4.18: Structural Basis of Ligand Affinities of Oxygen Carriers

The interaction of ligands, such as dioxygen, with metal complexes, such as iron-porphyrinato systems, and the means by which this interaction is characterized, have been covered in broad outline in the previous sections. As noted earlier, the affinities of hemoglobins for carbon monoxide and dioxygen span a wide range (see Table 4.2 and Figure 4.24). In this section the active site is examined in much finer detail than before in order to develop relationships between perturbations in structure and affinity (and hence function)—so called structure-function relationships. The reference point is the somewhat hypothetical situation where the dioxygen binder is in the gas phase and independent of interactions with solvent molecules, solute molecules, and itself, and where dioxygen, carbon monoxide, and other small molecules may bind without steric constraints—in other words, a state where intrinsic affinity is measured. In this section attention is focused exclusively on the hemoglobin family and on iron- and cobalt-porphyrinato systems. In recent years structural data on hemoglobin, myoglobin, and their derivatives have become available with a precision that permits meaningful comparison with the more precisely determined model or synthetic systems. In addition, the various hemoglobins and myoglobins, and especially the naturally occurring mutants of hemoglobin A (human Hb), have provided a sort of poor man's site-directed mutagenesis. Now the techniques of molecular biology permit the site of mutation to be selected, the altered gene to be inserted into *E. coli*, and the mutant protein to be expressed in large (mg) quantities. With the conditions for crystallization of hemoglobins now well-established, we can discover quite rapidly what structural perturbations are caused by the substitution of one amino acid for another, and can relate these to the perturbations in properties, such as cooperativity, dioxygen affinity, and kinetics of ligand binding.

The principles enunciated here are applicable generally to hemerythrin and hemocyanin; however, we currently lack the thermodynamic and especially structural data we would like to have for these systems.

Ligand Affinities in Hemoglobins and Their Models

The O2 affinities in biological carriers span five orders of magnitude, which at room temperature corresponds to a difference in the free energy of oxygen binding

$$\delta\Delta G = -RTln(K_{max}/K_{min}) = -RTln(P_{1/2min}/P_{1/2max}) \tag{4.47}$$

of about 6.0 kcal/mol. This wide range of O_2 and CO affinities has not yet been paralleled in synthetic systems; the values for O_2 affinity do not exceed those for R-state human hemoglobin. A selection of values from model systems is given in Table 4.5.[23,31,160-165] For the flat-open porphyrin system (Figure 4.23) the dioxygen ostensibly binds in an unconstrained manner, but is actually subject to solvent influences. In order to obtain thermodynamic constants on these "unhindered" systems, one must gather data at several low temperatures and then extrapolate to room temperature, or obtain them from kinetic measurements, $K = k_{on}/k_{off}$, at room temperature.

For the picket-fence porphyrins, dioxygen binds in a protected pocket that is deep enough to accommodate it and to prevent the dimerization that leads to irreversible oxidation, provided that there is a slight excess of base to ensure full saturation of the coordination sites on the unprotected face of the porphyrin.[72] Thus the picket-fence, the capped, and the bis-pocket porphyrins reversibly bind dioxygen at room temperature with little oxidation over many cycles. This stability facilitated isolation of crystals of a synthetic iron-dioxygen species of the picket-fence porphyrin. The capped porphyrin offers a more highly protected site. The low affinity these latter systems have for dioxygen indicates that the binding cavity is so small that repulsive steric interactions between coordinated dioxygen and the cap are unavoidable. The left-hand side of Figure 4.24 depicts on a logarithmic scale the range of O_2 affinities. Each power of 10 corresponds to around 1.2 kcal/mol at 25 °C.

The right-hand side of Figure 4.24 illustrates the range of affinities for CO binding. For many synthetic systems the CO affinities are orders of magnitude greater than in the biological systems that have an O_2 affinity similar to the synthetic; for example, see the entries for the picket-fence porphyrin. Comparison of the left- and right-hand sides of Figure 4.24 reveals that the strongest O_2 binder, hemoglobin *Ascaris*, is one of the weakest CO binders. The O_2 affinity of the picket-fence porphyrins is very similar to that of myoglobin, but, as will be detailed shortly, one cannot infer from this that the binding sites are strictly comparable. Indeed, similar affinities have been observed with a non-porphyrin iron complex.[121,162] Moreover, if the CO affinity of myoglobin paralleled that of the picket-fence porphyrins, some 20 percent of myoglobin (and hemoglobin) would be in the carbonmonoxy form (in contrast to the approximately 3 percent that occurs naturally), a level that could render reading this section while chewing gum physically taxing.[117]

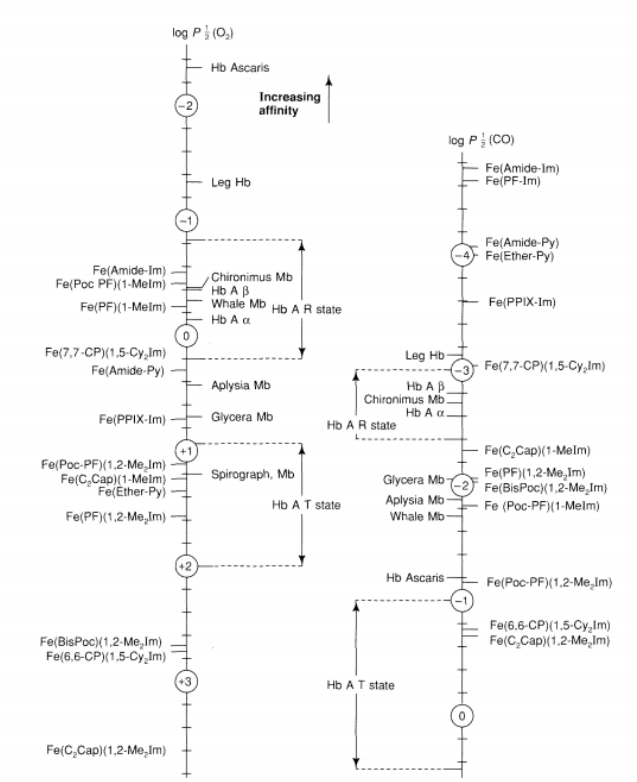

Figure 4.24 - CO and O_2 affinities of a selection of hemoglobins and model systems. Affinities are given as $P_{1/2}$, and the scale is logarithmic. One order of magnitude corresponds to 1.2 kcal/mol at 25 °C.

Table 4.5 - Thermodynamics and kinetics of ligand binding to synthetic oxygen carriers at 20-25 ºC

a) When available $P_{1/2}$ are from thermodynamic measurements, otherwise from k_{on}/k_{off}, where solubility of O_2 in toluene is 1.02 x 10^5 M/Torr and of CO in toluene is 1.05 x 10^{-2}; solubilities in benzene are very similar.

b) Some k_{off} are calculated from $K(O_2)$, $k_{on}(CO)$, and M.

Carrier	Dioxygen Binding					Carbon Monoxide Binding				
	$P_{1/2}(O_2)$ Torr	ΔH kcal/mol	ΔS eu	k_{on} $\mu M^{-1}s^{-1}$	k_{off} s^{-1}	$P_{1/2}(CO)$ Torr	ΔH kcal/mol	ΔS eu	k_{on} $\mu M^{-1}s^{-1}$	k_{off} s^{-1}
Toluene/ benzene solvent										
Picket fence, pocket										
Fe(PF-Im)	0.58	-16.3	-40	430	2,900	0.000022	—	—	36	0.0078
Fe(PF)(1,2-Me_2Im)	38	-14.3	-42	106	46,000	0.0089	—	—	1.4	0.14
Fe(Poc-PF)(1-MeIm)	0.36	—	—	2.2	9	0.0015	—	—	0.58	0.0086

Carrier	$P_{1/2}(O_2)$ Torr	Dioxygen Binding ΔH kcal/mol	ΔS eu	k_{on} $\mu M^{-1}s^{-1}$	k_{off} s^{-1}	$P_{1/2}(CO)$ Torr	Carbon Monoxide Binding ΔH kcal/mol	ΔS eu	k_{on} $\mu M^{-1}s^{-1}$	k_{off} s^{-1}
Fe(Poc-PF) (1, 2-Me_2Im)	12.6	-13.9	-28	1.9	280	0.067	—	—	0.098	0.055
Fe(Bis-Poc) (1, 2-Me_2Im)	508	-14.4	-47	—	—	0.0091	—	—	—	—
Cap										
Fe(C_2Cap) (1-MeIm)	23	-10.5	-28	—	—	0.0054	—	—	0.95	0.05
Fe(C_2Cap) (1, 2-Me_2Im)	4,000	-9.7	-36	—	—	0.20	—	—	—	—
Strapped										
Fe(7, 7-CP) (1, 5-Cy_2Im)	1.4	—	—	65	1,000	0.00091	—	—	6	0.05
Fe(6, 6-CP) (1, 5-Cy_2Im)	700	—	—	0.1	800	0.17	—	—	0.03	0.05
Flat open										
Fe(PPIX-Im)	5.6	—	—	62	4,200	0.00025	—	—	11	0.025
Bis-strapped										
Fe(Amide-Im)	0.29	—	—	310	620	0.000017	—	—	40	0.067
Fe(Amide-Py)	2.0	—	—	360	5,000	0.00009	—	—	35	0.03
Fe(Ether-Py)	18	—	—	300	40,000	0.0001	—	—	68	0.069
H_2O, alkylammonium micelles, pH 7.3										
Fe(PPIX-Im)	1.0	-14.0	-3.5	26	4.7	0.002	-17.5	-34	3.6	0.009
Fe(MPIX-Im)	0.57	—	—	22	23	0.0013	—	—	11	0.019

Carrier	$P_{1/2}(O_2)$ Torr	ΔH kcal/mol	Dioxygen ΔS eu	Binding k_{on} $\mu M^{-1}s^{-1}$	k_{off} s^{-1}	$P_{1/2}(CO)$ Torr	Carbon ΔH kcal/mol	Monoxide ΔS eu	Binding k_{on} $\mu M^{-1}s^{-1}$	k_{off} s^{-1}
Fe(MPIX -Py)	12.2	—	—	1	380	0.0021	—	—	12	0.035

There is a convenient index to summarize the extent to which CO (or O_2) binding is discriminated against for a given iron-porphyrin system. M is defined as the ratio of O_2 affinity (as $P_{1/2}$) to CO affinity for a particular system and experimental conditions:

$$M = \frac{P_{1/2}(O_2)}{P_{1/2}(CO)} \tag{4.48}$$

From Figure 4.24 and from Tables 4.2 and 4.5 the M values calculated may be somewhat arbitrarily divided into three classes: those where M > 2 x 10^4 (good CO binder); those where 2 x 10^2 < M < 2 x 10^4; and those where M < 2 x 10^2 (good O_2 binder). An analogous parameter, N, may be defined to summarize the differences in the O_2 affinity between an iron-porphyrin system and its cobalt analogue:

$$M = \frac{P_{1/2}(O_2—Co)}{P_{1/2}(O_2—Fe)}. \tag{4.49}$$

For the picket-fence porphyrins and for vertebrate hemoglobins N is in the range 10 to 250, whereas for the flat-open porphyrins and for some hemoglobins that lack a distal histidine (e.g., hemoglobin *Glycera* and hemoglobin *Aplysia*), N is at least an order of magnitude larger, indicating for these latter species that the cobalt analogue binds O_2 relatively poorly[167,168] (see Table 4.6).

Note that whereas the O_2 binding of the picket-fence porphyrins is similar to that for myoglobin, the kinetics of the process are very different; the synthetic system is more than an order of magnitude faster in k_1 and k_{-1} (often also referred to as k_{on} and k_{off}). On the other hand, O_2 binding to the pocket porphyrin is similar to that for the biological system. The factors by which ligand affinities are modulated, generally to the benefit of the organism, are subtle and varied, and their elucidation requires the *precise* structural information that is currently available only from x-ray diffraction experiments. Figure 4.25 shows the structural features of interest that will be elaborated upon in the next subsections.[110,169]

Table 4.6 - Relative affinities (M) of iron-porphyrinato systems for O_2 and CO, and relative affinities (N) for O_2 of iron and cobalt-porphyrinato systems.

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Compound	$P_{1/2}$(Fe—CO) Torr	$P_{1/2}$(Fe—O_2) Torr
H_2O, pH 7		
Whale Mb	0.018	0.51

Whale Mb (E7His → Gly)	0.0049	6.2	1,300
Aplysia Mb	0.013	2.7	5 × 10^12
Glycera Mb	0.00089	5.2	5 × 10^13
Fe(PPIX-Im)	0.002	1.0	500

Toluene/Benzene			
Fe(PF-Im)/Co(PF)(1-MeIm)	0.000022	0.58	27,400

M(PF)(1,2-Me$_2$Im)	0.0089	38	492,400
M(Bis-Poc)-(1,2-Me$_2$Im)	0.0091	508	55,800

Fe(PPIX-Im)/Co(PPIX)(1-Melm)	0.00025	5.6
M(C_2-Cap)(1-Melm)	0.0054	23

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