is released by damaged tissues, reacting with *prothrombin* (II), which, together with calcium (IV), forms *thrombin*, which converts fibrinogen into *fibrin* (I).

Coagulation factors

The remainder of the biochemical factors in the process of coagulation were largely discovered in the 20th century.

A first clue as to the actual complexity of the system of coagulation was the discovery of *proaccelerin* (initially and later called Factor V) by Paul Owren (1905-1990) in 1947. He also postulated its function to be the generation of accelerin (Factor VI), which later turned out to be the activated form of V (or Va); hence, VI is not now in active use.

Factor VII (also known as *serum prothrombin conversion accelerator* or *proconvertin*, precipitated by barium sulfate) was discovered in a young female patient in 1949 and 1951 by different groups.

Factor VIII turned out to be deficient in the clinically recognised but etiologically elusive hemophilia A; it was identified in the 1950s and is alternatively called *antihemophilic globulin* due to its capability to correct hemophilia A.

Factor IX was discovered in 1952 in a young patient with hemophilia B named Stephen Christmas (1947-1993). His deficiency was described by Dr. Rosemary Biggs and Professor R.G. MacFarlane in Oxford, UK. The factor is, hence, called Christmas Factor. Christmas lived in Canada, and campaigned for blood transfusion safety until succumbing to transfusion-related AIDS at age 46. An alternative name for the factor is *plasma thromboplastin component*, given by an independent group in California.

Hageman factor, now known as factor XII, was identified in 1955 in an asymptomatic patient with a prolonged bleeding time named of John Hageman. Factor X, or Stuart-Prower factor, followed, in 1956. This protein was identified in a Ms. Audrey Prower of London, who had a lifelong bleeding tendency. In 1957, an American group identified the same factor in a Mr. Rufus Stuart. Factors XI and XIII were identified in 1953 and 1961, respectively.

The view that the coagulation process is a "cascade" or "waterfall" was enunciated almost simultaneously by MacFarlane in the UK and by Davie and Ratnoff in the USA, respectively.

Nomenclature

The usage of Roman numerals rather than eponyms or systematic names was agreed upon during annual conferences (starting in 1955) of hemostasis experts. In 1962, consensus was achieved on the numbering of factors I-XII. This committee evolved into the present-day International Committee on Thrombosis and Hemostasis (ICTH). Assignment of numerals ceased in 1963 after the naming of Factor XIII. The names Fletcher Factor and Fitzgerald Factor were given to further coagulation-related proteins, namely prekallikrein and high-molecular-weight kininogen, respectively.

Factors III and VI are unassigned, as thromboplastin was never identified, and actually turned out to consist of ten further factors, and accelerin was found to be activated Factor V.

Other species

All mammals have an extremely closely related blood coagulation process, using a combined cellular and serine protease process. In fact, it is possible for any mammalian coagulation factor to "cleave" its equivalent target in any other mammal. The only nonmammalian animal known to use serine proteases for blood coagulation is the horseshoe crab.