Hemoglobin
Structure and Function

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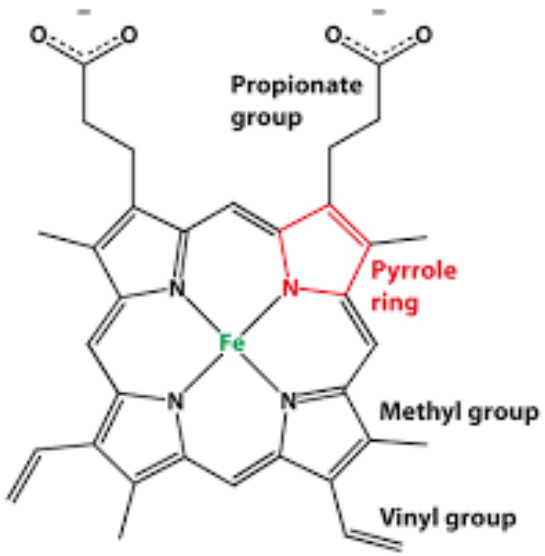
1. **Human hemoglobin A, present in adults, consists of four subunits: two α subunits and two β subunits.**
2. The α and β subunits are homologous and have similar three-dimensional structures.
3. The capacity of hemoglobin to bind oxygen depends on the presence of a bound prosthetic group called *heme*.
4. The heme group is responsible for the distinctive red color of blood.
5. The heme group consists of an organic component and a central iron atom.
6. The organic component, called *protoporphyrin*, is made up of four pyrrole rings linked by methene bridges to form a tetrapyrrole ring.
7. Four methyl groups, two vinyl groups, and two propionate side chains are attached.



Heme
(Fe-protoporphyrin IX)

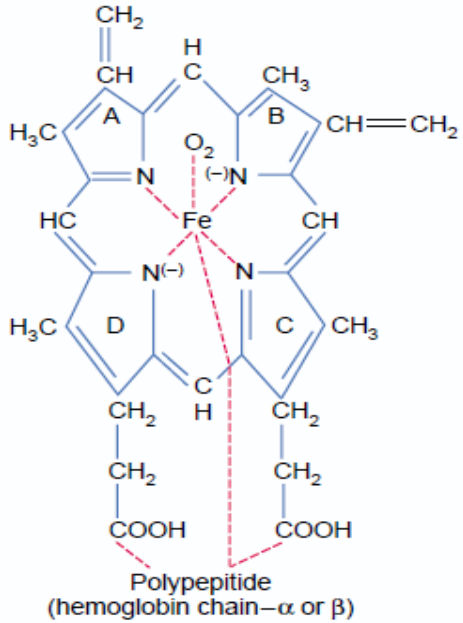


Figure 32–6

Basic structure of the hemoglobin molecule, showing one of the four heme chains that bind together to form the hemoglobin molecule.

1. **The iron atom lies in the center of the protoporphyrin, bonded to the four pyrrole nitrogen atoms**.
2. Under normal conditions, the iron is in the ferrous (Fe^{2+}) oxidation state. The iron ion can form two additional bonds, one on each side of the heme plane.
3. These binding sites are called the **fifth and sixth coordination sites**. In hemoglobin, the **fifth coordination site is occupied by the imidazole ring of a histidine residue from the protein**.
4. **In deoxyhemoglobin**, the **sixth coordination site remains unoccupied**.
5. The iron ion lies approximately 0.4 Å outside the porphyrin plane because iron, in this form, is slightly too large to fit into the well-defined hole within the porphyrin ring.
6. The binding of the oxygen molecule at the sixth coordination site of the iron ion substantially rearranges the electrons within the iron so that the ion becomes effectively smaller, allowing it to move into the plane of the porphyrin

COOPERATIVITY MODEL (Ref. – L. Stryer)

Oxygen Binding Markedly Changes the Quaternary Structure of Hemoglobin

The three-dimensional structure of hemoglobin is best described as a pair of identical αβ dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$) that associate to form the hemoglobin tetramer.

The deoxyhemoglobin, corresponds to the T state in the context of either the concerted or the sequential model for **hemoglobin cooperativity**. On oxygen binding, there are substantial changes in quaternary structure that correspond to the T-to-R state transition.

The $\alpha_1\beta_1$ and $\alpha_2\beta_2$ dimers rotate approximately 15 degrees with respect to one another. The dimers themselves are relatively unchanged, although localized conformational shifts do occur.

Consequently, the structural transition at the iron ion is directly transmitted to the other subunits.

The rearrangement of the dimer interface provides a pathway for communication between subunits, enabling the cooperative binding of oxygen.

Next open binding site has an affinity for oxygen more than 20-fold as great as that of fully deoxygenated hemoglobin binding its first oxygen.

Oxygenation of Hemoglobin Is Accompanied by Large Conformational Changes (Ref. – Harper)

The binding of the first O_2 molecule to deoxyHb shifts the heme iron towards the plane of the heme ring from a position about 0.6 nm beyond it (Figure 6–6). This motion is transmitted to the proximal (F8) histidine and to the residues attached thereto, which in turn causes the rupture of salt bridges between the carboxyl terminal residues of all four subunits. As a consequence, one pair of α/β subunits rotates 15 degrees with respect to the other, compacting the tetramer (Figure 6–7). Profound changes in secondary, tertiary, and quaternary structure accompany the high-affinity O_2-induced transition of hemoglobin from the low-affinity **T (taut) state** to the **R (relaxed) state.** These changes significantly increase the affinity of the remaining unoxygenated hemes for O_2, as subsequent binding events require the rupture of fewer salt bridges (Figure 6–8). The terms T and R also are used to refer to the low-affinity and high-affinity conformations of allosteric enzymes, respectively.

The three-dimensional structure of hemoglobin was solved using **X-ray crystallography** 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 (*Fig. 1b*) despite the fact that their amino acid sequences differ at 83% of the residues. This highlights a relatively common theme in protein structure:

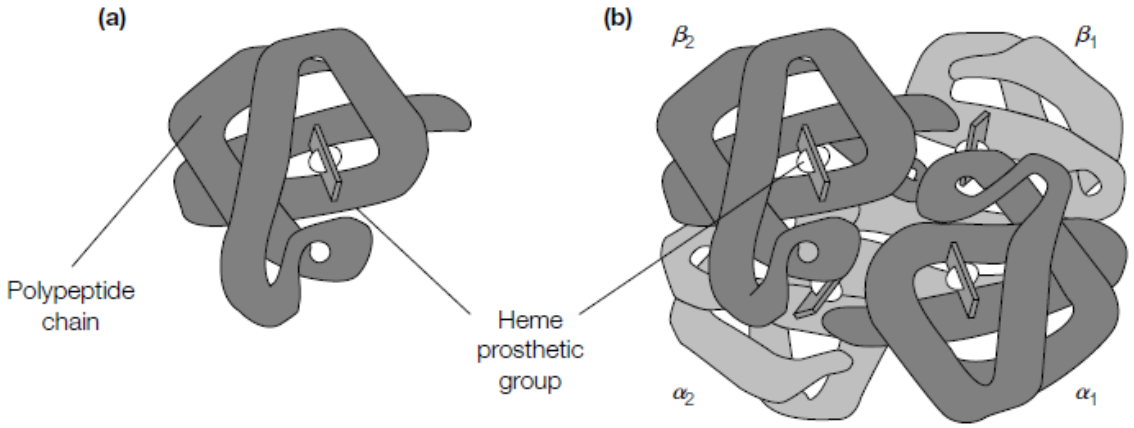
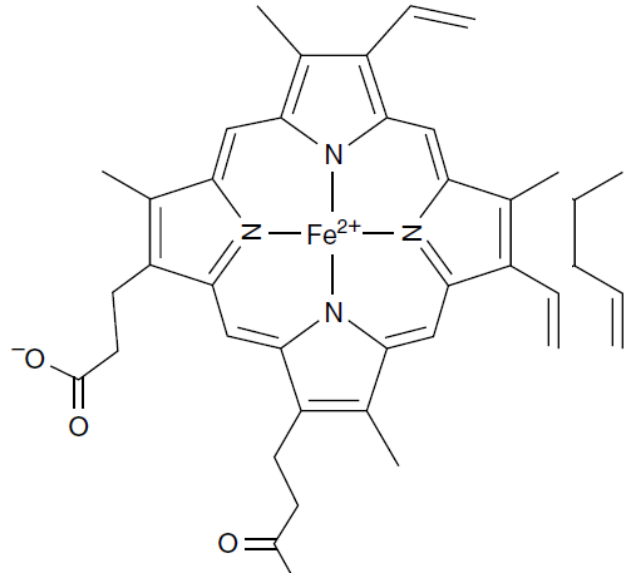


Fig. 1. Structure of (a) myoglobin and (b) hemoglobin, showing the α and β polypeptide chains.

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 ($\alpha_2\beta_2$; *Fig. 1b*). Each chain, like that in myoglobin, consists of eight α-helices and each contains a heme prosthetic group (*Fig. 1b*). Therefore, hemoglobin can bind four molecules of O_2. The four polypeptide chains, two α and two β, are packed tightly together in a tetrahedral array to form an overall spherically shaped molecule that is held together by multiple noncovalent interactions.



HEME & FERROUS IRON CONFER THE ABILITY TO STORE & TO TRANSPORT OXYGEN

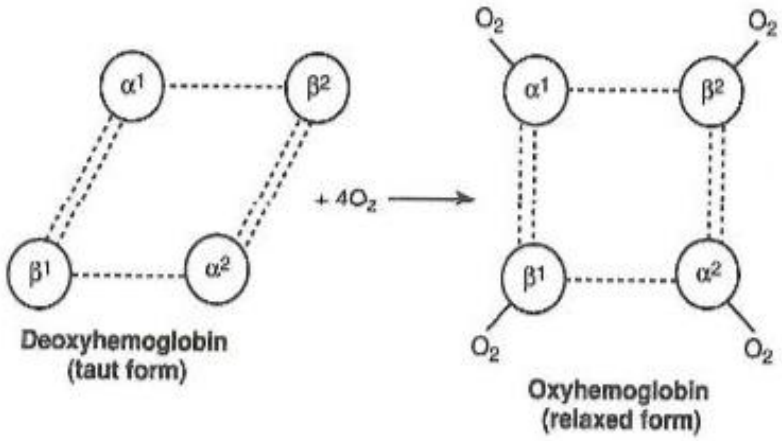
Myoglobin and hemoglobin contain **heme,** a cyclic tetrapyrrole consisting of four molecules of pyrrole linked by α-methylene bridges. This planar network of conjugated double bonds absorbs visible light and colors heme deep red. The substituents at the β-positions of heme are methyl (M), vinyl (V), and propionate (Pr) groups arranged in the order M, V, M, V, M, Pr, Pr, M (Figure 6–1). One atom of ferrous iron (Fe_2^+) resides at the center of the planar tetrapyrrole. Other proteins with metal-containing tetrapyrrole prosthetic groups include the cytochromes (Fe and Cu) and chlorophyll (Mg) (see Chapter 12). Oxidation and reduction of the Fe and Cu atoms of cytochromes is essential to their biologic function as carriers of electrons. By contrast, oxidation of the Fe_2^+ of myoglobin or hemoglobin to Fe_3^+ destroys their biologic activity.

THE ALLOSTERIC PROPERTIES OF HEMOGLOBINS RESULT FROM THEIR QUATERNARY STRUCTURES

The properties of individual hemoglobins are consequences of their quaternary as well as of their secondary and tertiary structures. The quaternary structure of hemoglobin confers striking additional properties, absent from monomeric myoglobin, which adapts it to its unique biologic roles. The **allosteric** (Gk *allos* "other," *steros* "space") properties of hemoglobin provide, in addition, a model for understanding other allosteric proteins (see Chapter 11).

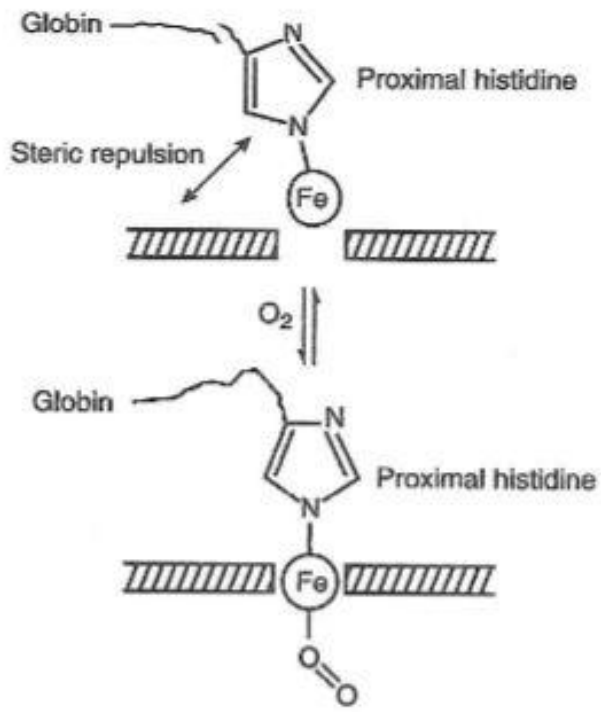
Hemoglobin Is Tetrameric

Hemoglobins are tetramers comprised of pairs of two different polypeptide subunits. Greek letters are used to designate each subunit type. The subunit composition of the principal hemoglobins are $\alpha_2\beta_2$ (HbA; normal adult hemoglobin), $\alpha_2\gamma_2$ (HbF; fetal hemoglobin), α_2S_2 (HbS; sickle cell hemoglobin), and $\alpha_2\delta_2$ (HbA_2; a minor adult hemoglobin). The primary structures of the β, γ, and δ chains of human hemoglobin are highly conserved.



Oxygenation of Hemoglobin Triggers Conformational Changes in the Apoprotein

Hemoglobins bind four molecules of O_2 per tetramer, one per heme. A molecule of O_2 binds to a hemoglobin tetramer more readily if other O_2 molecules are already bound (Figure 6–4). Termed **cooperative binding,** this phenomenon permits hemoglobin to maximize both the quantity of O_2 loaded at the P_{O_2} of the lungs and the quantity of O_2 released at the P_{O_2} of the peripheral tissues. Cooperative interactions, an exclusive property of multimeric proteins, are critically important to aerobic life.



HEMOGLOBIN: COOPERATIVE BINDING

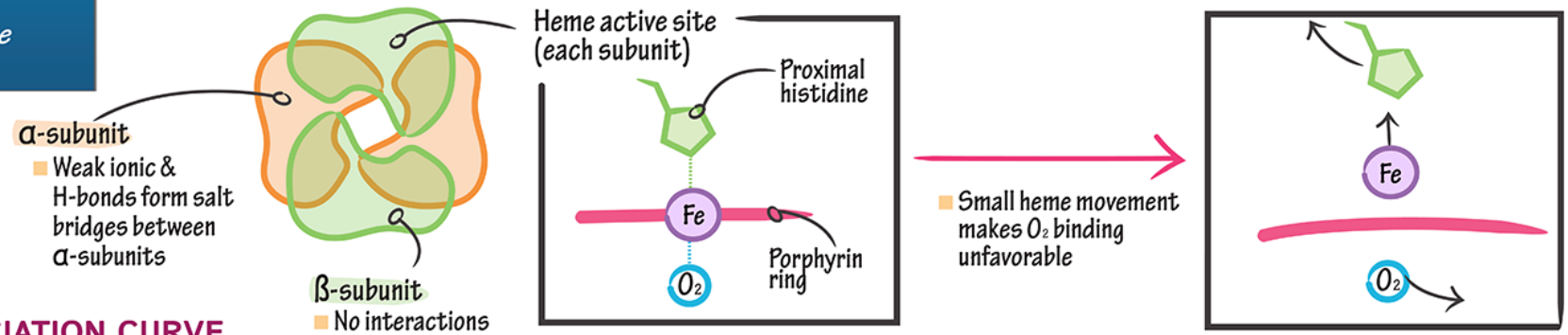
Cooperative Binding

✓ *Describes the unique interactions between heme groups in hemoglobin*

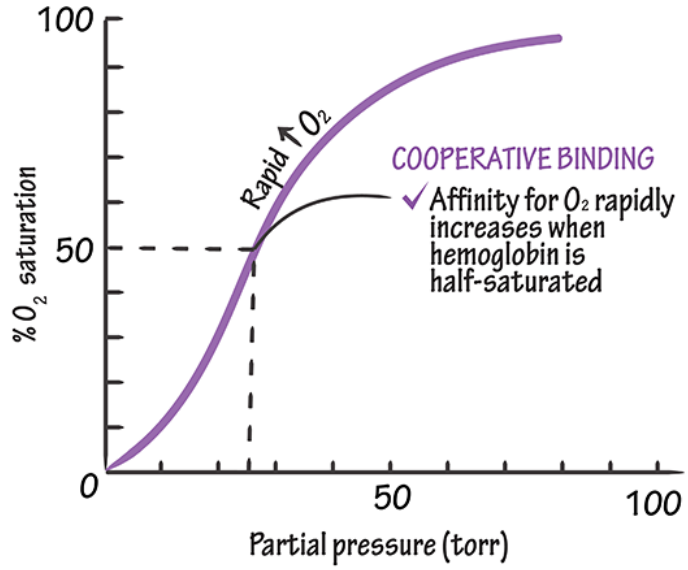
HEMOGLOBIN (Oxygenated)
R-form = "Relaxed" form

3D structure changes between R-form & T-form

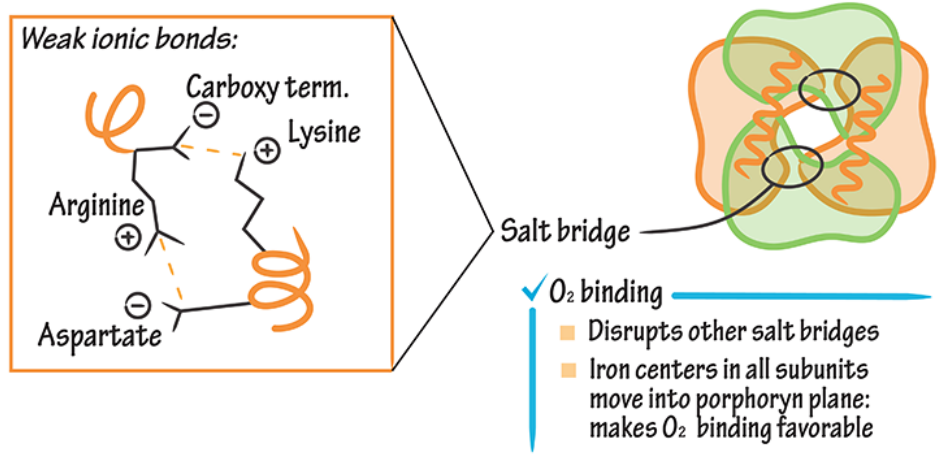
HEMOGLOBIN (Deoxygenated)
T-form = "Tense" form



HEMOGLOBIN DISSOCIATION CURVE



A Closer Look at R-form Hemoglobin



BOX 7-2 **PATHWAYS OF DISCOVERY** **(Ref. Voet, Voet and Pratt)**

Max Perutz and the Structure and Function of Hemoglobin

Max Perutz (1914–2002)

The determination of the three-dimensional structures of proteins has become so commonplace that it is difficult to appreciate the challenges that faced the first protein crystallographers. Max Perutz was a pioneer in this area, spending many years determining the structure of hemoglobin at atomic resolution and then using this information to explain the physiological function of the protein.

In 1934, two years before Perutz began his doctoral studies in Cambridge, J.D. Bernal and Dorothy Crowfoot Hodgkin had placed a crystal of the protein pepsin in an X-ray beam and obtained a diffraction pattern. Perutz tried the same experiment with hemoglobin, chosen because of its abundance, ease of crystallization, and obvious physiological importance. Hemoglobin crystals yielded diffraction patterns with thousands of diffraction maxima (called reflections), the result of X-ray scattering by the thousands of atoms in each protein molecule. At the time, X-ray crystallography had been used to determine the structures of molecules containing no more than around 40 atoms, so the prospect of using the technique to determine the atomic structure of hemoglobin seemed impossible. Nevertheless, Perutz took on the challenge and spent the rest of his long career working with hemoglobin.

In X-ray crystallography, the intensities and the positions of the reflections can be readily determined but the values of their phases (the relative positions of the wave peaks, the knowledge of which is as important as wave amplitude for image reconstruction) cannot be directly measured. Although computational techniques for determining the values of the phases had been developed for small molecules, methods for solving this so-called phase problem for such complex entities as proteins seemed hopelessly out of reach. In 1952, Perutz realized that the method of isomorphous replacement might suffice to solve the phase problem for hemoglobin. In this method, a heavy atom such as an Hg^{2+} ion, which is rich in electrons (the particles that scatter X-rays), must bind to specific sites on the protein without significantly disturbing its structure (which would change the positions of the reflections). If this causes measurable changes in the intensities of the reflections, these differences would provide the information to determine their phases. With trepidation followed by jubilation, Perutz observed that Hg-doped hemoglobin crystals indeed yielded reflections with measurable changes in intensity but no changes in position. Still, it took another 5 years to obtain the three-dimensional structure of hemoglobin at low (5.5-Å) resolution and it was not until 1968, some 30 years after he began the project, that he determined the structure of hemoglobin at near atomic (2.8-Å) resolution. In the meantime, Perutz's colleague John Kendrew used the method of isomorphous replacement to solve the structure of myoglobin, a smaller and simpler relative of hemoglobin. For their groundbreaking work, Perutz and Kendrew were awarded the 1962 Nobel Prize in Chemistry.

For Perutz, obtaining the structure of hemoglobin was only part of his goal of understanding hemoglobin. For example, functional studies indicated that the four oxygen-binding sites of hemoglobin interacted, as if they were in close contact, but Perutz's structure showed that the binding sites lay in deep and widely separated pockets. Perutz was also intrigued by the fact that crystals of hemoglobin prepared in the absence of oxygen would crack when they were exposed to air (the result, it turns out, of a dramatic conformational change). Although many other researchers also turned their attention to hemoglobin, Perutz was foremost among them in ascribing oxygen-binding behavior to protein structural features. He also devoted considerable effort to relating functional abnormalities in mutant hemoglobins to structural changes.

Perutz's groundbreaking work on the X-ray crystallography of proteins paved the way for other studies. For example, the first X-ray structure of an enzyme, lysozyme, was determined in 1965. The nearly 50,000 macromolecular structures that have been obtained since then owe a debt to Perutz and his decision to pursue an "impossible" task and to follow through on his structural work to the point where he could use his results to explain biological phenomena.

Perutz, M.F., Rossmann, M.G., Cullis, A.F., Muirhead, H., Will, G., and North, A.C.T., Structure of haemoglobin: A three-dimensional Fourier synthesis at 5.5 Å resolution, obtained by X-ray analysis. *Nature* **185,** 416–422 (1960).

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 2,3-bisphosphoglycerate 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 (*Fig. 5*).

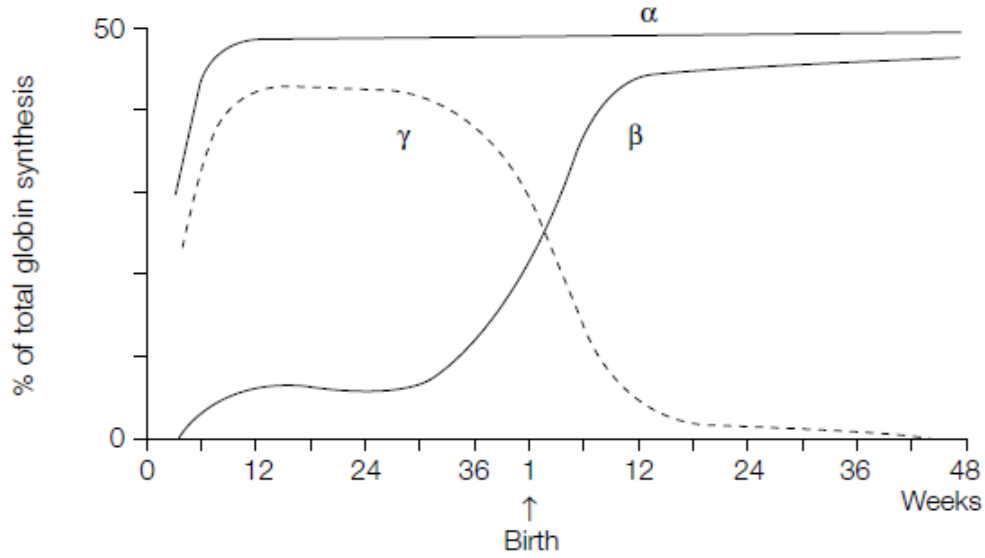


Fig. 5. The switch in human globin chain synthesis at birth.

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 valine is replaced with the hydrophobic isoleucine, or the polar serine is replaced with the polar asparagine), so-called **conservative substitutions**. In contrast, only a few residues have changed between species to a completely different residue (e.g. a hydrophobic leucine to a positively charged lysine or a negatively charged glutamate to a positively charged arginine), 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 **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 glutamic acid residue for a valine at position 6 of the β-chain, resulting in the substitution of a polar residue by a hydrophobic one. This **nonconservative substitution** of valine for glutamate 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 (*Fig. 6*). 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. 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 (see Topic I1).