Myoglobin

Myoglobin and hemoglobin are heme proteins whose physiological importance is principally related to their ability to bind molecular oxygen. Myoglobin is a monomeric heme protein found mainly in muscle tissue where it serves as an intracellular storage site for oxygen. During periods of oxygen deprivation oxymyoglobin releases its bound oxygen which is then used for metabolic purposes.

The tertiary structure of myoglobin is that of a typical water soluble globular protein. Its secondary structure is unusual in that it contains a very high proportion (75%) of α-helical secondary structure. A myoglobin polypeptide is comprised of 8 separate right handed α-helices, designated A through H, that are connected by short non helical regions. Amino acid R-groups packed into the interior of the molecule are predominantly hydrophobic in character while those exposed on the surface of the molecule are generally hydrophilic, thus making the molecule relatively water soluble.

Structure of Myoglobin with Heme

Each myoglobin molecule contains one heme prosthetic group inserted into a hydrophobic cleft in the protein. Each heme residue contains one central coordinately bound iron atom that is normally in the Fe^{2+}, or ferrous, oxidation state. The oxygen carried by heme proteins is bound directly to the ferrous iron atom of the heme prosthetic group. Oxidation of the iron to the Fe^{3+}, ferric, oxidation state renders the molecule incapable of normal oxygen binding. Hydrophobic interactions between the tetrapyrrole ring and hydrophobic amino acid R groups on the interior of the cleft in the protein strongly stabilize the heme protein conjugate. In addition a nitrogen atom from a histidine R group located above the plane of the heme ring is coordinated with the iron atom further stabilizing the interaction between the heme and the protein. In oxymyoglobin the remaining bonding site on the iron atom (the 6th coordinate position) is occupied by the oxygen, whose binding is stabilized by a second histidine residue.

Carbon monoxide also binds coordinately to heme iron atoms in a manner similar to that of oxygen, but the binding of carbon monoxide to heme is much stronger than that of oxygen. The preferential binding of carbon...
monoxide to heme iron is largely responsible for the asphyxiation that results from carbon monoxide poisoning.

Hemoglobin

Adult hemoglobin is a \([\alpha(2)\beta(2)]\) tetrameric hemeprotein found in erythrocytes where it is responsible for binding oxygen in the lung and transporting the bound oxygen throughout the body where it is used in aerobic metabolic pathways.

![Structure of Hemoglobin](image)

### Hemoglobin Genes

Each subunit of a hemoglobin tetramer has a heme prosthetic group identical to that described for myoglobin. The common peptide subunits are designated \(\alpha\), \(\beta\), \(\gamma\) and \(\delta\) which are arranged into the most commonly occurring functional hemoglobins.

Although the secondary and tertiary structure of various hemoglobin subunits are similar, reflecting extensive homology in amino acid composition, the variations in amino acid composition that do exist impart marked differences in hemoglobin's oxygen carrying properties. In addition, the quaternary structure of hemoglobin leads to physiologically important allosteric interactions between the subunits, a property lacking in monomeric myoglobin which is otherwise very similar to the \(\alpha\)-subunit of hemoglobin.

Comparison of the oxygen binding properties of myoglobin and hemoglobin illustrate the allosteric properties of hemoglobin that results from its quaternary structure and differentiate hemoglobin's oxygen binding properties from that of myoglobin. The curve of oxygen binding to hemoglobin is sigmoidal typical of allosteric proteins in which the substrate, in this case oxygen, is a positive homotropic effector. When oxygen binds to the first subunit of deoxyhemoglobin it increases the affinity of the remaining subunits for oxygen. As additional oxygen is bound to the second and third subunits oxygen binding is further, incrementally, strengthened, so that at the oxygen tension in lung alveoli, hemoglobin is fully saturated with oxygen. As oxyhemoglobin circulates to deoxygenated tissue, oxygen is incrementally unloaded and the affinity of hemoglobin for oxygen is reduced. Thus at the lowest oxygen tensions found in very active tissues the binding affinity of hemoglobin for oxygen is very low allowing maximal delivery of oxygen to the tissue. In contrast the oxygen binding curve for myoglobin is hyperbolic in character indicating the absence of allosteric interactions in this process.

The allosteric oxygen binding properties of hemoglobin arise directly from the interaction of oxygen with the iron atom of the heme prosthetic groups and the resultant effects of these interactions on the quaternary structure of the protein. When oxygen binds to an iron atom of deoxyhemoglobin it pulls the iron atom into the plane of the heme. Since the iron is also bound to histidine F8, this residue is also pulled toward the plane of the heme ring. The conformational change at histidine F8 is transmitted throughout the peptide backbone resulting in a significant change in tertiary structure of the entire subunit. Conformational changes at the subunit surface lead to a new set of binding interactions between adjacent subunits. The latter changes include disruption of salt bridges and formation of new hydrogen bonds and new hydrophobic interactions, all of which contribute to the new quaternary structure.
The latter changes in subunit interaction are transmitted, from the surface, to the heme binding pocket of a second deoxy subunit and result in easier access of oxygen to the iron atom of the second heme and thus a greater affinity of the hemoglobin molecule for a second oxygen molecule. The tertiary configuration of low affinity, deoxygenated hemoglobin (Hb) is known as the taut (T) state. Conversely, the quaternary structure of the fully oxygenated high affinity form of hemoglobin (HbO₂) is known as the relaxed (R) state.

In the context of the affinity of hemoglobin for oxygen there are four primary regulators, each of which has a negative impact. These are CO₂, hydrogen ion (H⁺), chloride ion (Cl⁻), and 2,3-bisphosphoglycerate (2,3BPG, or also just BPG). Some older texts abbreviate 2,3BPG as DPB. Although they can influence O₂ binding independent of each other, CO₂, H⁺ and Cl⁻ primarily function as a consequence of each other on the affinity of hemoglobin for O₂. We shall consider the transport of O₂ from the lungs to the tissues first.

In the high O₂ environment (high pO₂) of the lungs there is sufficient O₂ to overcome the inhibitory nature of the T state. During the O₂ binding-induced alteration from the T form to the R form several amino acid side groups on the surface of hemoglobin subunits will dissociate protons as depicted in the equation below. This proton dissociation plays an important role in the expiration of the CO₂ that arrives from the tissues (see below). However, because of the high pO₂ the pH of the blood in the lungs (≈7.4 – 7.5) is not sufficiently low enough to exert a negative influence on hemoglobin binding O₂. When the oxyhemoglobin reaches the tissues the pO₂ is sufficiently low, as well as the pH (≈7.2), that the T state is favored and the O₂ released.

\[4O_2 + Hb \rightleftharpoons nH^+ + Hb(O_2)_4\]

If we now consider what happens in the tissues, it is possible to see how CO₂, H⁺, and Cl⁻ exert their negative effects on hemoglobin binding O₂. Metabolizing cells produce CO₂ which diffuses into the blood and enters the circulating red blood cells (RBCs). Within RBCs the CO₂ is rapidly converted to carbonic acid through the action of carbonic anhydrase as shown in the equation below:

\[CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightarrow H^+ + HCO_3^-\]

The bicarbonate ion produced in this dissociation reaction diffuses out of the RBC and is carried in the blood to the lungs. This effective CO₂ transport process is referred to as isohydric transport. Approximately 80% of the CO₂ produced in metabolizing cells is transported to the lungs in this way. A small percentage of CO₂ is transported in the blood as a dissolved gas. In the tissues, the H⁺ dissociated from carbonic acid is buffered by hemoglobin which exerts a negative influence on O₂ binding forcing release to the tissues. As indicated above, within the lungs the high pO₂ allows for effective O₂ binding by hemoglobin leading to the T to R state transition and the release of protons. The protons combine with the bicarbonate that arrived from the tissues forming carbonic acid which then enters the RBCs. Through a reversal of the carbonic anhydrase reaction, CO₂ and H₂O are produced. The CO₂ diffuses out of the blood, into the lung alveoli and is released on expiration.

In addition to isohydric transport, as much as 15% of CO₂ is transported to the lungs bound to N-terminal amino groups of the T form of hemoglobin. This reaction, depicted below, forms what is called carboxyhemoglobin. As indicated this reaction also produces H⁺, thereby lowering the pH in tissues where the CO₂ concentration is high. The formation of H⁺ leads to release of the bound O₂ to the surrounding tissues. Within the lungs, the high O₂ content results in O₂ binding to hemoglobin with the concomitant release of H⁺. The released protons then promote the dissociation of the carbamino to form CO₂ which is then released with expiration.

\[CO_2 + Hb-NH_2 \rightleftharpoons H^+ + Hb-NH-COO^-\]

As the above discussion demonstrates, the conformation of hemoglobin and its oxygen binding are sensitive to hydrogen ion concentration. These effects of hydrogen ion concentration are responsible for the well known Bohr effect in which increases in hydrogen ion concentration decrease the amount of oxygen bound by
hemoglobin at any oxygen concentration (partial pressure). Coupled to the diffusion of bicarbonate out of RBCs in the tissues there must be ion movement into the RBCs to maintain electrical neutrality. This is the role of Cl\(^-\) and is referred to as the **chloride shift**. In this way, Cl\(^-\) plays an important role in bicarbonate production and diffusion and thus also negatively influences \(O_2\) binding to hemoglobin.

**Role of 2,3-bisphosphoglycerate (2,3-BPG)**

The compound 2,3-bisphosphoglycerate (2,3-BPG), derived from the glycolytic intermediate 1,3-bisphosphoglycerate, is a potent allosteric effector on the oxygen binding properties of hemoglobin. The pathway to 2,3BPG synthesis is diagrammed in the figure below.
The pathway for 2,3-bisphosphoglycerate (2,3-BPG) synthesis within erythrocytes. Synthesis of 2,3-BPG represents a major reaction pathway for the consumption of glucose in erythrocytes. The synthesis of 2,3-BPG in erythrocytes is critical for controlling hemoglobin affinity for oxygen. Note that when glucose is oxidized by this pathway the erythrocyte loses the ability to gain 2 moles of ATP from glycolytic oxidation of 1,3-BPG to 3-phosphoglycerate via the phosphoglycerate kinase reaction.

In the deoxygenated T conformer, a cavity capable of binding 2,3-BPG forms in the center of the molecule. 2,3-BPG can occupy this cavity stabilizing the T state. Conversely, when 2,3-BPG is not available, or not bound in the central cavity, Hb can be converted to HbO₂ more readily. Thus, like increased hydrogen ion concentration, increased 2,3-BPG concentration favors conversion of R form Hb to T form Hb and decreases the amount of oxygen bound by Hb at any oxygen concentration. Hemoglobin molecules differing in subunit composition are known to have different 2,3-BPG binding properties with correspondingly different allosteric responses to 2,3-BPG. For example, HbF (the fetal form of hemoglobin) binds 2,3-BPG much less avidly than HbA (the adult form of hemoglobin) with the result that HbF in fetuses of pregnant women binds oxygen with greater affinity than the mothers HbA, thus giving the fetus preferential access to oxygen carried by the mothers circulatory system.

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The Hemoglobin Genes

The α- and β-globin proteins contained in functional hemoglobin tetramers are derived from gene clusters. The α-globin genes are on chromosome 16 and the β-globin genes are on chromosome 11. Both gene clusters contain not only the major adult genes, α and β, but other expressed sequences that are utilized at different stages of development. The orientation of the genes in both clusters is in the same 5' to 3' direction with the earliest expressed genes at the 5' end of both clusters. In addition to functional genes, both clusters contain non-functional pseudogenes.

Hemoglobin synthesis begins in the first few weeks of embryonic development within the yolk sac. The major hemoglobin at this stage of development is a tetramer composed of 2 zeta (ζ) chains encoded within the α cluster and 2 epsilon (ε) chains from the β cluster. By 6-8 weeks of gestation the expression of this version of hemoglobin declines dramatically coinciding with the change in hemoglobin synthesis from the yolk sac to the liver. Expression from the α cluster consists of identical proteins from the α1 and α2 genes. Expression from these genes in the α cluster remains on throughout life.

Within the β-globin cluster there is an additional set of genes, the fetal β-globin genes identified as the gamma (γ) genes. The 2 fetal genes called Gγ and Aγ, the derivation of which stems from the single amino acid difference between the 2 fetal genes: glycine in Gγ and alanine in Aγ at position 136. These fetal γ genes are expressed as the embryonic genes are turned off.

Shortly before birth there is a smooth switch from fetal γ-globin gene expression to adult β-globin gene expression. The switch from fetal γ- to adult β-globin does not directly coincide with the switch from hepatic synthesis to bone marrow synthesis since at birth it can be shown that both γ and β synthesis is occurring in the
Given the pattern of globin gene activity throughout fetal development and in the adult the composition of the hemoglobin tetramers is of course distinct. Fetal hemoglobin is identified as HbF and includes both $\alpha_2\gamma_2$ and $\alpha_2\delta_2$. Fetal hemoglobin has a slightly higher affinity for oxygen than does adult hemoglobin. This allows the fetus to extract oxygen more efficiently from the maternal circulation. In adults the major hemoglobin is identified as HbA (more commonly HbA$\text{A}_1$) and is a tetramer of 2 $\alpha$ and 2 $\beta$ chains as indicated earlier. A minor adult hemoglobin, identified as HbA$\text{A}_2$, is a tetramer of 2 $\alpha$ chains and 2 $\delta$ chains. The $\delta$ gene is expressed with a timing similar to the $\beta$ gene but because the promoter has acquired a number of mutations its' efficiency of transcription is reduced.

The overall hemoglobin composition in a normal adult is approximately 97.5% HbA$\text{A}_1$, 2% HbA$\text{A}_2$ and 0.5% HbF.

### Hemoglobinopathies

A large number of mutations have been described in the globin genes. These mutations can be divided into two distinct types: those that cause qualitative abnormalities (e.g. sickle cell anemia) and those that cause quantitative abnormalities (the thalassemias). Taken together these disorders are referred to as the hemoglobinopathies. A third group of hemoglobin disorders include those diseases in which there is a persistence of fetal hemoglobin expression. These latter diseases are known collectively as hereditary persistence of fetal hemoglobin (HPFH).

Of the mutations leading to qualitative alterations in hemoglobin, the missense mutation in the $\beta$-globin gene that causes sickle cell anemia is the most common. The mutation causing sickle cell anemia is a single nucleotide substitution (A to T) in the codon for amino acid 6. The change converts a glutamic acid codon (GAG) to a valine codon (GTG). The form of hemoglobin in persons with sickle cell anemia is referred to as HbS.

The underlying problem in sickle cell anemia is that the valine for glutamic acid substitution results in hemoglobin tetramers that aggregate into arrays upon deoxygenation in the tissues. This aggregation leads to deformation of the red blood cell making it relatively inflexible and unable to traverse the capillary beds. Repeated cycles of oxygenation and deoxygenation lead to irreversible sickling. The end result is clogging of the fine capillaries. Because bones are particularly affected by the reduced blood flow, frequent and severe bone pain results. This is the typical symptom during a sickle cell "crisis". Long term the recurrent clogging of the capillary beds leads to damage to the internal organs, in particular the kidneys, heart and lungs. The continual destruction of the sickled red blood cells leads to chronic anemia and episodes of hyperbilirubinemia.

An additional relatively common mutation at codon 6 is the conversion to a lysine codon (AAG) which results in the generation of HbC.

Electrophoresis of hemoglobin proteins from individuals suspected of having sickle cell anemia (or several other types of hemoglobin disorders) is an effective diagnostic tool because the variant hemoglobins have different charges. An example of this technique is shown in the Figure below.

![Pattern of hemoglobin electrophoresis from several different individuals](http://themedicalbiochemistrypage.org/hemoglobin.../2010/03/24/1542)

Another effective tool to identify these genetic abnormalities and to identify hemoglobin S disease as well as for prenatal diagnosis is to use a combination of the RFLP and the PCR. An example of the use of these tools can be seen in the Molecular Tools of Medicine page.

In addition to the missence mutations that lead to HbS and HbC, a number of frameshift mutations leading to qualitative abnormalities in hemoglobin have been discovered. A 2-nucleotide insertion between codons 144 and
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145 in the β-globin gene results in the generation of hemoglobin Cranston. The insertion, which is near the C-terminus of the β-globin protein, results in the normal stop codon being out of frame and synthesis proceeding into the 3'-untranslated region to a fortuitous stop codon. The result is a β-globin protein of 157 amino acids.

In the hemoglobin Constant Spring variant, a mutation in the α-globin gene converts the stop codon (UAA) to a glutamine codon (CAA) so that the protein ends up being 31 amino acids longer than normal. The resultant α-globin protein in hemoglobin Constant Spring is not only qualitatively altered but because it is unstable it is a quantitative abnormality as well.

Because the globin gene loci contain clusters of similar genes there is the potential for unequal cross-over between the sister chromatids during meiosis. The generation of hemoglobin Gun Hill and Lepore hemoglobins are both the result of unequal cross over events. Hemoglobin Gun Hill is the result of a deletion of 15 nucleotides caused by unequal cross over between codons 91–94 of one β-globin gene and codons 96–98 of the other. Generation of Lepore hemoglobins results from unequal cross over between the δ-globin and β-globin genes. The resultant hybrid δβ gene is called Lepore and the βδ hybrid gene is called anti-Lepore. As indicated earlier, the promoter of the δ-globin gene is inefficient so the consequences of this unequal cross over event are both qualitative and quantitative.

The thalassemias are the result of abnormalities in hemoglobin synthesis and affect both clusters. Deficiencies in β-globin synthesis result in the β-thalassemias and deficiencies in α-globin synthesis result in the α-thalassemias. The term thalassaemia is derived from the Greek thalassa meaning “sea” and was applied to these disorders because of the high frequency of their occurrence in individuals living around the Mediterranean Sea.

In normal individuals an equal amount of both α- and β-globin proteins are made allowing them to combine stoichiometrically to form the correct hemoglobin tetramers. In the α-thalassemias normal amounts of β-globin are made. The β-globin proteins are capable of forming homotetramers (β₄) and these tetramers are called hemoglobin H. (HbH). An excess of HbH in red blood cells leads to the formation of inclusion bodies commonly seen in patients with α-thalassemia. In addition, the HbH tetramers have a markedly reduced oxygen carrying capacity. In β-thalassemia, where the β-globins are deficient, the α-globins are in excess and will form α-globin homotetramers. The α-globin homotetramers are extremely insoluble which leads to premature red cell destruction in the bone marrow and spleen.

With the α-thalassemias the level of α-globin production can range from none to very nearly normal levels. This is due in part to the fact that there are 2 identical α-globin genes on chromosome 16. Thus, the α-thalassemias involve inactivation of 1 to all 4 α-globin genes. If 3 of the 4 α-globin genes are functional, individuals are completely asymptomatic. This situation is identified as the “silent carrier” state or sometimes as α-thalassemia 2. Genotypically this situation is designated αα/– (where the dash indicates a non-functional gene) or α–/αα. If 2 of the 4 genes are inactivated individuals are designated as α-thalassemia trait or as α-thalassemia 1. Genotypically this situation is designated αα/α–. In individuals of African descent with α-thalassemia 1, the disorder usually results from the inactivation of 1 α-globin gene on each chromosome and is designated αα/αα. This means that these individuals are homozygous for the α-thalassemia 2 chromosome. The phenotype of α-thalassemia 1 is relatively benign. The mean red cell volume (designated MCV in clinical tests) is reduced in α-thalassemia 1 but individuals are generally asymptomatic. The clinical situation becomes more severe if only 1 of the 4 α-globin genes is functional. Because of the dramatic reduction in α-globin chain production in this latter situation, a high level of β₄ tetramer is present. clinically this is referred to as hemoglobin H disease. Afflicted individuals have moderate to marked anemia and their MCV is quite low, but the disease is not fatal. The most severe situation results when no α-globin chains are made (genotypically designated αα/αα). This leads to prenatal lethality or early neonatal death. The predominant fetal hemoglobin in afflicted individuals is a tetramer of γ-chains and is referred to as hemoglobin Bart's. This hemoglobin has essentially no oxygen carrying capacity resulting in oxygen starvation in the fetal tissues. Heart failure results as the heart tries to pump the unoxygenated blood to oxygen starved tissues leading to marked edema. This latter situation is called hydrops fetalis.

A large number of mutations have been identified leading to decreased or absent production of β-globin chains resulting in the β-thalassemias. In the most severe situation mutations in both the maternal and paternal β-globin genes leads to loss of normal amounts of β-globin protein. A complete lack of HbA is denoted as β-thalassemia. If one or the other mutations allows production of a small amount of functional β-globin then the disorder is denoted as β-thalassemia.

Both β⁰ and β⁺-thalassemias are referred to as thalassemia major, also called Cooley's anemia after Dr. Thomas Cooley who first described the disorder. Afflicted individuals suffer from severe anemia beginning in the first year of life leading to the need for blood transfusions. As a consequence of the anemia the bone marrow dramatically increases its' effort at blood production. The cortex of the bone becomes thinned leading to pathologic fracturing and distortion of the bones in the face and skull. In addition, there is marked
hepatosplenomegaly as the liver and spleen act as additional sites of blood production. Without intervention these individuals will die within the decade of life. As indicated, β-thalassemia major patient require blood transfusions, however, in the long term these transfusions lead to the accumulation of iron in the organs, particularly the heart, liver and pancreas. Organ failure ensues with death in the teens to early twenties. Iron chelation therapies appear to improve the outlook for β-thalassemia major patients but this requires continuous infusion of the chelating agent.

Individuals heterozygous for β-thalassemia have what is termed thalassemia minor. Afflicted individuals harbor one normal β-globin gene and one that harbors a mutation leading to production of reduced or no β-globin. Individuals that do not make any functional β-globin protein from 1 gene are termed β0 heterozygotes. If β-globin production is reduced at one locus the individuals are termed β+ heterozygotes. Thalassemia minor individuals are generally asymptomatic.

The term thalassemia intermedia is used to designate individuals with significant anemia and who are symptomatic but unlike thalassemia major do not require transfusions. This syndrome results in individuals where both β-globin genes express reduced amounts of protein or where one gene makes none and the other makes a mildly reduced amount. A person who is a compound heterozygote with α-thalassemia and ββ+-thalassemia will also manifest as thalassemia intermedia.

The primary cause of α-thalassemias is deletion, whereas, for β-thalassemias the mutations are more subtle. In β-thalassemias, point mutations in the promoter, mutations in the translational initiation codon, a point mutation in the polyadenylation signal and an array of mutations leading to splicing abnormalities have been characterized.

An interesting and common (up to 30% of persons from Southeast Asia) hemoglobinopathy that has both quantitative and qualitative characteristics is caused by the synthesis of hemoglobin E. Hemoglobin E arises due to a point mutation in codon 26 that changes glutamic acid (GAG) to lysine (AAG). Individuals with this mutation make only around 60% of the normal amount of β-globin protein. The reason for this is that the mutation creates a cryptic splice site such that 40% of the hemoglobin E mRNA is shorter by 16 nucleotides and does not give rise to detectable β-globin protein.

There are some individuals in whom the developmental timing of globin production is altered as a consequence of mutation. Persons with hereditary persistence of fetal hemoglobin, HPFH continue to make HbF as adults. Because the syndrome is benign most individuals do not even know they carry a hemoglobin abnormality. Many HPFH individuals harbor large deletions of the δ- and β-coding region of the cluster. There is no deletion of the fetal globin genes and by an as yet uncharacterized mechanism expression of these genes persists in adulthood.

As discussed above functional hemoglobin is a heterotetramer. Mutations in either the α-globin or the β-globin genes lead to quantitative and qualitative abnormalities in hemoglobin. Therefore, it should not be surprising that complex compound heterozygositles can result in offspring of individuals harboring different mutations.