Kinetic Studies on Reactions of Iron–Sulfur Proteins. 2. An Extension of the Range of Oxidants in the Reaction of Reduced Parsley 2-Fe Ferredoxin and Identification of Specific Binding Sites Using Redox Inactive $Cr(NH_3)_6^{3+}$ (and $Cr(en)_3^{3+}$)

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Abstract: The one-electron oxidations of reduced 2-Fe parsley ferredoxin, containing the $Fe_2S_2^*(SR)_4^{3-}$ center and in the Fe(II,III) state, with four additional oxidants ($Co(acac)_3$, $Co(C_2O_4)_3^{3-}$, $(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$, and $Pt(NH_3)_6^{4+}$) have been studied at pH 8.0 (Tris buffer), I = 0.10 M (NaCl). The range of charge on oxidants now extends from 5+ to 3−. The $Co(III)_2$ and Pt(IV) oxidants exhibit limiting-kinetic behavior consistent with a mechanism involving association followed by outer-sphere electron transfer: Fe(II,III) + oxidant $\rightleftharpoons$ Fe(II,III), oxidant (K) and Fe(II,III), oxidant $\rightarrow$ Fe(III,III) + product (k_{et}). The "dead-end" mechanism is also considered as an alternative. In terms of the former $K = 2.6 \times 10^4$ M^{-1} and $k_{et} = 214$ s^{-1} at 25 °C (both extrapolated from data at 0–7 °C) for $Co(III)_2$, and $K = 2.1 \times 10^4$ M^{-1} and $k_{et} = 3.29$ s^{-1} for $Pt(NH_3)_6^{4+}$ Overall rate constants k ($= Kk_{et}$) are for $Co(acac)_3$ 7.0×10^3 M^{-1} s^{-1} and for $Co(C_2O_4)_3^{3-}$ 3.9×10^3 M^{-1} s^{-1} at 25 °C. The temperature dependences were investigated. Association of a single redox inactive $Cr(NH_3)_6^{3+}$ (or $Cr(en)_3^{3+}$) with the protein blocks the reactions with $Co(III)_2$, $Pt(NH_3)_6^{4+}$, $Co(NH_3)_6^{3+}$, $Co(NH_3)_6Cl^{2+}$, and $Co(NH_3)_5(C_2O_4)^+$, K_{Cr} (25 °C) = 464 ± 14 M^{-1} for $Cr(NH_3)_6^{3+}$. It has no effect on the reaction with $Co(acac)_3$, and with Co(edta)$^-$ (K_{Cr} = 459 M^{-1}) results in an acceleration. Results are discussed in terms of three different reaction sites on the protein, which are selected according to ligand type on the oxidant.

Kinetic studies on the oxidation of reduced parsley (and spinach) 2-Fe ferredoxins, containing the units $Fe_2S_2^*(SR)_4^{3-}$, with $Co(NH_3)_6^{3+}$, (*dl*- and *d*-) $Co(en)_3^{3+}$, $Co(NH_3)_5Cl^{2+}$, $Co(NH_3)_5(C_2O_4)^+$, $Co(dmgH)_2(C_6H_5NH_2)_2^+$, and Co(edta)$^-$ have been reported previously,[1] and evidence consistent with the mechanism

$$\text{Fe(II,III)} + \text{oxidant} \overset{K}{\rightleftharpoons} \text{Fe(II,III),oxidant} \quad (1)$$

$$\text{Fe(II,III),oxidant} \xrightarrow{k_{et}} \text{Fe(III,III)} + \text{product} \quad (2)$$

involving protein–complex association has been presented. Other possible mechanisms were considered and are returned to later in this paper. The aim of the present study is first to extend the range of oxidants, and then to test the use of redox inactive $Cr(NH_3)_6^{3+}$ (and $Cr(en)_3^{3+}$) complexes as blocking agents for the redox process. Preliminary results for an X-ray crystallographic investigation[2] of the chloroplast ferredoxin *Spirulina platensis* (mol wt 10 890, 98 amino acids) have recently been reported, in which it is concluded that the 2-Fe active center is relatively close to the surface of the protein.

Experimental Section

The isolation of parsley 2-Fe ferredoxin containing the $Fe_2S_2^*(SR)_4^{2-}$ center, generation of the reduced form $Fe_2S_2^*(SR)_4^{3-}$ (using dithionite), adjustment of pH, kinetic measurements, and treatment of data were as previously described.[1]

Preparation of Complexes. Tris(acetylacetonate)cobalt(III) ($Co(acac)_3$),[3] potassium tris(oxalato)cobaltate(III) ($K_3[Co(C_2O_4)_3]\cdot 3.5H_2O$),[4] μ-amido-bis(pentaammine)cobalt(III) bromide ($[NH_3)_5CoNH_2Co(NH_3)_5]Br_5$),[5,6] hexaammineplatinum(IV) chloride ($[Pt(NH_3)_6]Cl_4\cdot H_2O$),[7] hexaamminechromium(III) chloride ($[Cr(NH_3)_6]Cl_3$),[8] and tris(ethylenediamine)chromium(III) chloride ($[Cr(en)_3]Cl_3\cdot 3H_2O$)[9] were prepared as described in the literature. The complexes $[Co(NH_3)_6]Cl_3$, $[Co(NH_3)_5Cl]Cl_2$, $[Co(NH_3)_5(C_2O_4)]Cl$, and $Na[Co(edta)]\cdot 4H_2O$ were as previously described.[1] Characterization of the Co(III) and Cr(III) complexes was by means of UV–visible spectra, Table I. Solutions of Cr(III) were generally made up ca. 15 min prior to kinetic measurements, and protected from light by Al foil to minimize photochemical decomposition. The Pt(IV) complex gave a shoulder λ 260 nm, ε 129 M^{-1}. Anal. Calcd for $[Pt(NH_3)_6]Cl_4\cdot H_2O$: H, 4.41; N, 18.39; Cl, 31.02. Found: H, 4.55; N, 18.15; Cl, 30.6. Sodium ferrocyanide (BDH, Analar) was used. Potassium tetrakis(oxalato)zirconate(IV) ($K_4[Zr(C_2O_4)_4]\cdot 5H_2O$) was prepared as described.[10] The latter can be recrystallized from H_2O without loss of oxalate and on titration with permanganate (0.02 M) at ca. 60 °C in 3:1 H_2SO_4–H_2O gives 51.5% oxalate (calcd value 51.1%). It was assumed that no oxalate was lost over 30-min periods in solution.

Stoichiometries. For the conditions investigated the primary product $Co(NH_3)_6^{3+}$ formed in the reaction with $(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$ as oxidant reacts relatively slowly,[11] and does not participate further in the reaction, which is therefore

$$\text{Fe(II,III)} + Co(III)_2 \rightarrow \text{Fe(III,III)} + \text{Co(III)} \quad (3)$$

Platinum(IV) complexes function as 2-equiv oxidants yielding Pt(II) products.[12,13] The reaction sequence is presumed to be

$$\text{Fe(II,III)} + \text{Pt(IV)} \rightarrow \text{Fe(III,III)} + \text{Pt(III)} \quad (4)$$

$$\text{Fe(II,III)} + \text{Pt(III)} \rightarrow \text{Fe(III,III)} + \text{Pt(IV)} \quad (5)$$

with Pt(III) as a transient and (5) rapid and non-rate-determining. First-order plots were linear to 80% completion consistent with this belief. Measured first-order rate constants were halved to allow for (5), giving values listed in the tables.

Kinetics. The procedure was as previously described.[1]

Results

First-order rate constants, k_{obsd}, for the reaction of zero charged and 3− oxidants $Co(acac)_3$, (0.4–6.4) $\times 10^{-3}$ M, and $Co(C_2O_4)_3^{3-}$, (0.15–3.0) $\times 10^{-3}$ M, with reduced 2-Fe ferredoxin, $Fe_2S_2^*(SR)_4^{3-}$ (Tables II and III),[14] give a linear dependence on oxidant concentration. From the temperature dependence activation parameters for second-order rate constants k ($= Kk_{et}$) are, with $Co(acac)_3$ as oxidant, $\Delta H^{\ddagger} = 6.28 \pm 0.85$ kcal mol^{-1} and $\Delta S^{\ddagger} = -19.6 \pm 2.9$ cal K^{-1} mol^{-1}, and with $Co(C_2O_4)_3^{3-}$, $\Delta H^{\ddagger} = 3.15 \pm 0.16$ kcal mol^{-1} and $\Delta S^{\ddagger} = -31.5 \pm 1.0$ cal K^{-1} mol^{-1}. For the positively charged oxidants $(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$ and $Pt(NH_3)_6^{4+}$, k_{obsd} values (Tables IV and V)[14] exhibit a nonlinear dependence on oxidant concentration, Figures 1 and 2. Reciprocal plots of

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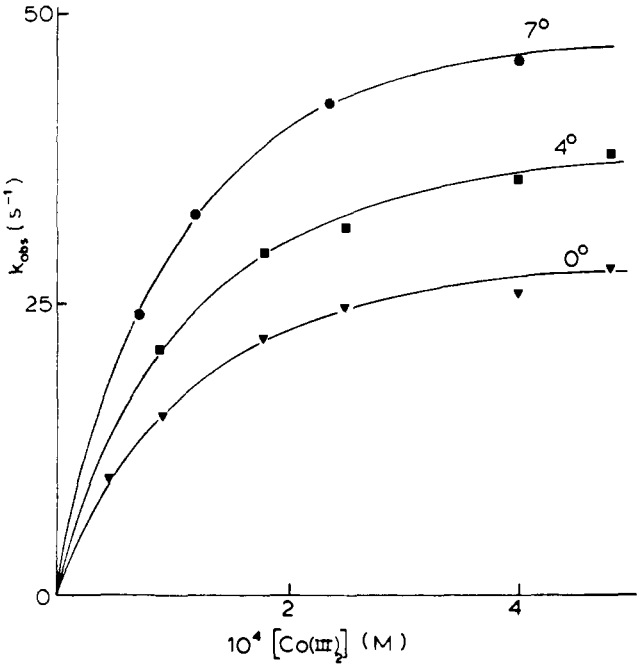

Figure 1. The nonlinear dependence of first-order rate constants, k_{obsd}, on oxidant concentration for the $(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$ oxidation of reduced parsley ferredoxin, $Fe_2S_2^*(SR)_4^{3-}$, pH 8.0 (Tris), I = 0.10 M (NaCl).

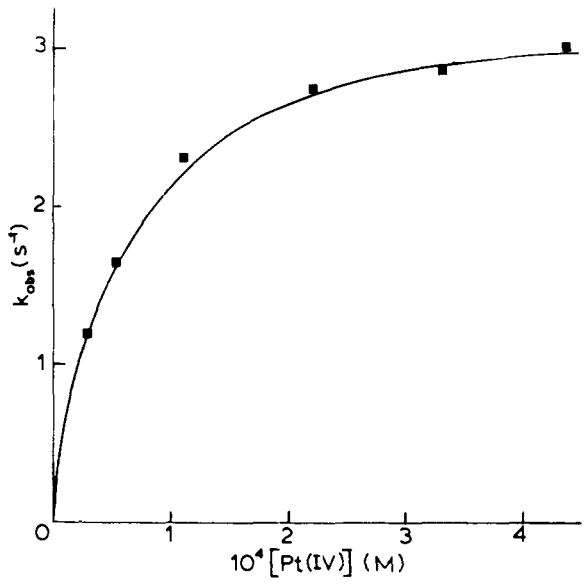

Figure 2. The nonlinear dependence of first-order rate constants (25 °C), k_{obsd}, on oxidant concentration for the $Pt(NH_3)_6^{4+}$ oxidation of reduced parsley ferredoxin, $Fe_2S_2^*(SR)_4^{3-}$, pH 8.0 (Tris), I = 0.10 M (NaCl).

$(k_{obsd})^{-1}$ against $[oxidant]^{-1}$ are linear consistent with

$$k_{obsd} = \frac{Kk_{et}[oxidant]}{1 + K[oxidant]} \quad (6)$$

which can be derived for (1) and (2). As in previous studies the intercept $(1/k_{et})$ and slope $(1/Kk_{et})$ allow K and k_{et} to be evaluated. The temperature dependence for $Co(III)_2$ was investigated over a limited range (0–7 °C) only, giving K (25 °C) = 2.64×10^4 M, $\Delta H°$ = ca. 5.6 kcal mol^{-1}, $\Delta S°$ = ca. 38.6 cal K^{-1} mol^{-1}, and k_{et} (25 °C) = 214 s^{-1}, $\Delta H^{\ddagger}_{et}$ = ca. 11.4 kcal mol^{-1}, $\Delta S^{\ddagger}_{et}$ = ca. −9.6 cal K^{-1} mol^{-1}. At 25 °C, with $Pt(NH_3)_6^{4+}$ as oxidant, $K = (2.1 \pm 0.2) \times 10^4$ M^{-1} and k_{et} = 3.29 ± 0.10 s^{-1}. The temperature dependence was not investigated.

Addition of redox inactive $Cr(NH_3)_6^{3+}$ or $Cr(en)_3^{3+}$ to the reaction of reduced 2-Fe ferredoxin with $Co(NH_3)_6^{3+}$ retards the redox process. A relatively small Co(III) concentration was used such that the Co(III) remained in large excess over the concentration of protein but K[Co(III)] ≪ 1. Varying amounts of the Cr(III) were used, Figure 3. First-order rate constants k_{obsd} (Table VI)[14] conform to

$$\frac{k_{obsd}}{[oxidant]} = \frac{Kk_{et}}{1 + K_{Cr}[Cr(III)]} \quad (7)$$

where $Kk_{et}(k)$ is the rate constant with no added Cr(III). This equation is consistent with a reaction sequence which may be expressed as

$$Fe(II,III) + Cr(III) \overset{K_{Cr}}{\rightleftharpoons} Fe(II,III),Cr(III) \quad (8)$$

$$Fe(II,III) + oxidant \overset{k}{\rightarrow} Fe(III,III) + product \quad (9)$$

From reciprocal plots, Figure 4, K_{Cr} values (Table VII) for the association of Cr(III) with the protein were obtained. Similarly with other ammine oxidants $(Co(NH_3)_5(C_2O_4)^+$ (Figure 5), $Co(NH_3)_5Cl^{2+}$, $(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$, and $Pt(NH_3)_6^{4+}$), inhibition was observed on addition of $Cr(NH_3)_6^{3+}$ and values of K_{Cr} (Table VII) were obtained. With $Co(acac)_3$ as oxidant $Cr(NH_3)_6^{3+}$ has no effect (Table VI),[14] and with $Co(edta)^-$ (Table VI)[14] there is a nonlinear increase in rate constants, k_{obsd}, with increasing concentration of $Cr(NH_3)_6^{3+}$, Figure 6. For the latter the reaction sequence

$$Fe(II,III),Cr(III) + oxidant \xrightarrow{k_{Cr}} Fe(III,III) + Cr(III) + product \quad (10)$$

is proposed, in which the protein-Cr(III) adduct is more reactive toward $Co(edta)^-$ than the protein itself. This scheme gives

$$\frac{k_{obsd}}{[Co(III)]} - k = \frac{(k_{Cr} - k)K_{Cr}[Cr(III)]}{1 + K_{Cr}[Cr(III)]} \quad (11)$$

which can be written in the form

$$\frac{[Co(III)]}{k_{obsd} - k[Co(III)]} = \frac{1}{(k_{Cr} - k)K_{Cr}}\frac{1}{[Cr(III)]} + \frac{1}{(k_{Cr} - k)} \quad (12)$$

Table I. Characterization of Complexes. Comparison of UV–Visible Peak Positions (λ) and Absorption Coefficients (ε) with Literature Values

complex	λ, nm (ϵ, M^{-1} cm^{-1})[a]	λ, nm (ϵ, M^{-1} cm^{-1})[b]	ref
$Co(acac)_3$	590	590	*c*
	514[d]	514[d]	*c*
	257 (3.4×10^4)[e]	257 (3.3×10^4)[e]	*f*
$Co(C_2O_4)_3^{3-}$	596 (167)	596 (165)	*g*
	420 (221)	420 (218)	*g*
	245 (2.2×10^4)	245 (2.1×10^4)	*g*
$(NH_3)_5CoNH_2$-$Co(NH_3)_5^{5+}$	505 (420)	505 (428)	5
	360 (705)	360 (708)	5
$Pt(NH_3)_6^{4+}$	260 (129)		
$Cr(NH_3)_6^{3+}$	462 (40)	462 (40)	*h*
	350 (33)	350 (33)	*h*
$Cr(en)_3^{3+}$	457 (73)	457 (75)	9
	351 (63)	351 (61)	9

[a] This work. [b] Literature values. [c] A. E. Finn, G. C. Hampson, and L. E. Sutton, *J. Chem. Soc.*, 1254 (1938). [d] Minimum. [e] Ethanol solvent. [f] R. H. Holm and F. A. Cotton, *J. Am. Chem. Soc.*, **80**, 5658 (1958). [g] J. Barrett and J. H. Baxendale, *Trans. Faraday Soc.*, **52**, 210 (1956). [h] G. Guastella and T. W. Swaddle, *Inorg. Chem.*, **13**, 61 (1974). [i] Shoulder.

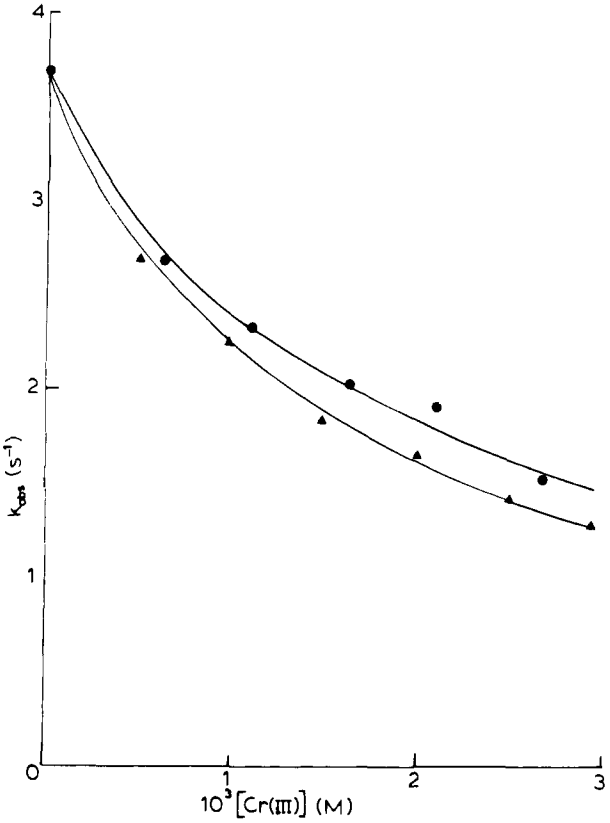

Figure 3. The Cr(III) inhibition of the $Co(NH_3)_6^{3+}$ oxidation of reduced parsley ferredoxin, $Fe_2S_2{}^*(SR)_4^{3-}$. Plot of k_{obsd} (25 °C) against Cr(III) concentration, (●) $Cr(NH_3)_6^{3+}$, (▲) $Cr(en)_3^{3+}$, $[Co(NH_3)_6^{3+}] = 2.0 \times 10^{-4}$ M, pH 8.0 (Tris), $I = 0.10$ M (NaCl).

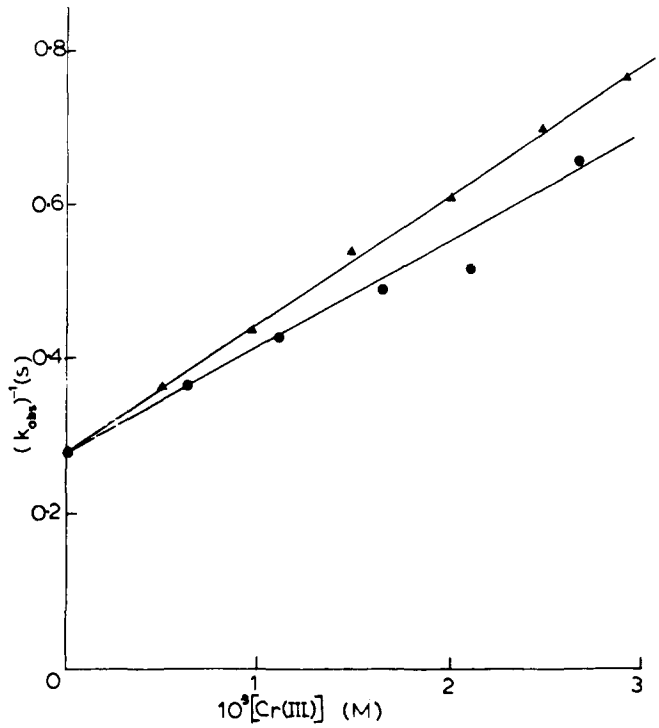

Figure 4. The Cr(III) inhibition of the $Co(NH_3)_6^{3+}$ oxidation of reduced parsley ferredoxin, $Fe_2S_2{}^*(SR)_4^{3-}$. Plot of $(k_{obsd})^{-1}$ (25 °C) against $[Cr(III)]^{-1}$, (●) $Cr(NH_3)_6^{3+}$, (▲) $Cr(en)_3^{3+}$, $[Co(NH_3)_6^{3+}] = 2.0 \times 10^{-4}$ M, pH 8.0 (Tris), $I = 0.10$ M (NaCl).

Table VII. Constants K_{Cr} (25 °C) for the Association of Redox-Inactive $Cr(NH_3)_6^{3+}$ (and in One Case $Cr(en)_3^{3+}$) to Reduced Parsley Ferredoxin, $Fe_2S_2{}^*(SR)_4^{3-}$, as Determined from Influence on Redox Process with Different Oxidants, pH 8.0 (Tris), $I = 0.10$ M (NaCl)

oxidant	influence of Cr(III)[a]	K_{Cr},[a] M
$Co(NH_3)_6^{3+}$	inhibits	476 (±54)
	inhibits[b]	590 (±18)[b]
$Co(NH_3)_5Cl^{2+}$	inhibits	462 (±15)
$Co(NH_3)_5C_2O_4^{+}$	inhibits	467 (±11)
$Co(acac)_3$	no effect	
$Co(edta)^-$	accelerates	459 (±10)
$Co_2(NH_3)_{10}(NH_2)^{5+}$	inhibits	305 (±29)[c]
$Pt(NH_3)_6^{4+}$	inhibits	474 (±14)

[a] $Cr(NH_3)_6^{3+}$ except for second entry. [b] $Cr(en)_3^{3+}$. [c] 7.0 °C. Values of K at 25.0 (average) and 7.0 °C give $\Delta H° = 4.1$ kcal mol^{-1} and $\Delta S° = 26$ cal K^{-1} mol^{-1}.

A plot of the left-hand side of (12) against $[Cr(III)]^{-1}$ is linear (Figure 6), and K_{Cr} and k_{Cr} can be evaluated. The value of K_{Cr} (459 ± 10 M^{-1}) is identical with those from the inhibition studies (Table VII), and $k_{Cr} = (2.2 \pm 0.1) \times 10^4$ M^{-1} s^{-1}. A similar study of the influence of $Cr(NH_3)_6^{3+}$ on the $Co(C_2O_4)_3^{3-}$ oxidation was precluded by precipitation which was observed on mixing these two complexes.

Discussion

Results for the three Co(III) oxidants $Co(acac)_3$, $Co(C_2O_4)_3^{3-}$, and $(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$ complement those for six Co(III) oxidants previously reported, and extend the range of charge types from 5+ to 3−. The oxidant $Pt(NH_3)_6^{4+}$ has also been included not only because it is a suitable highly charged reactant, but also because it is a 2-equiv oxidant which might display different redox behavior in its reactions with 2-Fe (1 equiv) and 8-Fe (2 equiv) ferredoxins.[15] The 5+, 4+, 3+, and (marginally) 2+ oxidants exhibit varying degrees of limiting kinetic behavior, so that K (Table VIII) and k_{et} (Table IX) as defined in (1) and (2) can be evaluated. Values of K exhibit a marked dependence on oxidant charge. No limiting kinetic behavior was detected with the 3-oxidant $Co(C_2O_4)_3^{3-}$ ($K < 50$ M^{-1} at 25 °C), and the 4− redox inactive complexes $Fe(CN)_6^{4-}$ ($\lesssim 3.0 \times 10^{-3}$ M) and $Zr(C_2O_4)_4^{4-}$ ($\lesssim 3.8 \times 10^{-3}$ M) did not inhibit the reaction, indicating no significant association of such negatively charged species. In contrast examples have been reported of both positive (3+) and negative (4−) complexes which give significant association (K) in analogous studies with blue copper proteins.[16-18] The amino acid composition of spinach 2-Fe ferredoxin, which on present evidence displays similar reaction characteristics to parsley 2-Fe ferredoxin,[1] has been determined. Based on individual amino acid charges at pH 7 the charge on the reduced spinach protein can be estimated to be

Table VIII. Values of K (25 °C), and (Where Measured) Corresponding Thermodynamic $\Delta H°$ and $\Delta S°$ for the Association of Complexes with Reduced Parsley Ferredoxin $Fe_2S_2{}^*(SR)_4^{3-}$, pH 8.0 (Tris), $I = 0.10$ M (NaCl)

complex	K, M	$\Delta H°$, kcal mol^{-1}	$\Delta S°$, cal K^{-1} mol^{-1}
$(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$	26 400[a]	5.6[b]	38.6[b]
$Pt(NH_3)_6^{4+}$	21 000		
$Co(NH_3)_6^{3+}$	998	10.2	47.9
$Co(en)_3^{3+}$	597	11.0	49.5
$Cr(NH_3)_6^{3+}$	464		
$Cr(en)_3^{3+}$	590		
$Co(NH_3)_5Cl^{2+}$	194	(2.6)[c]	(19.2)[c]

[a] Extrapolated from data at 0-7 °C. [b] Obtained from data over a limited temperature range, 0-7 °C. [c] Marginal effect only.

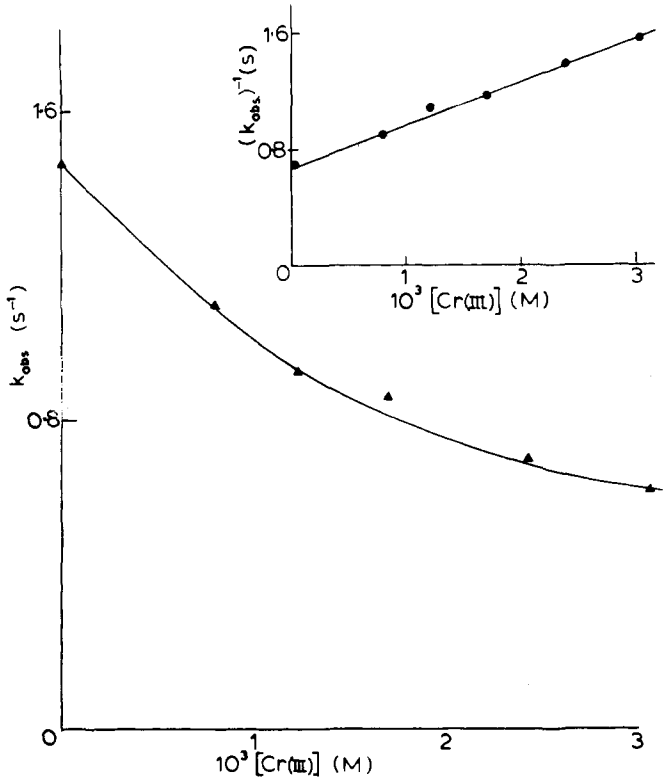

Figure 5. The $Cr(NH_3)_6^{3+}$ inhibition of the $Co(NH_3)_5(C_2O_4)^+$ oxidation of reduced parsley ferredoxin, $Fe_2S_2^*(SR)_4^{3-}$. The dependence of k_{obsd} (25 °C) on [Cr(III)], $[Co(NH_3)_5(C_2O_4)^+]$ = 2.5 × 10^{-4} M, pH 8.0 (Tris), I = 0.10 M (NaCl).

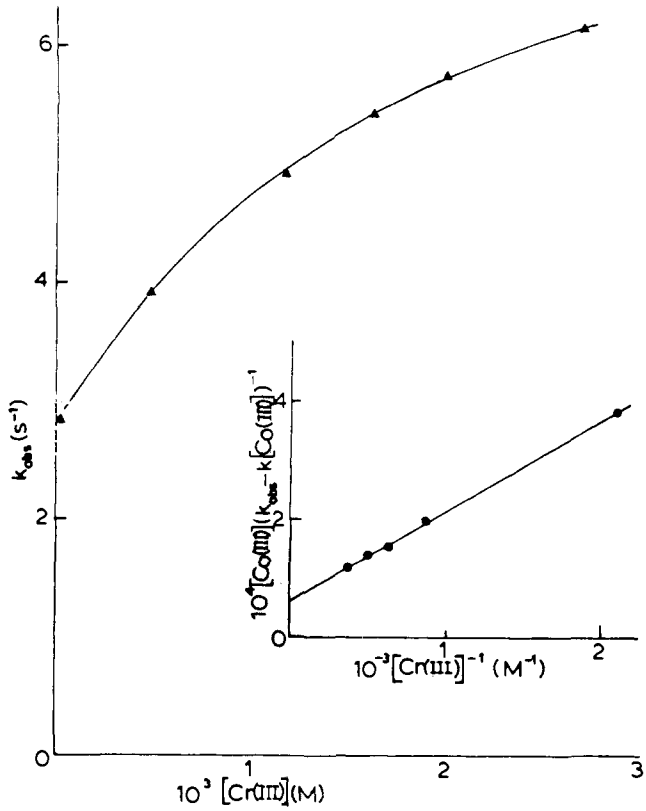

Figure 6. The $Cr(NH_3)_6^{3+}$ acceleration of the $Co(edta)^-$ oxidation of reduced parsley ferredoxin, $Fe_2S_2^*(SR)_4^{3-}$. The dependence of k_{obsd} (25 °C) on [Cr(III)], $[Co(edta)^-]$ 4.0 × 10^{-4} M, pH 8.0 (Tris), I = 0.10 M (NaCl).

17−.[19] Large net charges appear to be characteristic of all the plant ferredoxins so far isolated, although estimates are expected to be modified by internal compensatory effects such as H bonding, and should be regarded as maximum values. Experimental evidence for a substantial negative charge with parsley ferredoxin is obtained from the observation that this protein binds strongly to DEAE anion exchangers and requires >0.3 M Cl^- for elution. It would appear therefore that association with positively charged redox partners is favored and that the electrostatic contribution is a dominant factor.

The $Cr(NH_3)_6^{3+}$ (and in one case $Cr(en)_3^{3+}$) inhibition of the oxidation of reduced 2-Fe parsley ferredoxin with 5+, 4+, 3+, 2+, and 1+ ammine complexes is of interest for a number of reasons. Foremost is that from the dependence (7) a single redox inactive Cr(III) can completely block the reaction of these oxidants. Thus only one and not a number of sites on the protein surface appears to be utilized in these reactions.

The value of K for the 5+ $Co(III)_2$ oxidant (26 400 M^{-1}) is significantly larger than the value for 3+ $Co(NH_3)_6^{3+}$ (998 M^{-1}), suggesting that the protein sees more than the end-on aspect of the binuclear complex. This is confirmed by the observation that K for $Co(III)_2$ is slightly greater than for $Pt(NH_3)_6^{4+}$ (21 000 M^{-1}), Table VIII. For a sideways on electrostatic interaction of the 5+ $Co(III)_2$ complex clearly a fairly large surface area on the protein is involved. It would appear, however, that within this area there is a small, highly specific region at which electron transfer takes place. A small region is required; otherwise it is unlikely that a 3+ $Cr(NH_3)_6^{3+}$ complex against a ca. 17− background would completely block reaction with the 1+ complex $Co(NH_3)_5C_2O_4^+$ (which has a negative side), even if it were able to block the 2+ and 3+ oxidants. The magnitude of K_{Cr} for $Cr(NH_3)_6^{3+}$ (464 M^{-1}) as compared to K for $Co(NH_3)_6^{3+}$ (998 M^{-1}) suggests that the bond distance (and hence the electrostatic interaction) is important, Cr-N (2.06 Å)[20] being larger than Co-N (1.98 Å).[21] Ion-pairing constants (25 °C) for sulfate association with $Cr(NH_3)_6^{3+}$ (61 M^{-1}) and $Co(NH_3)_6^{3+}$ (93 M^{-1}),[22] I = 0.10 M ($NaClO_4$), exhibit a similar trend.

Table IX. Reactions of Reduced Parsley Ferredoxin, $Fe_2S_2^*(SR)_4^{3-}$. Kinetic Data for Electron Transfer within the Protein-Complex Adduct, pH 8.0, I = 0.10 M (NaCl), 25 °C

oxidant	k_{et}, s^{-1}	$\Delta H^{\ddagger}_{et}$	$\Delta S^{\ddagger}_{et}$
$(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$	214[a]	11.4[b]	−9.6[b]
$Pt(NH_3)_6^{4+}$	3.29		
$Co(NH_3)_6^{3+}$	19.2	8.5	−24.1
$Co(en)_3^{3+}$	2.7	10.0	−23.1
$Co(NH_3)_5Cl^{2+}$	2300	(5.8)[c]	(−23.8)[c]

[a] Extrapolated from data at 0-7 °C. [b] Obtained from data over a limited temperature range, 0-7 °C. [c] Marginal effect only.

With $Co(acac)_3$ as oxidant the presence of $Cr(NH_3)_6^{3+}$ associated to the proteins has no effect whatsoever. This complex must therefore be reacting at a different site on the protein surface. The oxidant $Co(edta)^-$, on the other hand, gains in reactivity when $Cr(NH_3)_6^{3+}$ is resident. There are two possible explanations of this latter effect. The first is that $Co(edta)^-$ uses the same site as $Cr(NH_3)_6^{3+}$, the enhancement resulting from the more favorable electrostatics when $Cr(NH_3)_6^{3+}$ is present. The second is that the presence of $Cr(NH_3)_6^{3+}$ activates an alternative site used by $Co(edta)^-$. We tend to exclude the first of these since $Co(NH_3)_5(C_2O_4)^+$ might have been expected to display similar behavior, or at least not give a complete switch-off in reactivity when $Cr(NH_3)_6^{3+}$ is resident. Also in further studies[23] on the reduction of 2-Fe ferredoxin in the Fe(III,III) state with positively charged $Cr(15\text{-ane}N_4)(H_2O)_2^{2+}$, where 15-ane$N_4$ is the macrocyclic ligand 1,4,8,12-tetraazacyclopentadecane, it has

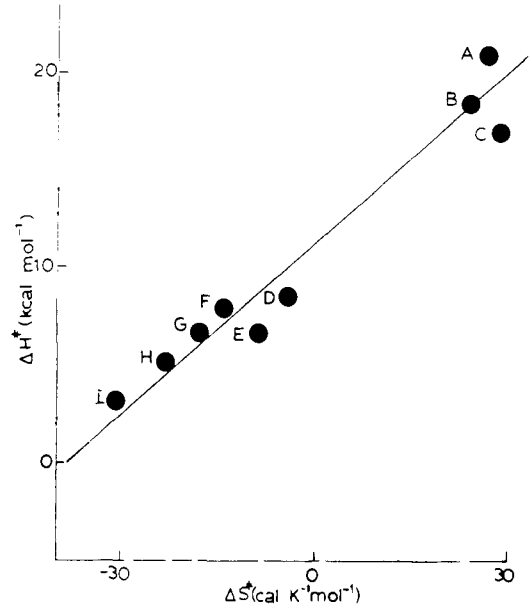

Figure 7. Plot of activation parameters $\Delta H^{\ddagger}$ against $\Delta S^{\ddagger}$ obtained for overall rate constants $k(=Kk_{et})$ for the oxidation of reduced parsley ferredoxin $Fe_2S_2{}^*(SR)_4{}^{3-}$. Oxidants: (A) $Co(en)_3{}^{3+}$, (B) $Co(NH_3)_6{}^{3+}$, (C) $(NH_3)_5CoNH_2Co(NH_3)_5{}^{5+}$, (D) $Co(NH_3)_5Cl^{2+}$, (E) $Co(dmgH)_2(C_6H_5NH_2)_2{}^{+}$, (F) $Co(NH_3)_5(C_2O_4)^{+}$, (G) $Co(acac)_3$, (H) $Co(edta)^{-}$, (I) $Co(C_2O_4)_3{}^{3-}$

been shown that $Cr(en)_3{}^{3+}$ associated with the protein accelerates the reaction. This result cannot be accounted for in terms of the first of these explanations. The suggestion is therefore that $Co(edta)^{-}$ is utilizing a different site to $Cr(NH_3)_6{}^{3+}$. It seems unlikely that $Co(edta)^{-}$ and $Co(acac)_3$ are using the same site in view of the influence of $Cr(NH_3)_6{}^{3+}$ on one but not the other of these reactions.

We have no information as to the precise locality of the different reaction sites on the protein. Both the $Co(NH_3)_6{}^{3+}$ and $Co(edta)^{-}$ oxidations are independent of pH in the range 7-9 investigated.[1] A further consequence of the observations of the effect of $Cr(en)_3{}^{3+}$ on the reduction with $Cr(15\text{-}aneN_4)(H_2O)_2{}^{2+}$ (and the conclusion that these complexes use different sites on the protein) is that charge alone is not determining which site is being utilized. Thus ligand type (NH_3 or RNH_2) would seem to be a common feature in the selection of the site blocked by $Cr(NH_3)_6{}^{3+}$.

The interpretation so far has been in terms of the mechanism (1)-(2), which is featured extensively in the biochemical literature. It is often not stressed sufficiently that other interpretations of limiting kinetic behavior are possible. The reaction scheme

$$\text{Fe(II,III)} \rightleftharpoons {}^*\text{Fe(II,III)} \quad (13)$$

$${}^*\text{Fe(II,III)} + \text{oxidant} \rightarrow \text{Fe(III,III)} + \text{product} \quad (14)$$

as well as

$$\text{Fe(II,III)} + \text{oxidant} \overset{K'}{\rightleftharpoons} \text{Fe(II,III), oxidant} \quad (15)$$

$$\text{Fe(II,III)} + \text{oxidant} \overset{k'}{\rightarrow} \text{Fe(III,III)} + \text{product} \quad (16)$$

sometimes referred to as the "dead-end" mechanism, give rate laws of the same form as (6). We have previously discussed the nonapplicability of (13)-(14), and do not consider this mechanism further.[1] The second possibility introduces binding at an alternative site on the protein, (15), the protein becoming redox inactive presumably as a result of conformational change. In other studies it is known that protonation can initiate such a change, and we have already reported one such

Table X. Summary of Overall Rate Constants k $(=Kk_{et})$ and Activation Parameters for the Oxidation of Reduced Parsley Ferredoxin, $Fe_2S_2{}^*(SR)_4{}^{3-}$, pH 8.0 (Tris), I = 0.10 M (NaCl)

oxidant	k (25 °C), M^{-1} s^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, cal K^{-1} mol^{-1}
$(NH_3)_5CoNH_2Co(NH_3)_5{}^{5+}$	5.6×10^6	17.0	29.0
$Co(NH_3)_6{}^{3+}$	1.9×10^4	18.7	23.8
$Co(en)_3{}^{3+}$	1.6×10^3	21.0	26.4
$Co(NH_3)_5Cl^{2+}$	4.1×10^5	8.4	−4.6
$Co(NH_3)_5C_2O_4{}^{+}$	5.7×10^3	8.0	−14.6
$Co(dmgH)(C_6H_5NH_2)_2{}^{+}$	7.4×10^5	6.7	−9.2
$Co(acac)_3$	7.0×10^3	6.28	−19.6
$Co(edta)^{-}$	7.2×10^3	5.2	−23.4
$Co(C_2O_4)_3{}^{3-}$	3.9×10^3	3.15	−31.5

instance in reactions of plastocyanin PCu[1].[17] The effect of redox inactive $Cr(NH_3)_6{}^{3+}$ on the $Co(edta)^{-}$ oxidation represents an instance (the first reported) in which an associated complex clearly induces a conformational change, in this case beneficial to the redox process. The feasibility of (15)-(16) cannot therefore be in question, where the consequences of interpretation in terms of this mechanism are that $K = K'$ and $k_{et} = k'/K'$. However, *for the present* we have chosen not to interpret in terms of (15)-(16) for the following reasons. Such a mechanism in this and other studies[1,17,18] requires (persistently!) (a) potentially redox-active pairs as in (15) to remain completely dormant, (b) the resulting "switch-off" in redox activity to be always complete and never partial, and (c) the unusual situation that association at the site inducing conformational change is extensive (and observable from the limiting kinetics), whereas association at the active site is not kinetically detectable. Furthermore, attention has been drawn previously to an instance involving (simultaneously) two redox-active complexes which react independently at different sites on the protein and do not impede each other.[18] This means that for mechanism (15)-(16) to be acceptable these complexes must be operating two quite independent "switch-off" mechanisms, which seems to be asking much in terms of versatility of the protein.

An interesting feature of the reactions of 2-Fe ferredoxins described here and those previously reported[1] is that the activation parameters for overall rate constants k $(=Kk_{et})$, Table X, exhibit trends with charge type, and give a moderately good isokinetic plot, Figure 7. The parameters for $Co(acac)_3$ and $Co(edta)^{-}$ conform as well as any other oxidant to this correlation, which does not therefore provide a means of distinguishing different reaction sites. Furthermore, an unweighted least-squares treatment gives the line illustrated, which corresponds to a temperature of 293.4 K. The implications are that at this temperature all the reactions have about the same rate constant k. The scatter introduces a spread in rate constant of around one order of magnitude. Despite the similarity in oxidant type, this is a remarkably narrow range of values bearing in mind that oxidant charges are varying from 5+ to 3−. As has already been pointed out, K values are higher for the more positive oxidants. Therefore, to compensate for the smaller K values obtained when the charge interaction is unfavorable, k_{el} must be correspondingly bigger. One contributing factor is the more favorable standard reduction potentials for the complexes $Co(C_2O_4)_3{}^{3-}$ (+0.57 V) and $Co(edta)^{-}$ (+0.38 V),[24] which happen to be negatively charged.

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Supplementary Material Available: A listing of rate constants, Tables II-VI (6 pages). Ordering information is given on any current masthead page.

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Unusual Structural and Reactivity Types for Copper(I). Synthesis and Structural and Redox Properties of Binuclear Copper(I) Complexes Which Are Probably Three Coordinate in Solution and Experience Intermolecular Metal–Metal Interactions in the Solid State

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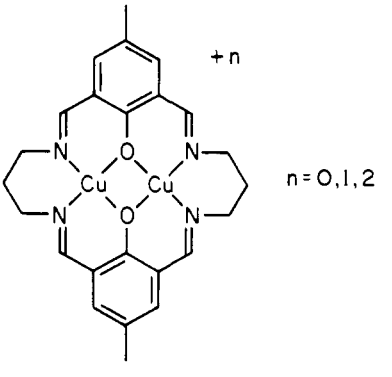
Abstract: Condensation of 2-hydroxy-5-methylisophthalaldehyde with 2-(2′-aminoethyl)pyridine, followed by addition of $Cu(CH_3CN)_4BF_4$ and pyrazolate, resulted in formation of a binuclear copper(I) complex, Cu^I_2ISOIM(Etpy)$_2$(pz). A complete crystal and molecular structural analysis showed each Cu(I) to be bound to the phenoxide oxygen as well as to one imine and one pyrazolate nitrogen. In addition each Cu(I) apparently experiences intermolecular copper–copper interactions, with an intermolecular Cu–Cu separation of 2.97 Å. The pyridine was found to be not coordinated to copper. Overall coordination about copper(I) is best described as highly distorted pyramidal, with long axial coordination. In the solid state Cu^I_2ISOIM(Etpy)$_2$(pz) exhibits an electronic absorption spectral band at 600 nm, which is not found in solution spectra, and which is attributed to the copper–copper interaction. Analogous Cu^ICu^I complexes were also prepared using a variety of amines instead of 2-(2′-aminoethyl)pyridine as side arms, and the 3,5-dimethylpyrazole or 7-azaindole anions as bridging groups. Amines without coordinating substituents also gave stable complexes; e.g., *tert*-butylamine gave Cu^I_2ISOIM(*t*-Bu)$_2$(pz). The latter complex did not exhibit an absorption at 600 nm in the solid state or in solution. It appears likely that Cu^I_2ISOIM(*t*-Bu)$_2$(pz) contains three-coordinate Cu(I) both in the solid state and in solution. Moreover, all Cu^ICu^I complexes examined may involve only three-coordinate Cu(I) in solution, yet they have exhibited no tendency to bind additional ligands, such as CO or pyridine. Most of the binuclear copper(I) complexes gave quasi-reversible electrochemical behavior, with two distinct, one-electron redox processes. The most positive reduction potentials obtained were $E_1^f = +0.239$, $E_2^f = +0.080$ V vs. NHE. Biological implications of the observed reactivities and redox properties of the new complexes are discussed.

Introduction

Protein binuclear copper sites effect remarkable reactions with dioxygen including reversible binding (hemocyanin),[1] activation (tyrosinase),[2] and reduction (laccase).[3] Structural information contrasting these active sites is limited but similarities are notable: sulfur ligands have been proposed but most studies suggest only nitrogen and/or oxygen coordination;[4] in the oxidized forms all three binuclear sites are strongly antiferromagnetically coupled;[5,6] the tyrosinase and laccase binuclear sites exhibit two-electron reductions at potentials which are rather high for the proposed all nitrogen/oxygen copper coordination.[6,7]

Model studies have addressed ligand environment(s), redox properties, magnetic interactions, and dioxygen binding in these protein active sites.[8-12] To help define protein structure/reactivity relationships we are endeavoring to catalog fundamental copper(I) coordination chemistry in relatively simple mononuclear and binuclear complexes. As discussed elsewhere, polydentate ligands, including macrocycles, can be utilized to minimize problems associated with both copper(II) and copper(I) substitution lability.[13,14] This approach has



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