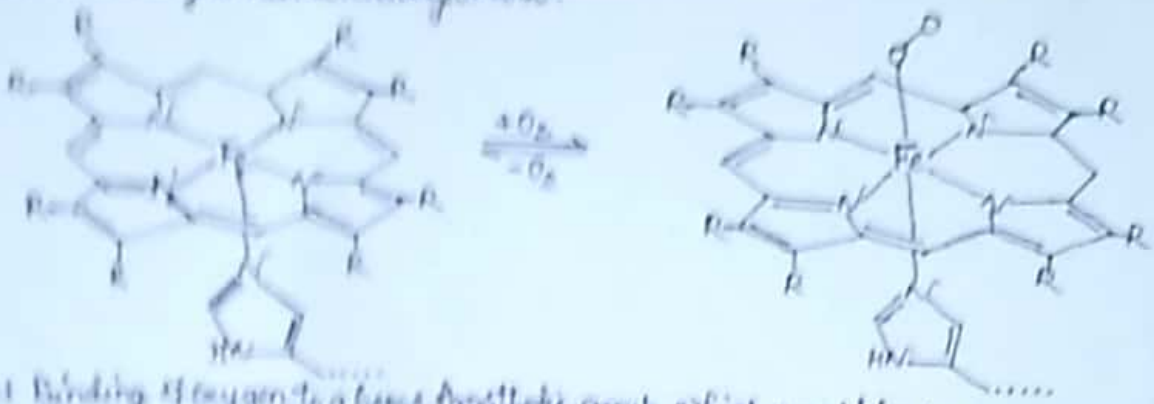
M.Sc Semester III, Paper: CC-11 (Bio Inorganic Chemistry)
Unit-3 "Transport & Storage of O_2" [By Dr. Birendra Kumar, Maharaja College]

Heme Proteins or Hemoproteins:

Hemoprotein is a protein that contains a heme prosthetic group. They are very large class of metalloproteins. The heme group confers functionality, which can include oxygen carrying, oxygen reduction, electron transfer and other processes. Heme is bound to the protein either covalently or non-covalently or both.



[Fig: Binding of oxygen to a heme prosthetic group, which could be part of a hemoprotein]

The heme consists of iron(Fe^{2+}) cation found at the center of the conjugate base of the porphyrin, as well as other ligands attached to the axial sites of the iron. The porphyrin ring is a planar dianionic, tetradentate ligand. The iron is typically Fe^{2+} or Fe^{3+}. One or two ligands are attached at the axial sites. The porphyrin ring has four nitrogen atoms that bind to the iron, leaving two other coordination positions of the iron available for binding to the histidine of the protein and a divalent atom.

Heme proteins probably evolved to incorporate the iron atom contained within the protoporphyrin IX ring of heme into proteins. As it makes heme protein responsive to molecules that can bind divalent iron(Fe^{2+}), this strategy has been maintained throughout evolution as it plays crucial physiological functions. Oxygen(O_2), nitric oxide(NO), carbon monoxide(CO) and hydrogen sulphide(H_2S) bind to the iron atom in heme groups, these molecules can modulate the activity/function of these heme proteins, affording signal transduction. Therefore, when produced in biologic systems (cells), these gaseous molecules are referred to as gastransmitters.

Because of their diverse biological functions and wide spread abundance, heme-Proteins are among the most studied biomolecules. Data on heme Proteins structure and function has been aggregated into the Heme Protein Database (HPD), a secondary database to the Protein Data Bank.

⇒ Roles of Heme proteins:

* Heme proteins have diverse biological functions including oxygen transport, which is completed via heme proteins including Hemoglobin, myoglobin, neuroglobin, cytoglobin and leghemoglobin.
* Some heme proteins - cytochrome P450s, Cytochrome c oxidase, ligninases, catalase and peroxidases are enzymes. They often activate O_2 for oxidation or hydroxylation.

* Heme proteins also enable electron transfes as they form part of the electron transport chain. Cytochrome 'a', Cytochrome 'b', and Cytochrome 'c' have such electron transfer functions.
* The sensory system also relies on some heme proteins including 'FixL', an oxygen sensor, CooA, a carbon monoxide (CO) sensor and soluble guanylyl cyclase.

⟹ **Haemoglobin:**

Haemoglobin, an example of heme proteins, which transfer oxygen in mammals. Haemoglobin is a quaternary protein that occurs in the red blood cell of mammals. In vertebrates, haemoglobin is found in the cytosol of red blood cells. It is sometimes referred to as the oxygen transport protein, in order to contrast it with myoglobin, which is stationary. In human body, about 65% of Fe(II) is present as haemoglobin (Hb). It is a red pigment which present in red blood cells (RBCs) in human body. It is a Fe(II) porphyrin, which contains about 0.95% of iron. Haemoglobin which has not taken up O_2 is called deoxy-haemoglobin (deoxy-Hb) or simply haemoglobin while the haemoglobin which has taken up O_2 is called Oxygenated haemoglobin or Oxyhaemoglobin (oxy-Hb).

⟹ **Structure of Haemoglobin:** The structure of Haemoglobin can be classified under two headings: (1) Structure of Heme, the prosthetic group (2) Structure of Globin, the protein part-apoprotein.

1. **Structure of Heme:** (a) It is an iron porphyrin. The porphyrins are cyclic compounds with tetra pyrrole structure.

(b) Four pyrrole rings, called (I) to (IV) are linked through methylene bridges or methylidene bridges.
(c) The outer carbon atoms which are not linked with the methylidene bridges are numbered 1 to 8.
(d) The methylidene bridges are designated as α, β, γ & δ respectively.
(e) Iron in the ferrous state (Fe^{2+}) is bound to the nitrogen atoms of the pyrrole rings.
(f) Iron is also linked internally (5th linkage) to the nitrogen of the imidazole ring of Histidine of the polypeptide chains.
(g) The propionic acid of 6th & 7th position of heme III & IV pyrroles are also linked to the amino acids 'Arg' and 'lys' of the polypeptide chain respectively.

2. **Structure of Globin:** The globin of haemoglobin is a protein which is composed of four parallel layers of closely packed polypeptide chains.
(b) Two of the chains (α-chains) have identical amino acid composition of 141 amino acids. The two other chains may be two of the four polypeptide chains designated as β, γ, δ & ε (epsilon). Each is having 146 amino acids.
(c) The total number of amino acids in globin is 574.
(d) α-chains have Val-Leu-Ser in N-terminal residues and Lys-tyr-Arg in C-terminal residues.
(e) β-chains have Val-His-Leu in N-terminal residues and Lys-tyr-His in C-terminal residues.
(f) γ-chains have Gly-His-Phe in N-terminal residues & Arg.-Tyr-His in C-terminal residues.
(g) Haemoglobin molecules and its sub units contain mostly hydrophobic amino acids.

internally and hydrophilic amino acids on their surfaces. So they form "Water pockets" (b) In "Heme pockets", α-subunits are of like necessary for entry of O_2 molecule but the entry of O_2 molecule in β-subunit is blocked by Valine residue.

⇒ Properties/functions of Haemoglobin: Reversible: 1. Oxyhaemoglobin: It forms oxyhaemoglobin in combination with O_2. When haemoglobin is exposed to air, it takes up two atoms of oxygen for each atom of Fe^{++} present. Thus, Haemoglobin will take up four molecules of O_2. In low oxygen tension, oxyhaemoglobin gives up O_2 readily. By this way, blood carries O_2 to different parts of the body.

2. Formation of Carbamino compound: It reacts with CO_2 forming carbamino compounds.

$Hb-NH_2 + CO_2 \longrightarrow$ Hb-NH COOH.

3. Reaction with CO: It forms Carboxyhaemoglobin. The resulting carboxy haemoglobin is stable and prevents the formation of oxyhaemoglobin. So inhalation of even small amounts of CO is highly dangerous.

4. Buffering action: One role of haemoglobin contains 38 histidine residues. Histidine exerts its buffering action through its basic imidazole ring. Hence, haemoglobin plays an important role in regulating the acid-base balance of blood.

5. Formation of methaemoglobin: Methaemoglobin is formed as a result of the oxidation of haemoglobin by the mild oxidising agent, potassium ferricyanide. The Fe^{++} (ferrous) ion is oxidised to the Fe^{+++} (ferric) ion. Methaemoglobin cannot carry oxygen in blood. It is also formed by the action of some drugs. This is found in the blood of some individuals owing to inborn errors of metabolism. It can be reduced to haemoglobin by Vit C which is used in the treatment of methaemoglobinemia.

6. Sulphaemoglobin: It is formed by the administration of certain drugs. It continues to remain in the blood and cannot be reconverted into haemoglobin.

7. Cyanomethaemoglobin: It is formed by the addition of Cyanide to methaemoglobin. It has a bright red colour.

8. Absorption Spectra: The different haemoglobin derivatives can be easily identified by their characteristic absorption spectra (a) Oxyhaemoglobin, two bands (one narrow and other wide in the green region) observed (b) In reduced haemoglobin, one single broad band in the green region observed. c) In carboxyhaemoglobin, two bands in the green region observed (d) In methaemoglobin, three bands (one in red & two in the green regions) observed. e) Sulphaemoglobin, three bands similar to methaemoglobin.

Functions: 1. Haemoglobin transports CO_2 and protons to the lungs after releasing O_2 to the tissues.

2. Haemoglobin can bind CO_2 directly when O_2 is released. CO_2 reacts with the amino terminal α-amino groups of the haemoglobin forming a carbamate and releasing protons.

$CO_2 + Hb-NH_2 \rightleftharpoons 2H^+ + Hb-NH-COO^-$

The amine terminal is converted from a positive to a negative charge favouring salt bridge formation between the α & β-chains.

3. At the lungs, haemoglobin is oxygenated being accompanied by expulsion and subsequent expiration of O_2. CO_2 is absorbed in blood and the carbonic anhydrase in

erythrocytes catalyses the formation of carbonic acid which is rapidly dissociated into bicarbonate and a proton.

$$CO_2 + H_2O \underset{}{\overset{\text{anhydrase}}{\rightleftharpoons}} H_2CO_3 \overset{\text{spontaneously}}{\rightleftharpoons} HCO_3^- + H^+$$

A buffering system absorbs these excess protons to avoid the increasing acidity of blood. Haemoglobin binds two protons for every four O_2 molecules.

4. In the lungs, the process is reversed, i.e., when O_2 binds to deoxygenated haemoglobin, protons are released and combines with bicarbonate forming carbonic acid which is exhaled. Thus, the binding of oxygen forces the exhalation of CO_2. This reversible phenomenon is called the Bohr effect.

⇒ **Bio Synthesis of Haemoglobin:**

1. The biosynthesis of haemoglobin takes place in the bone marrow in the erythroid cell during its development to erythrocyte.
2. It starts appearing at stage II (early normoblast) and the synthesis is complete when the cell reaches stage IV (late normoblast).
3. Iron in the ferrous state (Fe^{2+}) is incorporated into protoporphyrin to form heme.
4. The heme gets attached to the newly synthesised globin to form haemoglobin.
5. The iron of heme is coordinated to two imidazole nitrogen of histidine at position 38 & 72 in β-chains and 63 & 92 in α-chains.

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