Synthetic Analogs of Heme Proteins

Thomas J. Reinert Final Seminar December 9, 1986

The synthesis and study of small molecule analogues of the active sites of heme proteins can yield a more detailed understanding of the active site than can be obtained from the protein itself: it provides a basis for comparison of spectroscopic, structural, and reactivity properties of heme proteins, and may reveal previously unrecognized functions of the proteins [1]. The site of dioxygen binding within the hemoprotein is the five-coordinate iron (II) protoporphyrin-IX bound to the imidazole of a histidine residue; the dioxygen molecule reversibly binds at the sixth coordination site. The influence of the protein on the active site includes the 10^5 range in O_2 affinities found among heme proteins, the discrimination in binding O_2 vs. CO, and the cooperative nature of ligand binding in hemoglobin.

Several model complexes are capable of reversible CO and O_2 binding but quantitative comparisons among the model systems and to the protein reveal divergent values for observed affinities [1d,2]. The unaccountable discrepancies in ligand affinity among the model systems prompted us to perform a systematic examination [3] of the binding of CO to a simple iron porphyrin as a function of the steric demands of and hydrogen-bonding to the bound (proximal) imidazole and of solvent polarity.

A. Effects of the Proximal Imidazole

Since the iron-imidazole bond is the only covalent linkage between the heme and the protein matrix, a fundamental role in the proposed mechanisms by which the protein of hemoglobin controls the O_2 affinity. Two possible means by which the protein may affect the O_2 affinity of the active site are: sterically restricting the motion of the imidazole toward the porphyrin plane during coordination of the sixth ligand; and, altering the Lewis base strength (or donor ability) of the imidazole.

1. Proximal Steric Effects

The concept of steric hindrance of the proximal imidazole is a fundamental element of the Perutz model [4] of hemoglobin cooperativity. Many synthetic analogues for T-state Hb employ an imidazole functionalized at carbon-2; the steric contact between porphyrin plane and the substituent group of the imidazole serves as a simple approximation for the restricted motion of the imidazole imposed by the tertiary structure of the protein.

From an analogy to Tolman's steric model for phosphine ligands [5], we examined a series of imidazoles selected by steric size to probe the effect of sterics upon the initial binding of the imidazole to the iron porphyrin and the subsequent binding of CO to the five-coordinate complex.

Consistent with previous studies [6], the equilibrium constant associated with the binding of the hindered imidazole to the four-coordinate iron (II) porphyrin (K_B) decreases monotonically with increased steric bulk. Since the iron atom is closer to the center of the porphyrin plane in the six-coordinate complex, one could expect the increased steric demands of the imidazole would affect the binding of CO even more dramatically effected than the imidazole binding. As one initially introduces steric constraint on the bound imidazole, one can note the 300-fold increase in the $P_{1/2}(CO)$ (i.e., decreasing affinity), as expected. Proceeding through the steric series, however, the CO affinity does not decrease monotonically. The notable observation is that very minor steric hindrance of the axial imidazole provides nearly all of the control over the CO affinity, and the introduction of further steric demands do not dramatically alter the trans ligation.

2. Proximal Hydrogen Bonding

The modulation of the heme iron reactivity in peroxidases via deprotonation of the proximal imidazole has been proposed [7]. The suggestion of the role of the hydrogen bond to the proximal imidazole and cooperativity in hemoglobin has been related to observations of the effect of deprotonation of or hydrogen-bonding to coordinated imidazoles [8,9], including a decrease in the CO affinities of ferrous porphyrin imidazolate complexes [10].

Direct measurement of the CO affinity of Fe(TPP)(2-MeIm) (capable of hydrogen bonding) and Fe(TPP)(1,2-Me_2Im) (incapable of hydrogen bonding) in the presence of 1,10-phenanthroline revealed no significant influence of proximal hydrogen bonding on the CO affinity of the five-coordinate iron (II) porphyrin.

B. Solvent Polarity

In comparisons between heme proteins and synthetic analogues and among various synthetic analogues, the interactions between the model heme and the solvent present a potential problem for the interpretation any observed differences. It is generally accepted that increased solvent polarity will increase the O_2 affinity via stabilization of the expected charge separation. The effect of solvent on CO affinity is not certain; in anecdotal reports, one observes as the solvent polarity increases, CO affinities sometimes increase [11], and sometimes decreases [12].

In order to probe this apparent discrepancy, we measured various CO and O_2 affinities for sterically unprotected FeTPP and sterically protected FeTTPPP [13] as a function of increasing solvent polarity. The solvents selected, substituted aromatics, increase in polarity with nearly negligible differences in hydrogen bonding and coordination properties. We found that as the solvent polarity increased, the CO affinity decreased [FeTPP(1,2-Me_2Im) and FeTTPPP(1,2-Me_2Im)] while the O_2 affinity increased [FeTTPPP(1,2-Me_2Im)]. This difference indicates a mechanism by which heme proteins can discriminate between CO and O_2.

References

1. (a) Traylor, T. G. Accts. Chem. Res. 1981, 14, 102.
(b) Collman, J. P.; Brauman, J. I.; Iverson, B. L.; Sessler, J. L. Studies Org. Chem. 1983, 13, 37.
(c) Jameson, G. B.; Ibers, J. A. Comments Inorg. Chem. 1983, 2, 97.
(d) Suslick, K. S.; Reinert, T. J. J. Chem. Ed. 1985, 62, 974.

2. (a) Traylor, T. G.; Koga, N.; Deardurff, L. A. J. Am. Chem. Soc. 1985, 107 6504.
(b) Traylor, T. G.; Tsuchiya, S.; Campbell, D.; Mitchell, M.; Stynes, D.; Koga, N. J. Am. Chem. Soc. 1985, 107, 604.

3. Suslick, K. S.; Fox, M. M.; Reinert, T. J. J. Am. Chem. Soc. 1984, 106, 4522.

4. Perutz, M. F. Nature 1970, 228, 726.

5. Tolman, C. A. Chem. Rev. 1977, 77, 313.

6. (a) Wang, C. M.; Brinigan, W. S. Biochemistry 1979, 18, 4960.
(b) Yoshimura, T.; Ozaki, T. Bull. Chem. Soc. Jpn. 1979, 52, 2268.
(c) Neya, S.; Morishima, I.; Yonezawa, T. Biochemistry 1981, 20, 2610.

7. de Ropp, J. S.; Thanabal, V.; La Mar, G. N. J. Am. Chem. Soc. 1985, 107, 8268.

8. (a) Doeff, M. M.; Sweigart, D. A.; O'Brien, P. Inorg. Chem. 1983, 22, 851.
(b) Tondreau, G. A.; Sweigart, D. A. Inorg. Chem. 1984, 23, 1060.

9. (a) Quinn, R.; Nappa, M.; Valentine, J. S. J. Am. Chem. Soc. 1982, 104, 2588.
(b) Walker, F. A.; Lo, M. W.; Ree, M. T. J. Am. Chem. Soc. 1976, 98, 5552.

10. (a) Mincey, T.; Traylor, T. G. J. Am. Chem. Soc. 1979, 101, 765.
(b) Caron, C.; Mitschler, A.; Riviere, F.; Ricard, L.; Schappacher, M.; Weiss, R. J. Am. Chem. Soc. 1979, 101, 7401.

11. (a) Hashimoto, T.; Dyer, R. L.; Crossley, M. J.; Baldwin, J. E.; Basolo, F. J. Am. Chem. Soc. 1982, 104, 2101.
(b) Collman, J. P.; Brauman, J. I.; Iverson, B. L.; Sessler, J. L.; Morris, R. M.; Gibson, Q. H. ibid 1983, 105, 3052.

12. Traylor, T. G.; Mitchell, M. J.; Tsuchiya, S.; Campbell, D. H.; Stynes, D. V.; Koga, N. J. Am. Chem. Soc. 1981, 103, 5234.

13. Initial reports of the synthesis (Suslick, K. S.; Fox, M. M. J. Am. Chem. Soc. 1983, 105, 3507.) gave inconsistent yields; an improved synthesis of H_2TTPPP (based on a Rothemund condensation in quinoline) may be found in Cook, B. R.; Reinert, T. J.; Suslick, K. S. J. Am. Chem. Soc. 1986, 108, 7281.