Synthetic Fe-S Cluster Assembly with Rhodanese. D,L-Dihydrolipoate was used to generate sulfide via reaction 1 for the bulk of these studies because of its presumed biological relevance and because of solubility problems encountered with Fe(III)/dithiothreitol mixtures. As shown in Table I, at comparable reagent concentrations we obtain a four- to fivefold higher rate and 15-20% higher yield for formation of $[Fe_4S_4(SR)_4]^{2-}$ using $S_2O_3^{2-}$/rhodanese in place of sulfide. Since the rates were calculated from the decrease in absorbance at 620 nm due to the Fe(III)/D,L-dihydrolipoate complex, the four- to fivefold increase suggests an interaction between this complex and rhodanese, perhaps at a site close to that of generation of sulfide. The demonstrated hydrophobicity and structural flexibility near the active site of rhodanese might permit such an interaction.[29,30]

We have been unable to obtain $[Fe_2S_2(SCH_2CH_2OH)_4]^{2-}$ (which is unknown) under any of several conditions. Therefore, to examine the rhodanese-mediated formation of $[Fe_2S_2(SR)_4]^{2-}$, we are limited at this point to the PhSH/Triton mixture used previously to obtain $[Fe_2S_2(SPh)_4]^{2-}$ in the presence of tetraalkylammonium ions.[4,5] Due to inhibition of rhodanese activity we are also limited to Et_4N^+. Figure 5 shows that, for the thiosulfate/rhodanese/D,L-dihydrolipoate system, a significantly higher d^{2-}/t^{2-} ratio is obtained in the isolated solids when iron is supplied as Fe(II) (~0.7) instead of Fe(III) (~0.06). This higher proportion may mean that, with Fe(II), D,L-dihydrolipoate tends to favor formation of an Fe_2S_2 core as does *o*-xylene-α,α'-dithiolate.[27,28]

Comparisons to Cluster Assembly in Ferredoxins. Our limited success in selective assembly of $[Fe_2S_2(SR)_4]^{2-}$ with S^{2-} or $S_2O_3^{2-}$/rhodanese prevents us from making meaningful comparisons with assembly of [2Fe-2S] centers in ferredoxins.[11] However, our results are consistent with the idea[5] that [2Fe-2S] sites form as part of a general $[Fe(S\text{-}Cys)_4]^{2-} \rightarrow [Fe_2S_2(S\text{-}Cys)_4]^{2-} \rightarrow [Fe_4S_4(S\text{-}Cys)_4]^{2-}$ assembly pathway in ferredoxins.

Clostridium pasteurianum ferredoxin contains two [4Fe-4S] centers and can be reconstituted from the apoprotein with use of either S^{2-} or $S_2O_3^{2-}$/rhodanese. With use of S^{2-} in the presence of D,L-dihydrolipoate under comparable conditions, the rates of reconstitution of *C. pasteurianum* ferredoxin ($t_{1/2}$ = 0.3-7 min) determined previously in this laboratory[12] are 1-2 orders of magnitude faster than the rates of assembly of synthetic $[Fe_4S_4(SR)_4]^{2-}$ ($t_{1/2}$ = 80-130 min) determined in the present work. The rates for reconstitution measure the recovery of the native structure of the ferredoxin, which can be no faster than the assembly of its [4Fe-4S] centers. Therefore, from this comparison of rates we conclude that the *C. pasteurianum* ferredoxin polypeptide accelerates the assembly of its own clusters. This perhaps predictable result has not been previously subjected to a direct experimental test.

Whether or not rhodanese actually catalyzes cluster assembly remains an open question. One function of rhodanese in vivo may be to generate toxic sulfide from nontoxic substrates in proximity to iron and apoferredoxin, thereby minimizing harmful or unproductive diffusion of sulfide through the cell. The possibility mentioned in the introduction of feedback regulation of rhodanese activity by the ferredoxin, the abundance and ubiquity of rhodanese, and the efficiency of the enzyme in terms of both rates and yields all point to its involvement in the delivery of core sulfide in vivo. The synthetic results presented above provide further support for this involvement.

Acknowledgment. This research was supported by grants from the Italian Ministero della Publica Istruzione (F.B.) and the National Science Foundation (D.M.K., Grant No. PCM-8216447). The sojourn of F.B. to Iowa State University was made possible by funds from the U.S.-Italy Cooperative Science Program of the National Science Foundation (D.M.K., Grant No. INT-8300197).

Registry No. $(n\text{-}Pr_4N)_2[Fe_4S_4(SCH_2CH_2OH)_4]$, 99148-42-2; $(Et_4N)_2[Fe_4S_4(SPh)_4]$, 55663-41-7; $(Et_4N)_2[Fe_2S_2(SPh)_4]$, 55939-70-3; $FeCl_3$, 7705-08-0; $FeCl_2$, 7758-94-3; S^{2-}, 18496-25-8; S, 7704-34-9; rhodanese, 9026-04-4; thiosulfate, 14383-50-7; D,L-dihydrolipoate, 7516-48-5; dithiothreitol, 3483-12-3; ferrous ammonium sulfate, 10045-89-3; ferric ammonium citrate, 1185-57-5.

Supplementary Material Available: Figures 1-3, depicting rhodanese activity under the synthetic conditions used in this work and spectral time courses for formation of $[Fe_4S_4(SPh)_4]^{2-}$ and $[Fe_4S_4(SCH_2CH_2OH)_4]^{2-}$ in water using sulfide, and Table III, containing reagent concentrations for Figure 5 (5 pages). Ordering information is given on any current masthead page.

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Distal Histidine Coordination to Iron in Phthalocyanine-Reconstituted Myoglobin

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Reconstitution of apomyoglobin with tetracarboxy- (TcPc) and tetrasulfonated- (TsPc) (phthalocyanato)iron(II) complexes gives proteins containing both proximal and distal histidine bound to iron. MbTcPc binds isocyanides (RNC; R = benzyl, butyl, and tosylmethyl) at a rate independent of the concentration or nature of the isocyanide, $k = 1.7 \times 10^{-4}$ s^{-1} at 25 °C in phosphate buffer, pH 7. The rate-determining step in ligation to iron is proposed to involve a conformational change in which the E-helix is swung back approximately to its position in native Mb. Data for isocyanide and CO binding to $FeTsPcL_2$, L = methylimidazole, 2-methylimidazole, and pyridine, in water are similar to previously reported data for $FePcL_2$ in toluene. The rate constant for acid-catalyzed cleavage of the μ-oxo dimer $(FeTsPc)_2O$, $k = 5.6$ M^{-1} s^{-1} at $\mu = 0.11$, is over 100-fold slower than that for a similar water-soluble porphyrin dimer.

Introduction

Investigations of artificially reconstituted heme proteins in which the heme group is replaced with other metalloporphyrins[1,2] or metal complexes of phthalocyanines[2-4] or other tetradentate ligands[5] have led to an increased understanding of the role of the protein in modifying the reactivity of a metal complex. From a coordination chemist's perspective an apoprotein may be thought of as a rather large ligand that may introduce a number of effects by dominating the chemistry associated with the primary and secondary coordination spheres of the metal. A readily available protein such

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as myoglobin thus provides a very low-cost source of a well characterized "ligand", which can introduce effects that would otherwise require lengthy and elaborate synthetic work.

For example, conferring a pentacoordinate geometry on iron(II) porphyrins is readily accomplished by using apomyoglobin as the "ligand" but requires a sterically hindered ligand,[6] or a pentadentate porphyrin ligand[7] in "simple" complexes. Models designed to explore distal steric effects in hemoproteins have required ingenious and elaborate synthetic approaches[8-10] to mimic an effect easily demonstrated by using the native proteins synthesized by horses, whales, or bovines.

We have previously speculated on the cause of dramatic differences in the axial ligation properties of hemes and iron phthalocyanines[12,13] in terms of the nature of the pentacoordinate intermediate or more exactly the transition states in the dissociative substitution reactions of low-spin six-coordinate derivatives, FeN_4XY. Numerous examples of pentacoordinate hemes now exist, both high spin and low spin.[14] There are no examples to date of pentacoordinate iron(II) phthalocyanines. We therefore sought to confer a five-coordinate geometry on the iron phthalocyanine by using the same characteristics of apomyoglobin that give a pentacoordinate heme in deoxymyoglobin. Previous studies of phthalocyanine[2-4] reconstituted globins have not adequately described the axial ligation characteristics of these systems. Parallel studies of the aqueous chemistry of FeTsPc in water are also presented for comparison.

Experimental Section

FeTsPc and FeTcPc were prepared by literature methods[15-17] and purified as the Fe(III) dimer by dialysis vs. water and evaporation to dryness ($\epsilon_{630} = 9 \times 10^4$) or as $FeTsPc(MeIm)_2$, $\lambda_{max} = 672$ nm, by dialysis in the presence of 10^{-2} M MeIm and dithionite.

Apomyoglobin was prepared by Teale's method[18] from horse heart Mb (Sigma) and the reconstituted proteins MbTcPc and MbTsPc were prepared by injecting a solution of $(FeTsPc)_2O$ or $(FeTcPc)_2O$ in 0.1 M phosphate buffer, pH 7. A small amount of dithionite was added to reduce the iron to Fe(II). The solution was allowed to stand in air for 10 min to 1 h and then chromatographed on G35 Sephadex twice using 0.1 M pH 7 phosphate buffer as eluent. The resulting solutions typically had an absorbance at 678 nm of ~1.0 in 1-cm cells.

FeTsPc(CO). Addition of a minimum amount of sodium dithionite to $[Fe(TsPc)]_2O$ under 1 atm of CO results in reduction of the dimer and gives a spectrum assigned to the carbonyl ($\lambda_{max} = 666$ nm) complex. Rates and equilibria on addition of ligands L = MeIm, 2-MeIm, and py were obtained by standard spectrophotometric methods.[12]

Kinetics of Dimer Cleavage. Solutions of $(FeTsPc)_2O$ (~10^{-5} M) in 0.1 M aqueous $NaNO_3$ and $HNO_3 + NaNO_3$ were mixed in an Aminco DW2A UV–visible spectrophotometer with a stopped-flow accessory, and the absorbance at 632 nm was monitored with time. The visible spectrum of the product in the stopped-flow cuvette was obtained by using dual wavelength spectroscopy with $\lambda_2 = 500$ nm within 1 min of the stopped-flow kinetic run.

MbTcPc. Kinetics of isocyanide binding were obtained by difference spectroscopy. Pseudo-first-order rate constants were obtained by least-squares analysis at 440, 660, and 686 nm. Fluorescence measurements were made on a Varian SF330 spectrofluorometer.

Results and Discussion

Aqueous Chemistry of FeTsPc. Aqueous solutions of FeTsPc in air oxidize to $Fe^{III}TsPc$ species. Oxidation is suppressed in the presence of an excess of a ligand such as methylimidazole, which gives a well-characterized $FeTsPc(MeIm)_2$ species, $\lambda_{max} = 672$ nm. $Fe^{III}TsPc$ exists as a well-defined oxo dimer, $\lambda_{max} = 630$ nm, at alkaline pH and participates in a pH-, concentration-, and ionic-strength-dependent monomer–dimer equilibrium analogous to that found for iron tetraphenylporphyrinsulfonate (FeTPPS) and other Fe(III) complexes.[19,20] The monomeric form of $Fe^{III}TsPc$ is not well-behaved (in contrast to the FeTPPS system) at acidic pHs and appear to slowly aggregate and precipitate and possibly also undergo redox reactions. The product of acid cleavage of the oxo dimer gives initially two peaks at 640 and 680 nm at a clean first-order rate on the stopped-flow time scale.

Table I. Rate Constant vs. $[H^+]$ for Cleavage of $(FeTsPc)_2O$[a]

$[H^+]$, M	k_{obsd}, s^{-1} $\mu = 0.11$	$\mu = 0.55$
0.01	0.057	
0.025	0.14	
0.05	0.28	0.6
0.10		1.2
0.25		4.3
0.50		7.9

[a] $NaNO_3/HNO_3$, 25 °C.

Table II. Kinetic Data for the Reaction of $FeTsPc(MeIm)_2$ with 8.9×10^{-3} M BuNC

10^2[MeIm], M	$10^3 k_{obsd}$, s^{-1}	10^2[MeIm], M	$10^3 k_{obsd}$, s^{-1}
0	41	5.58	3.2
1.86	9.4	9.29	1.3
3.72	4.3		

The rate of cleavage of the dimer as a function of pH is given in Table I.

Kinetic data for other Fe(III) oxo dimers show acid-independent and acid-dependent paths,[19,20] $k_{obsd} = k_0 + k_H[H^+]$. For FeTPPS $k_0 = 41$ s^{-1} and $k_H = 840$ M^{-1} s^{-1} at $\mu = 0.1$ ($NaNO_3$). The $(FeTsPc)_2O$ μ-oxo dimer undergoes acid-dependent cleavage about 100-fold slower than the FeTPPS system, $k_H = 5.6 \pm 0.5$ M^{-1} s^{-1} at $\mu = 0.1$ and 16 ± 1 M^{-1} s^{-1} at $\mu = 0.5$. No significant acid-independent path was detected for the FeTsPc system. The slower reaction in the FeTsPc system is consistent with the generally greater inertness of axial ligand bonds in phthalocyanines compared to those in porphyrins.[13]

Ligand-Substitution Reactions of $Fe^{II}TsPc$. A simple dissociative mechanism has been demonstrated for iron phthalocyanine in noncoordinating solvents.[12,13,21] In aqueous solution, direct replacement of one ligand by another without intervention of an aquo complex is probably unknown for a dissociative mechanism. If the aquo complex is sufficiently labile, its presence will have no effect on the kinetics of the ligand-substitution reaction.

$$FeTsPc(MeIm)_2 + BuNC \rightarrow Fe(TsPc)(MeIm)(BuNC) + MeIm \quad (1)$$

The rate of reaction 1 was determined as a function of the ratio of MeIm/BuNC under pseudo-first-order conditions in both MeIm and BuNC. The data in Table II are consistent with a dissociative mechanism.

$$FeTsPc(MeIm)_2 \underset{k_{+N}}{\overset{k_{-N}}{\rightleftharpoons}} FeTsPc(MeIm) \overset{k_{+BuNC}}{\rightleftharpoons} FeTsPc(MeIm)(BuNC) \quad (2)$$

A least-squares fit of k_{obsd} vs. [MeIm]/[RNC] according to eq 3 gives values of $k_{-N} = 0.05 \pm 0.01$ s^{-1} and $k_{+N}/k_{+BuNC} = 2.5$

$$k_{obsd} = \frac{k_{-N}k_{+RNC}[RNC]}{k_{+N}[MeIm] + k_{+RNC}[RNC]} \quad (3)$$

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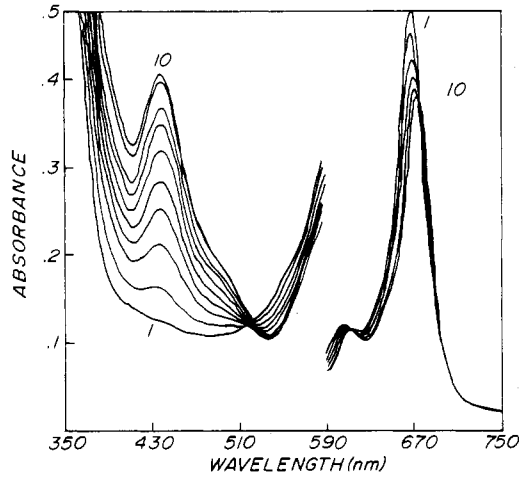

Figure 1. Spectral changes on addition of methylimidazole to an aqueous solution of FeTsPc(CO) under 1 atm of CO. Spectra 1–10 are for increasing added MeIm from 0 to 10^{-1} M. The absorbance scale for the 590–750-nm region is × 4 with spectra 6–9 omitted for clarity.

Table III. CO Binding Data for $L_2FeTsPc$ in Water and L_2FePc in Toluene

	$L_2FeTsPc^a$		L_2FePc^b	
L	K_{CO}	k_{-CO}, s^{-1}	K_{CO}	k_{-CO}, s^{-1}
MeIm	0.25	0.02	0.02	0.02
2-MeIm	70	>0.1	7.8	0.7
py	6	0.08	0.37	0.09
Im		0.02	0.04	0.02

[a] This work, aqueous solution, 25 °C. [b] Reference 13, toluene, 23 °C.

± 0.2. These results are similar to those for $FePc(MeIm)_2$ in toluene. The rate of dissociation of MeIm is somewhat faster in water (k_{-N} = 0.0013 s^{-1} for $FePc(MeIm)_2$ in toluene at 23 °C) consistent with solvent assistance in aqueous solution.

CO Binding. Equilibrium constants for CO binding to $FeTsPcL_2$ in water were determined by spectrophotometric titration for L = MeIm, py, and 2-MeIm. Spectral characteristics shown in Figure 1 are similar to those found for corresponding FePc complexes in toluene.[12] Rates of CO displacement were obtained by addition of excess ligand to the carbonyl complexes. Under these conditions, the pseudo-first-order rate constant is k_{-CO}.

$$FeTsPcL_2 + CO \overset{K_{CO}}{\rightleftharpoons} FeTsPc(L)(CO) + L \qquad (4)$$

Values of the equilibrium constant K_{CO} and dissociation rate k_{-CO} for FeTsPc in water are given in Table III along with comparable data for the FePc complexes studied in toluene solution. The trends in K_{CO} and k_{-CO} as a function of L are identical for the two systems. The value of K_{CO} is dependent on k_{-L}, which is in the order MeIm < py < 2-MeIm in FePc[12] and presumably also for FeTsPc. The greater k_{-CO} for the 2-MeIm derivative is a now well-known steric effect that mimics the proposed strain in T-state hemoglobin.[12,22] The value of k_{-CO} for L = Im is the same as that for MeIm in both water and toluene. This result indicates that hydrogen bonding of Im to water does not significantly alter the CO dissociation rate.[23] From the value of k_{-N} determined above, the ratio of addition rates k_{+MeIm}/k_{+CO} is calculated to be 10 from eq 5. This value is typical for heme[22]

$$K = \frac{k_{-N}k_{+CO}}{k_{+N}k_{-CO}} \qquad (5)$$

and other iron macrocycles. These results demonstrate that the spectral and ligation characteristics of FeTsPc in water are similar

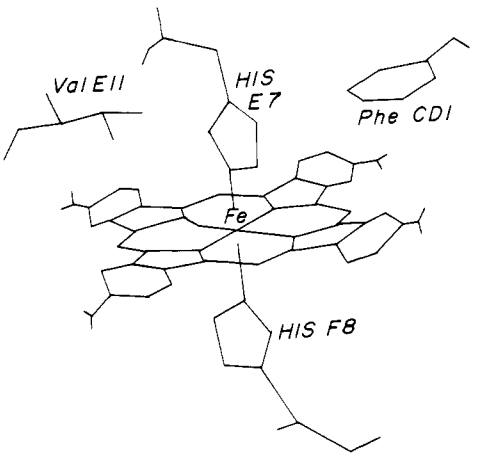

Figure 2. Proposed geometry of the active site in MbTcPc showing binding of both proximal (HIS-F-8) and distal (HIS-E-7) histidines.

Table IV. Kinetic Data for RNC Binding to MbTcPc[a]

	RNC	10^4[RNC], M	$10^4 k_{obsd}$, s^{-1}
MbTcPc	TMIC	1.6	1.7 (3)
	TMIC	1.6	2.0 (5)[b]
	TMIC[c]	1.6	1.7 (3)
	BzNC	1.6	1.5 (3)
	BuNC	100	1.5 (4)
	BuNC	10	1.9 (3)
	BuNC	5	1.5 (3)
	BuNC	160	1.4 (3)
MbTsPc	TMIC	1.6	1.5 (3)

[a] Aqueous phosphate buffer pH 7, 25 °C. [b] From absorption spectra; all others by difference spectroscopy. [c] In the presence of 10^{-2} M MeIm.

to those previously found for FePc in toluene. Corresponding results with FeTcPc were found to be qualitatively similar to those for FeTsPc.

MbTcPc. Solutions of MbTcPc with the iron reduced to the divalent state were obtained by addition of dithionite-reduced $(FeTcPc)_2O$ in 0.1 M base to apomyoglobin followed by Sephadex chromatography. The final protein solutions were stable for weeks at 5 °C in buffer or water and for a least 10 h at room temperature. ANS fluorescence demonstrated that the FeTcPc occupied the heme pocket.[5,24] MbTcPc solutions also display several characteristics that readily distinguish them from solutions of FeTsPc. (1) MbTcPc displays an absorbance peak at 440 nm, which is not present in FeTcPc unless a nitrogen base is added. (2) MbTcPc is stable in air, while solutions of reduced FeTcPc are reoxidized to the oxo dimer in air with the characteristic absorbance at 630 nm. (3) Excess dithionite has no effect on MbTcPc but results in reduction of $Fe^{II}TcPc$ to a red-violet anion (λ_{max} = 500 nm).[25] (4) Under 1 atm of CO, $FeTcPc(MeIm)_2$ forms a carbonyl complex with loss of absorbance at 440 nm while MbTcPc undergoes no spectral change under CO. These properties are all consistent with the hypothesis that the FeTcPc is occupying the heme site in a hydrophobic pocket. The similarity in the visible spectra of MbTcPc and $FeTcPc(MeIm)_2$ (especially the band at 440 nm) led us to suspect that a six-coordinate low-spin $FeTcPcN_2$ geometry was present in MbTcPc and that both proximal and distal histidines were bound to iron as shown in Figure 2 in contrast to Mb where only the proximal histidine is bound.[26] To test this hypothesis, we studied the kinetics of binding of isocyanides to MbTcPc and MbTsPc. In Mb, where a vacant coordination site is present, isocyanides, CO, and other ligands add by strictly second-order kinetics. The second-order rate constant, k_{+X}, has been shown to depend on steric effects with k_{+X} spanning several orders of magnitude: $k_{+CO} = 5 \times 10^5$ M^{-1}

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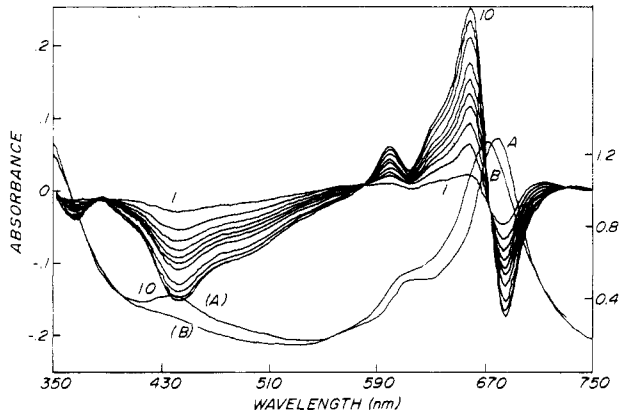
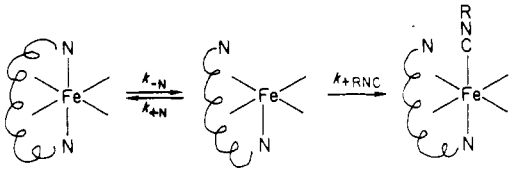


Figure 3. Spectral changes with time for the reaction of MbTcPc with BuNC. Difference spectra 1–10 use absorbance scale at left. Spectra A and B are the initial and final spectra and use the absorbance scale at right.

s^{-1};[27] $k_{+BuNC} = 3.7 \times 10^4$ M^{-1} s^{-1};[28] $k_{+TMIC} = 2 \times 10^2$ M^{-1} s^{-1}.[8] Quite different kinetic characteristics would be anticipated if a six-coordinate iron were present in the active site.

The rates of reaction of MbTsPc and MbTcPc with isocyanides were measured spectrophotometrically. A typical difference spectrum is shown in Figure 3. The observed pseudo-first-order rates are summarized in Table IV. The rates for MbTcPc and MbTsPc are similar. The important feature is that the rates are independent of *both* the concentration and nature of the isocyanide used. Furthermore the rate is not inhibited by 10^{-2} M MeIm. (The corresponding reaction of FeTsPc(MeIm)$_2$ is inhibited by more than 100-fold under comparable conditions (see eq 3). The lack of inhibition in the proteins is consistent with the expected inability of a bulky MeIm to bind in the protein pocket and also rules out the possibility that the FeTcPc is not occupying a buried sited in the protein.

Kinetic data for the reaction of MbTcPc with RNC is consistent with a mechanism in which the rate determining step involves a conformational change of the protein, which breaks the Fe–HIS-E-7 bond.



$$k_{obsd} = \frac{k_{-N}k_{+RNC}[RNC]}{k_{+N} + k_{+RNC}[RNC]} \quad (7)$$

For this mechanism, the pseudo-first-order rate constant is given by eq 7 in which RNC competes with the distal HIS-E-7 imidazole for the coordination site. For k_{obsd} to be independent of [RNC] we require

$$k_{+N} < k_{+RNC}[RNC] \quad (8)$$

If we assume that the intermediate five-coordinate conformation is similar to that of deoxymyoglobin, one might expect rates of addition of isocyanides to this protein to be similar to those of Mb. With this assumption, noting that for Mb, k_{+BuNC}[BuNC] = 3.70 at 10^{-4} M BuNC, an upper limit of about 1 may be set for k_{+N}. If the binding constant of HIS-E-7 to iron defined as

$$K_{HIS} = k_{+N}/k_{-N} \quad (9)$$

is small, then a significant fraction of the protein would exist as the five-coordinate conformation. This form is expected to react rapidly. Assuming we could detect 10% of a quickly reacting form (no evidence for a rapid initial absorbance change was detected except in old denatured samples), then $K_{HIS} \geq 10$. Assuming $k_{obsd} = k_{-N} = 1.7 \times 10^{-4}$ s^{-1}, the equilibrium constant may be used to place a lower limit on k_{+N}.

$$10 < K_{HIS} = \frac{k_{+N}}{1.7 \times 10^{-4}} \qquad k_{+N} > 10^{-3} \quad (10)$$

These two considerations suggest that k_{+N} is between 10^{-3} and 1 and K_{HIS} is between 10 and 10^3.

The rate-determining step k_{-N} would involve a conformational change in which the E-helix would swing back to a position resembling that in Mb.

Why is the distal histidine bound in MbTcPc but not in Mb? A straightforward explanation is found in the rates of dissociation of methylimidazole for simple iron porphyrins and phthalocyanines. $k_{-MeIm} = 1500$ s^{-1} for FeTPP(MeIm)$_2$ and 1.3×10^{-3} s^{-1} for FePc(MeIm)$_2$ in toluene. The 10^6 difference in rate corresponds to about 8 kcal/mol in bond strength. In MbTcPc then, the changes required to bring this residue within bonding distance must be less than 8 kcal/mol.

One may estimate an equilibrium constant for distal histidine coordination in Mb assuming the Fe–N bond strength is the *only* differences in Mb and MbTcPc. We estimate K_{HIS}^{Mb} is between 10^{-5} and 10^{-2}, consistent with the known fact that no significant six-coordinate form of Mb is detected at room temperature.

The failure of MbTcPc to bind CO is a consequence of the lower affinity of FePc for CO compared to isocyanides and the inhibition caused by the bound distal histidine residue.

Summary

Strong evidence for the coordination of HIS-E-7 to iron in MbTcPc is presented. This result illustrates how the conformation of a metalloprotein is a consequence of the requirements of both the metal prosthetic group and the protein. In Mb the low affinity of hemes for imidazole prevents the globin from acting as a trans-spanning bidentate ligand. In MbTcPc, the higher affinity of FePc for imidazoles induces conformational changes allowing both proximal and distal histidines to coordinate. Coordination of the distal histidine has been invoked by others in a rhodium myoglobin[29] and also in a variant hemoglobin, HbJAG, which lacks a proximal histidine,[30] and as an intermediate in the acid denaturation of Mb.[31] Kildahl[5] has speculated that distal histidine is also coordinated in an Fe(TAAB) reconstituted myoglobin. We note that k_{-MeIm} in FeTAAB(MeIm)$_2^{+2}$ is 6.9×10^{-3} s^{-1} at 25 °C in acetonitrile.[32] Distal histidine coordination may be anticipated in any reconstituted myoglobin when k_{-MeIm} is less than ~0.1 s^{-1}, providing peripheral contacts of the metal macrocycle are not also important[33] in determining the protein structure.

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