Kinetic Studies on Reactions of Iron–Sulfur Proteins. 1. Oxidation of Reduced Parsley (and Spinach) 2-Iron Ferredoxins with $Co(NH_3)_6^{3+}$, *dl*- and *d*- $Co(en)_3^{3+}$, $Co(NH_3)_5Cl^{2+}$, $Co(NH_3)_5C_2O_4^{+}$, $Co(dmgH)_2(C_6H_5NH_2)_2^{+}$, and $Co(edta)^{-}$. Evidence for Protein-Complex Association

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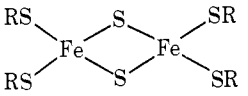
Abstract: Parsley (and spinach) ferredoxins containing the Fe(III,III) cluster, $Fe_2S_2^*(SR)_4^{2-}$, have been isolated by standard procedures. Reduction with dithionite gives Fe(II,III), and the kinetics of one-electron reoxidation with the Co(III) complexes $Co(NH_3)_6^{3+}$, *dl*-$Co(en)_3^{3+}$, $Co(NH_3)_5Cl^{2+}$, $Co(NH_3)_5(C_2O_4)^{+}$, $Co(dmgH)_2(C_6H_5NH_2)_2^{+}$, and $Co(edta)^{-}$ have been studied by the stopped-flow technique, $I = 0.10$ M (NaCl). The reactions of parsley and spinach $Fe_2S_2^*(SR)_4^{3-}$ with $Co(NH_3)_6^{3+}$ at 25 °C are independent of pH in the range 7.0–9.0 (Tris buffer). With $Co(NH_3)_6^{3+}$ (parsley and spinach) and $Co(en)_3^{3+}$ (parsley) a less than first-order dependence on oxidant (in large excess) is observed consistent with association followed by outer-sphere electron transfer: Fe(II,III) + Co(III) $\rightleftharpoons$ Fe(II,III), Co(III) (K) and Fe(II,III), Co(III) $\rightarrow$ Fe(III,III) + Co(II) (k_{et}). For the parsley reactant thermodynamic and activation parameters have been obtained from the temperature dependences of K and k_{et}. At 25 °C for the reaction of $Co(NH_3)_6^{3+}$, $K = 998$ M^{-1} (10.2, 47.9), $k_{et} = 19.2$ s^{-1} (8.5, −24.1); and for $Co(en)_3^{3+}$, $K = 597$ M^{-1} (11.0, 49.5), $k_{et} = 2.7$ s^{-1} (10.0, −23.1), where enthalpy and entropy terms, respectively, are given in brackets (kcal mol^{-1}, cal K^{-1} mol^{-1}). Rate constants for resolved *d*-$Co(en)_3^{3+}$ are identical with those for the racemic form. A less than first-order dependence on $Co(NH_3)_5Cl^{2+}$ was only marginally detectable. Other complexes of smaller charge gave no direct kinetic evidence for an association step with parsley $Fe_2S_2^*(SR)_4^{3-}$. However, trends in activation parameters for the overall rate constants $k(= Kk_{et})$ indicate a dependence on oxidant charge type.

Iron–sulfur proteins have emerged in recent years as an important class of redox metalloproteins, functioning in such diverse processes as respiration, photosynthesis, nitrogen fixation, biosynthesis, and degradative metabolism.[1-5] Different species of composition 1-Fe, 2-Fe, 4-Fe, and 8-Fe have been identified, and synthetic analogues of the active sites have been prepared. There is as yet no confirmation from X-ray crystallography of the nature of the active site of 2-Fe proteins which are obtained from a variety of sources including algae, higher plants, bacteria, and mammalian organs. However, physicochemical data are fully consistent with a structure $Fe_2S_2^*(SR)_4^{2-}$ (where SR denotes a coordinated cysteine of the amino acid chain), containing two tetrahedrally coordinated high-spin iron(III) atoms bridged by sulfide(2−) (S*).



This structure, which was originally proposed some 12 years ago, has become even more probable as a result of studies on the analogues.[6] The 2-Fe proteins function as one-electron carriers, reduction potential ca. −0.42 V,[7] utilizing Fe(III,III) and Fe(II,III) oxidation states:

$$Fe_2S_2^*(SR)_4^{2-} + e^- \rightleftharpoons Fe_2S_2^*(SR)_4^{3-} \qquad (1)$$

There is as yet no evidence for further reduction yielding forms isoelectronic with $Fe_2S_2^*(SR)_4^{4-}$. Mössbauer and ENDOR spectroscopy have clearly established that a structure containing trapped oxidation states, Fe(II) and Fe(III), respectively, applies in the case of $Fe_2S_2^*(SR)_4^{3-}$.[8a] With the 4-Fe proteins, on the other hand, the iron atoms are identical and an average oxidation state applies.[8b]

Rawlings et al.[9] have previously studied the oxidation of spinach $Fe_2S_2^*(SR)_4^{3-}$ with the Fe^{III}–(edta) complex. Studies on reactions of 1-Fe rubredoxin[10] and 4-Fe HIPIP have also been reported.[11-13] The studies reported herein are an attempt to understand further the redox reactivity of the 2-Fe proteins (mol wt ca. 10 500) obtained from parsley and (less extensively) spinach. The redox partners are small inorganic complexes, the redox behavior of which is fairly well understood. Long-term aims are to define specific reaction sites on the proteins and the influence of charge on reactivity. At this stage it is not, for example, clear to what extent overall and local protein charges are important, whether positive and negatively charged reactants utilize different sites on the protein, and what influence hydrophobic or hydrophilic ligands on the oxidant have on the proceedings.

The detection of protein-complex association followed by electron transfer, and the enthalpic and entropic parameters obtained for these individual steps, are highly relevant in an assessment of such factors.

Experimental Section

Isolation of Parsley Ferredoxin. This 2-Fe protein, containing $Fe_2S_2^*(SR)_4^{2-}$, was extracted from fresh leaves (12 kg) according to the method of Plesničar and Bendall.[14] A final purification was carried out using solutions buffered with 0.025 M Tris/0.02 M HCl, which had been deoxygenated by bubbling N_2 through for ca. 30 min. The crude ferredoxin solution was diluted to a total chloride concentration of ca. 0.2 M and loaded on to a column of Whatman DE23 cellulose (2.5-cm diameter, 50-cm height) previously equilibrated with 0.2 M NaCl. Ferredoxin was eluted using NaCl solution of increasing concentration 0.2–0.5 M (ca. 1 L), where fractions with absorbance (A) at wavelengths (nm) $A_{422}/A_{277} > 0.54$ were retained. The procedure was repeated, this time retaining fractions with $A_{422}/A_{277} > 0.59$. Finally the solution was diluted twice and loaded onto a smaller column (5-cm diameter, 2-cm height) from which it was eluted in concentrated form with 0.5 M NaCl. The yield was typically 20 mL of ca. 10^{-3} M ferredoxin having A_{422}/A_{277} of 0.61, in agreement with the literature value.[15]

Isolation of Spinach Ferredoxin. The 2-Fe ferredoxin was extracted and purified according to the procedure of Borchert and Wessels.[16] A concentrated solution was obtained in the same way as for parsley ferredoxins. The spinach ferredoxins gave A_{422}/A_{277} of 0.47, in satisfactory agreement with a literature value of 0.49.[16]

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Table I. Details of Absorption Maxima from UV–Visible Spectra of Oxidants and Comparison with Literature Values

complex	λ, nm	ϵ, M^{-1} cm^{-1}	λ, nm	ϵ, M^{-1} cm^{-1}	ref
$Co(NH_3)_6^{3+}$	339	46.4	473	57.1	*a*
	339	47	473	58	*b*
dl-$Co(en)_3^{3+}$	338	79.8	465	87.1	*a*
	338	78	465	87	*b*
$Co(NH_3)_5Cl^{2+}$	362	48.0	530	51.4	*a*
			530	50.1	*c*
$Co(NH_3)_5(C_2O_4)^+$			502	73	*a, d*
			502	74.3	*b*
$Co(dmgH)_2$-$(C_6H_5NH_2)_2^+$	352	1.17×10^4			*a*
	352	1.41×10^4			*e*
Co(edta)$^-$			535	320	*a, f*
			534	318	*g*

[a] This work; spectra same at pH 8.0 in Tris/HCl. [b] R. T. Wang and J. H. Espenson, *J. Am. Chem. Soc.*, **93**, 380 (1971). [c] Reference 23. [d] Minimum at 411 nm (ϵ 10.6 M^{-1} cm^{-1}). [e] Reference 24; shoulder at 248 nm (ϵ our sample 2.08×10^4 M^{-1} cm^{-1}). [f] Absorption measurements at λ 500 (ϵ 228) and 597 (119) rule out the quinquedentate edta complex; see also R. Dyke and W. C. E. Higginson, *J. Chem. Soc.*, 406, 1998 (1960). [g] L. Rosenheim, D. Speiser, and A. Haim, *Inorg. Chem.*, **13**, 1571 (1974).

Preparation of Oxidants. The complexes hexaamminecobalt(III) chloride, $[Co(NH_3)_6]Cl_3$,[17] tris(ethylenediamine)cobalt(III) chloride trihydrate, $[dl\text{-}Co(en)_3]Cl_3 \cdot 3H_2O$,[18] chloropentaamminecobalt(III) chloride, $[Co(NH_3)_5Cl]Cl_2$, oxalatopentaamminecobalt(III) chloride, $[Co(NH_3)_5(C_2O_4)]Cl$,[19] bis(aniline)bis(dimethylglyoximato)-cobalt(III) chloride tetrahydrate, $[Co(dmgH)_2(C_6H_5NH_2)_2]Cl \cdot 4H_2O$,[20] and sodium ethylenediaminetetraacetatocobaltate(III) tetrahydrate, $Na[Co(edta)] \cdot 4H_2O$,[21] were prepared according to existing or modified literature procedures. Samples containing $Co(NH_3)_6^{3+}$, *dl*-$Co(en)_3^{3+}$, and $Co(dmgH)_2(C_6H_5NH_2)_2^+$ were recrystallized from water (twice) until a constant UV–visible spectrum was obtained. To prepare $Co(NH_3)_5Cl^{2+}$ the carbonato complex $[Co(NH_3)_5CO_3]NO_3$[22] (6 g) was suspended in cold water (50 mL) and concentrated HCl (100 mL) added slowly with stirring. After effervescence had subsided the resultant solution was heated with stirring on a hot plate and boiled for ca. 10 min. The solution was cooled and the product filtered off. Recrystallization from 0.1 M HCl was repeated until a constant spectrum was obtained. The sample was stored over silica gel. The oxalato complex, $[Co(NH_3)_5C_2O_4]ClO_4 \cdot H_2O$[19] (4 g), was converted into the chloride salt by first dissolving in warm water (40°C, 100 mL). It was then treated batchwise with 3 × 20 mL of BDH Amberlite IR(4B) resin (chloride form), and the resin removed by filtration after ca. 1 min contact time. To the filtrate, excess ethanol (3:1 by volume) was added with stirring. The solid was filtered off and redissolved in cold water (25 mL). Ethanol (30 mL) was added with stirring, and the red solid was filtered off and washed with 1:1 water–ethanol (10 mL), ethanol (20 mL), and ether (20 mL) and stored over P_2O_5, yield 0.9 g. The IR spectrum gave no perchlorate bands (1140–1060 cm^{-1}).

Absorption coefficients were in good agreement with existing literature values (Table I), except in the case of $[Co(NH_3)_5Cl]Cl_2$ (agreement with ref 23 only) and the dmg complex $[Co(dmgH)_2$-$(C_6H_5NH_2)_2]Cl \cdot 4H_2O$, for which there was a 15% discrepancy.[24] Spectra for the latter were reproducible from different samples. Anal. Calcd for $CoC_{20}H_{36}N_6O_8Cl$: C, 41.2; H, 6.17; N, 14.4. Found: C, 41.4; H, 6.30; N, 14.5.

To obtain a sample of resolved $[d\text{-}Co(en)_3]I_3 \cdot H_2O$ the racemic $[dl\text{-}Co(en)_3]Cl_3 \cdot 3H_2O$ (8.5 g, 0.021 mol) was dissolved in H_2O at ca. 50°C (30 mL), and sodium *d*-tartrate dihydrate (Hopkin and Williams, GP reagent) (5.75 g, 0.025 mol) was added. The mixture was heated on a steam bath for ca. 10 min and filtered, and the residue was washed with a little hot water. The filtrate was evaporated to ca. 15 mL and allowed to stand for several hours in a cool place. Crystals of $[d\text{-}Co(en)_3][d\text{-}tartrate]Cl \cdot 5H_2O$ were filtered off, washed with 3:2 water/ethanol (20 mL), and recrystallized by dissolving in water at ca. 60 °C (8 mL) and cooling to 0 °C. The crystals were filtered off, washed with absolute ethanol (20 mL), and dried in air by suction, yield 2.1 g. To convert to the iodide salt 2 g (0.004 mol) was dissolved in hot water, and aqueous ammonia (0.1 mL) was added, followed by stirring with an excess of solid NaI (0.75 g) in hot water (3 mL). The solution was cooled to 0 °C (15 min) to obtain $[d\text{-}Co(en)_3]I_3 \cdot H_2O$ with a small amount of $[dl\text{-}Co(en)_3]I_3 \cdot H_2O$. After the sample was filtered and washed with ice-cooled 30% NaI (3 mL), ethanol (10 mL), and acetone (10 mL) it was dried by suction, yield 2.5 g. The product was purified by dissolving in hot water (15 mL), cooling, and filtering off the less soluble *dl* form. Crystals of $[d\text{-}Co(en)_3]I_3 \cdot H_2O$ were obtained after reducing the volume of the solution over P_2O_5. The specific rotation was determined by polarimetry (Bellingham and Stanley Model D) and found to be $[\alpha]^{20}_D$ +86° compared to a published value of 89° cm^3 dm^{-1} g^{-1}.[25] A composition of 98% *d* isomer was therefore indicated. The absorption coefficient at the 465-nm peak was ϵ 89 M^{-1} cm^{-1} in agreement with the value for the *dl* form (Table I).

Adjustment of pH. Solutions of reagent grade tris(hydroxymethyl)methylamine (Trizma), here referred to as Tris (Sigma Chemicals), in 0.010 M HCl were used to buffer solutions. The ionic strength was maintained at 0.10 M using Analar sodium chloride. Knowing log K_B = 8.09 at 25 °C (0.1 M KCl)[26a] for the protonation of Tris

$$\text{Tris} + H^+ \overset{K_B}{\rightleftharpoons} \text{Tris } H^+ \qquad (2)$$

the required amount of Tris was calculated from

$$\text{pH} = \log K_B + \log \frac{[\text{Tris}] - [\text{HCl}]}{[\text{HCl}]} \qquad (3)$$

Thus a solution of Tris (0.0181 M) in HCl (0.010 M), with NaCl (0.090 M), gave a pH of 8.00 at 25 °C which was then used to standardize a pH meter (Radiometer type PHM 4d) complete with calomel (type K401) and glass (type G202B) electrodes. Values of log K_B at other temperatures were 8.61 (7 °C), 8.44 (12 °C), 8.36 (15 °C), 8.28 (18 °C), 8.22 (20 °C), 7.94 (30 °C).[26b] Little variation of log K_B is observed with ionic strength, 8.09 (I = 0.10 M) and 8.074 ($I \rightarrow 0$), at 25 °C.[26]

Kinetic Measurements. Kinetic measurements were carried out using a Durrum-Gibson stopped-flow spectrophotometer. Nitrogen gas, which had previously been deoxygenated by passing through a 1.0 M NaOH solution of resublimed BDH pyrogallol (5 g/L), was bubbled through all reactant solutions for ca. 30 min. Except for some runs with $Co(dmgH)_2(C_6H_5NH_2)_2^+$ all reactions were carried out under pseudo-first-order conditions with oxidant in at least eightfold excess. With $Co(dmgH)_2(C_6H_5NH_2)_2^+$ as oxidant it was not always possible to use such a large excess owing to the rapidity of the reaction. Alternatively, by assuming pseudo-first-order behavior, and taking the initial slope of absorbance log ($A_\infty - A_t$) against time plots, rate constants could be evaluated with the oxidant in as little as fourfold excess. A similar modified procedure has been described previously by Corbett.[27]

The reduced ferredoxin was generated by initially loading the drive syringe of the stopped-flow apparatus with ca. 4×10^{-4} M sodium dithionite (BDH, GP reagent) made up by addition of degassed solutions at the required pH and ionic strength to solid dithionite under anaerobic conditions. After the relevant connecting valves were opened, a volume of this solution (ca. 1 mL) was pushed back into a solution of $Fe_2S_2^*(SR)_4^{2-}$ ferredoxin (10 mL of ca. 3.5×10^{-5} M) also at the required pH and ionic strength, which had been loaded into the storage syringe. Mixing was achieved by having a Teflon-coated stirrer in the storage syringe and applying (externally) a bar magnet. Dithionite reduction of spinach ferredoxin is known to be rapid.[28] The solution was allowed to stand for ca. 1 min before transferring to the drive syringe. It was found that a 1:1 mole ratio, i.e., a twofold excess, of $S_2O_4^{2-}$ was generally necessary to ensure >90% reduction of $Fe_2S_2^*(SR)_4^{2-}$ to $Fe_2S_2^*(SR)_4^{3-}$. The extent of the conversion could be judged from the amplitude of the subsequent stopped-flow trace. Trace amounts of oxygen remaining in the system are probably responsible for consumption of excess $S_2O_4^{2-}$. Variation of $S_2O_4^{2-}$ to a fivefold excess, and addition of excess Na_2SO_3 (product of the oxidation of $S_2O_4^{2-}$) to amounts in eightfold excess of that generated in the reduction process, had little (<5%) or no effect on observed rate constants. Concentrations of reduced ferredoxin were varied within the range (0.8–2.5) × 10^{-5} M without any effect on rate constants. Solutions of oxidants were made up at the required pH and ionic strength. The ionic strength was 0.10 M except for five $Co(NH_3)_6^{3+}$

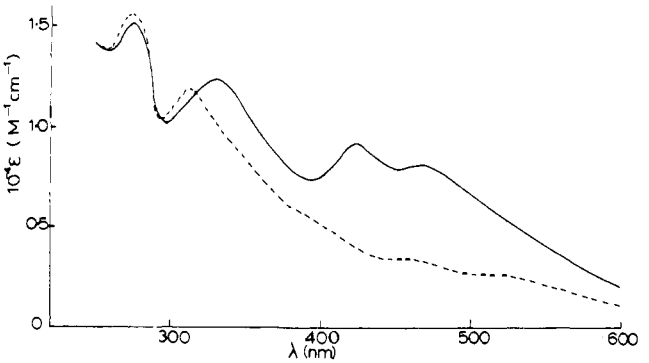

Figure 1. Spectra of oxidized, $Fe_2S_2^*(SR)_4^{2-}$ (—), and reduced, $Fe_2S_2^*(SR)_4^{3-}$ (- - - -), parsley ferredoxin at pH 8.0 (Tris), I = 0.10 M (NaCl).

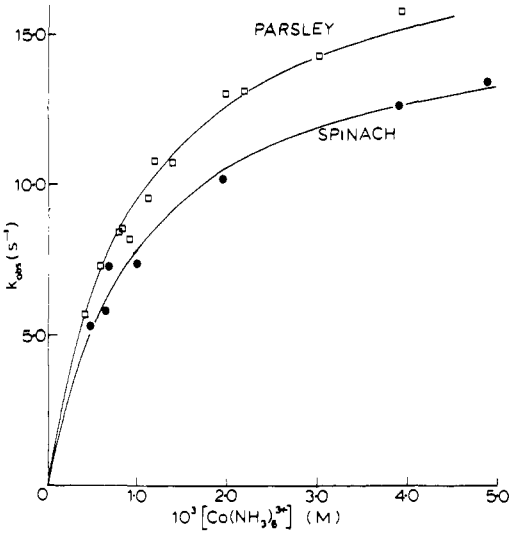

Figure 2. The oxidation of reduced parsley (□) and spinach (●) ferredoxin, $Fe_2S_2^*(SR)_4^{3-}$, with $Co(NH_3)_6^{3+}$. The variation of first-order rate constants k_{obsd} (25 °C) with $[Co(NH_3)_6^{3+}]$, pH 8.0 (Tris), I = 0.10 M (NaCl).

Table II. Effect of pH[a] on First-Order Rate Constants (25 °C) for the 1:1 Redox Reaction between Reduced Parsley and Spinach Ferredoxins $Fe_2S_2^*(SR)_4^{3-}$ and $Co(NH_3)_6^{3+}$, Tris Buffer, I = 0.10 M (NaCl)

pH	k_{obsd} (parsley),[b] s^{-1}	k_{obsd} (spinach),[c] s^{-1}
7.0	8.29	6.56
7.5	8.78	7.24
8.0	8.13	7.28
8.5	8.43	7.54
9.0	8.31	6.72

[a] pH of oxidized ferredoxin, $Fe_2S_2^*(SR)_4^{2-}$, adjusted ca. 1 h prior to $S_2O_4^{2-}$ reduction to $Fe_2S_2(SR)_4^{3-}$. [b] $[Co(NH_3)_6^{3+}] = 9.2 \times 10^{-4}$ M. [c] $[Co(NH_3)_6^{3+}] = 6.9 \times 10^{-4}$ M.

runs. The pH was adjusted to 8.0 except for those experiments in which the effect of varying the pH from 7.0 to 9.0 was tested.

The increase in absorbance corresponding to the oxidation of parsley $Fe_2S_2^*(SR)_4^{3-}$ (ϵ 4500 M^{-1} cm^{-1}) to $Fe_2S_2^*(SR)_4^{2-}$ (ϵ 9200 M^{-1} cm^{-1}) was monitored at 422 nm[7c] for all but the $Co(dmgH)_2(C_6H_5NH_2)_2^+$ system (λ 520 nm). Similar absorbance changes were observed for the spinach ferredoxin with ϵ 9400 M^{-1} cm^{-1} at 422 nm.[29] The Co(III) absorbance gives an appreciable background contribution in the studies with the dmg and edta complexes. Stopped-flow traces were photographed from a Tektronix 564 RM storage oscilloscope. Plots of log $(A_\infty - A_t)$ against time were generally linear to >90% reaction. First-order rate constants k_{obsd} were obtained from the slope (× 2.303). Variation of the wavelength of 440 nm with Co(edta)$^-$ as oxidant (last two entries of Table IX) had no effect on rate constants obtained.

Treatment of Data. A nonlinear least-squares program[30] was used to fit data to different dependences. Weighting factors $1/k$ or $1/k^2$ were acceptable, whichever gave the smallest standard deviations.

Results

Spectra of $Fe_2S_2^*(SR)_4^{3-}$ (reduced) and $Fe_2S_2^*(SR)_4^{2-}$ (oxidized) forms of parsley ferredoxin are shown in Figure 1. On reoxidation of $Fe_2S_2^*(SR)_4^{3-}$ the same spectrum is regenerated, with losses[15] depending on the time of storage in the reduced state (typically ca. 10% over 20 min). The spectrum regenerated is the same whether 1 equiv or excess of oxidant is used, consistent with a 1:1 stoichiometry[7,15] as in

$$Fe_2S_2^*(SR)_4^{3-} + Co(III) \rightarrow FeS_2^*(SR)_4^{2-} + Co(II) \quad (4)$$

The reduced form was used within 1 h of addition of dithionite.

With the oxidant in large excess, the kinetics conform to a rate law

$$\text{rate} = k_{obsd}[Fe_2S_2^*(SR)_4^{3-}] \quad (5)$$

No dependence of k_{obsd} on pH is observed over the range 7.0–9.0 investigated (Table II). A nonlinear dependence of k_{obsd} on concentration of oxidants is observed for the reactions of parsley (Table III[31]) and spinach ferredoxin (Table IV[31]) with $Co(NH_3)_6^{3+}$ (Figure 2). The dependence can be accounted for by a mechanism involving association (eq 6) followed by electron transfer (eq 7).

$$Fe(II,III) + Co(III) \overset{K}{\rightleftharpoons} Fe(II,III), Co(III) \quad (6)$$

$$Fe(II,III), Co(III) \xrightarrow{k_{et}} Fe(III,III) + Co(II) \quad (7)$$

This reaction sequence gives an expression

$$k_{obsd} = \frac{Kk_{et}[Co(III)]}{1 + K[Co(III)]} \quad (8)$$

A plot of $1/k_{obsd}$ against $1/[Co(III)]$ is linear (Figure 3), where the slope and intercept correspond to $1/Kk_{et}$ and $1/k_{et}$, respectively. From a nonlinear least-squares fit to eq 8 including data at different temperatures, thermodynamic parameters ΔH_0 and ΔS_0 (for K) and activation parameters $\Delta H^\ddagger$ and $\Delta S^\ddagger$ (for k_{et}) were obtained. For the reaction of parsley $Fe_2S_2^*(SR)_4^{3-}$ with $Co(NH_3)_6^{3+}$, K(25 °C) = 998 ± 39 M^{-1}, ΔH_0 = 10.2 ± 1.2 kcal mol^{-1}, ΔS_0 = 47.9 ± 4.0 cal K^{-1} mol^{-1}, and k_{et} (25 °C) = 19.2 ± 1.8 s^{-1}, $\Delta H^\ddagger_{et}$ = 8.5 ± 0.6 kcal mol^{-1}, $\Delta S^\ddagger_{et}$ = −24.1 ± 2.1 cal K^{-1} mol^{-1}. Data for the reaction of spinach ferredoxin with $Co(NH_3)_6^{3+}$ at 25 °C give K = 993 ± 112 M^{-1} and k_{et} = 15.9 ± 0.8 s^{-1}. All further studies reported are with parsley ferredoxin.

The 3+ complex $Co(en)_3^{3+}$ gives similar behavior (Figure 4), and from a study at three temperatures only (Table V),[31] K(25 °C) = 597 ± 40 M^{-1}, ΔH_0 = 11.0 ± 5.1 kcal mol^{-1}, ΔS_0 = 49.5 ± 17.1 cal K^{-1} mol^{-1}, and k_{et} (25 °C) = 2.7 ± 0.1 s^{-1}, $\Delta H^\ddagger_{et}$ = 10.0 ± 1.9 kcal mol^{-1}, $\Delta S^\ddagger_{et}$ = −23.1 ± 6.5 cal K^{-1} mol^{-1}. Rate constants for the reaction of resolved *d*-$Co(en)_3^{3+}$ were identical with those with the *dl* form (Figure 4, 25 °C), and have been included in the computation of the enthalpy and entropy terms.

The rapidity of the $Co(NH_3)_5Cl^{2+}$ oxidation limited the range of Co(III) concentration to $<10^{-3}$ M (Table VI).[31] Over this narrower range of concentrations some curvature is incident (Figure 5), but the case must be regarded as marginal. Assuming no curvature, and a strictly first-order dependence

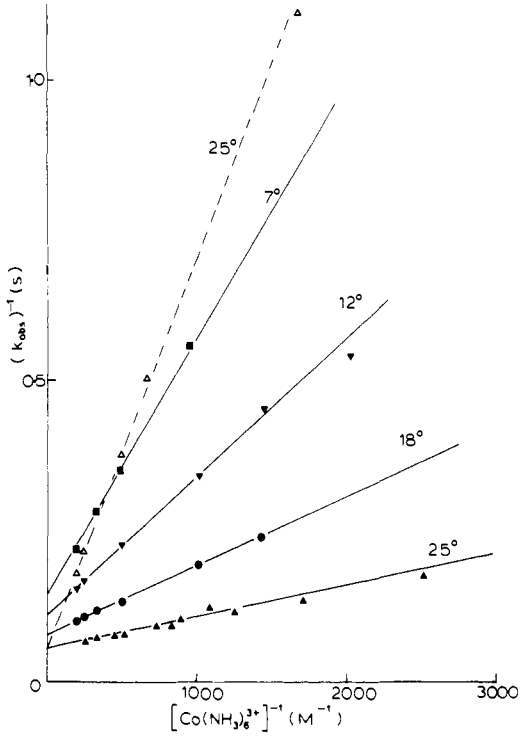

Figure 3. The oxidation of reduced parsley ferredoxin, $Fe_2S_2{}^*(SR)_4{}^{3-}$, with $Co(NH_3)_6{}^{3+}$. The dependence of first-order rate constants $(k_{obsd})^{-1}$ on $[Co(NH_3)_6{}^{3+}]^{-1}$ at temperatures 7–25 °C, pH 8.0 (Tris), I = 0.10 M (NaCl). Points for runs at pH 8.0 (Tris), I = 0.50 M (NaCl), at 25 °C are also indicated (△).

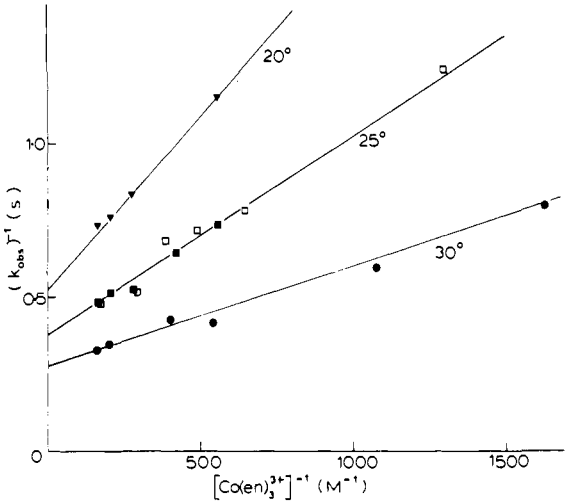

Figure 4. The oxidation of reduced parsley ferredoxin, $Fe_2S_2{}^*(SR)_4{}^{3-}$, with $Co(en)_3{}^{3+}$. The dependence of first-order rate constants $(k_{obsd})^{-1}$ on $[Co(en)_3{}^{3+}]^{-1}$, at temperatures 20–30 °C, pH 8.0 (Tris), I = 0.10 M (NaCl). Data points for *dl*-$Co(en)_3{}^{3+}$ (■) and *d*-$Co(en)_3{}^{3+}$ (□) at 25 °C are in good agreement.

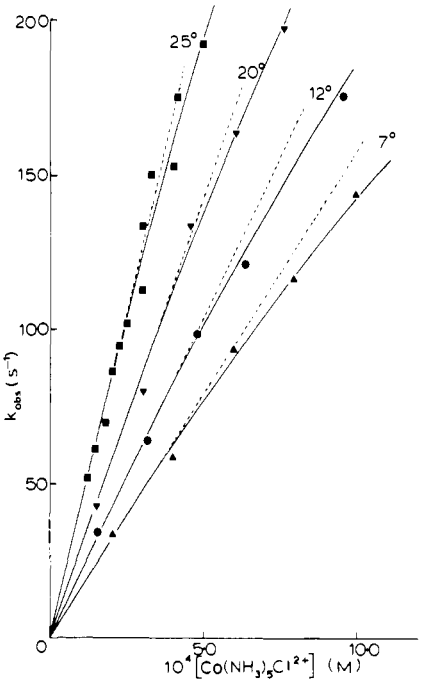

Figure 5. The oxidation of reduced parsley ferredoxin, $Fe_2S_2{}^*(SR)_4{}^{3-}$, with $Co(NH_3)_5Cl^{2+}$. The variation of first-order rate constants k_{obsd} with $[Co(NH_3)_5Cl^{2+}]$, pH 8.0 (Tris), I = 0.10 M (NaCl). Broken lines correspond to fit of data assuming no curvature.

on [Co(III)], second-order rate constants $k(k_{obsd}$ [Co(III)]) as defined in eq 9 are obtained.

$$\text{rate} = k[Fe_2S_2{}^*(SR)_4{}^{3-}][Co(III)] \quad (9)$$

At 25 °C $k = 4.1 \times 10^5$ M^{-1} s^{-1}, $\Delta H^{\ddagger} = 8.7 \pm 0.3$ kcal mol^{-1}, and $\Delta S^{\ddagger} = -3.7 \pm 0.9$ cal K^{-1} mol^{-1}. A fit to eq 8 is also possible giving K(25 °C) = 194 M^{-1}, $\Delta H_0 = 2.6 \pm 8.7$ kcal mol^{-1}, $\Delta S_0 = 19.2 \pm 30$ cal K^{-1} mol^{-1}; and k_{et} (25 °C) = 2300 s^{-1}, $\Delta H^{\ddagger}_{et} = 5.8 \pm 8.2$ kcal mol^{-1}, $\Delta S^{\ddagger}_{et} = -23.8 \pm 28.3$ cal K^{-1} mol^{-1}. Here and for the 3+ oxidants, when the condition K[Co(III)] ≪ 1 applies (e.g., [Co(III)] < 10^{-3} M, Figure 2), second-order rate constants k correspond to Kk_{et}.

Rate constants k_{obsd} for the reactions with $Co(NH_3)_5(C_2O_4)^+$, $Co(dmgH)_2(C_6H_5NH_2)_2{}^+$, and $Co(edta)^-$ as oxidants are given in Tables VII–IX, respectively.[31] The range of oxidant concentrations was increased to the limit possible, but in no case was a less than first-order dependence detected. Second-order rate constants at 25 °C (M^{-1} s^{-1}), $\Delta H^{\ddagger}$ (kcal mol^{-1}), and $\Delta S^{\ddagger}$ (cal K^{-1} mol^{-1}) are for $Co(NH_3)_5(C_2O_4)^+$ (5.7×10^3, 8.0 ± 0.4, -14.6 ± 1.3), $Co(dmgH)_2(C_6H_5NH_2)_2{}^+$ (7.4×10^5, 6.7 ± 0.3, -9.2 ± 1.0), and $Co(edta)^-$ (7.2×10^5, 5.2 ± 0.3, -23.4 ± 1.1).

Discussion

No pH dependence is observed (7.0–9.0 range) for the $Co(NH_3)_6{}^{3+}$ oxidation of parsley and spinach ferredoxins and the $Co(edta)^-$ oxidation of parsley ferredoxin, I = 0.10 M (NaCl). This is in accordance with electrochemical studies on ferredoxins[7c] which indicate little variation in reduction potential (pH 6–9). However, it is in contrast to the behavior observed by Gray and co-workers[9] for the Fe^{III}–(edta) oxidation of spinach ferredoxin, I = 0.10 M (Tris), pH 6.7–9.0, when rate constants decreased by a factor of ca. 10. A pK value of 7.2 ± 0.1 was obtained,[9] but the origin of the acid dissociation was not discussed. It can now be concluded that the ferredoxins do not exhibit an acid dissociation in this range, which suggests that the Gray value most likely orginates from the Fe^{III}–(edta) reactant. An X-ray crystal study has indicated a seven-coordinate structure, $Fe(edta)(H_2O)^-$,[32] and pK values of 7.49 (20 °C, I = 0.10 M KCl)[33] and 7.58 (25 °C, I = 1.0 M KCl)[34] have been determined for the acid dissociation yielding $Fe(edta)(OH)^{2-}$.[35] The hydroxo complex is known to form a μ-oxo species $[\{Fe(edta)\}_2O]^{4-}$, dimerization constant $10^{2.25}$.[34] Activation parameters obtained from second-order rate constants at pH 7.8, $\Delta H^{\ddagger} = 0.7$ kcal mol^{-1} and $\Delta S^{\ddagger} = -31$ cal K^{-1} mol^{-1}, therefore correspond to oxidation of spinach ferredoxin by 2^- and/or 4^- Fe^{III}–(edta) species. Whichever of these predominates, the activation parameters

Table X. Second-Order Rate Constants k ($= Kk_{et}$) at 25 °C and Activation Parameters for the 1:1 Redox Reactions of Reduced Parsley (and Spinach) Ferredoxin, $Fe_2S_2{}^*(SR)_4{}^{3-}$, with Cobalt(III) Oxidants, pH 8.0 (Tris), I = 0.10 M (NaCl)

oxidant	k, M^{-1} s^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, cal K^{-1} mol^{-1}
$Co(NH_3)_6{}^{3+}$	1.9×10^4	18.7	23.8
$Co(NH_3)_6{}^{3+}$ [a]	1.6×10^4		
$Co(en)_3{}^{3+}$ [b]	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
$Co(edta)^{-}$	7.2×10^3	5.2	−23.4

[a] Reaction with spinach ferredoxin. [b] Combined data for *dl* and *d* forms.

obtained would seem to continue the trend of decreasing enthalpy and entropy of activation with increasing negative charge as indicated in Table X.

With the 3+ oxidants $Co(NH_3)_6{}^{3+}$ and $Co(en)_3{}^{3+}$ a less than first-order dependence is observed with increased [Co(III)], e.g., Figure 2. The data conform to eq 8, which can alternatively be written in the empirical form

$$k_{obsd} = \frac{[Co(III)]}{A + B[Co(III)]}$$

To explain this dependence at least three mechanisms may be invoked. The first involves K and k_{et} as already outlined in eq 6 and 7. The second orginates from a protein activation process as in eq 10 and 11 and requires application of stationary-state kinetics

$$Fe(II,III) \rightleftharpoons Fe(II,III)^* \quad (10)$$

$$Fe(II,III)^* + Co(III) \rightarrow Fe(III,III) + Co(II) \quad (11)$$

If this mechanism were applicable it would yield identical forward rate constants for eq 10 regardless of the identity of the oxidant. Since this is not the case it can be dismissed. The third involves association at an alternative site to give a redox inactive form:

$$FE(II,III) + Co(III) \overset{K'}{\rightleftharpoons} Fe(III,III),Co(III) \quad (12)$$

$$Fe(II,III) + Co(III) \overset{k'}{\rightarrow} Fe(III,III) + Co(II) \quad (13)$$

The difficulty in distinguishing between mechanisms of the kind of eq 6, 7 and eq 12, 13 is well known. We have found, for example, that a series of runs at 25 °C, I = 0.50 M (NaCl), for the $Co(NH_3)_6{}^{3+}$ oxidation of $Fe_2S_2{}^*(SR)_4{}^{3-}$ gives a plot of $1/k_{obsd}$ against $1/[Co(III)]$ which has virtually the same intercept but a different slope as for data at I = 0.10 M; see Figure 3. For the reaction sequence of eq 6, 7 the slope and intercept correspond to $1/k_{et}K$ and $1/k_{et}$, respectively, giving K = 77 M^{-1}, k_{et} = 19.9 s^{-1}, compared to K = 998 M^{-1}, k_{et} = 19.2 s^{-1} at I = 0.10 M. Rate constants k_{et} for electron transfer within an adduct, eq 7, are not expected to be influenced by ionic strength, and the results obtained are certainly consistent with eq 6 and 7. For the reaction sequence of eq 12, 13 the intercept corresponds to K'/k' and the slope to $1/k'$. If this mechanism applies the large decrease in k' would have to be exactly balanced by a decrease in K'. Since the latter remains a possibility mechanism 12, 13 cannot be excluded on this evidence. However, in experiments involving a second complex, redox-inactive $Cr(NH_3)_6{}^{3+}$ blocks the reaction with $Co(NH_3)_6{}^{3+}$ but accelerates that with $Co(edta)^{-}$, and it is difficult to explain this behavior in terms of eq 12, 13.[36] For the present we choose therefore to interpret in terms of the simpler mechanism of eq 6, 7.

Table XI. Reactions of Reduced Parsley Ferredoxin, $Fe_2S_2{}^*(SR)_4{}^{3-}$. Thermodynamic Data for Protein-Complex Associations, pH 8.0 (Tris), I = 0.10 M (NaCl)

oxidant	K (25 °C), M^{-1}	ΔH_0, kcal mol^{-1}	ΔS_0, cal K^{-1} mol^{-1}
$Co(NH_3)_6{}^{3+}$	998	10.2	47.9
$Co(NH_3)_6{}^{3+}$ [a]	993		
$Co(en)_3{}^{3+}$ [b]	597	11.0	49.5
$Co(NH_3)_5Cl^{2+}$	194	(2.6)[c]	(19.2)[c]

[a] Reaction with spinach ferredoxin. [b] Data for *dl* and *d* forms combined. [c] Errors large ±8.7 (ΔH_0) and ±30 (ΔS_0).

Values of K (998 ± 39 M^{-1}) and k_{et} (19.2 ± 1.8 s^{-1}) for the $Co(NH_3)_6{}^{3+}$ oxidation of parsley ferredoxin are in very close agreement with K (993 ± 112 M^{-1}) and k_{et} (15.9 ± 0.8 s^{-1}) for the oxidation of spinach ferredoxin (all at 25 °C, I = 0.10 M). Amino acid sequences have been determined for a number of 2-Fe plant ferredoxins,[37] including spinach (but not parsley), and have indicated that many of the amino acids (ca. 50%), including the cysteines which bind the Fe_2S_2 core, are invariant and occupy identical positions in the peptide chain. Variations in amino acid composition for the two cases investigated are seen to have a negligible effect on the reactivity (k_{et}), as well as properties of the binding site (K).

The remaining discussion is confined to the studies with parsley ferredoxin. The 2+ oxidant $Co(NH_3)_5Cl^{2+}$ gave only marginal kinetic evidence for an association step, and association was not detected with the less positive reactants $Co(NH_3)_5(C_2O_4)^{+}$, $Co(dmgH)_2(C_6H_5NH_2)_2{}^{+}$, and $Co(edta)^{-}$. It is nevertheless our belief that all reactions proceed by association followed by electron transfer as in eq 6 and 7. Reactions conform to second-order kinetics when $K[Co(III)] \ll 1$, eq 8. Rate constants k in Table X therefore correspond to Kk_{et}. Activation parameters for 3+, 2+, 1+, and 1− oxidants in Table X give clear-cut trends which as far as we are aware have not been previously demonstrated on this scale for outer-sphere electron transfer between two inorganic complexes. At pH ca. 8 the overall charge on the ferredoxin is negative.[38] Association constants K are higher for the 3+ than the 2+ oxidants (Table XI), and both ΔH_0 and ΔS_0 are positive. Positive ΔS_0 values are to be expected from changes in degree of solvation when oppositely charged reactants associate. The trend in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ (Table X) can be accounted for by ΔH_0 and ΔS_0 becoming less positive as the charge on the oxidant decreases. In short the Coulombic charge interaction appears to be a major influence in this series of reactions.

Interpretation in terms of prior association as in eq 6 and 7 is also consistent with kinetic data for the reactions of the blue copper proteins plastocyanin with inorganic complexes.[39] This protein is also known to be negatively charged at the pHs investigated. With $Co(phen)_3{}^{3+}$ as oxidant K(25 °C) = 167 M^{-1}, ΔH_0 = 10 kcal mol^{-1}, and ΔS_0 = 45 cal K^{-1} mol^{-1}, and it is noted that ΔH_0 and ΔS_0 are of similar magnitude to those for the reaction of parsley ferredoxin with $Co(NH_3)_6{}^{3+}$. For the reduction of plastocyanin, PCu^{II}, with the negatively charged redox partner $Fe(CN)_6{}^{4-}$, association is also detected: K(25 °C) = 110 M^{-1}, ΔH_0 = −5.1 kcal mol^{-1}, and ΔS_0 = −7.8 cal K^{-1} mol^{-1}. This reaction therefore represents an instance in which K is favorable (ΔH_0 is dominant) in spite of the unfavorable electrostatics, and specific interactions over and above the electrostatic interaction clearly apply. Although the trend of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ in Table X suggests that electrostatic forces are effective and possibly dominant, it remains to assess for such reactions to what extent specific (chemical)

Table XII. Reactions of Reduced Parsley Ferredoxins $Fe_2S_2*(SR)_4^{3-}$. Kinetic Data for Electron Transfer within the Protein-Complex Adducts, pH 8.0 (Tris), I = 0.10 M (NaCl)

oxidant	k_{et} (25 °C), s^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, cal K^{-1} mol^{-1}
$Co(NH_3)_6^{3+}$	19.2	8.5	−24.1
$Co(NH_3)_6^{3+}$ [a]	15.9		
$Co(en)_3^{3+}$ [b]	2.7	10.0	−23.1
$Co(NH_3)_5Cl^{2+}$	2300	(5.8)[c]	(−23.8)[c]

[a] Reaction with spinach ferredoxin. [b] Data for *dl* and *d* forms combined. [c] Errors large, ±8.2 ($\Delta H^{\ddagger}$) and ± 28.3 ($\Delta S^{\ddagger}$).

interactions are contributing. For example, it is not clear whether values of ΔH_0 as high as 10 kcal mol^{-1} can be accounted for solely in terms of electrostatics.

The possibility of specific (i.e., nonidentical) interactions of *d*- and *l*-$Co(en)_3^{3+}$ with the protein was tested for in the present work by replacing racemic *dl*-$Co(en)_3^{3+}$ by resolved *d*-$Co(en)_3^{3+}$. There was no measurable effect on rate constants (Figure 4). Nor does the pendant carboxylate of $Co(NH_3)_5(C_2O_4)^+$ or the aromatic rings of $Co(dmgH)_2(C_6H_5NH_2)_2^+$ appear to introduce any readily detectable effect in terms of enhanced association.

Reduction potentials have been determined for the couples $Co(NH_3)_6^{3+,2+}$ (+0.06 V), $Co(en)_3^{3+,2+}$ (−0.26 V), $Co(edta)^{-,2-}$ (+0.38 V), and $Co(dmgH)_2(C_6H_5NH_2)_2^{+,0}$ (+0.06 V).[40,41] The thermodynamic driving force is consistent with the smaller k_{et} for $Co(en)_3^{3+}$ as compared to that for $Co(NH_3)_6^{3+}$ (Table XII). The strongest of these oxidants, $Co(edta)^-$, has one of the smaller overall rate constants (Table X) which could be indicative of a smaller protein-complex association constant K for this negatively charged reactant.

Bennett has recently reviewed electron-transfer reactions of the ferredoxins,[42] and discussed kinetic parameters for the oxidation of reduced HIPIP $Fe_4S_4*(SR_4)_4^{2-}$ species. The overall $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for reactions with $Co(phen)_3^{3+}$ and $Fe(CN)_6^{3-}$ as oxidant exhibit similar trends to those for the blue Cu proteins,[39] suggesting that there is a significant contribution from the association step. This and other aspects of reactions of ferredoxins and their analogues are under investigation.

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Supplementary Material Available: A listing of rate constants, Tables III-IX (9 pages). Ordering information is given on any current masthead page.

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