ring: PMn^{IV}–O–$Mn^{III}P^{\bullet+}$ (porphyrin π cation radical) or P-Mn^{III}–O–Mn^{III}P (porphyrin dication). These species with ring-centered oxidation would be susceptible to nucleophilic attack by water or hydroxide. Thus, the initial product would be an epoxide or a hydroxy species that would rapidly undergo further oxidation by remaining Mn(IV) dimers. As the pH is lowered, water instead of OH^- becomes the predominant axial ligand for Mn. This would favor rearrangement within the Mn(IV) dimer to species with Mn(III) and porphyrin-centered oxidation.

Acknowledgment. This work was supported by the Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy, under Contract No. DE-AC03-76SF00098. L.O.S. acknowledges the support of Associated Western Universities by a Summer Faculty Participation Award.

Registry No. $Mn^{III}P^+$, 70649-54-6; PMn^{IV}–O–$Mn^{IV}P^{2+}$, 114595-73-2; Mn^{II}P, 72924-08-4; O_2, 7782-44-7; 3-buten-1-ol, 627-27-0; maleic acid, 110-16-7; maleimide, 541-59-3.

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Kinetic Studies on Metal Removal from Transferrins by Pyrophosphate. Investigation on Iron(III) and Manganese(III) Derivatives

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Received March 27, 1987

The kinetics of metal removal from iron(III) transferrin by pyrophosphate (PP) has been studied at pH 7.4. A biphasic behavior is observed that is related to the presence of two metal sites. The dependence of the k_1 and k_2 values on PP concentration provides evidence of the existence of more than one pathway for the metal release reaction in both sites. The effect of chaotropic agents on the kinetic parameters has been studied. All the data are interpreted and rationalized on the grounds of a kinetic model that represents an extended version of the Bates mechanism. Kinetic data on manganese(III) transferrin are also shown.

Introduction

We have been recently studying the kinetics of release of metal ions from metallotransferrins in the presence of increasing amounts of pyrophosphate. The iron(III)–transferrin–carbonate derivative is the most representative compound of this class on account of its biological significance.[1,2] We had designed an experiment to measure the kinetics of release of iron under different solution conditions by using desferrioxamine B as a sink to ensure the complete removal of the metal.[3] The kinetics was followed through the decrease in intensity of the tyrosinate-to-metal charge-transfer bands. Such a decay was fit by an equation of the type

$$A_t = (A_0 - A_\infty)(0.5e^{-k_1 t} + 0.5e^{-k_2 t}) + A_\infty \qquad (1)$$

where A_t and A_0 are the absorbances, at the observation wavelength, at t and zero times, respectively, A_∞ is the absorbance at infinite time, and k_1 and k_2 are the first-order kinetic parameters for the two transferrin sites. This equation assumes that the two metal sites are completely independent from each other. This point has been addressed by several authors[4-7] and is proved a posteriori[6,7] to represent a reasonable assumption. The two kinetic constants for the two sites have been determined under various concentrations of nonsynergistic anions. We have used pyrophosphate (PP hereafter) as a scavenger anion—i.e. as an anion that increases the rate of release of the metal ion although its binding affinity for iron is weaker than that of transferrin itself—on the grounds of early experiments that demonstrated its efficiency in facilitating iron removal;[8-12] we have investigated also the effect of the chaotropic agent perchlorate on the kinetics of release. After this research program was well ahead, a paper by Harris et al. appeared[13] which shows that accurate measurements of the initial iron release rate in a large range of phosphonate concentrations reveal a previously unobserved profile of the average rate constant for release versus the pyrophosphate ligand concentration. The experimental data are rationalized on the grounds of a complex kinetic model consisting of both a saturation and a linear term; the former dominates at low scavenger concentration whereas the latter is prevalent at high scavenger concentration. This finding contradicts previous hypotheses and draws new interest to such a controversial topic. Indeed, despite the fact that in the last decade many reports have appeared in the literature on the reaction of iron removal from transferrin,[2] serious limitations to a general interpretation of the many reported results come from the extreme variability of the experimental conditions under which the kinetic experiments have been run as well as of the used data treatments.

In light of the latest developments, we report here the analysis of our experimental data, recorded under homogeneous conditions, in terms of two kinetic constants and their dependence on various solution conditions. Our study is a further attempt to rationalize the matter and to evaluate the specific relevance of the various factors on the overall process. In particular, we have studied the effect of both a representative scavenger agent (PP) and a representative chaotropic agent (perchlorate) on the kinetics of metal release and focused on their mutual interactions.

The data on the manganese(III)–transferrin–carbonate system are also discussed.

Experimental Part

Human serum transferrin (Tf hereafter) in the apo form was pur-

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(1) Aisen, P.; Listowsky, I. *Annu. Rev. Biochem.* **1980**, *49*, 357.
(2) Chasteen, N. D. *Adv. Inorg. Biochem.* **1983**, *5*, 201.
(3) Pollack, S.; Aisen, P.; Lasky, F. D.; Vanderhoff, G. *Br. J. Haematol.* **1976**, *34*, 231.
(4) Kretchmar, S. A.; Raymond, K. N. *J. Am. Chem. Soc.* **1986**, *108*, 6212.
(5) Baldwin, D. A. *Biochim. Biophys. Acta* **1980**, *623*, 183.
(6) Harris, W. R. *J. Inorg. Biochem.* **1984**, *21*, 263.
(7) Bertini, I.; Hirose, J.; Kozlowsky, H.; Luchinat, C.; Messori, L.; Scozzafava, A. *Inorg. Chem.* **1988**, *27*, 1081.
(8) Egyed, A. *Biochim. Biophys. Acta* **1975**, *411*, 349.
(9) Pollack, S.; Vanderhoff, G.; Lasky, F. D. *Biochim. Biophys. Acta* **1977**, *497*, 481.
(10) Kojima, N.; Bates, G. W. *J. Biol. Chem.* **1979**, *254*, 8847.
(11) Cowart, R. E.; Swope, S.; Loh, T. T.; Chasteen, N. D.; Bates, G. W. *J. Biol. Chem.* **1986**, *261*, 4607.
(12) Morgan, E. H. *Biochim. Biophys. Acta* **1979**, *580*, 312.
(13) Harris, W. R.; Rezvani, A. B.; Bali, P. K. *Inorg. Chem.* **1987**, *26*, 2711.

0020-1669/88/1327-2405$01.50/0

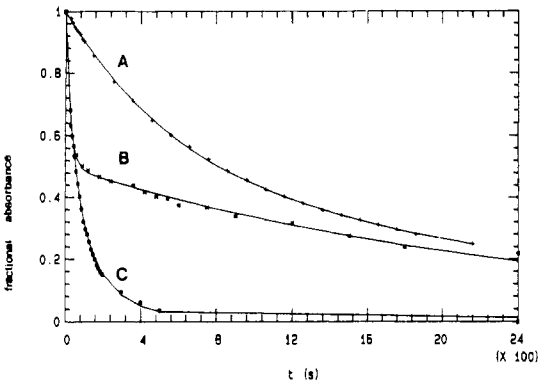


Figure 1. Experimental profiles of the percentual decay of absorbance versus time for iron transferrin (A), iron transferrin plus 1 M ClO_4^- (B), and manganese transferrin (C) after addition of 0.1 M PP. The best-fitting curves according to eq 1 are also reported. Conditions: 5×10^{-5} M protein; 0.1 M pyrophosphate; 50 mM Tris-HCl buffer, pH 7.4; 1 mM HCO_3^-; 2.5×10^{-4} M DFB; 25 °C. No DFB was added in the case of manganese transferrin. The absorbance decay was followed at 330 nm in the case of the iron derivatives and at 430 nm in the case of the manganese derivative.

chased from Sigma Chemical Co. as a lyophilized powder and further purified according to the standard procedure.[14] The protein concentration was estimated through UV absorption at 280 nm (ϵ_M = 92 300). Desferrioxamine B (DFB hereafter) was purchased from Ciba Geigy (DESFERAL). All the other reagents were of analytical grade.

The Fe^{III}_2–Tf–$(CO_3)_2$ derivative was prepared according to known procedures.[15] The Mn^{III}_2–Tf–$(CO_3)_2$ derivative was prepared by adding stoichiometric amounts of manganese(II) chloride to millimolar solutions of apotransferrin in the presence of 20 mM synergistic anion concentration at pH 9 (0.1 M Tris buffer) and then oxiding the samples with a 5-fold stoichiometric amount of hydrogen peroxide at 4 °C for 2 days. The complete formation of the Mn(III) derivatives was monitored by following the development of the absorption and CD bands typical of the manganese(III) derivative.[16,17] The samples were then adjusted to the desired pH by addition of aliquots of hydrochloric acid and diluted to the appropriate final concentration.

Kinetic measurements were performed in the following way: 800 μL of a 5.6×10^{-5} M protein solution was reacted with a 2-fold stoichiometric amount of DFB (only in the case of iron derivatives) and variable amounts of a 0.2 M sodium pyrophosphate solution buffered at pH 7.4. The final solution conditions for all the experiments were 5×10^{-5} M protein concentration, 1 mM [HCO_3^-], 50 mM Tris-HCl buffer, and pH 7.4. (In order to obtain the higher concentration values of sodium pyrophosphate, the volume ratios of the starting solutions were appropriately modified.)

The reaction was performed in a 1-mL cuvette of a Cary 17 D spectrophotometer, thermostated at 25 °C; absorption changes were followed at 330 nm for the iron derivatives and 430 nm for the manganese derivatives. In each experiment data collection was started 10 s after mixing and carried on up to disappearance of at least 80% of the initial absorption values.

Data analysis was performed by using standard programs for desk-top computers. Spectrophotometric data were fitted to eq 1 by using a four-parameter (namely A_0, A_∞, k_1, k_2) nonlinear regression fitting procedure. Reproduction of the experimental data was usually very good as shown in Figure 1. The errors in k_1 and k_2 values were around ±5%.

Results

Typical experimental profiles for metal release from iron– and manganese–transferrin–carbonate systems are reported in Figure 1 together with the calculated best-fitting curves; the profile of an experiment performed in the presence of 1 M sodium perchlorate is also shown. We have followed the decay in absorbance of the bands typical of the metalloprotein down to 10–20% residual absorption values. This allowed us to obtain good estimates of the rate constants of each site (k_1 and k_2 of eq 1) and to differentiate their behavior.

The plots of k_1 and k_2 versus pyrophosphate concentration, determined for iron transferrin at zero perchlorate concentration (0.05 Tris-HCl, pH 7.4; 1 mM [HCO_3^-]), are shown in Figure 2. It appears that the dependence of the kinetic constants on the concentration of pyrophosphate is of saturating type at low pyrophosphate concentration whereas it is linear in [PP] at higher concentration in agreement with the empirical Harris equation (see eq 2 in the Discussion).[13] This holds for both sites. The data on iron transferrin substantially agree with the average values found by Harris.[13]

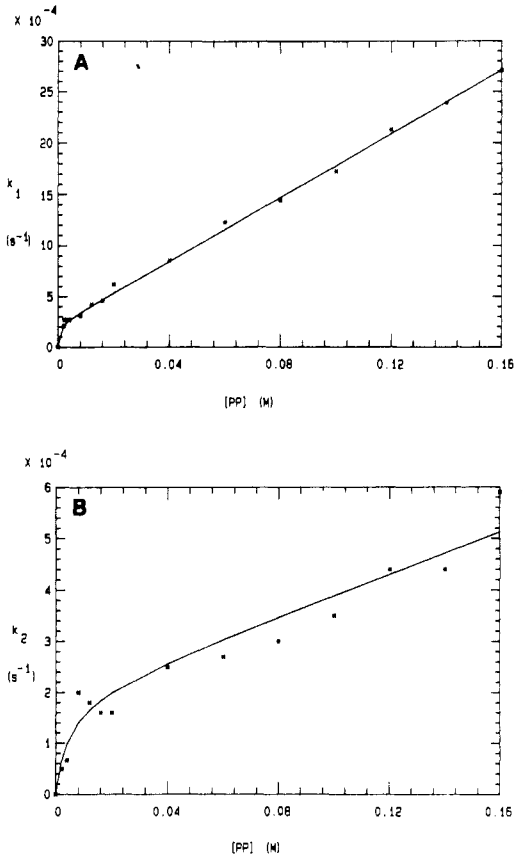


Figure 2. Dependence of k_1 (A) and k_2 (B) values on pyrophosphate concentration for iron transferrin. Conditions are as in Figure 1. The best-fitting curves calculated according to eq 2 are shown. The best-fitting parameters were as follows: $K_M = 0.6 \pm 0.3$ mM, $k_{max} = (2 \pm 1) \times 10^{-4}$ s^{-1}, and $k' = (1.55 \pm 0.02) \times 10^{-2}$ s^{-1} M^{-1} for the C-terminal site (k_1); $K_M = 5 \pm 2$ mM, $k_{max} = (2 \pm 1) \times 10^{-4}$ s^{-1}, and $k' = (2.0 \pm 0.3) \times 10^{-3}$ s^{-1} M^{-1} for the N-terminal site (k_2).

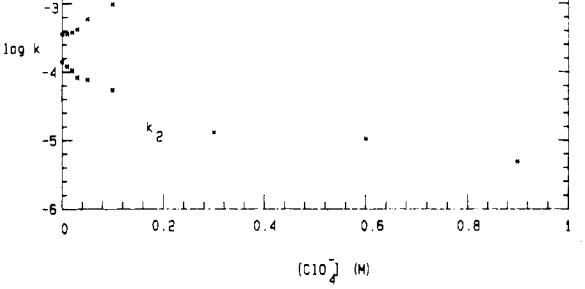


Figure 3. Dependence of k_1 and k_2 on perchlorate concentration for iron transferrin, in the presence of 10 mM pyrophosphate. The k values are reported on a logarithmic scale. Other conditions are as in Figure 1.

(14) Bates, G. W.; Schlabach, M. R. *J. Biol. Chem.* **1973**, *248*, 3228.
(15) Schlabach, M. R.; Bates, G. W. *J. Biol. Chem.* **1975**, *250*, 2182.
(16) Tomimatsu, Y.; Kinti, S.; Scherer, S. R. *Biochemistry* **1976**, *15*, 4918.
(17) Gaber, B. P.; Miskowski, V.; Spiro, T. G. *J. Am. Chem. Soc.* **1974**, *96*, 6868.

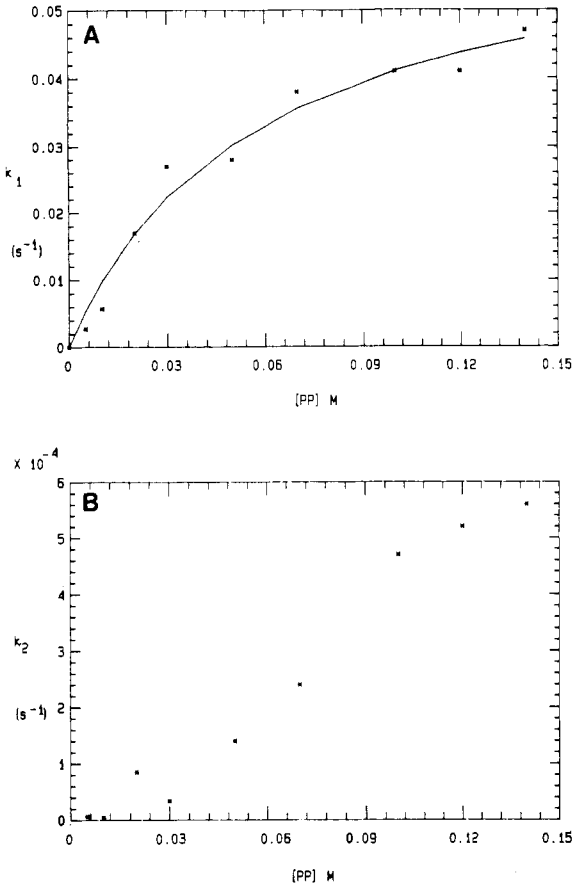


Figure 4. Dependence of k_1 (A) and k_2 (B) on PP concentration for iron transferrin in the presence of 1 M sodium perchlorate. Best fitting of k_1 to a simple hyperbolic curve is shown. Other conditions are as in Figure 1.

For a single pyrophosphate concentration (10 mM) the effect of a chaotropic agent like perchlorate has been investigated. The concentration of PP has been chosen to correspond roughly to the point where the saturating part of the profile has already been reached. It appears (Figure 3) that, in the presence of increasing concentrations of sodium perchlorate, iron release from one site is progressively accelerated and from the other site is slowed down. It should be noted that these experiments have been performed with 45 mM chloride ions from the buffer. Previous experiments have shown that chloride has the effect of increasing the metal release rate of the C-terminal site and of decreasing that of the N-terminal site.[18] It was also shown that perchlorate had a similar effect.[19] The latter experiment had been performed in the presence of EDTA, probably acting both as a scavenger and as a sink.

The effect of perchlorate alone has also been investigated; the kinetics of iron exchange between transferrin and DFB is very slow and is accelerated by less than 1 order of magnitude by addition of perchlorate. Values ranging between 10^{-6} and 10^{-5} s^{-1} can be estimated for one site at increasing perchlorate concentrations in the 0–1 M interval; for the other site the rate constant is always much lower than 10^{-6} s^{-1}. Analogous data on the effect of sodium chloride alone had been previously reported.[18]

Finally, the dependence of k_1 and k_2 on [PP] in the presence of 1 M perchlorate has been determined (Figure 4). k_1 shows a linear dependence on [PP] at low concentration and a clear decrease in slope at high concentration; the data can be fitted to a simple hyperbolic curve with $k_{max} = (6.4 \pm 0.6) \times 10^{-2}$ s^{-1} and $K_M = 56 \pm 13$ mM (Figure 4A). The profile of k_2 does not provide any evidence of a saturating behavior (Figure 4B).

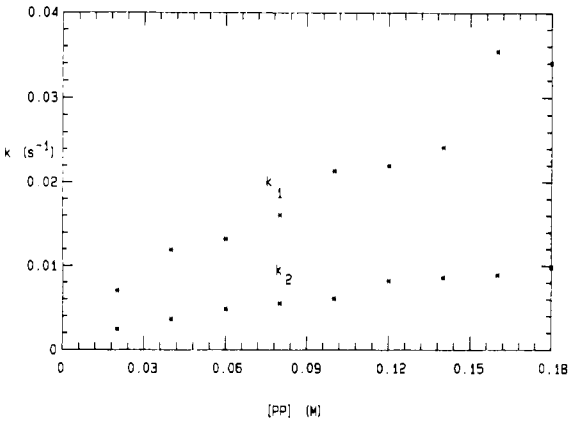


Figure 5. Dependence of k_1 and k_2 on pyrophosphate concentration for manganese(III) transferrin. k' values of $(1.7 \pm 0.1) \times 10^{-1}$ s^{-1} M^{-1} for k_1 and $(4.6 \pm 0.2) \times 10^{-2}$ s^{-1} M^{-1} for k_2 could be estimated according to eq 2. Conditions are as in Figure 1.

The data on manganese(III)–transferrin–carbonate, analyzed in the same way, show that the rate constants are higher than those of iron by about 1 order of magnitude. The dependence on [PP] is analogous to that of iron (Figure 5).

Discussion

In order to proceed with the discussion it is important to relate k_1 and k_2 to the actual sites of the protein. Assignment of the k_1 and k_2 values to the C-terminal and N-terminal sites of the protein could be straightforwardly deduced from inspection of their dependence on perchlorate concentration in the presence of 10 mM PP. Since it is known that the release kinetics of the C-terminal site is increased by an increase in $[Cl^-]$ or $[ClO_4^-]$, whereas the contrary happens for the N-terminal site,[2,19] it can be concluded that under the present experimental conditions (i.e. in the presence of 50 mM Tris-HCl buffer) the k_1 values, being always the larger ones, can always be referred to the C-terminal site.

Both sites exhibit a dependence of the kinetic parameters versus [PP] that can be considered as the sum of a saturative and of a linear term; this substantially confirms the recent finding by Harris et al.,[13] who had analyzed the same reaction and fitted the experimental data with an equation of the type

$$k_{obsd} = k_{max}[L]/(K_M + [L]) + k'[L] \quad (2)$$

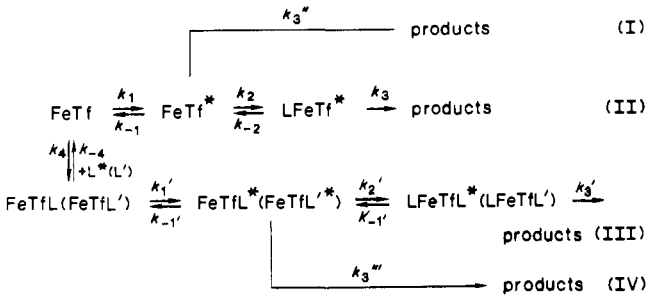
Although the distinction between a single saturative process with a high K_M value and the sum of a saturating process with low K_M and a linear term may be, under certain conditions, a quite subtle one, its relevance to the molecular mechanism is noteworthy. Indeed, this implies that more than a single pathway is operative in both sites for the iron removal reaction in contrast with the previous proposals.[20,21]

The first term of eq 2, of saturative type, can be considered[13] as arising from direct interaction of PP with the metal in the "open" conformation of transferrin as in the classical Bates mechanism (the transition from a closed to an open form is the rate-limiting step for the metal release reaction);[20] the meaning of the second term remains to be clarified.

The most reasonable hypothesis is that the latter term arises from the interaction of PP (which is also a good lyotropic agent) with positively charged allosteric sites which affect the thermodynamic and kinetic properties of the chromophore. Indeed, the presence and the importance of such sites have been recently demonstrated through spectroscopic and chemical modification studies.[22-24] This hypothesis is also supported by the data on the

(18) Williams, J.; Chasteen, N. D.; Moreton, K. *Biochem. J.* **1982**, *201*, 527.
(19) Baldwin, D. A.; de Sousa, D. M. R. *Biochem. Biophys. Res. Commun.* **1981**, *99*, 1101.
(20) Cowart, R. E.; Kojima, N.; Bates, G. *J. Biol. Chem.* **1982**, *257*, 7560.
(21) Carrano, C. J.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 5401.
(22) Price, E. M.; Gibson, J. *J. Biol. Chem.* **1972**, *247*, 8031.
(23) Folajtar, D. A.; Chasteen, N. D. *J. Am. Chem. Soc.* **1982**, *104*, 5775.
(24) Thompson, C. P.; McCarty, B. M.; Chasteen, N. D. *Biochim. Biophys. Acta* **1986**, *870*, 530.

Scheme I



interaction between PP and perchlorate (see below).

On the basis of such a hypothesis a comprehensive scheme for the mechanism of metal removal from each site can be written as shown in Scheme I. L refers to a species that can act both as a scavenger and a lyotropic agent whereas L′ refers to a species that can act only as a lyotropic agent.

The above mechanistic scheme retains the main concept of the Bates mechanism, according to which the rate-limiting step for the overall reaction is represented by a conformational transition of the protein from a closed to an open conformation (FeTf → FeTf* and FeTfL → FeTlL* in our scheme).[20] In addition, our mechanism makes explicit the concept that this rate-limiting step is affected by the occupancy of the allosteric sites by nonsynergistic anions. For the sake of clarity this circumstance is expressed by the presence of only two parallel pathways, the first for the MTf form and the second for the MTfL (MTfL′) form, even if, in principle, there could be as many pathways as the number of the $MTfL_n$ ($MTfL'_n$) species.

The spontaneous dissociation rates of the iron transferrin adducts are also explicated in the above mechanism (k_3'' and k_3'''; branches I and IV). However, in the case of the iron derivative, these terms have been directly measured by us and found to be small with respect to the kinetic constants of the "ligand-assisted" mechanism; so, they can be neglected.

The analytical expression of the $k_{1,2}$ values that can be deduced from the above scheme is

$$k_{obsd} = k_1k_2k_3[L]/\{k_{-1}k_{-2} + k_{-1}k_3 + (k_2k_3 + K_Ak_{-1}k_{-2} + K_Ak_{-1}k_{-3})[L] + K_Ak_2k_3[L]^2\} + K_Ak_1'k_2'k_3'[L]^2/\{k_{-1}'k_{-2}' + k_{-1}'k_3' + (k_2'k_3' + K_Ak_{-1}'k_{-2}' + K_Ak_{-1}'k_3')[L] + K_Ak_2'k_3'[L]^2\} \quad (3)$$

where $K_A = k_4/k_{-4}$, i.e. the affinity constant of L (L′) for FeTf.

This complex equation can be reduced to eq 2 if the quadratic terms in the denominators are small with respect to the linear terms and the zero-order term in the second part is also small with respect to the linear term. This provides a theoretical support to the Harris analysis. The linear dependence on [L] at high L concentrations would correspond to the progressive increase of the molar fraction of the FeTfL species.

Additional information on the reaction of iron removal can be deduced from the analysis of the dependence of the kinetic parameters on increasing concentrations of sodium perchlorate (L′). A direct visualization of the mutual interactions of PP and ClO_4^- can be obtained from three-dimensional plots in which the k_1 and k_2 values are reported versus PP and perchlorate concentrations (Figure 6). All the values are expressed on a logarithmic scale.

From inspection of these plots the following observations can be made:

(i) The rate of iron release from the C-terminal site (Figure 6A) is drastically enhanced by the addition of increasing concentrations of sodium perchlorate; this should reflect the progressive formation of a FeTfL′ species for which the rate-limiting conformational transition is faster than for the native derivative ($k_1' > k_1$). The affinity constant of perchlorate for iron transferrin is low, possibly corresponding to the presence of multiple equilibria of the type $MTf + nL' \rightleftarrows MTfL_n'$.

(ii) Addition of increasing concentrations of sodium perchlorate in the presence of 10 mM sodium pyrophosphate causes a net decrease of the rate constant of the N-terminal site (Figure 6B). This is indicative of reversible inhibition by perchlorate of the PP attack to the metal. In other words, k_2'/k_{-2}' of PP for the N-terminal site is apparently decreased by the presence of perchlorate.

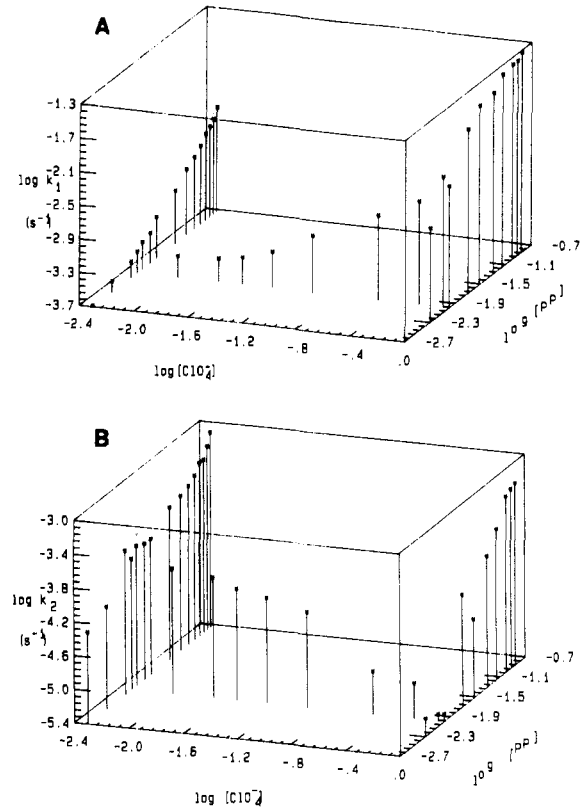


Figure 6. Three-dimensional plots showing the dependence of k_1 (A) and k_2 (B) values of iron transferrin on both pyrophosphate and perchlorate concentration. All values are reported on logarithmic scales. Data are deduced from the experiments shown in Figures 2–4.

(iii) The dependence of k_1 on PP concentration, in the presence of 1 M sodium perchlorate, exhibits a saturative behavior with a K_M value around 60 mM. This finding can be interpreted by assuming that the FeTfL′ species is largely predominant with respect to FeTf. Under these conditions only branch III of the mechanistic scheme is operative and a hyperbolic dependence on [PP] is expected. The higher value of K_M may be ascribed to a lower affinity of pyrophosphate for the FeTfL′ species possibly due to electrostatic repulsions. This is in agreement with point ii above.

(iv) Increasing the PP concentration at fixed 1 M sodium perchlorate concentration produces a rapid increase of k_2 that could reflect the progressive displacement of perchlorate from the metal site or from the channel that permits access to it. Indeed, at the highest PP concentrations the inhibitory effect of perchlorate is almost canceled out.

All these data can be accounted for on the basis of the above-proposed kinetic scheme; whereas PP behaves at the same time as a scavenger and as a lyotropic agent, perchlorate behaves only like a lyotropic agent with an efficacy similar to that of PP. So, when the relative concentrations of the two agents are modified appropriately, one of the two effects of PP can be canceled. This is immediately reflected in the kinetic parameters since the two terms of the Harris empirical equation respectively reflect the two actions of PP on iron transferrin.

A further matter of complication arises from the fact that perchlorate, in the N-terminal site, hampers drastically the access of PP to the metal. This results in a net differentiation of the two sites and in a clearly biphasic kinetics. This behavior is indicative of the presence of some important structural differences between the two sites at the level of the immediate surroundings of the metal. Indeed, it has been recently shown how positively charged residues like lysine and histidine not directly coordinated

to the metal are capable of binding nonsynergistic anions and of affecting the reactivity of the metal.[24,25] A different spatial arrangement of these clusters of positive charges in the two sites could account for the intersite differences. Refinement at higher resolution of the X-ray three-dimensional structure, which is now available for iron lactoferrin at 3.2-Å resolution,[26] could give the appropriate answers.

The results of the kinetic experiments on other metallotransferrins such as manganese(III) transferrin and copper(II) transferrin give us the opportunity of discussing the role played by the metal in the overall process.

In the case of manganese(III) transferrin, the profiles of the kinetic parameters versus PP concentration strictly resemble those observed for iron transferrin. Again, the data may be reproduced quite well with eq 2. The major difference between the two series of data resides in the fact that for the same values of PP concentration both of the kinetic constants of the manganese derivative are about 1 order of magnitude larger than the corresponding kinetic constants of iron transferrin. This suggests that the same mechanism of metal release is operative for both derivatives and that the substitution of iron(III) with the more labile manganese(III) ion only causes an increase of the intrinsic rate of the conformational transition.

An analogous interpretative scheme could hold also for the previously investigated copper derivative,[7] even if in the latter case the separation of the linear term from the hyperbolic one is more questionable. This could be ascribed to the fact that for copper transferrin the terms k_1 and k_1' are of comparable magnitude.

Concluding Remarks

The present study suggests that accurately determined experimental data obtained for the iron removal reaction from transferrin by pyrophosphate exhibit a complex pattern and cannot be rationalized on the grounds of simple kinetic models. Indeed, the previous models proposed by Raymond[21] and Bates[20] appear unable to account for them. An extension of the Bates model is needed to interpret the data and to provide a mechanistic support to the empirical Harris equation. In this framework a satisfactory and exhaustive interpretation of the data of PP as well of the effects of chaotropic agents and metal substitution is given. The role of residues of the metal binding cavity that are not directly coordinated to the metal but lie in its immediate surroundings is emphasized, and intersite differences are stressed.

Registry No. PP, 14000-31-8; perchlorate, 14797-73-0.

(25) Thompson, C. P.; Grady, J. K.; Chasteen, N. D. *J. Biol. Chem.* **1986**, *261*, 13128.
(26) Anderson, B. F.; Baker, H. M.; Dodson, E. J.; Norris, G. E.; Rumball, S. V.; Waters, J. M.; Baker, E. N. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1769.

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Synthesis and Characterization of Technetium(V) Complexes with Amine, Alcoholate, and Chloride Ligands

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Received January 28, 1988

Two general routes for the preparation of technetium complexes with aromatic amine, chloride, and alkoxide ligands are presented. The reactions of the tetrachlorooxotechnetate(V) anion of $(n\text{-}Bu_4N)[TcOCl_4]$ with pyridine in tetrahydrofuran/alcohol solvent or of the dioxotetrakis(pyridine)technetium(V) cation of $[TcO_2(py)_4]Cl$ with LiCl in sulfuric acid/alcohol both yield a neutral alkoxydichlorooxobis(pyridine)technetium(V) complex. The same isomer appears to result from either route. The analogous thiazole derivatives may be prepared in a like manner. The complex chloro(1,2-ethanediolato)oxo(1,10-phenanthroline)technetium(V), $TcOCl(C_2H_4O_2)(C_{12}H_8N_2)$, is prepared from $(n\text{-}Bu_4N)[TcOCl_4]$ with 1,2-ethanediol and 1,10-phenanthroline in methanol. This complex was characterized by IR, UV/vis, and ^1H and ^{99}Tc NMR spectroscopies and by fast atom bombardment mass spectrometry. Further insight into the bonding in this complex comes from the single-crystal X-ray structure determination. The space group is monoclinic, $P2_1/c$, with $a = 7.440$ (2) Å, $b = 8.928$ (3) Å, $c = 21.355$ (4) Å, $\beta = 92.48$ (2)°, $V = 1417.2$ (7) Å^3, and $Z = 4$. The structure was solved by standard methods and refined to $R = 0.051$ and $R_w = 0.036$ based on 1859 reflections. The oxo and chloride ligands are mutually cis in the highly distorted octahedral coordination sphere. The unusually long Tc–Cl bond length (2.418 (2) Å) is attributed to a trans influence exerted by the coordinated diolate. This effect combined with short lengths for the C–C bond (1.491 (1) Å) and for the O–Tc–O linkage (1.924 (4), 1.902 (3) Å) suggests partial multiple bonding between the technetium and the diolate.

Introduction

Since the discovery[2] of *trans*-$[TcO_2(py)_4]^+$, there has been considerable interest in oxotechnetium(V) complexes with aromatic amine ligands. Some of these compounds have been investigated as potential ^{99m}Tc diagnostic radiopharmaceutical agents,[3] and they have been used as ligand substitution reagents for the preparation of novel species.[4]

Clarke[5] recently reported a series of mixed-ligand complexes with stoichiometry $TcOX_2(A)_2(OR)$ (A = 4-cyanopyridine, 4-nitropyridine; X = Cl, Br; R = Me, Et) related to the compound $TcOCl_2(bpy)(OEt)$,[6] previously prepared in our laboratory. It had been postulated that these complexes can only be isolated when an electron-withdrawing amine is used. It was speculated, how-

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(2) Kusina, A. F.; Oblova, A. A.; Spitsyn, V. I. *Russ. J. Inorg. Chem. (Engl. Transl.)* **1972**, *17*, 1377.
(3) Seifert, S.; Münze, R.; Johannsen, B. In *Technetium in Chemistry and Nuclear Medicine*; Deutsch, E., Nicolini, M., Wagner, H. N., Jr., Eds.; Cortina International: Verona, Italy, 1983; pp 19–23.
(4) Trop, H. S.; Jones, A. G.; Davison, A. *Inorg. Chem.* **1980**, *19*, 1993.
(5) Fackler, P. H.; Kastner, M. E.; Clarke, M. J. *Inorg. Chem.* **1984**, *23*, 3968.
(6) Davison, A.; Jones, A. G.; Abrams, M. J. *Inorg. Chem.* **1981**, *20*, 4300.

0020-1669/88/1327-2409$01.50/0