4.19: Structures Relevant to Liganded Hemoglobins

Stereochemistry of the Active Site

Before the advent of techniques that enabled the preparation and stabilization of oxyhemoglobin crystals, key information on the probable structure of oxyhemoglobin and thence on the mechanism of cooperativity was extrapolated from structures of methemoglobin derivatives[11] and from various five- and six-coordinate cobalt-porphyrinato complexes.[110,112,113] The structures of these met derivatives have proved to be similar to that of oxyhemoglobin, at least in the stereochemistry of the metalloporphyrinato species and for the protein tertiary and quaternary structure as well.

Two synthetic iron-dioxygen adducts built from the picket-fence porphyrins have been structurally characterized.[172,187] The high, effectively fourfold symmetry of the binding pocket in these systems results in fourfold disorder of the angularly coordinated dioxygen molecule, and precludes the precise and accurate measurements of the Fe—O—O angle and O—O separation that are grist to the theoretical mills.* Figure 4.28B illustrates the stereochemistry for one conformer. Subsequently, the structures of several dioxygen adducts of biological oxygen carriers have been determined.[183,188-191] Although the dioxygen moiety is usually ordered, the precision is tantalizingly just less than that needed to decide whether the apparently more-linear geometry seen for oxyerythrocruorin[183] and oxyhemoglobin[190] is significantly different from that for oxymyoglobin[188] and therefore attributable to the water molecule or imidazole that is hydrogenbonded to the coordinated dioxygen ligand. Nonetheless, several interesting differences emerge.

The axial base in oxymyoglobin and oxyhemoglobin is almost eclipsed; that is, $\phi_1 \approx 0°$. The axial base has moved from a tilted position in deoxyhemoglobin to a symmetric one in oxyhemoglobin. In the absence of steric constraints, the iron atom is essentially in the center of the porphyrin plane for Fe(PF)(1-MeIm)(O_2), oxymyoglobin, and oxyhemoglobin. For the 2-methyl analogue, Fe(PF)(2-MeIm)(O_2), the iron remains significantly out of the plane, as also appears to occur for oxyerythrocruorin.

In the structure of Fe(TPP)(Py)(CO), Figure 4.30, a model for carbonyl hemoglobins, the iron atom is in the plane and the Fe—C ≡O bond is linear and perpendicular, as expected.[118] Not so for carbonyl hemoglobins, where the blob of electron density that is identified with the coordinated carbon monoxide lies substantially off the normal to the porphyrin. We return to this point shortly. In general, with the exception of the coordinated ligand, the structures of sixcoordinate low-spin hemoglobins, whether Fe^{II} or Fe^{III}, are similar. Indeed, the refined structures of oxy- and carbonmonoxyhemoglobin are superimposable within experimental uncertainties, except in the immediate vicinity of the diatomic ligand. Some metrical details are given in Table 4.8.[11c,118,121,172,183,187,188,190-192]

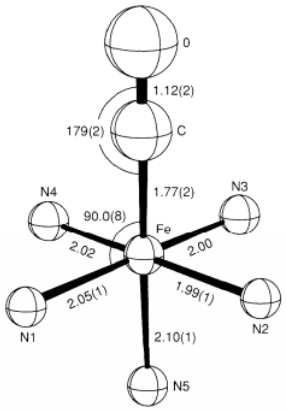


Figure 4.30: Molecular structure of Fe(TPP)(py)(CO), a model for unhindered coordination of CO.[118]

Table 4.8 - Metrical details of selected liganded hemoglobins and their modelsa a) See Figure 4.25 for definition of symbols. b) Alternative interpretation of EXAFS data.

Compound	Resol. Å	Fe-N_p Å	Fe-porph Å	Doming Å	Fe-L_1 Å	Fe-L_2 Å	Fe-XY deg.	$\varnothing_1$ deg.	$\varnothing_2$ deg.	Tilt deg.
Dioxygen Adducts										

Compound	Resol. Å	Fe-N_p Å	Fe-porph Å	Doming Å	Fe-L_1 Å	Fe-L_2 Å	Fe-XY deg.	$\varnothing_1$ deg.	$\varnothing_2$ deg.	Tilt deg.
Fe(PF)(1-MeIm)(O_2)	—	1.98(1)	-0.03	0.02	2.07(2)	1.75(2)	131(2)	20	45	0
Fe(PF)(2-MeIm)(O_2)	—	1.996(4)	0.09	0.02	2.107(4)	1.898(7)	129(1)	22	45	7
MbO_2	1.6	1.95(6)	0.18(3)	0.01	2.07(6)	1.83(6)	115(4)	1	~0	4
	EXAFS	2.02(2)	—	—	2.06(2)	1.80(2)	123(4) 148(8)[b]	—	—	—
ErO_2	1.4	2.04	0.38	-0.08	2.1	1.8	150	7	3	—
HbO_2 α	2.1	1.99(5)	0.12(8)	0.04	1.94(9)	1.66(8)	153(7)	11	0	3
HbO_2 β		1.96(6)	-0.11(8)	0.11	2.07(9)	1.87(3)	159(12)	27	45	5
	EXAFS	1.99(2)		—	2.05(2)	1.82(2)	122(4) 143(8)[b]	—	—	—
[α-FeO_2]$_2$[β-Fe]$_2$ α	2.1	2.04(4)	0.19(5)	0.17(5)	2.24(10)	1.82(4)	153(4)	6	—	11
[α-FeO_2]$_2$[β-Fe]$_2$ β			0.3	—	2.2	—	—	—		
Carbon monoxy Adducts										
MbCO	1.5	1.97(3)	0.00	0.03	2.2	1.9	140	—	30	—
	EXAFS	2.01(2)	—	—	2.20(2)	1.93(2)	127(4) 145(8)[b]	—	—	—
ErCO	1.4	2.01	-0.11	-0.10	2.1	2.2	161(9)	7	—	1
HbCO α	2.2	2.02	-0.10	—	1.95	1.83	175(15)	—	—	—
HbCO β		2.03	-0.10	—	2.20	1.70	171(15)	—	—	—
[α-Ni][β-FeCo] α	2.6	—	—	—	—	—	—	—	—	—
[α-Ni][β-FeCo] β			0.15	0.12	2.23(5)	—	—	—	—	—
Fe(TPP)(Py)(CO)		2.02(3)	-0.02	-0.02	2.10(2)	1.77(2)	179(2)	45	—	~0
	EXAFS	2.02(2)	—	—	2.09(2)	1.81(2)	138(6) 180(11)[b]	—	—	—

Compound	Resol. Å	Fe-N_p Å	Fe-porph Å	Doming Å	Fe-L_1 Å	Fe-L_2 Å	Fe-XY deg.	$\varnothing_1$ deg.	$\varnothing_2$ deg.	Tilt deg.
Fe(poc)(1,2-Melm)(CO)		1.973(8)	0.001	—	2.079(5)	1.768(7)	172.5(6)	—	—	—
Fe(C_2Cap)(1-Melm)(CO)		1.990(7) 1.988(13)	0.01 0.02	—	2.043(6) 2.041(5)	1.742(7) 1.748(7)	172.9(6) 175.9(6)	—	—	—

Interactions of Coordinated Ligands with Distal Groups

Without exception to date (but see footnote 1 in Reference 168), in structurally characterized oxyhemoglobins, the coordinated dioxygen ligand is hydrogen-bonded to the distal histidine or to a water molecule—even though theoretical calculations show that hydrogen bonding would destabilize M—O_2 moieties.[192] This universal observation of hydrogen bonding in these biological systems is consistent with notions that electron density accumulates on the dioxygen molecule upon coordination. Given the errors associated with atomic positions (at best, ±0.20 Å) the x-ray crystallographic evidence could be equivocal, since hydrogen atoms on the distal imidazole are not observed. There are at least three lines of evidence that support the existence of a specific $O_2 \bullet \bullet \bullet$ HN interaction. First, the EPR spectrum of cobalt oxyhemoglobin indicates that the coordinated dioxygen is hydrogen-bonded to something.[177-179] Second, and more directly, in the neutron-diffraction structure of oxymyoglobin,[189] where hydrogen and especially deuterium nuclides scatter strongly, the imino hydrogen or deuteron was located on the nitrogen atom closest to the coordinated dioxygen, as illustrated in Figure 4.31A. In contrast, in the neutron-diffraction structure of carbonmonoxymyoglobin, the alternative imidazole tautomer was observed (Figure 4.3IB).[125,189] The absence of hydrogen bonding of the distal imidazole residue with the coordinated CO molecule is consistent with other lines of evidence that there is little accumulation of electron density on the carbonyl ligand. Third, but less directly, genetically engineered mutants have been produced in which the distal histidine has been replaced by glycine—sperm whale Mb E7His → Gly, and HbαE7His → Gly and HbA βE7His → Gly.[35b,192] For the myoglobin mutant, the O_2 binding rate constant at room temperature increases by an order of magnitude, but the dissociation rate constant increases by two orders of magnitude, leading to a decrease in affinity of more than an order of magnitude, as derived from k_{on}/k_{off}. This leads to an estimate of the free energy associated with hydrogen bonding of

$$\Delta G = -RTlog\left[\frac{P_{1/2}(O_2)\cdot Native}{P_{1/2}(O_2)\cdot Mutant}\right] = 1.5kcal/mol. \qquad (4.19.1)$$

In addition, this mutant myoglobin autooxidizes rapidly compared to the native one. On the other hand, the affinity for CO is greatly increased, leading to a value of M for the mutant of 1300, compared to 16 for the native. Thus the distal histidine stabilizes a coordinated O_2 ligand by hydrogen bonding and destabilizes a coordinated CO ligand by steric clash.

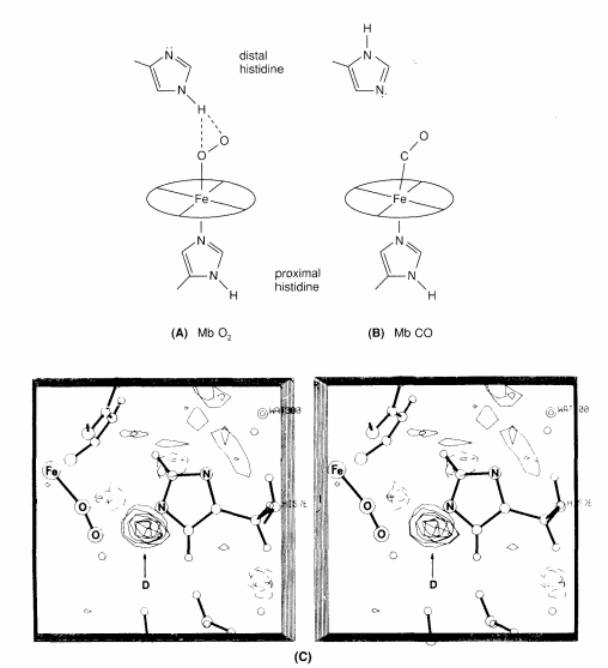
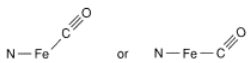


Figure 4.31 - (A) The hydrogen bonding interaction between coordinated dioxygen and the distal histidine. (B) The interaction of coordinated carbon monoxide and the distal histidine, showing the absence of hydrogen bonding. (C) Stereodiagram of a neutron-difference density map for oxymyoglobin. The refined structure showing the FeO_2 and distal histidine moieties is superimposed. The imidazole deuterium atom is arrowed.[189] Reproduced with permission from S. E. V. Phillips and B. P. Schoenborn, Nature **292** (1981), 81-82.

A similar discrimination is seen for the α chains of the hemoglobin mutant in the binding of the fourth O_2 or CO molecule. For the β chains little difference is seen relative to the native protein: hydrogen bonding between the distal histidine and the coordinated dioxygen ligand appears to be much weaker in β chains, as evidenced by longer N(H) • • • O separations than those seen in the α chains. Comparison of the crystal structures of the native and mutant $\alpha_2(\beta E7His \rightarrow Gly)_2$ structures reveals negligible changes in the distal environment, except for that occasioned by the replacement of $—CH_2—C_3N_2H_3$ (histidine side chain) by —H (glycine side chain).

Studies of hemoglobin mutants where the nonpolar distal residue βValE11 ($—CH(CH_3)_2$) is replaced by alanine ($—CH_3$), isoleucine ($—CH(CH_3)CH_2CH_3$), and leucine ($—CH_2CH(CH_3)_2$) reveal that this valine offers steric hindrance to oxygen binding in the T state.

Whereas the angularly coordinated O_2 ligand fits comfortably around the distal histidine, a perpendicular and linear CO moiety cannot. Either the distal histidine rotates out of the way, or the CO tilts off axis, or the Fe—C≡O group bends, or some combination of these occurs. Notwithstanding the absence of bent M—CO moieties in the inorganic literature, reports of strongly bent M—CO groups appear in the biochemical literature.[122-127] The controversy is illustrative of the synergistic interplay of data from models and proteins, and the importance of examining a problem with a miscellany of techniques. The molecular orbital model of ligand-metal interactions presented in Figure 4.22 does not preclude a bent M—C≡O moiety on symmetry grounds. Groups related to CO can bend; the normally linearly coordinated SCN^- moiety has been observed[194] to become strongly bent under severe steric stress, with an Fe—N—CS of 140°. Unfortunately, the resolution in protein crystal structures is not sufficient to distinguish unequivocally a linear tilted stereochemistry from a bent one or from a combination of tilt and bend. Studies by the XANES technique have been interpreted in terms of a bent Fe—C≡O moiety (150°)



(4.50)

both in MbCO[127] and in the CO adduct of a chelated heme in micelles, the latter being an especially surprising result. From EXAFS data on a number of carbonyl adducts, *two* interpretations were offered: linear or moderately bent (150°) FeCO moieties for unhindered model systems, and moderately bent or strongly bent (130°) FeCO moieties for hindered synthetic and biological systems.[195] In the crystal structure of MbCO at 1.5 Å resolution,[122] the CO group is disordered, and Fe—C≡O angles of 120° and

140° were proposed, although the alternative model of tilted, nearly linear Fe—C≡O stereochemistry could not be eliminated, and is indeed far more likely to account for the off-axis nature of the oxygen position. Vibrational spectroscopy confirms the existence of two major configurations, and indicates a third minor configuration of the Fe—C≡O moiety in MbCO.[196] An elegant infrared study of the polarization of reattached carbon-monoxide molecules following photolysis of MbCO by linearly polarized light at 10 K gave tilt angles of the CO vector with respect to the heme normal of 15(3)°, 28(2)°, and 33(4)° for the three conformational substates;[196b] the former two values were confirmed in a similar study at room temperature.[196c] Note that these studies do not yield the tilt of the Fe—C bond to the heme normal.

In three synthetic compounds with severe steric hindrance, the extent of bending and tilting of the Fe—CO moiety is small. In one nonporphyrinic system the Fe—C≡O group is bent by 9.4(5)° and tilted by 4.2°.[121a] In Fe(PocPF)(1,2-Me_2Im)(CO) the Fe—C≡O angle is 172.5(6)° and modest tilting of the Fe—CO group and substantial buckling of the porphyrin ring are apparent.[121b] In Fe(C_2Cap)(1-MeIm)(CO) the two independent Fe—C≡O angles are 172.9(6)° and 175.9(6)° and modest tilting of the Fe—CO group is again apparent.[121d]

From a detailed analysis of the force constants describing the vibrational spectroscopy for the Fe—CO moiety, values of 171° for the Fe—CO angle, 9.5° for the tilt, and 11° for porphyrin buckling were calculated for MbCO.[121c] These results are particularly important, for in a model complex very closely related to Fe(Poc-PF)(1,2-Me_2Im)(CO), just mentioned, an EXAFS study[195] suggested an Fe—C≡O bond angle of 127(4)°; that same study ascribed an Fe—C≡O bond angle of around 130° to MbCO. The structure of carbonmonoxyhemoglobin, $Hb(CO)_4$, now is interpreted in terms of a nearly linear tilted geometry.[192] Clearly the geometry of attachment of CO to hemoglobins is perturbed by the surroundings of the ligand-binding site and hence the affinity of hemoglobins for CO is also perturbed. Unfortunately, a clear resolution of the geometry of the Fe—CO moiety in MbCO does not exist yet.