B3 Myoglobin and hemoglobin

Key Notes

Oxygen-binding proteins

Hemoglobin and myoglobin are the two oxygen-binding proteins present in large multicellular organisms. Hemoglobin transports oxygen in the blood and is located in the erythrocytes; myoglobin stores the oxygen in the muscles.

Myoglobin

Myoglobin, whose three-dimensional structure was solved by X-ray crystallography, is a globular protein made up of a single polypeptide chain of 153 amino acid residues that is folded into eight α-helices. The heme prosthetic group is located within a hydrophobic cleft of the folded polypeptide chain.

Hemoglobin

Adult hemoglobin (HbA) has quaternary structure as it is made up of four polypeptide chains: two α-chains and two β-chains ($\alpha_2\beta_2$), each with a heme prosthetic group. The individual polypeptides of hemoglobin have a three-dimensional structure almost identical to the polypeptide chain of myoglobin.

Binding of oxygen to heme

The heme prosthetic group consists of a protoporphyrin IX ring and a central Fe^{2+} atom, which forms four bonds with the porphyrin ring. In addition, on one side of the porphyrin ring the Fe^{2+} forms a bond with the proximal histidine (His F8); a residue eight amino acids along the F-helix of hemoglobin. The sixth bond from the Fe^{2+} is to a molecule of O_2. Close to where the O_2 binds is the distal histidine (His E7), which prevents carbon monoxide binding most efficiently.

Allostery

Hemoglobin is an allosteric protein. The binding of O_2 is cooperative; the binding of O_2 to one subunit increases the ease of binding of further O_2 molecules to the other subunits. The oxygen dissociation curve for hemoglobin is sigmoidal, whereas that for myoglobin is hyperbolic. Myoglobin has a greater affinity for O_2 than does hemoglobin. Oxyhemoglobin has a different quaternary structure from deoxyhemoglobin. As O_2 binds to the Fe^{2+} it distorts the heme group and moves the proximal histidine. This in turn moves helix F and alters the interactions between the four subunits. H^+, CO_2 and 2,3-bisphosphoglycerate (BPG) are allosteric effectors, promoting the release of O_2 from hemoglobin. BPG binds in the central cavity between the four subunits.

Fetal hemoglobin Hemoglobin F (HbF), which consists of two α-chains and two γ-chains ($\alpha_2\gamma_2$), is present in the fetus. HbF binds BPG less strongly than HbA and thus has a higher affinity for O_2, which promotes the transfer of O_2 from the maternal to the fetal circulation.

Hemoglobinopathies Hemoglobinopathies are diseases caused by abnormal hemoglobins. The best characterized of these is the genetically transmitted, hemolytic disease sickle-cell anemia. This is caused by the nonconservative substitution of a Glu by a Val, resulting in the appearance of a hydrophobic sticky patch on the surface of the protein. This allows long aggregated fibers of hemoglobin molecules to form, which distort the shape of the red blood cells. Heterozygotes carrying only one copy of the sickle-cell gene are more resistant to malaria than those homozygous for the normal gene.

Related topics (B2) Protein structure and function
(D5) Regulation of enzyme activity
(M4) Hemes and chlorophylls

Oxygen-binding proteins

Hemoglobin is one of two **oxygen-binding proteins** found in vertebrates. The functio of hemoglobin is to carry O_2 in the blood from the lungs to the other tissues in the bod in order to supply the cells with the O_2 required by them for the oxidative phosphoryla tion of foodstuffs (Section L2). The hemoglobin is found in the blood within the **eryth rocytes** (red blood cells). These cells essentially act, amongst other things, as a sac fo carrying hemoglobin, since mature erythrocytes lack any internal organelles (nucleu mitochondria, etc.). The other O_2-binding protein is **myoglobin**, which stores the oxyge in the tissues of the body ready for when the cells require it. The highest concentration of myoglobin are found in skeletal and cardiac **muscle**, which require large amounts of O because of their need for large amounts of energy during contraction (Section B5).

Myoglobin

Myoglobin is a relatively small protein of mass 17.8 kDa made up of 153 amino acids in single polypeptide chain. It was the first protein to have its **three-dimensional structur** determined by **X-ray crystallography** (Section B2) by John Kendrew in 1957. Myoglobi is a typical **globular protein** in that it is a highly folded compact structure with most o the hydrophobic amino acid residues buried in the interior and many of the polar resi dues on the surface. X-ray crystallography revealed that the single polypeptide chain o myoglobin consists entirely of **α-helical secondary structure** (Section B2). In fact, ther are eight α-helices (labeled A–H) in myoglobin (Figure 1a). Within a hydrophobic crevic formed by the folding of the polypeptide chain is the **heme prosthetic group** (Figure 1a) This nonpolypeptide unit is noncovalently bound to myoglobin and is essential for th biological activity of the protein (i.e. the binding of O_2).

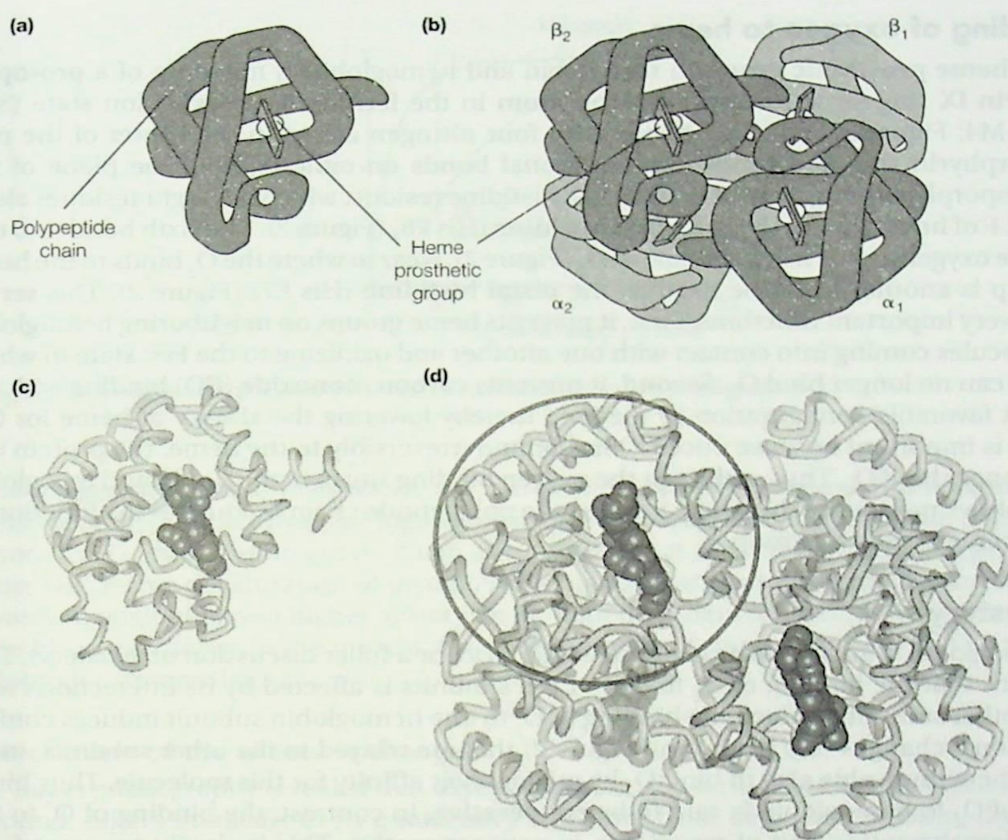

Figure 1. Structure of (a) myoglobin and (b) hemoglobin, showing the α- and β-polypeptide chains. C_α-backbone traces of (c) human myoglobin and (d) human hemoglobin, showing the α-helices, the heme prosthetic group in space-filling representation and how the monomer of myoglobin maps onto the structure of hemoglobin (circle).

Hemoglobin

The three-dimensional structure of hemoglobin was solved using **X-ray crystallography** (Section B2) in 1959 by Max Perutz. This revealed that hemoglobin is made up of four polypeptide chains, each of which has a very similar three-dimensional structure to the single polypeptide chain in myoglobin (Figure 1b) despite the fact that their amino acid sequences differ at 83% of the residues. This highlights a relatively common theme in protein structure: that very different primary sequences can specify very similar three-dimensional structures. The major type of hemoglobin found in adults (HbA) is made up of two different polypeptide chains: the **α-chain** that consists of 141 amino acid residues, and the **β-chain** of 146 residues ($\alpha_2\beta_2$; Figure 1b). Each chain, like that in myoglobin, consists of eight α-helices and each contains a heme prosthetic group (Figure 1b). Therefore, hemoglobin can bind four molecules of O_2. The four polypeptide chains are packed tightly together in a tetrahedral array to form an overall spherically shaped molecule that is held together by multiple noncovalent interactions (Section B2).

Binding of oxygen to heme

The **heme prosthetic group** in myoglobin and hemoglobin is made up of a **protop** **phyrin IX** ring structure with an **iron atom** in the ferrous (Fe^{2+}) oxidation state (S tion M4; Figure 2). This Fe^{2+} bonds with four nitrogen atoms in the center of the p toporphyrin ring and forms two additional bonds on either side of the plane of protoporphyrin ring. One of these is to a histidine residue, which lies eight residues alo helix F of hemoglobin, the **proximal histidine** (His F8) (Figure 2). The sixth bond is to o of the oxygen atoms in a molecule of O_2 (Figure 2). Near to where the O_2 binds to the her group is another histidine residue, the **distal histidine** (His E7) (Figure 2). This serv two very important functions. First, it prevents heme groups on neighboring hemoglob molecules coming into contact with one another and oxidizing to the Fe^{3+} state in whi they can no longer bind O_2. Second, it prevents **carbon monoxide** (CO) binding with th most favorable configuration to the Fe^{2+}, thereby lowering the affinity of heme for C This is important because once CO has bound irreversibly to the heme, the protein ca no longer bind O_2. Thus, although the oxygen binding site in hemoglobin and myoglobi is only a small part of the whole protein, the polypeptide chain modulates the function o the heme prosthetic group.

Allostery

Hemoglobin is an **allosteric protein** (Section D5 for a fuller discussion of allostery). This means that the binding of O_2 to one of the subunits is affected by its interactions with the other subunits. In fact, the binding of O_2 to one hemoglobin subunit induces conformational changes (see below and Figure 2) that are relayed to the other subunits, making them more able also to bind O_2 by raising their affinity for this molecule. Thus binding of O_2 to hemoglobin is said to be **cooperative**. In contrast, the binding of O_2 to the single polypeptide unit of myoglobin is **noncooperative**. This is clearly apparent from

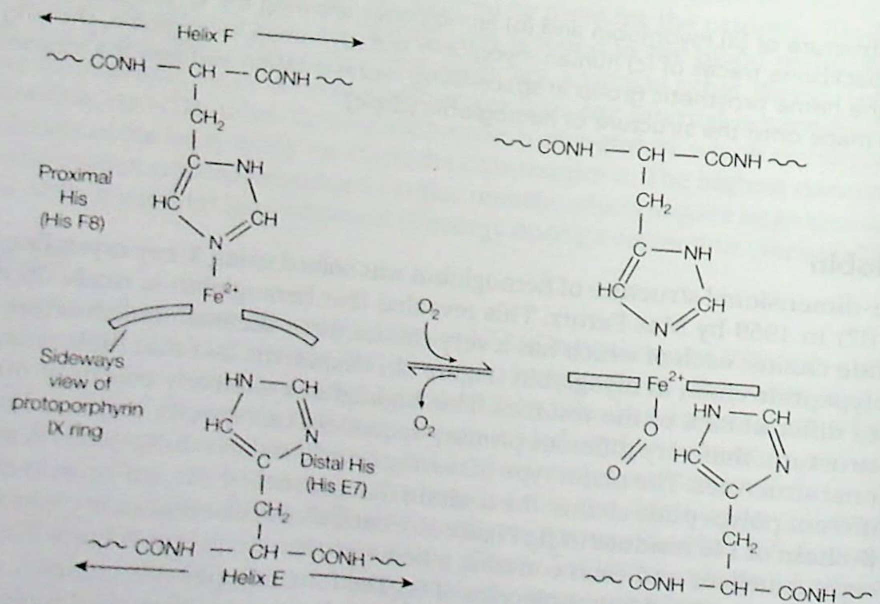

Figure 2. Binding of O_2 to heme. The Fe^{2+} of the protoporphyrin ring is bonded to His F8 but not to His E7, which is located nearby. As the heme Fe^{2+} binds O_2, helix F moves closer to helix E (see the text for details).

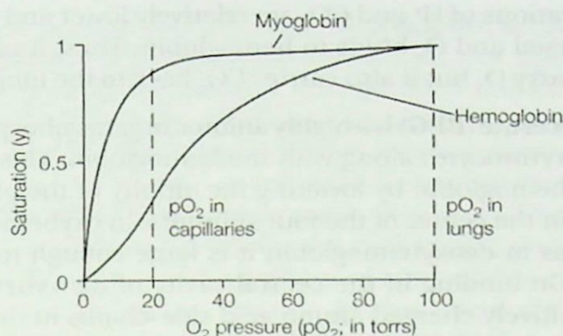

Figure 3. Oxygen dissociation curves for hemoglobin and myoglobin.

the **oxygen dissociation curves** for the two proteins: that for hemoglobin is **sigmoidal**, reflecting this cooperative binding, whereas that for myoglobin is **hyperbolic** (Figure 3). From the O_2 dissociation curve, it can also be seen that for any particular oxygen pressure the degree of saturation of myoglobin is higher than that for hemoglobin. In other words, myoglobin has a higher affinity for O_2 than does hemoglobin. This means that in the blood capillaries in the muscle, for example, hemoglobin will release its O_2 to myoglobin for storage there.

Mechanism of the allosteric change

X-Ray crystallography revealed that **oxyhemoglobin**, the form that has four O_2 molecules bound, differs markedly in its **quaternary structure** from **deoxyhemoglobin**, the form with no O_2 bound. In the absence of bound O_2, the Fe^{2+} lies slightly to one side of the porphyrin ring, which itself is slightly curved (Figure 2). As a molecule of O_2 binds to the heme prosthetic group it pulls the Fe^{2+} into the plane of the porphyrin ring (Figure 2), flattening out the ring in the process. Movement of the Fe^{2+} causes the **proximal histidine** to move also. This, in turn, shifts the position of helix F and regions of the polypeptide chain at either end of the helix. Thus, movement in the center of the subunit is transmitted to the surfaces, where it causes the ionic interactions holding the four subunits together to be broken and to re-form in a different position, thereby altering the quaternary structure, leading to the cooperative binding of O_2 to Hb.

The Bohr effect

The binding of O_2 to hemoglobin is affected by the concentration of **H^+ ions** and **CO_2** in the surrounding tissue; the Bohr effect. In actively metabolizing tissue, such as muscle, the concentrations of these two substances are relatively high. This effectively causes a shift of the O_2 dissociation curve for hemoglobin to the right, promoting the release of O_2. This comes about because there are H^+ binding sites, primarily His146 in the β-chain, which have a higher affinity for binding H^+ in deoxyhemoglobin than in oxyhemoglobin. An increase in CO_2 also causes an increase in H^+ due to the action of the enzyme **carbonic anhydrase**, which catalyzes the reaction:

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

In addition, CO_2 can react with the primary amino groups in the polypeptide chain to form a negatively charged carbamate. Again, this change from a positive to a negative charge favors the conformation of deoxyhemoglobin. On returning in the blood to the

lungs, the concentrations of H^+ and CO_2 are relatively lower and that of O_2 higher, so tha the process is reversed and O_2 binds to hemoglobin. Thus, it can be seen that not onl does hemoglobin carry O_2 but it also carries CO_2 back to the lungs where it is expelled.

2,3-Bisphosphoglycerate (BPG) is a highly anionic organic phosphate molecule (Figure 4 that is present in erythrocytes along with the hemoglobin. This molecule promotes th release of O_2 from hemoglobin by lowering the affinity of the protein for O_2. BPG binds in the small cavity in the center of the four subunits. In oxyhemoglobin this cavity is too small for it, whereas in deoxyhemoglobin it is large enough to accommodate a single molecule of BPG. On binding in the central cavity of deoxyhemoglobin it forms ionic bonds with the positively charged amino acid side-chains in the β-subunits, stabilizing the quaternary structure. H^+, CO_2 and BPG are all **allosteric effectors** (Section D5) as they favor the conformation of deoxyhemoglobin and therefore promote the release of O_2. Because these three molecules act at different sites, their effects are additive.

Fetal hemoglobin

In the fetus there is a different kind of hemoglobin, **hemoglobin F** (HbF), which consists of two α-chains and two γ-chains ($\alpha_2\gamma_2$), in contrast to adult hemoglobin (HbA, $\alpha_2\beta_2$). HbF has a **higher affinity** for O_2 under physiological conditions than HbA, which optimizes the transfer of oxygen from the maternal to the fetal circulation across the placenta. The molecular basis for this difference in O_2 affinity is that HbF binds BPG less strongly than does HbA. Near birth, the synthesis of the γ-chain is switched off, and that of the β-chain (which is present in HbA) is switched on.

Hemoglobinopathies

Comparison of the primary sequences of hemoglobin chains from more than 60 different species reveals that only nine residues in the polypeptide chain are **invariant** (i.e. the same) between all of the species. These nine residues include the **proximal and distal histidines**, which are essential for the correct functioning of the protein. Many of the other residues are replaced from one species to another by residues with similar properties (e.g. the hydrophobic Val is replaced with the hydrophobic Ile, or the polar Ser is replaced with the polar Asn), so-called **conservative substitutions**. In contrast, only a few residues have changed between species to a completely different residue (e.g. a hydrophobic Leu to a positively charged Lys or a negatively charged Glu to a positively charged Arg), so-called **nonconservative substitutions**, since this type of change could have a major effect on the structure and function of the protein.

Several hundred **abnormal hemoglobins** have been characterized, giving rise to the so-called **hemoglobinopathies**. Probably the best characterized hemoglobinopathy is

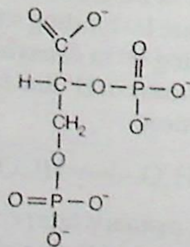

Figure 4. 2,3-Bisphosphoglycerate.

sickle-cell anemia (sickle-cell hemoglobin; HbS). This disease is characterized by the patient's erythrocytes having a characteristic sickle or crescent shape. The molecular basis for this disease is the change of a Glu for a Val at position 6 of the β-chain, resulting in the substitution of a polar residue by a hydrophobic one. This **nonconservative substitution** of Val for Glu gives HbS a **sticky hydrophobic patch** on the outside of each of its β-chains. In the corner between helices E and F of the β-chain of deoxy-HbS is a hydrophobic site that is complementary to the sticky patch (Figure 5). Thus the complementary site on one deoxy-HbS molecule can bind to the sticky patch on another deoxy-HbS molecule, resulting in the formation of **long fibers** of hemoglobin molecules that distort the erythrocyte. Electron microscopy (Section A4) has revealed that the fibers have a diameter of 21.5 nm and consist of a 14-stranded helix. Multiple polar interactions, in addition to the critical interaction between the sticky patches, stabilize the fiber. In oxy-HbS the complementary site is masked, so the formation of the long fibers occurs only when there is a high concentration of the deoxygenated form of HbS.

Sickle-cell anemia is a **genetically transmitted**, hemolytic disease. The sickled cells are more fragile than normal erythrocytes, lysing more easily and having a shorter half-life, which leads to severe anemia. As sickle-cell anemia is genetically transmitted, **homozygotes** have two copies of the abnormal gene whereas **heterozygotes** have one abnormal and one normal copy. Homozygotes often have a reduced life-span as a result of infection, renal failure, cardiac failure or thrombosis, due to the sickled cells becoming trapped in small blood vessels leading to tissue damage. In contrast, heterozygotes are usually not symptomatic as only approximately 1% of their erythrocytes are sickled, compared with approximately 50% in a homozygote. The frequency of the sickle gene is relatively high in certain parts of Africa and correlates with the incidence of **malaria**. The reason for this is that heterozygotes are protected against the most lethal form of malaria, whereas normal homozygotes are more vulnerable to the disease. Inheritance of the abnormal hemoglobin gene can now be monitored by recombinant DNA techniques (Section I1).

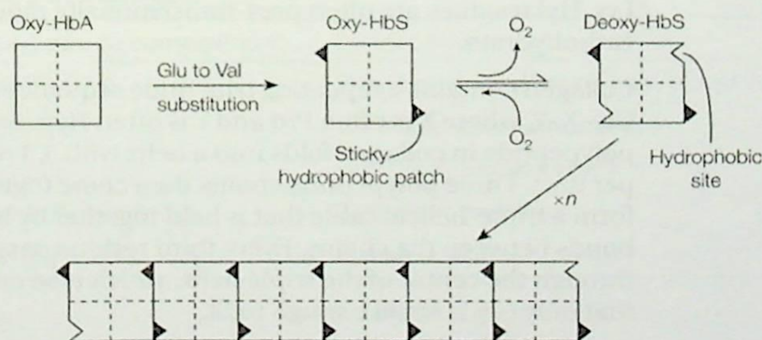

Figure 5. Molecular basis for the aggregation of deoxyhemoglobin molecules in sickle-cell anemia.