$(OAc)_4(cyt)_2$ complex, it can be concluded that the site of cross-linking in the horse protein is His 33.

Selectivity. This and a previous study[9] from this laboratory have already shown the potential versatility of transition-metal complexes as cross-linking reagents for proteins. Whereas the complexes $PtCl_4^{2-}$ and *trans*-$[Pt(2\text{-}Fpy)_2Cl_2]$ are specific toward methionine side chains (in cytochrome *c* it is Met 65), the complex $Rh_2(OAc)_4$ is specific toward histidine side chains, provided the imidazole ring is accessible from solution. This selectivity of the bimetallic reagent, observed herein with cytochromes *c*, is likely to be found in experiments with other proteins that lack exposed cysteine residues. When such residues are present—the number of proteins containing them is relatively small—they will probably be the binding sites.

The selectivity of both the platinum and dirhodium reagents is consistent with the known affinity of these metals toward ligands. In particular, aromatic amines, imines, and other molecules with trigonal nitrogen atoms are the most common axial ligands in $Rh_2(RCOO)_4L_2$ complexes, and structures of many such adducts have been determined crystallographically.[50-55]

VI. This Study in the Context of Others

Discovery of the antitumor activity of $Rh_2(RCOO)_4$ complexes[14-19,26] spurred the research into their interactions with biomolecules. Although some of these studies involved macromolecules (nucleic acids and proteins),[20,21,23,24] most of them dealt with complexes of the corresponding small molecules (nitrogenous bases and amino acids).[22,27,47,48,85] In other studies, the dirhodium complexes were chosen as typical transition-metal compounds and their interactions with a vitamin[57] and with a model for a DNA-binding drug[86] were examined. Although the investigations of model complexes proved informative and useful in various ways, a concern was voiced about their relevance to the study of complexes with large biomolecules.[31] The enhanced hydrolytic stability of $Rh_2(OAc)_4(cyt)_2$ over that of the model complexes may be an example of such incomplete analogy between amino acids and proteins as ligands for transition metals. Both the relevance[24,27] and the irrelevance[25] of axial ligation to the antitumor effects of $Rh_2(RCOO)_4$ have been argued. The importance of size and of structural complexity of the biopolymers for their interactions with $Rh_2(RCOO)_4$ should be considered in the study of this question.

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Registry No. $Rh_2(OAc)_4$, 15956-28-2.

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Effects of Anions on the Removal of Iron from Transferrin by Phosphonic Acids and Pyrophosphate

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It has been reported previously that phosphonate ligands can remove the ferric ion from human serum transferrin by parallel reaction pathways, one which shows saturation behavior and one which is first-order with respect to the competing ligand (Harris, W. R.; et al. *Inorg. Chem.* **1987**, *26*, 2711–2716). In the present work the rates of iron removal from diferric transferrin by the chelating agents pyrophosphate (PP_i), nitrilotris(methylenephosphonic acid) (NTP), and *N*,*N*-bis(phosphonomethyl)glycine (DPG) have been measured in 0.1 M, pH 7.4 hepes buffer containing 200 mM concentrations of the sodium salt of the following anions: fluoride, chloride, nitrate, thiocyanate, bicarbonate, sulfate, and perchlorate. All these anions increase the rate constant for the first-order pathway for iron removal. The effects of anions on iron removal do not correlate with the lyotropic number of the anion or the iron-binding affinity of the anion. Instead, there appears to be a very specific effect of perchlorate on the first-order process. This observation supports the hypothesis of a selective anion-binding site near the C-terminal binding site of transferrin. The anions have varying effects on the saturation pathway for iron removal. The anions tend to increase the maximum rate constant for the saturation pathway for PP_i. However, the anions virtually eliminate the saturation pathway for NTP and tend to reduce the maximum rate of iron removal from this pathway by DPG. It is proposed that the high salt concentrations interfere with the binding of the phosphonic acids as anions to cationic amino acid residues near the iron-binding site. Initial rates of iron removal by PP_i and NTP have been measured as a function of the chloride and perchlorate concentrations. Both anions produce a linear increase in the initial rates of iron removal from diferric transferrin. Rates of iron removal by PP_i and NTP from both forms of monoferric transferrin have also been measured. Both chloride and perchlorate accelerate the rate of iron removal from C-terminal monoferric transferrin but reduce the rate of iron removal from N-terminal monoferric transferrin.

Introduction

Transferrin is the mammalian protein that carries ferric ion through the blood between sites of uptake, utilization, and storage.[1-3] The protein consists of two major lobes, each containing a single high-affinity iron-binding site. Although these sites are very similar, they are not identical. A distinctive feature of transferrin is the requirement of a synergistic anion for effective metal binding. Under physiological conditions the anion is (bi)carbonate, which appears to bind simultaneously to the ferric ion and to cationic groups on the protein to form an Fe–HCO_3-Tf ternary complex.

The iron binding constants of Tf are $10^{20.7}$ and $10^{19.4}$ at atmospheric CO_2 and pH 7.4.[4] It is not clear how cells overcome this high binding affinity to procure their iron. Exchange of iron at pH 7.4 with low-molecular-weight ligands tends to be quite slow,[5-7] and the mechanism by which this exchange takes place is still not completely clear. A better understanding of iron removal from transferrin takes on added importance because of the need

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for a more effective drug for the treatment of the lethal iron overload often associated with chronic transfusion therapy for certain types of anemias.[8]

Recent studies have shown that pyrophosphate (PP_i),[9,10] several phosphonic acids,[10,11] the catecholate ligand 1,5,10-tris(5-sulfo-2,3-dihydroxybenzoyl)-1,5,10-triazadecane (LICAMS),[12,13] and acetohydroxamic acid (AHA)[14] can remove ferric ion with half-lives of 5–80 min. Iron removal by the ligands LICAMS,[12,13] aerobactin,[7] and AHA[14] shows complete saturation kinetics with respect to the ligand concentration. Saturation has been attributed to a slow conformational change in ferric transferrin from a closed, inert form into an open, reactive form.[9,14]

We recently reported that PP_i as well as several aminophosphonic acids can remove iron by parallel saturation and first-order pathways.[10] This conclusion was based on the observation that plots of the apparent first-order rate constant k_{obsd} vs [ligand] could not be described by simple saturation kinetics, but rather fit the equation

$$k_{obsd} = \frac{k'[L]}{1 + k''[L]} + k'''[L] \quad (1)$$

However, an alternative explanation for eq 1 could be that the increasing ligand concentration is changing the ionic strength of the solution enough to cause a gradual increase in k'. This possibility is suggested by recent work of Kretchmar and Raymond, which shows that the rate constants for LICAMS are sensitive to ionic strength and tend to decrease dramatically at low ionic strength.[15]

This paper reports kinetic studies of iron removal from diferric transferrin by PP_i and two tripodal phosphonic acids, nitrilotris(methylenephosphonic acid) (NTP) and *N*,*N*-bis(phosphonomethyl)glycine (DPG) in the presence of 200 mM concentrations of various anions. The results indicate that the first-order term in eq 1 is not an artifact of changes in ionic strength but that the relative importance of the saturation and first-order pathways is affected by several anions.

Experimental Section

Reagents. Purified human serum transferrin was purchased from Sigma and further purified as previously described.[16] Monoferric C-terminal and diferric transferrin were prepared by adding 1 or 2 equiv of iron as a weakly acidic solution of ferric ion with a 2-fold excess of NTA. N-Terminal monoferric transferrin was prepared from diferric transferrin as described by Baldwin and de Sousa,[28] except that *N*-(2-hydroxyethyl)piperazine-*N*'-ethanesulfonic acid (hepes) was substituted for Tris as the buffer. All iron–transferrin solutions were eluted through a 1.5 × 30 cm Sephadex G-25 column with 0.1 M, pH 7.4 hepes buffer. The solutions were further purified and concentrated by washing with the same buffer in an Amicon ultrafiltration cell fitted with an XM-50 membrane.

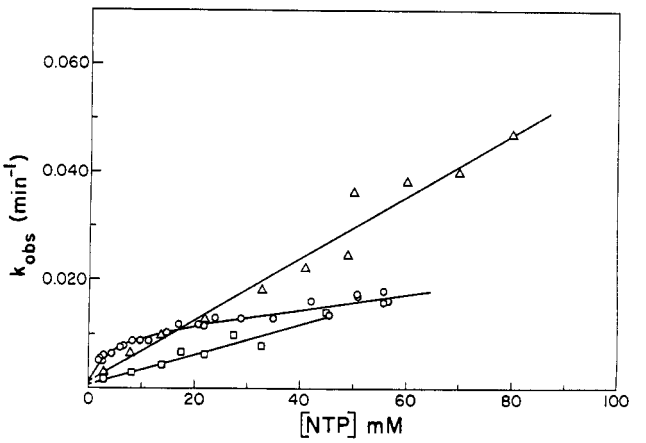


Figure 1. Variation in the apparent first-order rate constant for iron removal from diferric transferrin as a function of the concentration of NTP for 25 °C, pH 7.4, 0.1 M hepes buffer containing no added salt (○), 200 mM thiocyanate (□), and 200 mM perchlorate (△).

The tetrasodium salt of pyrophosphate was purchased from Sigma and used as received. The syntheses of the ligands NTP and DPG have been recently reported.[10] All anion solutions were prepared from reagent grade sodium salts.

Procedures. Visible absorbance measurements were made with a Varian 2290 UV–vis spectrophotometer equipped with a jacketed sample cell holder connected to an external circulating water bath. All solutions were maintained at 25 °C and contained 0.10 M hepes, which was adjusted to pH 7.4 by the addition of concentrated NaOH. All pH values were measured with a Corning Model 150 pH meter equipped with either an Orion or Fisher combination electrode standardized with commercial pH 4 and pH 7 buffers.

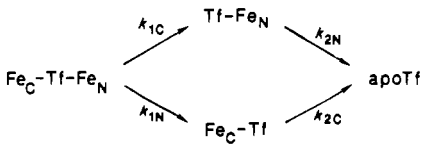
Results

Ferric transferrin has a strong iron–phenolate charge-transfer band at 465 nm. Since the ferric–phosphonate complexes have virtually no absorbance at this wavelength, the rate of iron removal is easily followed by monitoring the decrease in the 465-nm band. The absorbance values are converted to a reaction coordinate defined as

$$R_t = \frac{A_t - A_\infty}{A_0 - A_\infty} \quad (2)$$

where A_0 and A_∞ are the absorbance values at zero and infinite times, respectively, and A_t is the absorbance at time t.

A general scheme describing iron removal from the two sites of Tf is shown as follows:



We have previously shown[10] that if one assumes noncooperativity, i.e. that $k_{1C} = k_{2C}$ and $k_{1N} = k_{2N}$, then one can derive that

$$R_t = 0.5e^{-k_C t} + 0.5e^{-k_N t} \quad (3)$$

At the beginning of the iron removal reaction, when both sites are equally occupied by iron, the apparent first-order rate constant for iron removal k_{obsd} is the arithmetic mean of k_C and k_N. Since the site-specific rate constants are usually different, the degree of saturation of the two sites will begin to differ as the reaction

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Table I. Rate Constants for the Removal of Ferric Ion from Transferrin by Chelating Agents in the Presence of 200 mM Concentrations of Various Anions[a]

	PP_i		NTP		DPG	
anion	10^3k_{max}	k'''	10^3k_{max}	k'''	10^3k_{max}	k'''
none	18 ± 4	0.82 ± 0.03	9.8 ± 2.9	0.14 ± 0.02	9.4 ± 2.0	0.067 ± 0.013
SCN^-	29 ± 3	0.91 ± 0.04	0.7 ± 1.4	0.28 ± 0.03	3.7 ± 0.8	0.31 ± 0.02
F^-	17 ± 3	1.07 ± 0.06	2.8 ± 1.4	0.33 ± 0.04	5.4 ± 3.6	0.34 ± 0.10
NO_3^-	30 ± 3	1.08 ± 0.06	1.4 ± 1.0	0.30 ± 0.02	4.9 ± 1.0	0.30 ± 0.02
Cl^-	22 ± 3	1.23 ± 0.07	1.9 ± 1.5	0.37 ± 0.02	6.3 ± 1.1	0.35 ± 0.02
HCO_3^-	37 ± 7	1.28 ± 0.12	10.5 ± 2.2	0.30 ± 0.04	5.8 ± 3.3	0.52 ± 0.05
SO_4^{2-}	39 ± 6	1.87 ± 0.13	2.7 ± 1.0	0.38 ± 0.02	10.0 ± 1.4	0.34 ± 0.03
ClO_4^-	17 ± 7	3.48 ± 0.13	1.1 ± 3.2	0.57 ± 0.04	7.1 ± 2.5	0.71 ± 0.03

[a] All rate constants are expressed in units of min^{-1}.

proceeds and deviations from first-order behavior may be observed. In this study k_{obsd} values have been calculated as the slopes of plots of ln R_t vs t using no more than the initial 30% of the reaction.

Salt Effects on Ligand Dependence. Values of k_{obsd} were measured at ligand concentrations from 5 to 80 mM in 0.10 M hepes buffer and in the same buffer containing 200 mM concentrations of the following salts: NaF, Na_2SO_4, NaSCN, NaCl, $NaHCO_3$, $NaClO_4$, and $NaNO_3$. The data from simple hepes buffer have been previously fit to eq 1 to obtain values for k', k'', and k'''. When the salts were added, the degree of curvature in the plots of k_{obsd} vs ligand concentration is reduced. In addition, fewer data are available for each of the salt solutions compared to the data set for hepes buffer alone, so that it is not possible at this time to refine independent values of k' and k'' for each anion. Instead, values of k_{obsd} have been measured above the saturation point for diferric transferrin, i.e. under conditions where $k''[L] \gg 1$, so that eq 3 reduces to the linear eq 4, where k_{max}

$$k_{obsd} = k_{max} + k'''[L] \quad (4)$$

$= k'/k''$ and represents the maximum rate constant for the saturation pathway. Values of k_{max} and k''' have been calculated from plots of k_{obsd} vs the concentration of ligand. In some cases the k_{obsd} value at the lowest ligand concentration falls below the line because the system has not reached saturation, and these points have not been included in the calculations of k''' and k_{max}.

Representative plots of k_{obsd} vs [NTP] are shown in Figure 1. The values for k_{max} and k''' for iron removal by NTP are shown in Table I. For all the anions except perchlorate, there is an increase in k''' from 0.14 to 0.30–0.38 min^{-1}. The plot for thiocyanate in Figure 1 is representative of sulfate, fluoride, nitrate, and chloride. The k''' in the presence of perchlorate is significantly larger at 0.57 ± 0.04 min^{-1}. The value of k_{max} drops to essentially zero for solutions containing 200 mM concentrations of most of the anions. Only for bicarbonate is the absolute value of k_{max} significantly different from zero. The k_{max} in the presence of bicarbonate is unchanged from the value measured in hepes buffer alone.

The effects of salts on the k''' of DPG are qualitatively similar to those observed with NTP. Most of the salts increase k''' from 0.067 to 0.31–0.35 min^{-1}. In most cases there is no significant difference in the k''' values for NTP and DPG in the presence of a given anion. The two exceptions are perchlorate and bicarbonate. Perchlorate results in a k''' of 0.71 ± 0.03 min^{-1}, while bicarbonate produces a k''' of 0.52 ± 0.05 min^{-1}. Both constants are significantly greater than the corresponding values for iron removal by NTP. The salts tend to decrease the k_{max} values of DPG, although the effect is not as marked as that for NTP. In some cases the uncertainties in k_{max} are so large that it is difficult to assess the significance of the decrease. The k_{max} value for sulfate is essentially unchanged from the hepes-only value.

Representative plots of k_{obsd} vs [PP_i] are shown in Figure 2. The salts cause a somewhat wider dispersion of the k''' for PP_i than for NTP and DPG. For most of the salts, k''' falls in the range of 0.91–1.28 min^{-1}. Sulfate is somewhat above this range at 1.87 ± 0.13 min^{-1}. By far, the greatest effect is seen with perchlorate, which produces a k''' value of 3.48 ± 0.13 min^{-1}. Unlike those with DPG and NTP, there is no decrease in the k_{max} values with PP_i. Fluoride, chloride, and perchlorate have no

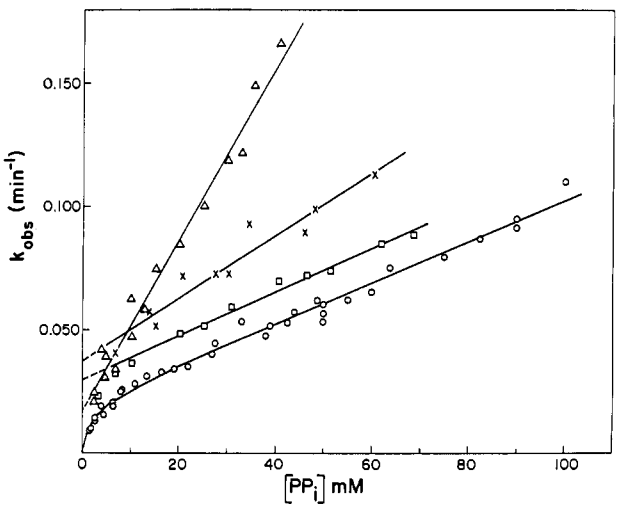

Figure 2. Variation in the apparent first-order rate constant for the removal of iron from diferric transferrin as a function of the concentration of PP_i for 25 °C, pH 7.4, 0.1 M hepes buffer containing no added salt (○) and 200 mM thiocyanate (□), bicarbonate (×), and perchlorate (△).

Table II. Effect of Anions on the Apparent First-Order Rate Constant for Iron Removal from Monoferric Transferrins by 72 mM Ligand

ligand	k_C, min^{-1}	k_N, min^{-1}
PP_i	0.062	0.057
PP_i + 100 mM ClO_4^-	0.150	0.019
PP_i + 800 mM Cl^-	0.300	0.052
NTP	0.012	0.015
NTP + 100 mM ClO_4^-	0.065	0.0038
NTP + 900 mM Cl^-	0.069	0.0069

detectable effect on k_{max}. Thiocyanate, nitrate, bicarbonate, and sulfate increase k_{max}, but the greatest impact of the added salts on the overall rate of iron removal is related to increases in k'''.

Salt Dependence at Fixed Ligand Concentration. The removal of ferric ion by 72 mM NTP and PP_i was followed in hepes buffer containing 0–900 mM sodium chloride and from 0 to 140 mM sodium perchlorate. The initial rates of iron removal were used to calculate the pseudo-first-order rate constant k_{obsd}. The results shown in Figure 3 indicate that, for both ligands, k_{obsd} increases linearly with increasing perchlorate concentration. There is also a linear increase in k_{obsd} with increasing concentration of chloride ion. However, much larger chloride concentrations are required to produce equivalent changes in rate constants.

The effect of perchlorate and chloride on the rate constants for iron removal from both N- and C-terminal monoferric transferrins have also been measured. These constants correspond to k_{2C} and k_{2N} in the general scheme for iron removal. Table II shows the values for these constants measured in hepes only, hepes plus 100 mM perchlorate, and hepes plus 800 or 900 mM chloride. Both anions greatly accelerate the rate of iron removal from the C-terminal site. In contrast, chloride has little effect on the removal of iron from the N-terminal site by PP_i but reduces the rate of iron release to NTP. Perchlorate significantly reduces the rate

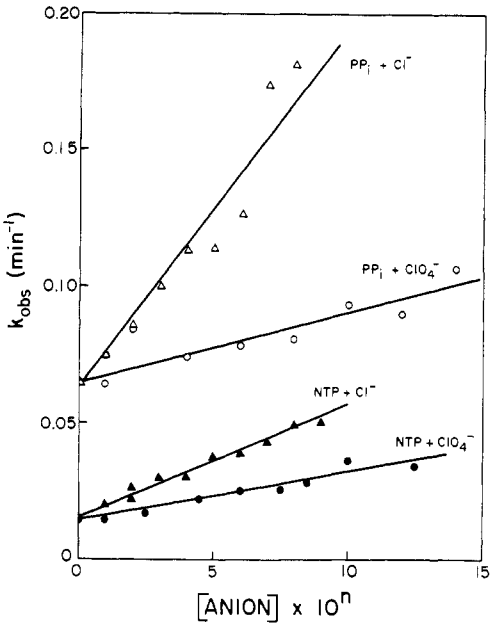

Figure 3. Variation in k_{obsd} for iron removal from diferric transferrin as a function of the concentration of perchlorate ($n = -2$) and chloride ($n = -1$) for 25 °C, pH 7.4, 0.1 M hepes buffer. Open symbols represent iron removal by 72 mM PP_i in the presence of perchlorate (○) or chloride (△). Closed symbols represent iron removal by 72 mM NTP in the presence of perchlorate (●) or chloride (▲).

constant for the N-terminal site with both ligands. Thus, the increase in k_{obsd} as a function of the salt concentration can be attributed entirely to faster iron release from the C-terminal site.

Discussion

The effects of the various anions on the plots of k_{obsd} vs concentration of ligand indicated that the nonzero slope at high ligand concentration is not an artifact of increasing ionic strength. The addition of a high background of electrolyte would be expected to dampen any changes in k_{obsd} related just to changes in ionic strength. The opposite is observed. The slope, which corresponds to k''', increases in the presence of all anions. Furthermore, the magnitude of this increase does not correlate with ionic strength. The greatest effect is observed for perchlorate, even though the dianion sulfate results in a much higher ionic strength.

We have also attempted to interpret these results in terms of the lyotropic series, an empirical ranking of the relative effectiveness of salts for denaturing or altering the activity or state of aggregation of a protein.[17] However, the anion effects do not parallel the lyotropic numbers for these anions, which decrease as $SCN^- > ClO_4^- > NO_3^- > Cl^- > SO_4^{2-}$, $HCO_3^- > F^-$.[18] Although perchlorate is the most active anion and also lies high in the lyotropic series, thiocyanate and nitrate have similar lyotropic numbers as perchlorate, yet have relatively little influence on the iron removal rate constants. Significant kinetic effects are more often seen for bicarbonate and sulfate, which lie in the middle of the lyotropic series. This observation differs from the studies on iron removal by EDTA, in which Baldwin reported that anion effects did follow the lyotropic series.[5]

The observation that perchlorate consistently shows the greatest effect on k''' supports the hypothesis that cationic groups near the binding site form a specific anion-binding site. Chasteen has noted that there is an abundance of cationic groups near the metal-binding sites.[1] Studies on tyrosine modification by periodate have suggested that there is a site that is specific for a tetrahedral arrangement of oxygen atoms such as that in perchlorate.[18-20] One should note that the oxoanions bicarbonate and sulfate also tend to have significant effects on the iron removal kinetics. Penner et al. have shown that chemical modification of a key arginine residue near the metal-binding site slows the rate of iron removal by EDTA and PP_i.[19] Thompson et al. have shown that either the acylation of readily accessible lysine residues or the addition of perchlorate accelerates removal of iron by PP_i from the C-terminal site but markedly decreases the site-specific rate constant for the N-terminal site.[21]

Folajtar and Chasteen[22] have reported the equilibrium constants for the binding of a series of anions to diferric transferrin. This anion binding alters the EPR spectra of the two bound ferric ions and might also affect their reactivity toward chelating agents. However, the data reported here argue against a role of this nonsynergistic anion binding as a major factor in the iron-release kinetics. There is no correlation between the effect on the kinetics and the nonsynergistic anion binding constants,[22] which follow the order $SCN^- > ClO_4^- > Cl^- > SO_4^{2-} \sim F^- \sim 0$. The k_{max} and k''' values at 200 mM anion indicate that ClO_4^- has by far the greatest effect and that thiocyanate and fluoride have similar, weak effects on these rate constants. In addition, the values of k_{obsd} do not show the expected saturation as a function of Cl^- and ClO_4^- concentrations. The reported equilibrium constants for nonsynergistic anion binding indicate that ferric transferrin should be 90% saturated by about 100 mM perchlorate and 300 mM chloride. If this binding were a critical component of the kinetic effect, then one would expect to see sigmoidal curves in Figure 3, reflecting the change in the fraction of diferric transferrin present as the anion complex.

It has been suggested several times that iron release might involve substitution of the bicarbonate anion by the entering chelating agent.[6,23-26] We have previously suggested that k''' might correspond to a reaction pathway involving slow substitution of the synergistic bicarbonate anion by a functional group of the incoming ligand, followed by rapid release of the ferric chelate.[10] All the anions tested in this study increase k''', which would be consistent with the disruption of bicarbonate–protein linkages by high concentrations of other anions.

The rate of iron removal from serum transferrin by PP_i[9] and acetohydroxamic acid[14] appears to be limited by a conformational change in ferric transferrin from an unreactive, closed form to a more labile, open conformation. Thus, the k_{max} value for PP_i should correspond to the forward rate constant of this conformational change. Surprisingly, perchlorate does not appear to alter this rate, while the rate constant is essentially doubled by nitrate, bicarbonate, and sulfate. The strong effects on the rates of iron removal routinely observed for the perchlorate ion are related entirely to the marked acceleration in the first-order pathway associated with k'''. Thus, they may be related more to destabilization of the anion-binding site than to conformational changes at the metal-binding site.

We have previously reported that for NTP and DPG, the rate-limiting step corresponding to k_{max} is not the protein conformational change, but rather the dissociation of an intermediate L–Fe–HCO_3–Tf quarternary complex.[10] For iron removal by NTP, 200 mM anion virtually eliminates this reaction pathway. Most anions also reduce the k_{max} values for DPG. We initially hoped to relate this reduction in the rates of iron removal to the iron-binding affinity of the anions. This expectation was based on the assumption that the entering ligand was binding to ferric transferrin by coordination to the aquated site on the iron and that anions like thiocyanate and fluoride, which have appreciable iron-binding affinities, would be much more effective at blocking this site. However, the effect on k_{obsd} appears to have no relation to iron-binding affinity.

We now believe that the decrease in k_{max} for NTP and DPG may be related to interference with the binding of the phosphonic acids *as anions* to the protein. This would involve a reduction in k'' in eq 1, such that saturation is reached only at high ligand concentrations. The relatively small degree of curvature that this would introduce into the plots of k_{obsd} vs [L] would not be easy to detect. This model of anion binding could explain the lack of a spectroscopically distinct intermediate during iron removal by the phosphonic acids.

Previous studies on iron removal by PP_i and EDTA have indicated that the N-terminal site is the more labile.[21,27,29] These

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appear to conflict with the data reported here that show that the C-terminal site releases iron more quickly at 72 mM concentrations of ligand. However, the published data on iron removal by PP_i were collected at a lower pH (6.9), which may selectively labilize the N-terminal site. In addition, the earlier studies have been conducted by using 1-3 mM concentrations of PP_i, with EDTA or desferrioxamine as a thermodynamic sink for the released iron. At low concentrations of PP_i, the predominant reaction pathway is the saturation process. The salt effects on the monoferric transferrins reported here were measured at 72 mM ligand, where the predominant reaction pathway is the first-order process (k'''). The apparent inversion of the relative lability of the two sites toward PP_i may be related to the change in the predominant iron removal pathway for high and low concentrations of PP_i. Additional data at low ligand concentration are needed for a phosphonic acid chelating agent with greater iron-binding affinity than PP_i, so that the iron is completely removed even at low ligand concentrations.

The effects of chloride and perchlorate on iron removal from monoferric transferrins are generally consistent with previous studies.[27,28] In all cases studied thus far, perchlorate accelerates iron release from the C-terminal site and retards iron release from the N-terminal site. Chloride also consistently accelerates iron release from the C-terminal site. However, the effect of chloride on the N-terminal site is not consistent. Baldwin and de Sousa reported that chloride causes only a minor decrease in the rate constant for iron removal from N-terminal monoferric transferrin by EDTA. Chasteen et al. report a sharp decrease in the rate constant for iron removal by 1 mM PP_i from the N-terminal site of diferric transferrin. We observe a significant reduction in the rate constant for iron removal by NTP but only a minor decrease in the rate constant for 72 mM PP_i. It appears that iron release from the N-terminal site is more sensitive to factors such as temperature and the identity and concentration of the competing ligand.

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Registry No. PP_i, 14000-31-8; NTP, 6419-19-8; DPG, 2439-99-8; SCN^-, 302-04-5; ClO_4^-, 14797-73-0; NO_3^-, 14797-55-8; Cl^-, 16887-00-6; SO_4^{2-}, 14808-79-8; HCO_3^-, 71-52-3; F^-, 16984-48-8; Fe, 7439-89-6.

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Unusual Hyperporphyrin Spectra for Bis(thiolato)rhodium(III) Octaethylporphyrins and Generation of the Superoxide Anion by the Complexes

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Bis(thiolato) complexes of rhodium(III) octaethylporphyrin (Rh^{III}(OEP)) exhibiting a "split Soret band" with peaks at about 370 nm and 450 nm, classified as a "hyperporphyrin spectrum", were prepared with Rh^{III}(OEP)Cl, aliphatic or aromatic thiol compounds, and tetrabutylammonium hydroxide in organic solvents. The structure of the complexes was characterized by comparing their electronic absorption spectra with the spectra of thiolato adducts of cytochrome P-450 and its cobalt-substituted enzymes, bis(thiolato)iron(III)-, cobalt(III)-, and Cr(III)-porphyrin complexes reported previously. These complexes were stable under air at room temperature, their apparent half-lives being over 30 min, monitored by the intensities of the split Soret band. The split Soret band correlated well with the dielectric constants of alcohols as solvents and with the Hammett σ values of the substituents of thiophenol derivatives as axial ligands on Rh^{II}(OEP) in benzene and acetone solvents. Under the same conditions observed for the formation of the hyperporphyrin spectra, the generation of the free superoxide anion was detectable by ESR spectrometry at 77 K. On the basis of the results, the possible reaction mechanism generating the superoxide anion by a bis(thiolato)-Rh^{III}(OEP) complex was proposed.

Introduction

Cytochrome P-450 enzymes (P-450) catalyze the incorporation of one of the two atoms of molecular oxygen into a wide variety of organic compounds.[1] In activation of molecular oxygen, P-450 is unusual and unique in that the axial fifth ligand trans to the molecular oxygen binding to iron(III) protoporphyrin IX (protoheme) has been revealed to be a negatively charged cysteinato sulfur, on the basis of recent X-ray structure analysis.[2] P-450 in the reduced state exhibits an unusual split Soret band at 360 and 450 nm in the presence of CO. These bands have been classified as a unique hyperporphyrin (HP) spectrum resulting from a mixture of two transitions: sulfur p' → porphyrin π^* and porphyrin $\pi \rightarrow \pi^*$ (p' being one of two lone-pair sulfur orbitals).[3] Characterization of this thiolato-ferrous heme-CO axial coordination mode was achieved with chemical model complexes by Stern and Peisach,[4] followed by Collman and Sorell,[5] Chang and Dolphin,[6] and Caron et al.[7] These model complexes played an important role in showing that a cysteinyl thiolato ligand is present in the ferrous P-450-CO complex.

On the other hand, similar unusual HP spectra due to bis(thiolato)iron and cobalt protoheme complexes provided by exogenous thiolato binding at the sixth axial position of Fe(III)[8,9]

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