CHAPTER

37 BIOINORGANIC CHEMISTRY

Bioinorganic chemistry is a fast developing branch of chemistry which explores the role of metallic (and some non-metallic) elements in biological systems. We shall, however, confine our discussion to the role of only the metallic elements. Metals such as Na, K, Ca, Mg, whose ions are present in biological systems in bulk quantities, are called **bulk metals** and metals such as Fe, Cu, Co, Zn, Cr, Mn, Mo, W, Ni, etc., whose ions are present in trace amounts, are called **trace metals**. Both the categories of metals are essential for sustaining life. There is a large number of biochemicals containing metal ions which play a significant role in biological systems. The most important amongst these are myoglobin and haemoglobin, the compounds containing iron.

MYOGLOBIN AND HAEMOGLOBIN

Both myoglobin and haemoglobin are *metal porphyrins* which contain the 'haeme' group in their structure. The haeme group consists of an iron atom which is coordinated to four nitrogen atoms of porphyrin ring, as shown in Fig. 1.

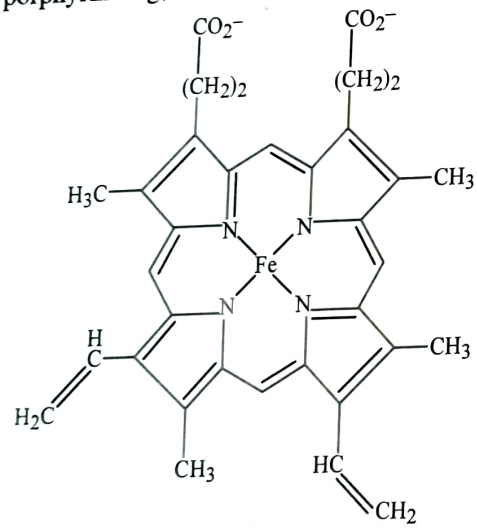

Fig. 1. The haeme group present in myoglobin and haemoglobin.

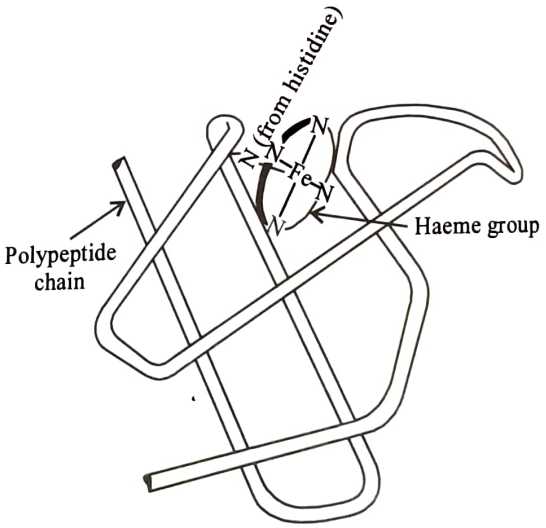

Fig. 2. Coordination of coiled polypeptide chain with the haeme group.

In myoglobin, the haeme group is embedded in a crevice formed by the coiling of its polypeptide chain containing 150–160 amino acid residues. The polypeptide chain is coordinated to iron atom of the haeme group through the N atom of one of its histidine groups, as shown in Fig. 2.

The molar mass of myoglobin is about 17000. Myoglobin which has not taken up oxygen is called *deoxymyoglobin* or simply myoglobin.

Haemoglobin, which has a molar mass of about 64500, comprises of four myoglobin-like (not myoglobin) sub units. None of the polypeptide chains of these sub units has exactly the same sequence of groups as are present in the polypeptide of myoglobin. Nevertheless, the polypeptide chains of both haemoglobin and myoglobin coil in a similar manner to produce crevices for accommodating the heme

group. Each sub unit of haemoglobin contains a polypeptide chain and a heme group coordinated through the N atom of the histidine group of its polypeptide chain. The four sub units of haemoglobin are linked with one another through salt bridges present between the four polypeptide chains. These salt bridges are formed due to electrostatic interaction between the $-NH_3^+$ and $-COO^-$ groups present on all the four polypeptide chains of haemoglobin. Each polypeptide chain consists of a peptide backbone with various side chains having a number of cationic groups (such as $-NH_3^+$), anionic groups (such as $-COO^-$) and nonpolar aliphatic and aryl groups. It is now believed that these salt bridges between the polypeptide chains of haemoglobin introduce **strain** in the molecule.

The oxygenated haemoglobin is called *oxyhaemoglobin*. Haemoglobin which has not taken up oxygen is called *deoxyhaemoglobin* or simply haemoglobin.

The five-coordinated high spin Fe(II) present in myoglobin and haemoglobin lies about 0·5 Å above the plane containing the four coordinated nitrogens of the porphyrin ring and the coordinated N atom of the histidine group.

The approach to the sixth coordination site of Fe(II) is sterically overcrowded by groups from the side chains attached with the main coiled polypeptide chain surrounding the haeme group. The probable structure of the heme group present in haemoglobin and myoglobin is shown in Fig. 3.

Both myoglobin and haemoglobin are paramagnetic due to the presence of unpaired electrons in Fe(II).

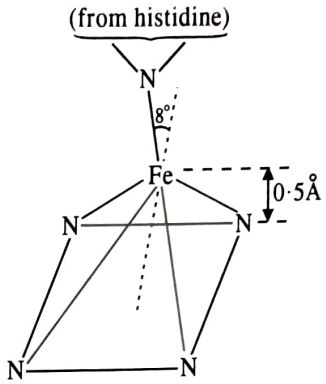

Fig. 3. Probable structure of the haeme unit present in myoglobin and haemoglobin.

Role of Myoglobin and Haemoglobin in Biological Systems

Oxygenation. Haemoglobin picks up oxygen from the lungs and carries it to the muscle tissues via the circulatory system. The oxygenated haemoglobin then transfers this oxygen to the myoglobin present in the muscle tissues where it remains stored as such till it is transferred to other oxygen acceptors for performing metabolic actions in the biological system. Apart from acting as oxygen carrier, haemoglobin has an additional function of carrying CO_2 from worked up muscle tissues to the lungs. This is done by certain side chains present on the polypeptide chains of haemoglobin. The heme groups of haemoglobin are not involved in flushing out CO_2 from the muscles.

Oxygenation of myoglobin (Mb) may be represented as

$$\underset{\text{Myoglobin}}{Mb} + O_2 \rightleftharpoons \underset{\text{Oxymyoglobin}}{MbO_2}$$

so that

$$K = \frac{[MbO_2]}{[Mb][O_2]} \quad \ldots(1)$$

If f is the fraction of myoglobin molecules bearing oxygen and p is the equilibrium partial pressure of oxygen, then, Eq. 1 may be written as

$$K = \frac{f}{(1-f)p} \quad \ldots(2)$$

or

$$f = \frac{Kp}{1+Kp} \quad \ldots(3)$$

The constant K is called the **binding constant** of myoglobin for oxygen.

Haemoglobin (Hb), with its four myoglobin-like sub units, exhibits more complex behaviour. It approximately follows Eq. 4 :

$$K = \frac{[Hb(O_2)_4]}{[Hb][O_2]^n} \quad \ldots(4)$$

so that

$$f = \frac{Kp^n}{1+Kp^n} \quad \ldots(5)$$

The exponent n is called the **Hill constant**. Its exact value depends upon the pH of the biological system. If the value of n is 1, it would mean that the oxygen intake by one heme group of haemoglobin is totally independent of the oxygen intake of its other three heme groups.

If the value of n is 4, it would imply that only Hb and $Hb(O_2)_4$ remain as the ultimate participants in the oxygenation of haemoglobin. The value of n ranging between 1 and 4 means that the attachment of oxygen to one haeme group of haemoglobin progressively increases its tendency to bind with the subsequent heme groups of haemoglobin.

The phenomenon where the addition of oxygen to one haeme group facilitates its addition to the other haeme groups of haemoglobin is known as **cooperativity effect**. Thus, a value of 4 for n would represent the maximum cooperativity effect.

A graph showing percent oxygen saturation of myoglobin and haemoglobin as a function of partial pressure of oxygen is given in Fig. 4.

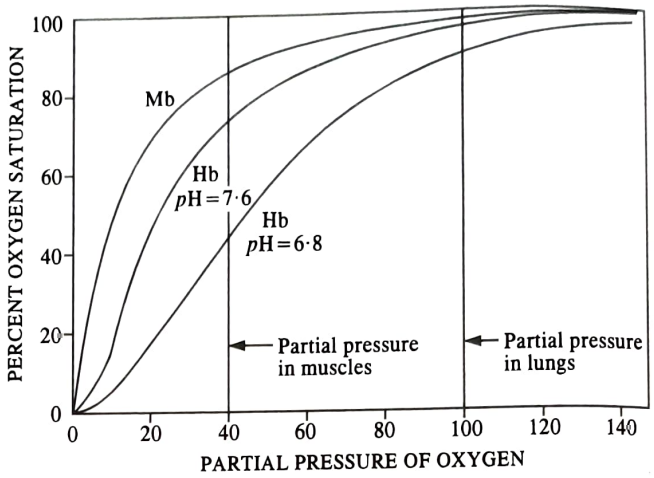

Fig. 4. A plot of percent oxygen saturation of myoglobin and haemoglobin vs partial pressure of oxygen.

It can be seen from the graph that at the partial pressure of oxygen prevailing in the lungs (which is around 100–120 mm Hg), both haemoglobin and myoglobin are almost completely saturated with oxygen. However, at the low partial pressure of oxygen prevailing in the muscles (20–40 mm Hg), haemoglobin is a much poorer oxygen binder compared to myoglobin. Hence in the muscle tissues where myoglobin is already present, oxygenated haemoglobin passes on its oxygen to myoglobin. The working muscles consume this oxygen to produce energy and carbon dioxide.

The binding power of haemoglobin with oxygen is pH-dependent. This is called **Bohr effect.** The binding power of haemoglobin decreases with decrease in pH. Hence, the transfer of oxygen from oxygenated haemoglobin to myoglobin is more efficient in the working muscles where the CO_2 concentration is higher than that in the resting muscles. CO_2, being acidic, decreases the pH.

Explanation for Cooperativity Effect in Haemoglobin

The following explanation is generally offered for the cooperativity effect in haemoglobin.

In hemoglobin, the haeme group is dome-shaped having the iron atom about 0·5 Å out of the porphyrin plane and the Fe—N bond of histidine residue of polypeptide chain about 8° off the perpendicular to the porphyrin plane, as shown above in Fig. 3.

When an oxygen molecule binds to the iron atom of a haeme group through its vacant sixth coordination site, the iron atom becomes low spin and, therefore, becomes smaller in radius (the low spin Fe(II) as well as Fe(III) have smaller radii than that of the high spin Fe(II)). As a result, the low spin iron moves towards the porphyrin plane and *just* fits in the hole generated by the four coordinating nitrogens of the porphyrin plane. This automatically pulls the coordinated histidine to move by about 0·5 Å towards the plane thereby making the Fe—N (histidine) bond vertical. These changes in the heme unit due to its coordination with O_2 form the trigger of cooperativity phenomenon.

The movement of iron atom and the coordinated histidine towards the porphyrin plane results in the movement of the whole polypeptide chain of which the histidine group is a part. This results in the breaking of some of the interpolypeptide salt bridges. As already mentioned, the presence of salt bridges introduces strain in the haemoglobin molecules. Hence the rupture of these salt bridges relaxes the constrained haemoglobin molecule. Conformational changes that subsequently occur in the polypeptide chains of the relaxed molecule increase the sizes of the crevices engulfing the remaining haeme groups

with the result that the sixth coordination sites of the iron atoms in the other haeme groups become more approachable to the attacking oxygen molecules. There is thus a progressive increase in the binding constants of the successive oxygenation reactions of haemoglobin that follow the first oxygenation reaction, as shown below :

$$Hb + O_2 \overset{K_1}{\rightleftharpoons} HbO_2$$

$$HbO_2 + O_2 \overset{K_2}{\rightleftharpoons} Hb(O_2)_2$$

$$Hb(O_2)_2 + O_2 \overset{K_3}{\rightleftharpoons} Hb(O_2)_3$$

$$Hb(O_2)_3 + O_2 \overset{K_4}{\rightleftharpoons} Hb(O_2)_4$$

$$K_4 > K_3 > K_2 > K_1$$

The above explanation of cooperativity effect is called **trigger mechanism**.

It may be of interest to know that the Fe—O_2 bond in both oxyhaemoglobin and oxymyoglobin is bent, as shown in Fig. 5. This is due to the fact that the sixth coordination site of iron, being sterically overcrowded as discussed earlier, does not have enough space for a straight Fe—O—O link.

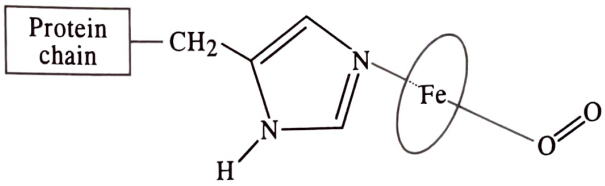

Fig. 5. Structure of oxyhaemoglobin and oxymyoglobin.

Both oxygenated haemoglobin [$Hb(O_2)_4$] and oxygenated myoglobin [MbO_2] are diamagnetic in character. This observation can be explained either by formulating that in the heme group of both oxyhaemoglobin and oxymyoglobin, a singlet oxygen is bound to low spin iron(II) or by assuming that each heme group in these compounds contains an $Fe^{III}—O_2^-$ link in which the only unpaired electron of low spin Fe(III) and odd electron on O_2^- are strongly coupled antiferromagnetically. However, none of the two explanations are universally accepted.

It is interesting to note that a haeme group, which is without a polypeptide chain, takes up an oxygen molecule to finally yield a stable μ-oxoproduct, as shown in Fig. 6.

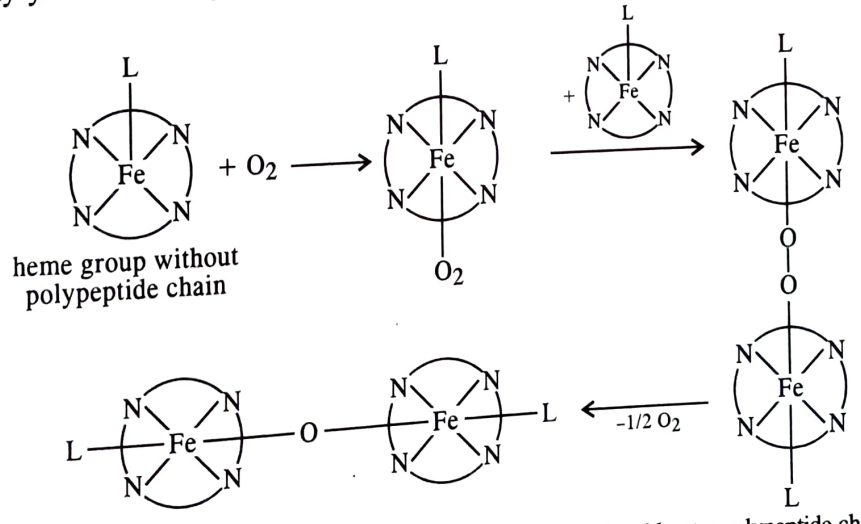

Fig. 6. Formation of μ-oxoproduct from a haeme group which is without a polypeptide chain

This reaction is irreversible. The essential condition for the formation of the μ-oxoproduct is that the two reacting heme groups should be able to come in contact with each other. Nature, however, avoids such irreversible oxidation process by surrounding the heme groups in haemoglobin and myoglobin by bulky polypeptide chains so that the direct contact between the heme groups becomes impossible. This explains why the oxygen intake by haemoglobin and myoglobin is completely reversible.