4.9: General Structural Features that Modulate Ligand Activity

There are many ways in which ligand affinity may be perturbed (Figure 4.25). It is convenient to divide these into two groups, referred to as distal and proximal effects.[163] *Proximal* effects are associated with the stereochemistry of the metalloporphyrinato moiety and the coordination of the axial base, and thus their influence on O_2 and CO affinity is indirect. *Distal* effects pertain to noncovalent interactions of the metal-porphyrinato skeleton and the sixth ligand (O_2, CO, etc.) with neighboring solvent molecules, with substituents, such as pickets or caps, on the porphyrin, and with the surrounding protein chain. The distal groups that hover over the O_2-binding site engender the most important distal effects. For convenience, the effects of crystal packing and the protein matrix on porphyrin conformation will be discussed among the proximal effects, although as nonbonded interactions they properly are distal effects.

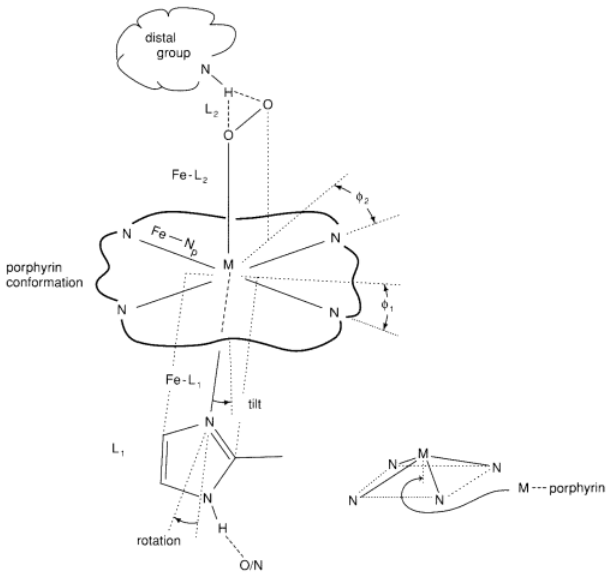

Figure 4.25 - Structural parameters and features that determine the affinity of oxygen carriers.

To a first approximation, the effects of substituents on the porphyrin ring, as transmitted through bonds to the metal center, do not perturb the ligand binding properties as much as do distal effects.[170] Thus substituents, such as vinyl and propionic-acid groups on protoporphyrin IX and o-pivalamidophenyl pickets, are ignored; one porphyrin is much like another. At the end of this subsection the various ways ligand affinity may be modulated will be summarized in an augmented version of Figure 4.3.

Proximal Effects

Few molecules have had their conformational properties characterized as exhaustively as have metalloporphyrins.

a. Porphyrin Conformation and M-N_p Separations

The cyclic aromatic 24-atom porphyrinato skeleton offers a tightly constrained metal-binding site. The conformation of least strain is planar, and the radius of the hole of the dianion is close to 2.00 Å,[110] leading to metal-porphyrinato nitrogen-atom separations, M-N_p, of 2.00 Å if the metal is centered in the square plane defined by the four porphyrinato nitrogen atoms. Small deviations from planarity are generally observed and attributed to crystal packing effects; large deviations may be induced by bulky substituents on the porphyrin skeleton, especially at the *meso* positions, by the crystal matrix,[65] or by the highly anisotropic protein matrix. The 2.00 Å radius hole neatly accommodates low-spin (S = 0) and intermediatespin (S = 1) iron(II), low-spin (S = $\frac{1}{2}$) iron(III), and cobalt(II) and cobalt(III) ions.[91]* With few exceptions the metal is centered in or above the central hole for mononuclear porphyrin species; only rarely do M-N_p bonds show a significant (though still small) scatter about their mean value.

* In order to accommodate smaller ions, such as nickel(II), the porphyrin skeleton may contract by ruffling, with little loss of aromaticity; like a pleated skirt the pyrrole rings rotate alternately clockwise and counterclockwise about their respective M-N_p vectors. This distortion leaves the four porphyrinato nitrogen atoms, N_p, still coplanar. Alternatively, the porphyrin skeleton may buckle to give a saddle conformation; the N_p atoms may acquire a small tetrahedral distortion in this process. M-N_p bonds as short as 1.92 Å have been observed. Metals with one or two electrons in their $3d_{x^2-y^2}$ orbital have a radius larger than 2.00 Å. In order to

accommodate them in the plane of the porphyrin, the porphyrin skeleton expands. M-N_p separations as long as 2.07 Å may occur with the metal still centered in the plane of the N_p atoms.[110]

b. M • • • Porph Displacement

For five-coordinate complexes the magnitude of the displacement of the metal from the plane of the four nitrogen atoms, M • • • porph, is a consequence of the electronic configuration of ML_5 complexes. Of course, the effect is augmented if the $3d_{x^2-y^2}$ orbital (directed along M-N_p bonds, Figure 4.16) is occupied. Compare a displacement of 0.14 Å for Co(TPP)(1,2-Me_2Im) (no $3d_{x^2-y^2}$ occupancy)[111] with 0.43 Å for Fe(PF)(2-MeIm) ($3d_{x^2-y^2}$ occupied).[169] For six-coordinate complexes where the two axial ligands, L_1 and L_2, are different, the M • • • porph displacement usually reflects relative *trans* influences.

Generally, displacement of the metal from the plane of the porphyrinatonitrogen atoms is within 0.04 Å of the displacement from the 24-atom mean plane of the entire porphyrin skeleton. On occasions this second displacement may be much larger, for example in Fe(TPP)(2-MeIm), where it is 0.15 Å larger[110] than it is for Fe(PF)(2-MeIm). This effect is called *doming*, and it is usually attributed to crystal packing forces. Interaction of the porphyrin with protein side chains leads to considerable doming or folding of the heme in vertebrate hemoglobins.

c. M-L Separations

The metal-axial ligand separations, M-L (when more than one, L_1 denotes the heterocyclic axial base), are dependent on the nature of the ligand, L. When L_1 and L_2 are different, the M-L separations are sensitive to the relative *trans* influences of L_1 and L_2 as well as to steric factors. For example, for Fe(TPP)(1-Melm)$_2$, the Fe—N_{Im} bond length is 2.016(5) Å,[110] whereas for Fe(TPP)(1-Melm)(NO) it is 2.180(4) Å.[111]For sterically active ligands, such as 2-methylimidazole compared to 1-methylimidazole (4.34), the longer Co—N_{Im} bond occurs for the 2-Melm ligand because of steric clash between the 2-methyl group and the porphyrin.[111]It is possible that combinations of intrinsic bonding and steric factors may give rise to a double minimum and two accessible axial ligand conformations (Figure 4.26). This situation seems to occur in the solid state for Fe(PF)(2-Melm)(O_2)•EtOH, where a short Fe—N_{Im} and a long Fe—O bond are observed both from the structure revealed by single-crystal x-ray diffraction methods and by EXAFS data. On the other hand, for solvate-free Fe(PF)(2-MeIm)(O_2) and for Fe(PF)(1,2-Me_2Im)(O_2), the EXAFS patterns are interpreted in terms of a short Fe—O and long Fe—Im bond.[171]

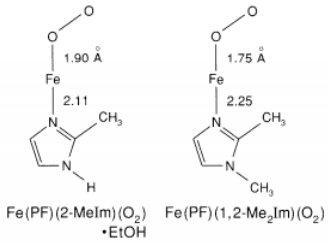

Figure 4.26 - Two arrangements of axial ligands. The right-hand side features a short L_2 and long L_1; the left-hand side the opposite.

d. The Angle ϕ

This parameter is the minimum angle that the plane of the axial base (e.g., pyridine, substituted imidazole, etc.) makes with a plane defined by the N_p, M, and L_1 atoms (Figure 4.25).[65] If there are two axial ligands, e.g., 1-methylimidazole and O_2, then, as before, the angle the axial base makes is denoted ϕ_1 and the other angle ϕ_2. For a linear CO ligand bound perpendicularly to the porphyrin plane, ϕ_2 is undefined. Note that the orientation of the second ligand is influenced by distal effects.

When ϕ = 0, the axial base eclipses a pair of M-N_p bonds; contacts with the porphyrin are maximized. When ϕ = 45°, contacts are minimized. Unless the axial base has a 2-substituent, however, the contacts are not excessively close for any value of ϕ. With a 2-methyl substituent, the contacts are sufficiently severe that the M-N_{L1} vector is no longer perpendicular to the porphyrin plane, and the imidazole group is rotated so that the M-N_{L1} vector no longer approximately bisects the imidazole C—N—C bond angle, as illustrated Figure 4.25.[110,172]

Distal Effects

Distal effects arise from noncovalent interactions of the coordinated dioxygen, carbon monoxide, or other ligand with its surroundings. The protein matrix, the pickets, and the caps are functionally equivalent to an anisotropic solvent matrix that contains a variety of solutes. The limits of this simplification are illustrated in the following example. The electronically similar cobalt

meso-, deutero-, and protoporphyrin IX complexes bind dioxygen with similar affinities under identical solvent conditions. When they are embedded in globin, larger differences in affinity and changes in cooperativity are observed.[170] These effects are attributed to the slightly different nestling of the porphyrin molecules in the cleft in hemoglobin or, in the generalization introduced, to slightly different solvation effects.

Interaction of the coordinated O_2 or CO molecule with solvent molecules or with the protein has a profound influence on kinetics and thermodynamics (see Figure 4.24, and Tables 4.2 and 4.5). As discussed earlier, there is accumulation of negative charge on the dioxygen ligand. The possibility then arises for stabilization of coordination through hydrogen bonding or dipolar interactions with solute molecules,[175] porphyrin substituents (such as amide groups in the picket-fence porphyrins[176] and some species of strapped porphyrins[161]), or with protein residues* (such as histidine).[167,177-179]

Destabilization of coordinated ligands and lowered affinity can result if the coordinated ligand is unable, through steric clash, to achieve its optimum stereochemistry or if the closest neighboring groups are electronegative, as are the ether and ester linkages on capped porphyrins.[31,180] We will describe in detail in the next subsection (III.C) the fascinating variety of means by which ligand binding is modulated by distal amino-acid residues.

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* For *Glycera* $CoMbO_2$ no change in EPR parameters occurs on substituting D_2O for H_2O.[168] No hydrogen bond between O_2 and a distal group comparable in strength to that in whale $CoMbO_2$ was inferred.

Approximate Contribution of Proximal and Distal Effects to Ligand Affinity

Dissimilar systems may show similar affinities for a ligand as a result of a different mix of the proximal and distal effects enumerated above. These effects are not all of equal magnitude, and an attempt is made here to show the increment in free energy that occurs if the effect is manifest in the deoxy or liganded state of Figure 4.3. Increasing the free energy of the deoxy state while holding that of the liganded state constant leads to an increase in affinity. The reference state is gaseous Fe(TPP)(1-MeIm). The magnitude and sign of these effects are shown in Figure 4.27. For the coordination of alkylisocyanide molecules to hemoglobin, the steric effects of different alkyl groups have been quantified.[35] Lowered affinity occurs with increasing alkyl chain length, with the exception of methyl isocyanide.

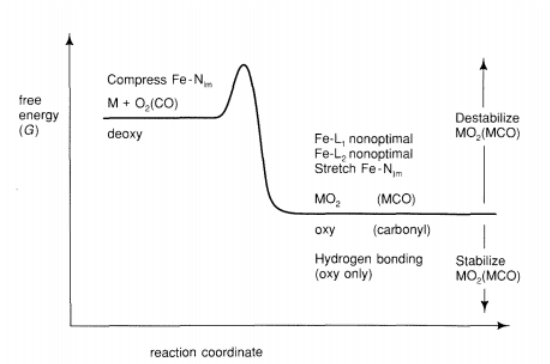

Figure 4.27 - Proximal and distal effects on the ligand affinities.
