Hemoglobin:

Hemoglobin abbreviated Hb, is the iron-containing oxygen-transport metalloprotein in the red blood cells of the blood in vertebrates and other animals. Hemoglobin transports oxygen from the lungs or gills to the rest of the body.

Structure:
The name hemoglobin is the concatenation of heme and globin, each heme group contains an iron atom, and this is responsible for the binding of oxygen through ion-induced dipole forces. In humans, the hemoglobin molecule is an assembly of four globular protein subunits.

X-ray Analysis of Hemoglobin:
After 3-dimensional X-ray analysis of reduced hemoglobin, the resolution attained was 5.5 Å; which shows the course of the polypeptide chains and the positions of the heme groups but does not allow individual amino acid residues to be seen. Each subunit is composed of a protein chain tightly associated with a non-protein heme group, chemically the white units are known as α chains and the black as the β chains(Fig1)(3).

A heme group consists of an iron (Fe) ion held in a heterocyclic ring, known as a porphyrin. The iron ion, which is the site of oxygen binding, bonds with the four nitrogens in the center of the ring, which all lie in one plane. The iron is also bound strongly to the globular protein via the imidazole ring of the F8 histidine residue below the porphyrin ring. A sixth position can reversibly bind oxygen, completing the octahedral group of six ligands. When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron.

Figure 1: Complete hemoglobin molecule. The heme groups are indicated by grey disks.

Oxygen-linked conformational changes in hemoglobin, using the spin-label technique (EPR): The purpose of this is to understand the spin labels depend on structure of label
A molecule of protein, for that isolated spin-labeled $\beta$-subunit were prepared from labeled human hemoglobin \textbf{A1}. The labeled $\beta$-chains were eluted with pH 6.7 phosphate buffer. Isolated labeled $\beta$-chains from human hemoglobin show resonance spectra (Fig. 2)

The paramagnetic resonance spectra of spin-labeled oxyhemoglobin is simply described by the spin Hamiltonian

$$\mathcal{H} = \beta_s \mathbf{g} \cdot \mathbf{H}_0 + \hbar \mathbf{S} \cdot \mathbf{I} + g_\beta \mu I \cdot \mathbf{H}_0,$$

The conformational change which the resonance spectra show involves the paramagnetic portion of the label which is at the surface of the $\alpha$-subunit, and it is entirely reasonable neighboring amino acid side chains are similarly involved. The label resonance spectrum is affected by $\alpha$-$\beta$-subunit interactions as evidenced by the effect of adding $\alpha$-chains to labeled $\beta$-chains. (2)

![Graphical representation of paramagnetic resonance of oxy and deoxy $\beta$-chains of human spin labeled hemoglobin](image)

\textit{Figure 2: Paramagnetic resonance of oxy and deoxy $\beta$-chains of human spin labeled hemoglobin}

\textbf{Protein-oxygen binding:}

Hb binds up to 4 $\text{O}_2$. Named \textit{deoxy-} Hb \textit{and oxy-} Hb & can also be oxidized to form \textit{met-} Hb in which the Fe is oxidized to Fe(III). The protein plays an important role in protecting the heme - heme group on its own in solution which will form a $\mu$-oxo dimer. In the deoxy form of Hb the Fe$^{\text{II}}$ exists as a high spin complex with $S=2$ ground state with 4 unpaired electrons while the Oxy form of Hb has $S=0$ ground state assignment.

\textit{Figure 3: Formation of the $\mu$ - oxo dimer}
Figure 4: Hemoglobin – ligand binding.
HS Fe tetrapyrrole is a little unusual - due to the incomplete fit of the Fe in the pocket due to the domed macrocycle (out-of-plane).
Binding of O₂ provides a relatively light 'pull' which is sufficient to affect spin crossover. This results in a contraction of the metal and a movement of the metal by about 20pm into the binding pocket of the coordinating macrocycle which now binds tightly. This has been termed an entatic state - the protein is poised for binding to O₂. Binding of O₂ causes the Fe to move - this causes the coordinating his to move which causes a change in the conformation of the protein.

Two states R and T. R has higher affinity for O₂ than T state (4-5).

<table>
<thead>
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<th>Bond Order</th>
<th>2</th>
<th>1.5</th>
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<tr>
<td>Bond Length</td>
<td>121 pm</td>
<td>128 pm</td>
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<td>Vibration</td>
<td>1560 cm⁻¹</td>
<td>1150-1100 cm⁻¹</td>
<td>850-740 cm⁻¹</td>
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</table>
Pauling and Coryell explained the diamagnetism by invoking a low spin Fe$^{II}$ bound to an $^1O_2^-$. Both the Fe and $^1O_2$ are diamagnetic. $O_2$ acts as a donor and Fe as an acceptor.

Weiss proposed a different model - a single electron transfer from the Fe$^{II}$ to $O_2$ to from Fe$^{III}$ and a superoxide radical anion $O_2^-$. Both the LS Fe$^{III}$ and the $O_2^-$ have $S=1/2$. The observed diamagnetism can then be explained by a strong antiferromagnetic coupling. In addition, vibrational frequency of the bound $O_2$ at 1100 cm$^{-1}$ is consistent with $O_2^-$. This can be shown as

The magnetic properties of hemoglobin and its metabolites are dependent on their content of iron. Iron in most of its simple ionic compounds contains paramagnetic electrons, and can cause shortened proton T1 relaxation in aqueous solution by proton–electron dipole–dipole relaxation enhancement which is the ability of paramagnetic electrons to enhance the return of the local proton magnetization vector to equilibrium.
The electronic structure of iron and the paramagnetism of hemoglobin

The electrons in each shell can be further divided into subshells by slight differences in energy. Each subshell is further subdivided into individual orbitals. Each orbital can contain a maximum of two electrons. When two electrons occupy the same orbital, their spins will always assume opposite orientations. This spin pairing neutralizes the paramagnetic properties of those two orbital electrons. Unpaired electrons in an inner, only partly filled 3d subshell of iron cause its compounds to frequently be paramagnetic.

Both oxyhemoglobin and deoxyhemoglobin, the predominant forms of heme iron in intact red blood cells, contain iron in its ferrous (+2 oxidation) state. In the ferrous iron of deoxyhemoglobin, the six inner 3d subshell electrons are distributed into five orbitals, leaving four of the five orbitals with one unpaired electron thus deoxyhemoglobin is paramagnetic molecule, containing four unpaired paramagnetic electrons.

Oxyhemoglobin is diamagnetic. The explanation of this striking difference in magnetic behavior derives from a subtle change in the four paramagnetic 3d orbital electrons of the iron atom of deoxyhemoglobin, caused by the binding of oxygen. The highly electronegative oxygen in oxyhemoglobin produces a strong perturbation of the paramagnetic 3d electrons of the iron, raising the energy of two of the five orbitals above that of the other three orbitals. All of the four unpaired electrons then spin pair with one another as they occupy the two remaining unfilled lower energy 3d orbitals (Fig. 2). Thus, in its most stable electronic state, the oxyhemoglobin molecule contains no unpaired orbital electrons, and is diamagnetic. In the language of the inorganic chemist, ferrous iron in oxyhemoglobin is low spin, and ferrous iron in deoxyhemoglobin is high spin. The low spin ferrous iron of oxyhemoglobin has a slightly smaller ionic radius than the high spin iron of deoxyhemoglobin, and it has been hypothesized that the former “fits” better into the center of the porphyrin moiety, thus causing a conformational change in the shape of the globin molecule of that particular hemoglobin subunit. This shape change alters the properties of the other three hemoglobin subunits, causing their affinity for oxygen binding to increase. This is the property of cooperativity, by which oxygen binding by one of the four subunits of the hemoglobin molecule facilitates oxygen binding by the other three. Cooperativity is the property responsible for allowing hemoglobin to become maximally oxygenated in the lungs while maximally releasing its bound oxygen in the relatively hypoxic capillaries. It is this same conformational change in the globin protein, as well as the off-center position of its heme iron
Figure 8: Magnetic susceptibility diagram of hemoglobin

Hemoglobin Energy Level diagrams.

The five-coordinate deoxy-Hb has a quintet ground state (5T2), but the low-energy excited states, a triplet (3T1) d2, dxy, dzy, dz2 and a singlet (1A1) d2, dxy, d2, place the triplet at 2500 cm⁻¹ above the almost degenerate 1A1, 5T2 pair, whereas in others the singlet is located 16,000 cm⁻¹ above the quintet. Many of the excited quintet states of Hb were experimentally assigned, and these results with crystal field calculations were used to generate a detailed energy level diagram for Hb, HbO2, and HbCO.

The HbCO ground state is 1A1, and the empty d2z orbital is usable in bonding to the ligand, identifying the states 1T1, derived from in the range 16,000 to 17,850 cm⁻¹. These transitions involve promotion of an electron from dz or dzy into the antibonding d2z orbital, and they correlate with excited states of Hb and CO. The Hb in HbO2 thereby has the 3T1 configuration. If, after excitation to the 1Q state by 530-nm light, HbX undergoes conventional non-radiative relaxation dynamics, significant excited state populations would exist only in the lowest electronic states of a given spin multiplicity. The spin-orbit interaction influences the initial partitioning of the excitation energy of the system. The 1Q state has no first-order spin-orbit mixing with any quintet so that lower energy singlets and triplets are the ones reached in the initial relaxation. Some of these and subsequent relaxations are expected to be improbable because of the widely different electronic configurations involved. The lowest-energy ligand field states of HbCO (5T2 and 3T1) might act as bottlenecks in the relaxation. Thus 3T1 is favored, but there is a substantial spin-orbit mixing of 3T1 and 5T2 so that if 5T2 were the lowest excited state it would very likely become populated. For HbO2 the so-called charge transfer states are (5T2) of HbO2 is near the 1Q state, but not a strong candidate to have the lowest and the most likely candidates for relaxation bottlenecks. The lowest quintet significant population soon after pumping 1Q. It appears more likely that 1Q will couple with other singlets and with triplets. The second-order spin-orbit interaction necessary to mix 1Q and 5T2 involves orbital prohibition so that this intercombination is expected to be improbable.
Fig 9: Simplified energy level diagram showing spin-orbit mixing and orbital correlations.
Conclusion:
Hemoglobin is a heme containing protein which contains 4 subunits, which is shown by X-ray analysis. The heme binds oxygen, then called as oxyhemoglobin. With the help of (EPR) the spin labels depend on structure of label molecule of protein, for that isolated spin-labeled β-subunit and Paramagnetic resonance of oxy and deoxy β-chains of human spin labeled hemoglobin is studied. The oxygen – protein binding is studied with the phenomenon called as magnetic susceptibility which include the coupling, bond order, vibrational frequencies, spin states and concluded that oxyhemoglobin is diamagnetic while deoxyhemoglobin is paramagnetic. The property of cooperativity which facilitates oxygen binding to other 3 subunits while oxygen is bonded to one subunit. The energy level diagram is studied which show spin-orbit mixing and orbital correlations. Hemoglobin plays crucial role in physiology and this paper help in better understanding its working.
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