4.3: Detailed Structures of Hemoglobins and Model Systems

With thermodynamic background and general structural features relevant to ligand affinity enumerated, attention may now be turned to the detailed structural aspects of the active site and its surroundings. As was shown crudely in Figure 4.3, the ligand affinity of an iron porphyrin may be perturbed either by modulating the structure of the deoxy material or by modulating the structure and surroundings of the liganded material or both. The model systems provide the reference points against which the protein structures may be compared.

Structures Relevant to Deoxy Hemoglobins

The structure of the picket-fence porphyrin compound, Fe(PF)(2-MeIm), is shown in Figure 4.28.[172] Minus the pickets, it is essentially a magnified view of the active site of deoxymyoglobin, shown in Figure 4.29.[181] Some metrical details of these structures, of a very similar unsubstituted tetraphenylporphyrin,[110] and of several other deoxyhemoglobins[11c,182-185] are listed in Table 4.7. In general they are all similar, but important differences exist.

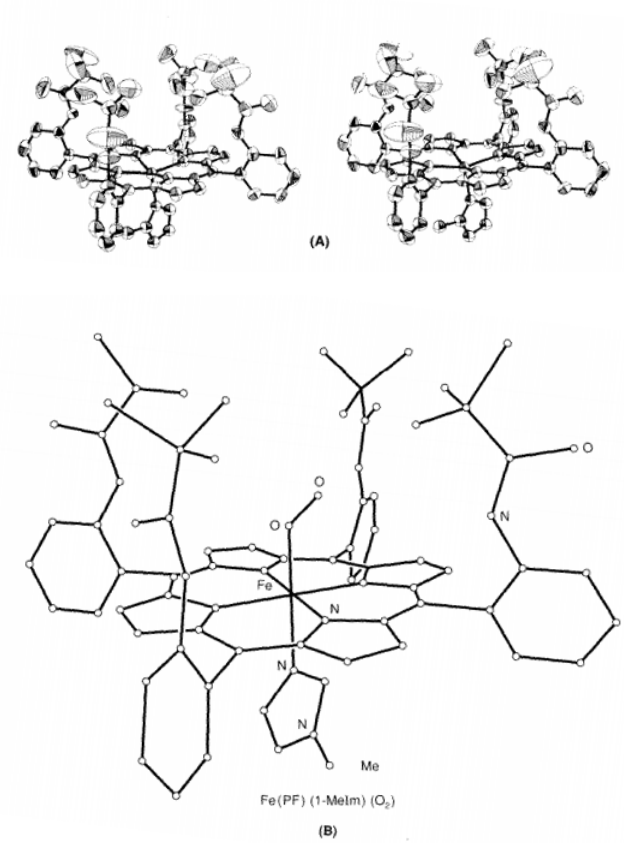

Figure 4.28 - (A) Stereodiagram of the structure of Fe(PF)(2-MeIm).[172] (B) Structure of Fe(PF)(1-MeIm)(O_2).[187]

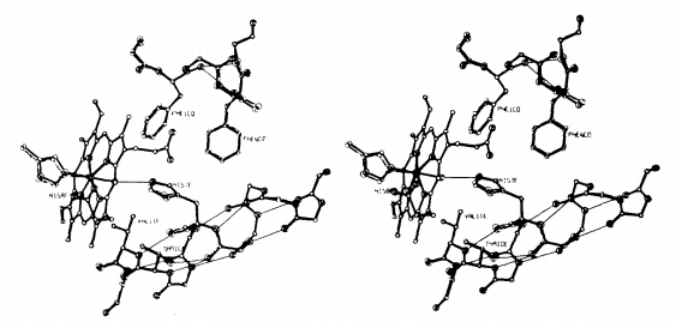

Figure 4.29 - Structure of metmyoglobin and deoxymyoglobin at 2.0 Å resolution near the heme.[181a] Solid bonds are for metmyoglobin; open bonds for deoxymyoglobin. Note the water molecule coordinated to the iron center and hydrogen bonded to the distal imidazole group in metmyoglobin. Reproduced with permission from T. Takano, J. Mol. Biol. **110** (1977), 569-584.

Table 4.7 - Metrical detalis of deoxyhemoglobins and their models[a]

a) See Figure 4.25 for definition of symbols.

b) From a difference refinement of CoHb vs. Hb, where the difference in metal-to-porphyrin-plane separation was 0.24(2) Å and the difference in M-N_{Im} was 0.13(4) Å. Doming is similar to Hb.

Compound	Resol. (Å)	Fe-N_p (Å)	Fe • • • Porp (Å)	Doming (Å)	Fe—N_{Im} (Å)	ϕ (deg)	Tilt (deg)
Fe(PF)(2-Melm)	—	2.072(5)	0.43	0.03	2.095(6)	22.8	9.6
Fe(TPP)(2-Melm)	—	2.086(6)	0.40	0.13	2.161(5)	7.4	10.3
Mb	1.4	2.03(10)	0.42	0.08	2.22	19	11
Er • • • H_2O	1.4	2.02	0.17	-0.06	2.25	7	3
HbA (α • • • H_2O)	1.74	2.08(3)	0.40(5)	0.16(6)	2.16(6)	18(1)	12(2)
HbA (β • • • H_2O)	1.74	2.05(3)	0.36(5)	0.10(6)	2.09(6)	24(1)	11(2)
CoHb[b]	2.5	—	0.14(5)	0.13	2.24(6)	—	—
Co(TPP)(1-MeIm)	—	1.977(6)	0.13	0.01	2.157(3)	3.8	0
Co(TPP)(1,2-Me_2Im)	—	1.985(3)	0.15	0.05	2.216(2)	10	—

In all structures, except deoxyerythrocruorin,[183] the iron atom is displaced about 0.4 to 0.5 Å from the plane of the porphyrin toward the axial base. For deoxyerythrocruorin the displacement is less than half this, perhaps because the water molecule is weakly coordinated to the iron center.

An imidazole group from a histidine residue—the distal histidine E7 in position 7 on helix labeled E—hovers over the binding site for most vertebrate hemoglobins, except for genetically engineered mutants of human hemoglobin (βE7His → Gly), pathological mutant hemoglobins, such as hemoglobin Zürich (βE7His → Arg), and some others, such as elephant hemoglobin. Long believed to be noncoordinating, this distal histidine may, in fact, coordinate weakly to the Fe center at low temperature.[159] In the α chains of human deoxyhemoglobin, hemoglobin A, a water molecule is found in the binding cavity.[182] For many years the binding cavity has been referred to as the hydrophobic pocket—literally, water-hating. Although many hydrophobic groups, such as valine, leucine, isoleucine, and phenylalanine are positioned over the porphyrin, the immediate environment around the binding site is, in fact, polar, with the distal histidine and associated water molecules, as well as the heme group itself. As will be shown in the next section, the label "hydrophobic pocket" becomes more misleading when the interaction of coordinated ligands with distal groups is examined.

The orientation of the axial base, angle ϕ_1, is similar for Fe(PF)(2-MeIm) and for several vertebrate deoxyhemoglobins. On the other hand, Fe(TPP)(2- Melm) and deoxyerythrocruorin have a similar eclipsed axial-base orientation. At least for five-coordinate species, where the iron center is substantially out of the porphyrin plane, orientation of the axial base does not invariably induce structural perturbations, e.g., doming, in the porphyrin skeleton.

The conformation of the protein chain is such that the proximal histidine in deoxyhemoglobin coordinates in a slightly tilted manner,[182,186] comparable to the tilt that the sterically active 2-methyl substituent induces in the synthetic systems.[172] Clearly, coordination of the histidine to the heme in a symmetric manner, as would be expected in the absence of the protein constraints, does *not* produce the conformation of lowest free energy for the *whole* molecule.