4.17: Stereochemical Changes Upon Ligation

Upon binding a second axial ligand, the iron center together with the axial base move toward the plane of the porphyrin, initiating a change in spin state from high-spin to low-spin when the sixth ligand is O_2 or CO or any other strong ligand with an even number of valence electrons. Given these general features, what are the structural differences between systems that bind O_2 with high affinity and those that bind O_2 with low affinity? The answers to this question are relevant to understanding at the molecular level the mechanism of cooperativity, where a low-affinity conformation, the T state, and a high-affinity conformation, the R state, are in dynamic equilibrium in one tetrameric molecule. In looking at crystallographic data one sees a particular conformation frozen in the crystal, usually the one of lowest free energy among many in equilibrium in the solution state. The R $\rightleftharpoons$ T equilibrium for hemoglobin is moderately rapid, at 4 x 10^3 s^{-1}; hemocyanin also switches quaternary conformations with a similar rate constant.[4]

Human hemoglobins are a heterogeneous group. Many mutants are known, and several have been structurally characterized. A structural alteration that affects the equilibrium between R and T states has a marked effect on ligand affinity and cooperativity in hemoglobin. If a specific amino-acid substitution destabilizes the T state, then the transition to the R state will occur earlier in the ligation process, and the hemoglobin will have an increased oxygen affinity. Hemoglobin Kempsey is an example. In this mutant an aspartic acid on the β chain is replaced by asparagine. Conversely, if the R state is destabilized, then the hemoglobin will have a lowered oxygen affinity. Hemoglobin Kansas is an example. Here an asparagine on the β chain has been replaced by threonine.[9]

Structural Changes in Normal-affinity Systems

It was proposed earlier that the molecule Fe(PF)(1-MeIm)(O_2) in the solid or solution state was a fair approximation to the reference gas-phase molecule. The axial base, although not oriented for minimization of contacts with the porphyrin (i.e., ϕ = 45°), is well-removed from an eclipsing orientation where $\phi = 0 \pm 10°$; the Fe atom is centered in the plane of a highly planar porphyrin; the O_2 ligand is oriented for minimization of contacts with the porphyrin, and its geometry is largely unconstrained by distal groups (the pickets); no groups are hydrogen-bonded to the axial base. The major difference from the reference state is that there is a significant attractive interaction between the electronegative dioxygen moiety and the amide groups on the pickets, and a smaller repulsive interaction with the picket t-butyl groups.[176]

For the CO adduct, contacts with the pickets are all at ideal van der Waals' separations and the Fe—CO moiety is free to assume its normal linear geometry. For CO binding the reference molecule is again the carbonyl adduct of the iron picket-fence porphyrinato molecule. In contrast to O_2 binding, there are no specific distal effects, such as hydrogen bonding, by which CO affinity may be increased; there remain many ways, as with O_2 binding, by which CO affinities may be reduced. Thus, the CO binder with highest affinity is the iron picket-fence porphyrin.

The O_2 affinities of myoglobin, R-state hemoglobin, and the Fe(PF)(1-MeIm) system are similar. However, the means by which this is achieved are different, and this difference is reflected most clearly in the kinetics of binding and release of O_2, which for Mb are much slower. The similarities and differences are summarized in Table 4.9, which is culled from Tables 4.2, 4.4, and 4.5.

Table 4.9 - Comparison of the picket-fence porphyrin system with Mb.

a) Solution state studies on Fe(PF-Im). Structural details on Fe(PF)(1-Melm)(O_2) and Fe(PF)(2-Melm).

Characteristic	Mb	Fe(PF-Im)[a] Fe(PF)(1-MeIm)	Fe(Poc-PF) (1-MeIm)
$P_{1/2}(O_2)$, Torr	0.7	0.58	0.36
$P_{1/2}$(CO), Torr	0.018	0.000022	0.0015
$k_{on}(O_2)$, $\mu M^{-1}s^{-1}$	15	430	2.2
$k_{off}(O_2)$, s^{-1}	10	2,900	9
k_{on}(CO), $\mu M^{-1}s^{-1}$	0.50	36	0.58
k_{off}(CO), s^{-1}	0.015	0.0078	0.0086
Solvent	H_2O/PO_4^{3-}	toulene	toulene
Local Environment	His (H_2O) polar, protic	H-N(amide) polar, aprotic	H-N (amide) phenyl

Characteristic	Mb	Fe(PF-Im)[a] Fe(PF)(1-MeIm)	Fe(Poc-PF) (1-MeIm)
O_2 • • • distal, Å	2.97(18)	NH 4.06(5) CH_3 2.67(6)	
CO • • • distal, Å (calc.)	2.7	NH 5.0 CH_3 3.3	
Fe-N_{Im}(deoxy), Å	2.22	2.095(6)	
Fe-N_{Im}(oxy), Å	2.07(6)	2.07(2)	
Fe-O, Å	1.83(6)	1.75(2)	
ϕ_{Im} (deoxy), deg	19°	22.8°	
ϕ_{Im} (oxy), deg	1°	20°	
ϕ_{O2}, deg	~0°	45°	
Fe • • • porph(deoxy), Å	0.42	0.43	
Fe • • • porph(oxy), Å	0.18	-0.03	

For the cobalt-dioxygen derivative, the putative hydrogen bonding between the dioxygen and the amide groups of the pickets assumes greater importance because the coordinated dioxygen is substantially more negative. Again the picket-fence porphyrin, being structurally characterized, is the reference system. Although no Co picket-fence porphyrin structures have been determined, the structures may be predicted with confidence from the iron analogues together with related structures of Co^{II} and Co^{III} tetraphenylporphyrinato systems.*

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* For $CoHbO_2$, single-crystal EPR spectra have been interpreted in terms of a nearly triangularly coordinated O_2,[197] although a crystal structure of $CoMbO_2$ shows a bent CoOO group.[198] There is no precedent for this triangular arrangement in any Co^{III}-superoxo (O_2^{1-}) system, whereas there are many for angularly coordinated O_2 in electronically not dissimilar square-planar Schiff-base systems.[66] Regardless of geometry, the picket amide • • • O_2 contacts do not change substantially.

Structural Changes in Low-affinity Systems

The 2-methyl substituent on 2-methylimidazole is not sterically active in the five-coordinate structures Fe(PF)(2-MeIm) and Fe(TPP)(2-MeIm), since the iron atom is displaced from the plane of the porphyrin by the expected amount and the Fe—N_{Im} bond is unstretched and similar to that in deoxyhemoglobin (low O_2 affinity) and deoxyMb (higher O_2 affinity). Moreover, resonance Raman measurements also indicate little strain† in this bond.[200] In other words, there is no "tension at the heme," a key concept in early discussions of cooperativity before structures on model systems and high-resolution, refined protein structures became available.[11a] On moving into the plane of the porphyrin upon oxygenation, the 2-methyl substituent prevents the Fe-imidazole group from achieving its optimum geometry with the iron at the center of the porphyrin hole, as seen in the structure of Fe(PF)(1-MeIm)(O_2). Thus, the sterically active 2-methyl substituent leads to lowered O_2 (and CO) affinity relative to the 1-methyl analogue. In metrical terms the lowered affinity is reflected in an increase in the sum of the axial bond lengths from 1.75 + 2.07 = 3.82 Å to 1.90 + 2.11 = 4.01 Å.

In the crystal structure of Fe(C_2Cap)(1-MeIm)(CO) the cap is about 5.6 Å from the porphryin plane.[121d] Hence, in the crystal structures of the free base H2(C_2Cap)[201a] and FeCI(C_2Cap)[201b] species, in which the cap is screwed down to approximately 4.0 Å from the porphyrin plane, considerable conformational rearrangement of the cap and the four chains attaching it to the porphyrin is needed to provide room for a small ligand such as CO. This is even more pronounced in a Co(C_3Cap) complex where the cap is only 3.49 Å from the mean porphyrin plane.[202] Thus not only is affinity for CO lowered, but some additional discrimination against it is induced, since a linear, perpendicular coordination creates considerable strain energy elsewhere in the molecule.

For the pocket porphyrin (Figure 4.23), structural data are available on the carbonyl adduct.[121b]The CO ligand is unable to achieve the linear perpendicular geometry seen in the high-affinity picket-fence porphyrin derivative, Fe(PF)(1-MeIm)(CO),[110]and distortion of the porphyrin core is greater. In the pocket-porphyrin system, O_2 affinity is unaffected, but CO affinity is lowered.

The crystal structure of partially oxygenated hemoglobin, $[\alpha\text{-}FeO_2]_2[\beta\text{-}Fe]_2$,[191a] reveals that the quaternary structure, except in the immediate vicinity of the α hemes, which have O_2 coordinated, resembles that of T-state deoxyhemoglobin rather than R-state liganded hemoglobin. In accord with the low affinity of T-state hemoglobin, the Fe—N_{Im} bonds for the six-coordinate α-hemes at 2.37 Å are significantly longer than those in fully oxygenated R-state oxyhemoglobin, $[\alpha\text{-}FeO_2]_2[\beta\text{-}FeO_2]_2$ in the notation above, (1.94 (α-hemes) and 2.06 Å (β hemes)) and that found in oxymyoglobin (2.07 Å). In contrast to the R-state structure and oxyMb, the a-hemes are folded as seen in the deoxy parent, leaving the Fe still substantially displaced (0.2 Å) from the plane of the four pyrrole nitrogen atoms. The deoxyhemoglobin T-state quaternary structure also has been observed in two other partially liganded hybrid hemoglobins, $[\alpha\text{-}FeCO]_2[\beta\text{-}Mn(II)]_2$[203] and $[\alpha\text{-}Ni]_2[\beta\text{-}FeCO]_2$.[191d] Again, structural changes upon coordination do not propagate beyond the immediate vicinity of the liganded heme to the critical $\alpha_1\beta_2$ interfaces.

Note that although the crystal structure of hemoglobin A reveals that access to the binding site for the β chains is blocked by groups at the entrance to the cavity above the iron center, this does not prevent facile access to the binding site; the rate of O_2 binding is slowed by a factor of only five. A similar situation occurs also for vertebrate myoglobins.

The large structural differences that exist between deoxy (T) and oxy (R) hemoglobin and the much smaller differences between deoxy (T) and partially liganded (T) hybrid hemoglobin are shown in Figure 4.32.[203]

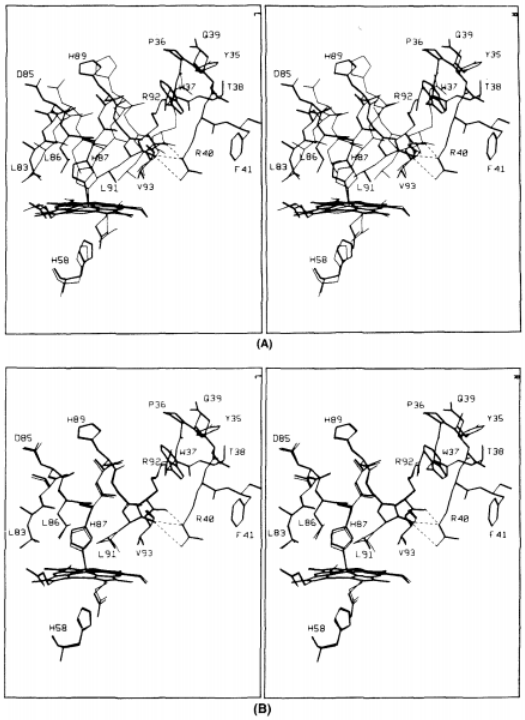

Figure 4.32 - The effects of changes in ligation and in quaternary structure on stereochemistry in the vicinity of α hemes:[203] (A) Stereodiagram of the large structural differences between deoxy T-state (heavy lines) and oxy R-state hemoglobin (light lines). (B) Stereodiagram of the small structural differences between deoxy T-state (heavy lines) and partially liganded T-state $[\alpha\text{-}FeCO]_2[\beta\text{-}Mn]_2$ hemoglobin (light lines). Reproduced with permission from A. Arnone et al., J. Mol. Biol. **188** (1986), 693-706.

Because of the steric hindrance afforded by the distal histidine, all biological systems have low affinity for CO relative to the picket-fence porphyrins, with the exception of mutants where the distal histidine has been replaced by glycine. Thus low affinity to CO is associated primarily with the inability of the Fe-CO group to achieve its preferred linear geometry perpendicular to the porphyrin.

Low-affinity O_2 binding in the hemoglobins appears to be associated with the inability of the Fe-proximal histidine unit to move into the plane of the porphyrin and less so to distal effects, such as a cavity too small to accommodate the coordinated ligand. The blocked access to the site affects the kinetics but not necessarily the thermodynamics of ligand binding, as evidenced by the structure of T-state $[\alpha\text{-}FeNi]_2[\beta\text{-}FeCO]_2$[191d] Some similarities between the structures and properties of partially oxygenated (T-state) $[\alpha\text{-}FeO_2]_2[\beta\text{-}Fe]_2$ hemoglobin and Fe(PF)(2-MeIm)(O_2) are provided in Table 4.10. In the synthetic systems low O_2 affinity can be induced by 2-methyl substituents—a restraint on the movement of the Fe-imidazole moiety analogous to that provided by the protein chain. A second means is by distal effects, such as caps and straps.

Table 4.10 - Comparison of the low-affinity picket-fence porphyrin system with low-affinity (T-state) partially liganded hemoglobin.

a) Ligand binding to Fe(PF)(1,2-Me_2Im)

Characteristic	HbA^T $[\alpha\text{-}FeO_2]_2[\beta\text{-}Fe]_2$	Fe(PF)(2-MeIm) Fe(PF)(1,22-Me_2Im)[a]
$P_{1/2}(O_2)$, Torr	46, first O_2	38
$P_{1/2}$(CO), Torr	~0.7, 1st CO	0.0089
$k_{on}(O_2)$, $\mu M^{-1}s^{-1}$	2.9(α)	106
$k_{off}(O_2)$, s^{-1}	183(β)	46,000
k_{on}(CO), $\mu M^{-1}s^{-1}$	0.099	1.4
k_{off}(CO), s^{-1}	0.09	0.14
Solvent	Tris buffer, pH 7, no 2,3-DPG	toluene
Local Environment	histidine polar	H-N(amide) polar, aprotic
O_2 • • • distal, Å	?	NH 3.88 CH_3 2.77(3)
CO • • • distal, Å (calc.)		NH 4.9 CH_3 3.5
Fe-N_{Im}(deoxy), Å	2.13(6) (average of α and β	2.095(6)
Fe-N_{Im}(oxy), Å	2.24(10)	2.107(4)
Fe-O, Å	1.82(4)	1.898(7)
ϕ_{Im} (deoxy), deg	21(3) (average of α and β	22.8
ϕ_{Im} (oxy), deg	~6	22.2
ϕ_{O2}, deg	?	45
Fe • • • porph(deoxy), Å	0.38(5) (average of α and β	0.43
Fe • • • porph(oxy), Å	0.19(5)	0.09

† Shortly (10^{-9}-10^{-12} s) after a ligand dissociates, a large difference in ν(Fe—N_{Im}) between R and T structures is observed, prior to relaxation to the equilibrium R and T conformations.[199]

Structural Changes in High-affinity Systems

Few structural data are available for high-affinity oxygen carriers. The crystal structures of two leghemoglobin derivatives, a monomeric myoglobin-like oxygen carrier found in the nitrogen-fixing nodules of legumes, are known at 2.0 and 3.3 Å.[204,205] The binding pocket appears more open, perhaps allowing H_2O to enter and partake in stronger hydrogen bonding than that offered by the distal imidazole. Consistent with this notion is the more rapid rate of autoxidation observed for oxyleghemoglobin. *Aplysia* oxymyoglobin, which lacks a distal histidine, also autoxidizes rapidly,[204] although a distal arginine further along the helix E, E10Arg, fulfills the role of the distal histidine by hydrogen bonding to the sixth ligand, at least in the fluoride derivative, met-MbF.

Although no structural data are available, a tenfold increase in O_2 affinity was observed between an ester-strapped porphyrin, offering no hydrogen-bonding possibilities, and its conformationally very similar amide analogue. O_2 • • • amide hydrogen bonding was demonstrated by means of NMR shift data (Zn and Fe—CO complexes vs. the Fe—O_2 complex) and from infrared spectroscopy, which showed shifted amide N—H absorptions.[166]

The *specific* structural features that lead to the extraordinarily high affinity for O_2 and low affinity for CO in hemoglobin *Ascaris* remain unidentified. This high affinity is due to an *extremely* slow dissociation rate of O_2 of only $0.1 s^{-1}$; in most hemoglobins the rate is about 10 to 2,500 s^{-1} (Table 4.2). Dioxygen binding is thus close to irreversible. Figure 4.27 shows that hydrogen bonding to the coordinated dioxygen ligand, unrestrained motion of the Fe-proximal histidine group into the plane of the porphyrin, hydrogen bonding to the proximal histidine, and, in the deoxy form, compression of the Fe—N_{Im} bond and decrease in the out-of-plane displacement of the Fe atom will all increase O_2 affinity over that of a system where these effects are absent.

When hydrogen bonding is impossible, as in various synthetic systems (Table 4.5) as well as hemoglobin *Glycera* and Mb(E7His → Gly), O_2 affinity is much lower than when hydrogen bonding can occur (see Table 4.6), especially for the cobalt analogues. But caution is needed in the absence of complete structural information: the lowered affinity of *Aplysia* hemoglobin had been attributed to the lack of a distal histidine and its attendant hydrogen-bonding capabilities. However, the crystal structure reveals that an arginine residue, normally directed out into the solution, is capable of folding back into the ligand-binding pocket and of hydrogen bonding to ligands at the sixth site. In oxyhemerythrin the hydrogen bonding of the coordinated hydroperoxy group to the oxo bridge linking the two iron atoms (Figure 4.10B), described in Section II.F.1, may not only increase the stability of oxyhemerythrin,[146] but also facilitate electron transfer that occurs in dioxygen binding.[205]
