g) was added and the suspension stirred at 23 °C for 5 h. The solvents were removed by evaporation in vacuo. Toluene was added and removed by evaporation and the product was crystallized from MeOH-ether to give IIa (24.2 g, 90% yield), mp 142-145.5 °C; R_f 0.49 (J). Anal. Calcd for $C_8H_{18}ClNO_2$: C, 49.10; H, 9.27; N, 7.16; Cl, 18.14. Found: C, 48.85; H, 9.28; N, 7.16; Cl, 18.00. δ $CDCl_3$ (ppm) 1.3-1.9 (m, 8 H), 2.3 (t, 2 H, $CH_2C{=}O$), 3.05 (t, 2 H, CH_2N), 3.66 (s, 3 H, OMe); IR $CHCl_3$ 5.75 μ.

Cbz-1/2Dsu-Phe-D-Trp-Lys(iNoc)-Thr(OBz)-1/2Dsu-Aha-OMe (XIIIa). A solution of 1.72 g (1.55 mmol) of XII and 370 mg (1.71 mmol) of IIa in 10 mL of degassed DMF was cooled to -20 °C and DPPA (0.40 mL, 1.86 mmol) was added followed by DIPEA (0.31 mL). The solution was maintained at -20 °C for 96 h followed by 48 h at 5 °C. During this period additional DPPA (0.08 mL) and IIa (33 mg) were added followed by DIPEA (0.4 mL) to keep the pH at 7.0 (moist pH paper, range 6-8). The resultant mixture was filtered to remove the precipitated $(PhO)_2PO_2H$ and the filtrate evaporated in vacuo to an oil which was triturated with two 30-mL portions of ether, air-dried, and washed in portions with a total of 150 mL of H_2O which after drying in vacuo gave XIIIa (1.83 g, 95% yield) and amino acid analysis in Table IV.

H-1/2Dsu-Phe-D-Trp-Lys(iNoc)-Thr-1/2Dsu-Aha-OMe (XIVa). XIIIa (1.9 g, 1.5 mmol) was moistened with 4 mL of anisole in a Teflon vessel into which HF (40 mL) was introduced by distillation and the mixture held at 0 °C for 30 min. The HF was removed by evaporation in vacuo at 0 °C. The oily residue was triturated with two portions of 20% EtOAc-petroleum ether and the granular solid was collected by filtration to give XIVa (1.6 g, 100%), R_f 0.45 (K), 0.4 (D), 91.3% pure by LC, 7.80 min (F).

***cyclo*-(Aha-1/2Dsu-Phe-D-Trp-Lys(iNoc)-Thr-1/2Dsu) (XVa).** To a solution of XIVa (1.6 g, 1.5 mmol) in degassed DMF (32 mL) was added 95% NH_2NH_2 (16 mL), and the solution allowed to stand for 100 min at 23 °C. After the solvents were evaporated in vacuo, two 20-mL portions of DMF were added and evaporated in vacuo. The residue was triturated three times with 50-mL portions of EtOAc to give crude hydrazide which was purified by chromatography on silica gel 60 (200 g) packed in solvent K. Elution with 2 L of solvent K was followed by solvent F. Fractions containing product were identified by TLC and combined to give single spot hydrazide XIVc (820 mg, 54%), R_f 0.2 (K), 0.55 (J); $Aha_{1.00}Phe_{0.99}D\text{-}Trp_{0.89}Lys_{0.99}Thr_{1.00}Dsu_{1.03}$.

To a solution of XIVc (800 mg, 0.78 mmol) in 16 mL of degassed DMF and 5.92 M HCl in THF (1.0 mL, 5.92 mmol) was added isoamyl nitrite (0.13 mL, 0.96 mmol) at -25 °C and the solution stirred for 1 h. The solution was added to 4.1 L of degassed, cold DMF. HBT·H_2O (4 g) was added; the solution was neutralized with DIPEA (7 mL) to an apparent pH 7.0 (moist pH paper, range 6-8) and stored at -20 °C for 24 h followed by 24 h at 5 °C, during which period additional DIPEA (2 mL) was added to maintain pH 7.0. The solvent was evaporated in vacuo, and the residue was triturated with a total of 200 mL of 5% $NaHCO_3$ solution to give, after drying, 500 mg (65%) of crude product XVa.

***cyclo*-(Aha-1/2Dsu-Phe-D-Trp-Lys-Thr-1/2Dsu) (Ib).** A suspension of 400 mg (0.4 mmol) of crude XVa and 313 mg of 10% Pd/C in a mixture of 30 mL of 50% HOAc and 30 mL of EtOH was hydrogenated for 2 h (40 psig) in a Parr shaker. The mixture was filtered and the filtrate evaporated in vacuo. The crude product was purified by filtration through a Sephadex G-50F column (5 × 100 cm) eluted with 50% HOAc, collecting 16-mL fractions. Monomeric product was eluted in fractions 91-106 and dimeric product was present in fractions 80-91. Fractions containing monomeric product were combined and passed through a Sephadex G25F column (5 × 100 cm) eluted with 2 N HOAc. Fractions 95-100 (20 mL each) were shown to contain product by TLC and combined to give 105 mg (29%) of Ib, $[\alpha]^{23}_D$ -12.6 (*c* 0.1, 50% HOAc), having a molecular weight of 977 (calcd 858). An additional 55 mg (15%) of product (97.6% pure, LC) was obtained from side fractions.

Trypsin Cleavage of Ia, Ib, and Ic. To a suspension of 1 mg of peptide in 0.5 mL of 0.046 M, pH 8.1, 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride buffer containing 0.015 M $CaCl_2$ was added an aqueous solution of Trypsin-TPCk (0.1 mL (666 units)); the mixture was stirred at 37 °C. Aliquots (20 μL) were removed at designated time intervals, acidified with 5 μL of acetic acid, and analyzed by HPLC using a Waters μBondapak C-18 column having a Whatman Co. PELL ODS C-18 guard column using 0.0085 M H_3PO_4/CH_3CN as the mobile phase. Ia and Ib were eluted at 8.90 and 9.40 min and their hydrolysis products at 2.27 and 4.00 min, respectively, using a buffer: CH_3CN ratio of 72:28. Ic and its hydrolysis product were eluted at 12.57 and 5.37 min using a buffer: CH_3CN ratio of 80:20. Ia was 50% hydrolyzed after 45 min. Ib and Ic showed 12 and 13% hydrolysis after 24 h.

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Kinetic Studies on Reactions of Iron-Sulfur Proteins. 3. Oxidation of the Reduced Form of *Clostridium pasteurianum* 8-Iron Ferredoxin with Inorganic Complexes. Observation of Single-Stage Kinetics for a Difunctional Protein Reactant

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Abstract: The kinetics of the 2-equiv oxidation of reduced *C. pasteurianum* 8-Fe ferredoxin, which contains one-electron active $Fe_4S_4{*}(SR)_4^{3-}$ clusters (average Fe oxidation state 2.25), with a range of oxidants $(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$, $Pt(NH_3)_6^{4+}$, $Co(NH_3)_6^{3+}$, $Co(en)_3^{3+}$, $Co(NH_3)_5Cl^{2+}$, $Co(acac)_3$, $Co(edta)^-$, $Co(cydta)^-$, and $Co(C_2O_4)_3^{3-}$, have been studied at pH 8.0 (Tris buffer), I = 0.10 M (NaCl). All the reactions give a single kinetic step which can be accounted for in terms of statistically related biphasic schemes, in which the difunctional fully reduced 8-Fe(rr) protein reacts at twice the rate of the monofunctional half-reduced 8-Fe(or) form. Rate constants listed are for the oxidation of 8-Fe(or) to 8-Fe(oo). The oxidants $Pt(NH_3)_6^{4+}$, $Co(NH_3)_6^{3+}$, and $Co(en)_3^{3+}$ exhibit limiting kinetic behavior, consistent with a mechanism involving association (K) followed by outer-sphere electron transfer (k_{et}). With $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ the temperature dependences for K give $\Delta H°$ and $\Delta S°$ values which suggest a predominantly electrostatic interaction. Overall rate constants $k(=Kk_{et})$ are reported for all other oxidants, including $(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$, which reacted too rapidly for a full study.

Ferredoxins isolated from bacteria are known to contain $Fe_4S_4{*}(SR)_4$ clusters,[1] where S* represents inorganic sulfide and SR a cysteine amino acid residue of the polypeptide chain. Such clusters also occur in complex enzymes, including xanthine oxidase

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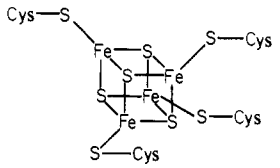

and nitrogenase,[2] and have been detected in membrane-bound proteins of plant chloroplasts.[3] The 8-Fe bacterial ferredoxins have a single polypeptide chain of ca. 55 amino acids (mol wt ca. 6000),[4] and contain two 4-Fe clusters each of which displays similar properties, e.g., spectra,[5,6] to the single 4-Fe cluster in the ferredoxins from *B. stearothermophilus* and *B. polymixa*.[7] Reduction potentials for the one-electron-active 4-Fe clusters are very similar to those for the 2-Fe ferredoxins, i.e., ca. −0.4 V.[5] In addition high-potential (ca. 0.35 V) 4-Fe proteins are known, and form a separate category.[4,8]

The structure of the 8-Fe ferredoxin from *Peptococcus aerogenes* (oxidized form) has been determined,[9] and the centers of the two (distorted) cubes have been shown to be ca. 12 Å apart. Other 8-Fe ferredoxins including that from *C. pasteurianum* exhibit similarities in amino acid sequencing, the homology being generally 40–50%.[4] Amino acid conservation is observed for the cysteine residues which bind the Fe_4S_4* clusters and consistently occupy the same positions. For each 8-Fe ferredoxin a marked similarity exists between the sequences of the first and second halves of the primary structures.[10] On the basis of known properties an analogous structure and shape (oblate ellipsoid) are expected for the 8-Fe ferredoxin from *C. pasteurianum*.[11]

Data from Mössbauer and ESR spectroscopy, although complicated by the presence of two redox centers, is consistent with each Fe_4S_4* cluster in oxidized 8-Fe ferredoxin consisting formally of two Fe(II) and two Fe(III) ions coupled antiferromagnetically to produce a resultant zero-spin $S = 0$.[12-14] However, all Fe atoms are equivalent and the oxidation state of each one can be regarded as 2.5+ assuming bridging sulfides to be formally 2−. Each cluster on acceptance of one electron becomes ESR active with a resultant spin $S = {}^1/_2$.[13] Mössbauer results at 195 K show almost complete delocalization of the added electron between four Fe atoms, in contrast to the distinct Fe(II) and Fe(III) sites observed under similar conditions for reduced Fe_2S_2*$(SR)_4$ proteins. Charges of 2− and 3− may be assigned to each of the Fe_4S_4*$(SR)_4$ clusters in the oxidized and reduced states, respectively.

The bacterial ferredoxins function as electron carriers in many diverse metabolic processes including nitrogen fixation. This paper is concerned with the electron-transfer properties of the difunctional 8-Fe protein from *C. pasteurianum*. A range of inorganic complexes and techniques are used as previously for studies with the unifunctional 2-Fe protein.[15,16] The different oxidized/reduced states of the 8-Fe protein are denoted by 8-Fe(oo), 8-Fe(or), and 8-Fe(rr).

Experimental Section

Isolation of Protein. Ferredoxin from *C. pasteurianum*[17] was isolated using a modification of the procedure described by Thompson et al.[12] *C. pasteurianum* cells (500 g), which had been stored at ca. −20 °C, were suspended in ca. 1200 mL of 0.024–0.020 M Tris HCl at 0 °C. The suspension was homogenized for ca. 5 min in a Waring commercial blender. The homogenate was then sonicated (to rupture cell membranes) in 100-mL portions for ca. 5 min using a Rapidis Soniprobe. During sonication, the beaker containing the mixture was placed in an ice–acetone mixture (−10 °C) to dissipate the heat generated. The combined sonicated mixture was centrifuged at $(2 \times 10^4)g$ for 2 h (2 °C) after which the residue was discarded. The supernatant liquid was then stirred for ca. 5 min with dry Whatman DEAE 23 cellulose (15 g). The cellulose was filtered off using a large-capacity sinter funnel (porosity 1 or 2) and the residue transferred as a slurry to a suitably sized column. The column was washed with ca. 200 mL of 0.1 M NaCl buffered with 0.024–0.020 M Tris HCl. Ferredoxin (and rubredoxin) were then eluted with similarly buffered 0.8 M NaCl (ca. 100 mL).

The protein was precipitated by the addition of solid $(NH_4)_2SO_4$ (BDH, reagent grade, 0.6 g/mL) to the stirred solution, and the pH lowered to 5.8 by the dropwise addition of 1 M acetic acid. The suspension was then centrifuged at $(4 \times 10^4)g$ for 30 min and the colorless supernatant liquid discarded. All subsequent procedures (which were air-free) were carried out using NaCl solutions bufferd with 0.025–0.020 M Tris HCl. The brown sediment was dissolved in the minium quantity of 0.15 M NaCl and dialyzed overnight against 2 × 3000 mL of the same buffer. The dialyzed solution was loaded onto a column of DEAE 23 (50-cm height, 2.5-cm diameter) equilibrated with 0.15 M NaCl, and the column was washed with 0.25 M NaCl (ca. 100 mL) to elute the rubredoxin as a pinkish solution. The ferredoxin was then eluted using NaCl solution of increasing concentration, 0.25–0.50 M (ca. 500 mL), and fractions with absorbance ratios $A_{390}/A_{285} > 0.73$ were pooled. Final purification was achieved by diluting the ferredoxin to a total Cl^- concentration of 0.15 M, and loading onto a column of DEAE 23 (50-cm height, 1.5-cm diameter) equilibrated with 0.15 M NaCl. After the solution was washed with ca. 100 mL of 0.15 M NaCl, the ferredoxin was eluted using NaCl solution of increasing concentration (0.2–0.5 M), retaining fractions with $A_{390}/A_{285} > 0.79$. Finally the pure protein was diluted (ca. threefold) and loaded onto a small column of DEAE 23 (2-cm height, 1.5-cm diameter) equilibrated with 0.15 M NaCl. It was eluted as a very dark solution with 0.5 M NaCl. The yield from 500 g of cells was typically 20 mL of 10^{-3} M solution having $A_{390}/A_{285} = 0.80$ in excellent agreement with published values.[5] Protein concentrations were determined from the absorbance at the 390-nm peak, ϵ 3.0×10^4 M^{-1} cm^{-1} (per mol of protein).

Preparation of Complexes. The following complexes were obtained by literature methods as indicated: hexaamminecobalt(III) chloride, $[Co(NH_3)_6]Cl_3$;[18] tris(ethylenediamine)cobalt(III) chloride trihydrate, $[Co(en)_3]Cl_3 \cdot 3H_2O$;[19] chloropentaamminecobalt(III) chloride, $[Co(NH_3)_5Cl]Cl_2$;[20] sodium ethylenediaminetetraacetatocobaltate(III) tetrahydrate, $Na[Co(edta)] \cdot 4H_2O$;[21] potassium cyclohexanediaminetetraacetatocobaltate(III) monohydrate, $K[Co(cydta)] \cdot 3H_2O$;[22] tris(acetylacetonate)cobalt(III), $[Co(acac)_3]$;[23] potassium tris(oxalato)cobaltate(III), $K_3[Co(C_2O_4)_3] \cdot 3 \cdot 5H_2O$;[24] μ-amido-bis(pentaammine)cobalt(III) bromide, $[(NH_3)_5CoNH_2Co(NH_3)_5]Br_5$;[25] hexaammineplatinum(IV) chloride, $[Pt(NH_3)_6]Cl_4 \cdot H_2O$.[26] UV–visible peak positions and absorption coefficients as previously listed[15,16] were used as a criterion of purity.

Stoichiometry. The stoichiometry for the oxidation of fully reduced 8-Fe ferredoxin with $Co(NH_3)_6^{3+}$ was determined at 25.0 °C, pH 8.0 (Tris), $I = 0.10$ M (NaCl). A solution of ferredoxin (3.2 mL of 3.1×10^{-5} M) was reduced with sodium dithionite solution (3.1×10^{-3} M)

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which was added from a Hamilton gas-tight microsyringe (0.1-mL capacity), until further addition brought no further change in absorbance at 420 nm. Final additions were carried out with aliquots of ca. 0.005-mL volume, to prevent the addition of excess $S_2O_4^{2-}$. Aliquots (ca. 0.01 mL) of $Co(NH_3)_6^{3+}$ solution (2.9×10^{-3} M) were then added by microsyringe until complete reoxidation (as judged by no further absorbance increase at 420 nm) had occurred. A stoichiometry of 1.9 mol of $Co(NH_3)_6^{3+}$ to 1 mol of ferredoxin was found:

$$\text{8-Fe(rr)} + 2\text{Co(III)} \rightarrow \text{8-Fe(oo)} + 2\text{Co(II)} \quad (1)$$

consistent with a net two-electron reaction of 8-Fe(rr).

Similar stoichiometries were assumed for other mononuclear Co(III) oxidants. It was necessary in the case of the binuclear cobalt(III) complex, $Co(III)_2$, to determine the consumption of $Co(III)_2$ and whether 2Co(III) + 2Co(II) or 2Co(II) were formed.[27] The former corresponds to a stoichiometry of 2 mol of $Co(III)_2$ per 8-Fe(rr), and the latter 1 mol of $Co(III)_2$ per 8-Fe(rr). The final product was 8-Fe(oo). To determine the final composition with regard to Co(III) the following procedure was adopted. Excess of dithionite (ca. 20%) was first added to 15 mL of protein solution (2.3×10^{-5} M). After 2–3 min for reduction of the protein an accurately measured volume of $Co(III)_2$ was added (0.5 mL, Hamilton syringe), with stirring, to give $[Co(III)_2] = 6.5 \times 10^{-5}$ M. The redox process was rapid (<1 min), and the mixture was then passed down a DEAE 23 column. The protein was held by the column and the cationic products were collected in 10-mL fractions. The concentration of $Co(III)_2$ was determined spectrophotometrically using the absorbance bands at 300 (ϵ 3040 M^{-1} cm^{-1}) and 360 nm (ϵ 708 M^{-1} cm^{-1}),[26] which are much more intense than absorbance bands of related mononuclear Co(III) or Co(II) complexes. It was found that 2.04 mol of $Co(III)_2$ were consumed per 8-Fe(rr). The reaction therefore conforms to the equation

$$\text{8-Fe(rr)} + 2\text{Co(III)}_2 \rightarrow \text{8-Fe(oo)} + 2\text{Co(III)} + 2\text{Co(II)} \quad (2)$$

A stoichiometry as in (3) is assumed for the reaction of Pt(IV):

$$\text{8-Fe(rr)} + \text{Pt(IV)} \rightarrow \text{8-Fe(oo)} + \text{Pt(II)} \quad (3)$$

since Pt(III) (if formed) is a highly reactive intermediate.

Kinetic Studies. Protein ($2–3 \times 10^{-5}$ M) was dialyzed (Sigma 250-7U dialysis sacks) for 3 days against 0.090 M NaCl buffered with 0.0100–0.0108 M Tris HCl (3 × 1000 mL). This was carried out at 0 °C under oxygen-free conditions. The dialysis medium was chosen so that the ionic strength (0.10 M) was equal to that used in most kinetic studies. The pH (ca. 7.0 when measured at 25 °C) was subsequently adjusted, usually to pH 8.0 at 25 °C, by addition of small amounts of Tris.[15] A Radiometer pH meter (Type PHM 4d) complete with calomel (Type K401) and glass (Type G202B) electrodes was used to measure pHs. Prior to use all ferredoxin solutions were kept oxygen-free by passing N_2 (preferably scrubbed with alkaline pyrogallol) across the solution surface. All studies unless otherwise stated were at $I = 0.10$ M (NaCl). A Durrum-Gibson stopped-flow spectrophotometer was used to monitor reactions. The procedure for generating reduced protein (ca. 10 mL of ca. 1×10^{-5} M) by dithionite reduction[28] (0.4 mL of ca. 4×10^{-4} M $Na_2S_2O_4$) in the storage syringe of the stopped-flow was as described previously.[15] Assessment of the extent of reduction from the amplitude of subsequent stopped-flow traces showed the ferredoxin to be ca. 100% reduced. A few test runs with the ferredoxin <50% reduced yielded the same results. The total concentration of 8-Fe protein was varied within the range ($0.3–0.6) \times 10^{-5}$ M. Excess $S_2O_4^{2-}$ (up to ca. fivefold) was also found to have little (<5%) or no effect on rates of oxidation with $Co(NH_3)_6^{3+}$ and $Co(edta)^-$.

Reactions were studied with the oxidant in at least tenfold excess, there being minor deviations from this condition with the $Co(III)_2$ oxidant. The oxidation of reduced ferredoxin was monitored at 420 nm, although test runs were also carried out at 390 and 440 nm with no observable difference in kinetic behavior. At 420 nm the reduced and oxidized protein have ϵ's of 1.1×10^4 and 2.7×10^4 M^{-1} cm^{-1}, respectively, Figure 1. For experiments in which the pH was varied, all solutions were adjusted to the required pH prior to kinetic runs.

The extent of reaction as judged by the total absorbance change (extrapolation of first-order plots to time $t = 0$) was consistent with complete oxidation from the fully reduced protein. Single-amplitude kinetics were observed in all cases. First-order plots of log of absorbance changes ΔA against time were generally linear to >85% completion, Figure 2, where slopes (×2.303) gave rate constants k_{obsd}. A nonlinear least-squares computer analysis[29] was used to fit rate constants to different dependences. Weighting factors $1/k_{obsd}$ or $1/k_{obsd}^2$ were used, whichever gave the smaller standard deviation.

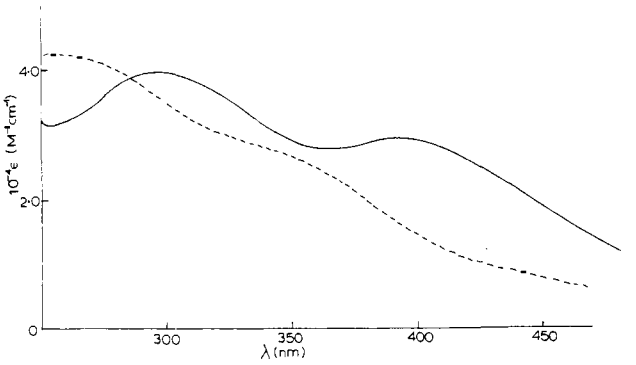

Figure 1. Spectra of *C. pasteurianum* 8-Fe ferredoxin oxidized (—) and reduced (---) at pH 8.0 (Tris), $I = 0.10$ M (NaCl).

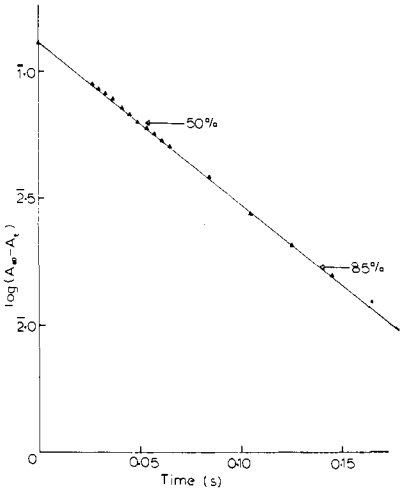

Figure 2. Illustration of the linearity of first-order plots for a run with $[8\text{-Fe(rr)}] = 4.0 \times 10^{-6}$ M, $[Co(NH_3)_6^{3+}] = 3.9 \times 10^{-4}$ M, pH 8.0 (Tris), $I = 0.10$ M (NaCl), temperature 25.0 °C.

Results

Rate Laws. Absorbance changes with the oxidant in large excess of protein conform to the rate law

$$\log (A_\infty - A_t) = (k_{obsd}t/2.303) + \text{constant} \quad (4)$$

with all Co(III) oxidants, including $Co(III)_2$, which behaves as a 1-equiv oxidant (see stoichiometry experiments). An allowance was made for the different stoichiometry (3) with $Pt(NH_3)_6^{4+}$ as oxidant, where k_{obsd} is defined by

$$\log (A_\infty - A_t) = 2k_{obsd}t/2.303 + \text{constant} \quad (5)$$

For the reaction with $Co(NH_3)_6^{3+}$ as oxidant, the dependence of k_{obsd} on $[Co(NH_3)_6^{3+}]$, Table I,[30] is illustrated in Figure 3. The data obtained gave a good fit to

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

where K and k_{et} are as defined in the equations

$$\text{protein} + \text{oxidant} \overset{K}{\rightleftharpoons} \text{protein,oxidant} \quad (7)$$

$$\text{protein,oxidant} \xrightarrow{k_{et}} \text{products} \quad (8)$$

From data at different temperatures the following are obtained: $K(25\ °C) = 466 \pm 26$ M^{-1}, $\Delta H° = 0.3 \pm 1.1$ kcal mol^{-1}, $\Delta S° = 13.4 \pm 3.8$ cal K^{-1} mol^{-1}; $k_{et}(25\ °C) = 98 \pm 8$ s^{-1}, $\Delta H_{et}^{\ddagger} = 15.3 \pm 0.8$ kcal mol^{-1}, $\Delta S_{et}^{\ddagger} = 1.9 \pm 12.7$ cal K^{-1} mol^{-1}.

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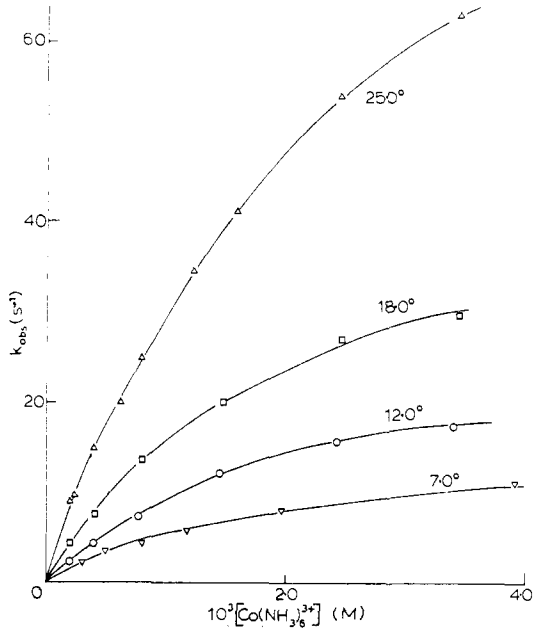

Figure 3. The variation of k_{obsd} with $[Co(NH_3)_6^{3+}]$ for the oxidation of reduced 8-Fe ferredoxin, pH 8.0 (Tris), I = 0.10 M (NaCl).

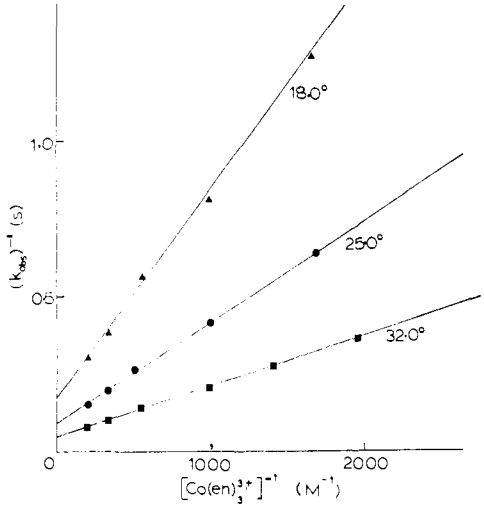

Figure 4. The dependence of first-order rate constants k_{obsd} on $[Co(en)_3^{3+}]$ for the oxidation of reduced 8-Fe ferredoxin, pH 8.0 (Tris), I = 0.10 M (NaCl).

With $Co(en)_3^{3+}$ as oxidant similar behavior is observed, Table II,[30] where the linearity of the reciprocal plot of $(k_{obsd})^{-1}$ against $[Co(III)]^{-1}$ is illustrated, Figure 4. From the temperature variation K(25 °C) = 261 ± 13 M^{-1}, ΔH = 0.9 ± 1.7 kcal mol^{-1}, $\Delta S°$ = 13.9 ± 5.6 cal K^{-1} mol^{-1}; k_{et}(25 °C) = 11.8 ± 0.8 s^{-1}, $\Delta H^{\ddagger}_{et}$ = 16.1 ± 1.2 kcal mol^{-1}, $\Delta S^{\ddagger}_{et}$ = 0.5 ± 4.0 cal K^{-1} mol^{-1}. Data with $Pt(NH_3)_6^{4+}$ as oxidant, Figure 5, gave K = 2400 ± 82 M^{-1} and k_{et} = 111 ± 10 s^{-1} at 25 °C.

All other oxidants give first-order rate constants, k_{obsd} (Table III), with a strictly first-order dependence on [oxidant]. The temperature dependence of rate constants k_{obsd} was investigated for the oxidation with $Co(edta)^-$, Table IV,[30] and second-order rate constants k ($=k_{obsd}/[Co(III)]$) give $\Delta H^{\ddagger}$ = 7.6 ± 0.3 kcal mol^{-1} and $\Delta S^{\ddagger}$ = −14.4 ± 1.0 cal K^{-1} mol^{-1}. Rate constants k are equivalent to Kk_{et} as defined in (7) and (8). The $Co(III)_2$ oxidant would have been expected to conform to (6) with $K[Co(III)_2]$ effective in the denominator. However, owing to the rapidity of the reaction (even at 7 °C), a sufficiently high range of $[Co(III)_2]$ could not be investigated, and $K[Co(III)_2]$ remained ≪1. An upper limit of $K < 4 \times 10^3$ M^{-1} with $k_{et} > 200$ s^{-1} is obtained at 7 °C.

pH Dependences. No meaningful dependence of k_{osbd} on pH was observed for the $Co(NH_3)_6^{3+}$ oxidation of 8-Fe(rr) at a relatively low value for $[Co(NH_3)_6^{3+}]$ (2×10^{-4} M), when $K[Co(III)] \ll 1$ and $k_{obsd}/[Co(III)]$ in (6) corresponds to Kk_{et}. The possibility that K and k_{et} exhibit independent variations with pH (in such a way as to compensate) was investigated by carrying out six runs at each of the pHs 7.0, 7.5, and 8.5 (25 °C, Tris buffer) to supplement existing data at pH 8.0 (Table V).[30] Small (±7%) trends only were detected, which are probably not meaningful and stem largely from experimental error. Rate constants k(25 °C) were also independent (±4%) of pH for the oxidants $Co(acac)_3$ and $Co(edta)^-$.

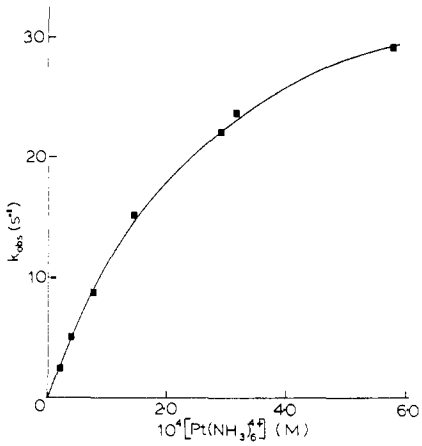

Figure 5. The variation of k_{obsd} with $[Pt(NH_3)_6^{4+}]$ for the oxidation of reduced 8-Fe ferredoxin, pH 8.0 (Tris), I = 0.10 M (NaCl).

Table III. First-Order Rate Constants[a] for 1:2 and in One Case with $Pt(NH_3)_6^{4+}$ 1:1 Redox Reactions between Reduced *C. pasteurianum* 8-Fe Ferredoxin, Fe_8S_8*(rr), and a Range of Oxidants, pH 8.0 (Tris), I = 0.10 M (NaCl), λ = 420 nm

$10^4[Co_2(NH_3)_{10}NH_2^{5+}]/M$[b]	0.21, 0.40, 0.77, 1.34
k_{obsd}/s^{-1}	17.0, 37.0, 76, 132
$10^4[Co(NH_3)_5Cl^{2+}]/M$[c]	0.51, 0.99, 1.53
k_{obsd}/s^{-1}	25.8, 50, 78
$10^4[Co(acac)_3]/M$	0.42, 0.84, 1.68, 3.36, 5.40, 7.80
k_{obsd}/s^{-1}	1.8, 3.2, 5.5, 10.4, 16.9, 23.8
$10^4[Co(edta)^-]/M$	5.0, 8.0, 12.1, 16.1, 20.1, 25.2
k_{obsd}/s^{-1}	5.6, 9.1, 13.0, 16.5, 23.8, 28.7
$10^4[Co(cydta)^-]/M$	1.4, 2.8, 5.5, 11.0, 22.0, 46
k_{obsd} s^{-1}	1.5, 3.0, 5.3, 9.5, 18.0, 36
$10^4[Co(C_2O_4)_3^{3-}]/M$	2.0, 4.0, 7.0, 10.0, 20.0
k_{obsd}/s^{-1}	1.04, 2.04, 3.0, 4.8, 9.5
$10^4[Pt(NH_3)_6^{4+}]/M$	0.20, 0.40, 0.75, 1.45, 2.90, 3.15, 5.80
k_{obsd}/s^{-1}	2.55, 5.0, 8.8, 15.0, 22.0, 23.8, 29.4

[a] Rate constants are for the reaction of 8-Fe(or). Multiply (×2) to obtain rate constants for the oxidation of 8-Fe(rr) (see Discussion). [b] 7.0 °C. [c] 20.0 °C; pH 7.0 (10^{-2} M phosphate); I = 0.10 M ($NaClO_4$).

Ionic Strength. The variation of k with ionic strength, I, was investigated for the oxidant $Co(edta)^-$ (low charge). At 25 °C, [8-Fe(rr)] = ca. 5×10^{-6} M, $[Co(edta)^-] = 1.0 \times 10^{-4}$ M, pH 8.0 (Tris), 10^3k (M^{-1} s^{-1}) values with I (quantity in paretheses) made up using NaCl (M) were 5.34 (0.001), 6.36 (0.003), 7.13 (0.0055), 7.63 (0.008), 8.10 (0.10).

Discussion

The difunctional nature of the protein and manner of the oxidation of 8-Fe(rr) through 8-Fe(or) to 8-Fe(oo) are of interest in this study. The dithionite-reduced protein, 8-Fe(rr), serves as a 2-equiv (net) reductant with each 4-Fe cluster one-electron active. All the complexes investigated, with the possible exception of $Pt(NH_3)_6^{4+}$, function as one-electron oxidants.

The single-stage kinetic process which is observed implies that (a) the intermediate 8-Fe(or) rapidly disproportionates in a non-rate-determining step, thus avoiding slow oxidation of 8-Fe(or) by the complex, or (b) that k_1 for the oxidation of 8-Fe(rr) is very much less than k_2 for the oxidation of 8 Fe(or) (i.e., there is cooperativity) or (c) that statistical kinetics apply, with equal (or

approximately equal) $\Delta\epsilon$ absorbance changes for the two steps and $k_1 = 2k_2$.[31] Fast disproportionation would not necessarily be detectable in (c).

It has been demonstrated in pulse radiolysis experiments that 8-Fe(or), concentration ca. 10^{-6} M, can be generated by reaction of 8-Fe(oo) with e_{aq}^-. No evidence was obtained[32] for a disproportionation step, which if dominant must at this level of 8-Fe(or) concentration have a rate constant $> 10^8$ M^{-1} s^{-1}. Furthermore, EPR spectra at 12 K distinguish between 8-Fe(rr) and 8-Fe(or) forms of *C. pasteurianum*, and are consistent with the retention of 8-Fe(or) in partially dithionite-reduced solutions.[33] Possibility (a) can be excluded, therefore.

The second possibility (b) with $k_2 \gg k_1$ can also be excluded. Thus the reaction of $Co(NH_3)_5Cl^{2+}$ with 8-Fe(or) generated by pulse radiolysis,[32] rate constant $k_2 = 4.1 \times 10^5$ M^{-1} s^{-1} at 18 °C (pH 7.0, $I = 0.10$ M), is of the same magnitude as the rate constant (5.1×10^5 M^{-1} s^{-1}) obtained from the single amplitude change in a stopped-flow study of the reaction of $Co(NH_3)_5Cl^{2+}$ with 8-Fe(rr) under identical conditions.

Possibility (c) involving statistical kinetics is, on the other hand, an acceptable interpretation. It is reasonable that identical absorbance changes, $(\epsilon_2 - \epsilon_1) = (\epsilon_3 - \epsilon_2)$, are observed at each Fe_4S_4* cluster:

$$\text{8-Fe(rr)}(\epsilon_1) \xrightarrow{k_1} \text{8-Fe(or)}(\epsilon_2) \xrightarrow{k_2} \text{8-Fe(oo)}(\epsilon_3) \qquad (9)$$

and that $k_1 = 2k_2$. Substitution of these relationships into the general equation for biphasic kinetics yields[34]

$$A_t = \epsilon_3 C_0 + (\epsilon_1 - \epsilon_3)C_0 e^{-k_1 t} + (\epsilon_2 - \epsilon_3)[k_1 C_0/(k_2 - k_1)](e^{-k_1 t} - e^{-k_2 t}) \qquad (10)$$

where C_0 is the initial concentration of 8-Fe(rr) in a 1-cm path length cell. Absorbance changes (A) can therefore be expressed as

$$A_\infty - A_t = 2(\epsilon_2 - \epsilon_1)C_0 e^{-k_2 t} \qquad (11)$$

Accordingly rate constants for the overall reaction (1) obtained from the single-stage kinetics, eq 4, correspond to true rate constants for the monofunctional reactant 8-Fe(or).[35] Rate constants k_{obsd} correspond therefore to the second stage of reaction k_2, and have to be multiplied by the statistical factor (2) to obtain k_1. From analog computer simulations it has been shown that 20% deviations from statistical absorbance ratios would probably go undetected (as curvature) in first-order plots.[36] A factor of 2 difference in rate constants from the 2:1 statistical factor is clearly observable, but ca. 20% deviation would again probably go undetected.

The question of communication between the Fe_4S_4*$(SR)_4$ clusters of 8-Fe proteins is now considered. ^1H NMR studies (30 °C) on the *C. acidi urici* 8-Fe protein have indicated that a fast electron exchange (fast on the ^1H NMR time scale) is taking place between oxidized and reduced iron–sulfur clusters.[37] *C. pasteurianum* ferredoxin exhibits similar properties. Intramolecular as well as intermolecular exchange may be occurring.[38] Now second-order rate constants (pseudo-first-order values k_1 and k_2 divided by oxidant concentrations) are known to be made up of two components. These correspond to the intial association of the two reactants in a rapid equilibration step (K) followed by electron transfer (k_{et}). For a reaction not exhibiting limiting kinetics the relationship $k = Kk_{et}$ holds. The statistical ratio, $k_1 = 2k_2$, can originate from either K or k_{et} depending on whether reaction occurs at one or two sites on the protein. Various possibilities are indicated in Figure 6, where in the latter two communication (electron delocalization) over both clusters is indicated. Clusters with "half-electron" occupancy are assumed to react at half the rate of fully reduced clusters.

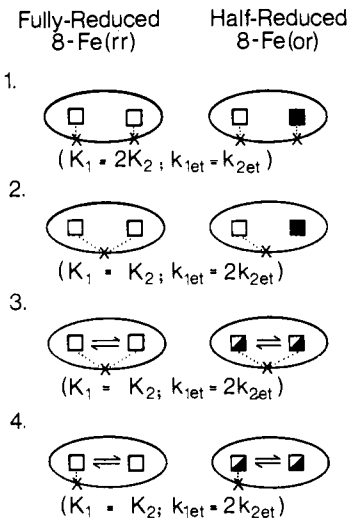

Figure 6. Schematic representation of alternative ways in which 8-Fe ferredoxin might react with involvement of one or two reaction sites (×) with and without communication between Fe_4S_4* clusters (□). Statistical values of K for association and k_{et} for electron transfer are indicated in each case.

Table VI. A Comparison of Overall Rate Constants k ($=Kk_{et}$) and Activation Parameters for the Oxidation of *C. pasteurianum* 8-Fe[a] and Parsley 2-Fe Reduced Ferredoxins, Respectively, at pH 8.0 (Tris), $I = 0.10$ M (NaCl)

reaction	k(25 °C), M^{-1} s^{-1}	$\Delta H^{\ddagger}$, kcal mol^{-1}	$\Delta S^{\ddagger}$, cal K^{-1} mol^{-1}
8-Fe + $Co(NH_3)_6^{3+}$	4.6×10^4	15.8	15.3
2-Fe + $Co(NH_3)_6^{3+}$	1.9×10^4	18.7	23.8
8-Fe + $Co(en)_3^{3+}$	3.1×10^3	17.0	14.4
2-Fe + $Co(en)_3^{3+}$	1.6×10^3	21.0	26.4
8-Fe + $Co(acac)_3$	3.1×10^4		
2-Fe + $Co(acac)_3$	7.0×10^3	6.3	−19.6
8-Fe + $Co(edta)^-$	1.1×10^4	7.6	−14.4
2-Fe + $Co(edta)^-$	7.2×10^3	5.2	−23.4
8-Fe + $Co(C_2O_4)^{3-}$	4.8×10^3		
2-Fe + $Co(C_2O_4)^{3-}$	3.9×10^3	3.15	−31.5
8-Fe + $Co(III)_2$	9.6×10^5 [b]		
2-Fe + $Co(III)_2$	5.6×10^5 [b]	17.0	29.0
8-Fe + $Pt(NH_3)_6^{4+}$	2.64×10^5		
2-Fe + $Pt(NH_3)_6^{4+}$	6.9×10^4		

[a] Rate constants are for the reaction of 8-Fe(or). Multiply (×2) to obtain the rate constant for the oxidation of 8-Fe(rr). [b] 7 °C.

It is known that the first and second halves of the amino acid sequences are related by a rotation of almost 180°, and that the 8-Fe ferredoxins display a remarkable intramolecular symmetry.[10] There are grounds therefore for supposing that two approximately equivalent sites could be utilized as in 1 of Figure 6. However, schemes 3 and 4, utilizing only one reaction site, cannot be entirely excluded at this time. It is hoped that it will be possible to comment further by investigating the inner-sphere reduction of 8-Fe(oo) with Cr(II) complexes, and determining (a) the number of moles of Cr(III) retained by the protein and (b) whether one or two kinetic stages are observable.

Discussion as to the utilization of one or two sites on the protein introduces the question of the feasibility of a single-stage two-electron change. The dicobalt(III) complex has the capacity to react by such a pathway, but from stoichiometry and product determination does not do so. This is perhaps not surprising in view of the inherent sluggishness of mononuclear one-electron Co(III) to Co(II) changes, due to the need for accompanying changes in electron spin.[39] The Pt(IV) oxidant is in a different

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Table VII. A Comparison of Association Constants (K) and Enthalpy and Entropy Terms for Association Prior to Electron Transfer in the Reactions of *C. pasteurianum* 8-Fe[a] and Parsley 2-Fe Reduced Ferredoxins, Respectively, at pH 8.0 (Tris), I = 0.10 M (NaCl)

reaction	K(25.0 °C), M^{-1}	ΔH°, kcal mol^{-1}	ΔS°, cal K^{-1} mol^{-1}
8-Fe + $Co(NH_3)_6^{3+}$	466	0.3	13.4
2-Fe + $Co(NH_3)_6^{3+}$	998	10.2	47.9
8-Fe + $Co(en)^{3+}$	261	0.9	13.9
2-Fe + $Co(en)_3^{3+}$	597	11.0	49.5
8-Fe + $Pt(NH_3)_6^{4+}$	2400		
2-Fe + $Pt(NH_3)_6^{4+}$	2100		

[a] Association constants are for the reaction of 8-Fe(or). Multiply (×2) to obtain association constants for the oxidation of 8-Fe(rr) if scheme 1 of Figure 6 applies.

category, but unfortunately there is no way of distinguishing between one- and two-electron changes with this oxidant. Since no two-electron outer-sphere electron transfer reactions have yet been identified,[40] and $Pt(NH_3)_6^{4+}$ must react in an outer-sphere manner, we opt here for the sequential changes Pt(IV) → Pt(III) → Pt(II), where reaction of the Pt(III) intermediate with the protein is assumed to be relatively rapid. Accordingly the measured rates, $-d[8\text{-Fe(rr)}]/dt$, have been divided by 2 to give k_{obsd} and hence the monofunctional rate constant k_2. This interpretation is supported by the rate constants, Table VI, determined for the 8-Fe(rr) and 2-Fe(r) reactions with $Pt(NH_3)_6^{4+}$. Thus the reactivity pattern observed is not sufficiently different from that with other oxidants as to suggest that the 8-Fe(rr) reaction is benefiting by introduction of a two-electron pathway.

As in the corresponding study with the reduced 2-Fe protein,[15,16] the more highly charged positive reactants $Pt(NH_3)_6^{4+}$, $Co(NH_3)_6^{3+}$, and $Co(en)_3^{3+}$ give limiting kinetics. Had it been possible to study the $(NH_3)_5CoNH_2Co(NH_3)_5^{5+}$ oxidant over a sufficiently wide range of concentrations, this reaction would almost certainly have displayed limiting kinetics also. The mechanism (7) and (8) is consistent with the rate-law dependence (6). As in previous studies there are two alternative mechanisms, one of which can be firmly excluded.[15] The second (the so-called "dead-end" mechanism) is more difficult to exclude in each and every case. This point has been extensively discussed elsewhere,[16] and the same considerations apply here also. General applicability of the second alternative seems unlikely, and for the present we interpret in terms of (7) and (8).

The incidence of limiting kinetics and implications as far as reaction scheme (9) is concerned are of further interest. The satisfactory linearity of rate plots should again be stressed. Thus for the seven 25 °C runs for the reaction of $Co(NH_3)_6^{3+}$, Figure 3, first-order plots were linear to 4, 3, 3, 2, 4, 4, and 4 half-lives, respectively, with increasing Co(III) concentrations. If situation 1 in Figure 6 applies, then at high oxidant concentrations binding of two oxidant molecules to each protein will give a limiting rate constant of $2k_{et}$, i.e., twice that observed for the monofunctional reactant 8-Fe(or). On a statistical basis the equilibrium constant for association of the second Co(III) to 8-Fe(rr) will be a quarter that of the first. Although it will not change the observed kinetic behavior, small contributions from the pathway involving association of a second Co(III) to 8-Fe(rr) will be effective at the higher Co(III) concentrations in this study.

Similar thermodynamic ΔH° and ΔS° values are obtained for the association of $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ with the 8-Fe protein, Table VII. Although these oxidants might have been expected to differ somewhat regarding their propensities to engage in certain specific interactions (H bonding for NH_3 ligands and possibly hydrophobic interactions involving the methylene groups of ethylenediamine), these are clearly not utilized. The respective values for ΔH° (0.3 and 0.9 kcal mol^{-1}) and ΔS° (13 and 14 cal K^{-1} mol^{-1}) are fully consistent with electrostatic interactions, and incidentally are remarkably similar to values observed in two reactions where 3+,4− and 3+,3− ion–ion interactions are known to be effective.[41,42] The electrostatic interaction is milder, however, than in the case of the 2-Fe ferredoxins, where for the same two oxidants similar values of ΔH° (10.2 and 11.0 kcal mol^{-1}) and ΔS° (48 and 50 cal K^{-1} mol^{-1}) are obtained. It is concluded that for each protein $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$ use the same site, and that this site has a smaller local negative charge for the 8-Fe than the 2-Fe protein. The overall net charge for the 8-Fe ferredoxin from *C. pasteurianum* is estimated to be −11 for 8-Fe(or) from the charge on individual amino acids at pH ca. 7, which compares with a value of −17 for reduced 2-Fe spinach ferredoxin (a similar value is expected for parsley ferredoxin, the amino acid composition of which is not known). It must be recalled, however, that the 8-Fe ferredoxins are smaller with molecular weights ca. 6000 compared to ca. 10 500 for 2-Fe ferredoxins.[4] A comparison of K values obtained for the reaction of $Pt(NH_3)_6^{4+}$ with 8-Fe and 2-Fe proteins is also made in Table VII.

Table VIII. A Comparison of Enthalpy and Entropy Terms Corresponding to k_{et} in the Reactions of *C. pasteurianum* 8-Fe[a] and Parsley 2-Fe Reduced Ferredoxins, Respectively, at pH 8.0 (Tris), I = 0.10 M (NaCl)

reaction	k_{et}(25 °C), s^{-1}	$\Delta H_{et}^{\ddagger}$, kcal mol^{-1}	$\Delta S_{et}^{\ddagger}$, cal K^{-1} mol^{-1}
8-Fe + $Co(NH_3)_6^{3+}$	98	15.3	1.9
2-Fe + $Co(NH_3)_6^{3+}$	19.2	8.5	−24.1
8-Fe + $Co(en)_3^{3+}$	11.8	16.1	0.5
2-Fe + $Co(en)_3^{3+}$	2.7	10.0	−23.1
8-Fe + $Pt(NH_3)_6^{4+}$	111		
2-Fe + $Pt(NH_3)_6^{4+}$	3.29		

[a] Rate constants apply to electron transfer within either of adducts 8-Fe(rr), oxidant and 8-Fe(or), oxidant.

While evidence for precursor adduct formation was not obtained with the other oxidants, it seems reasonable to assume that they also proceed with association followed by electron transfer, with K[Co(III)] ≪ 1 and $k = Kk_{et}$. The overall activation parameters (for k) were determined for the reaction with $Co(edta)^-$, $\Delta H^{\ddagger}$ = 7.6 kcal mol^{-1} and $\Delta S^{\ddagger}$ = −14 cal K^{-1} mol^{-1}, which compared to those for the $Co(edta)^-$ oxidation of the 2-Fe ferredoxin ($\Delta H^{\ddagger}$ = 5.2 kcal mol^{-1}, $\Delta S^{\ddagger}$ = −23.4 cal K^{-1} mol^{-1}) suggest that electrostatics are again a contributing factor.

The near-zero values of $\Delta S_{et}^{\ddagger}$ corresponding to electron transfer within the adducts with $Co(NH_3)_6^{3+}$ (1.9 cal K^{-1} mol^{-1}) and $Co(en)_3^{3+}$ (0.5 cal K^{-1} mol^{-1}) contrast sharply with the large negative values observed with the 2-Fe ferredoxin (Table VIII). The enthalpy $\Delta H_{et}^{\ddagger}$ terms are ca. 5 kcal mol^{-1} greater than for the 2-Fe ferredoxin. Electron transfer within a more compact assembly is indicated. Strong similarities probably exist between the tertiary structures of *P. aerogenes* and *C. pasteurianum* ferredoxins and it is likely that Fe_4S_4* clusters of both proteins lie close (< ca. 5 Å) to the surface at either end of the protein.[11] They are predominantly surrounded by hydrophobic aliphatic residues, but it is noteworthy that one of the cysteine residues of each cluster is exposed to solvent. Consequently electron transfer between an Fe_4S_4* cluster (3− charge) and substrate might occur with the cysteinyl S atom a lead-in group. Examination of the primary structure of *C. pasteurianum* ferredoxin reveals three aspartate residues in fairly close sequence at positions 33, 35, and 39 lying in the vicinity of cysteines 37, 40, and 43 which will form most of the coordination sphere of one Fe_4S_4* cluster. It is possible that this constitutes in part the negative binding site for $Co(NH_3)_6^{3+}$ and $Co(en)_3^{3+}$.

No significant dependence of rate constants on pH over the range 7.0–8.5 is observed for the oxidations of 8-Fe(rr) by Co-

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$(NH_3)_6^{3+}$, $Co(acac)_3$, and $Co(edta)^-$. A more detailed investigation for $Co(NH_3)_6^{3+}$ has also demonstrated that K and k_{et} show little or no variation with pH. Metalloproteins containing uncoordinated histidines are expected to exhibit acid dissociation pK_a values in this region of pH, and will affect the reactivity if situated close to the site at which electron transfer occurs. Proteins with no such histidines can also respond to pH changes in this region, however.[43] Interestingly no pH profiles have been detected with either the 2-Fe (parsley)[15] or 8-Fe (*C. pasteurianum*) proteins which have so far been investigated. No histidines are present in the 8-Fe protein. The amino acid composition of the parsley ferredoxin has not yet been reported, but spinach 2-Fe ferredoxin is known to contain a single histidine at position 90[44] which is conserved in at least five known 2-Fe amino acid sequences.

The trend of rate constants with ionic strength for the $Co(edta)^-$ oxidation of 8-Fe(rr) is in the direction expected for a reaction of two negatively charged reactants. Present understanding of ionic-strength dependences is such that we do not at this stage feel entitled to attempt to estimate the numerical value of the charge on the protein which is relevant to this reaction. The effect of ionic strength on K and k_{et} for the $Co(NH_3)_6^{3+}$ oxidation of the reduced 2-Fe protein has previously been reported,[15] when k_{et} was shown to be independent of an increase in I from 0.10 to 0.50 M.

Acknowledgment. We thank Dr. K. Rao for a sample of *C. pasteurianum* ferredoxin for preliminary studies in connection with this study. Discussions relating to statistical kinetic schemes with Dr. J. Chipperfield and Professor J. H. Espenson were particularly helpful. A Royal Society Research Grant for the purchase of an MSE Hi-spin 21 centrifuge is acknowledged, and also loan of equipment for the isolation of the metalloprotein from Dr. R. Herbert. Finally we are grateful to the Science Research Council (London) for postdoctoral (to R.A.H.) and postgraduate (to F.A.A.) awards. This work was carried out at the University of Leeds.

Supplementary Material Available: A listing of rate constants, Tables I, II, IV, and V (5 pages). Ordering information is given on any current masthead page.

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Communications to the Editor

Origin of Macrocyclic Enthalpy

Sir:

The extra stability imparted to complexes of ligands such as polyamines upon cyclization has been termed[1] the macrocyclic effect. The results of Paoletti et al.[2] indicate an equal contribution from enthalpy and entropy to the stabilization of the complex of cyclam (see Figure 1 for nature of ligands) relative to that of 2,3,2-tet with Ni(II). Busch et al.[3] explain the effect in terms of *multijuxtapositional fixedness*, which is a kinetic explanation, and not strictly applicable to thermodynamics. It is generally agreed that the entropy contribution arises from the smaller configurational entropy of the macrocycle, and it is the enthalpy contribution that requires explanation. Margerum et al.[4] suggested that it arose from steric hindrance to solvation of the nitrogen donor atoms, which are oriented in the "hole" in the center of the ligand. Paoletti et al.[2] and McDougall et al.[5] suggested that it arose because the ligand was 'preoriented' or 'prestrained', i.e., the unfavorable energy contribution arising from the increase in U, the conformational potential energy, of the ligand, normally accompanying complex formation, was smaller in the macrocycle because it was already in the conformation required for the complex. Metal ions experience a stronger ligand field (LF) from macrocycles than the linear analogues,[2,3] provided that the 'hole' is not too large for the metal ion. Busch et al.[3,6] explained this as constriction of the metal ion by the ligand.

We have reported[7-9] a series of empirical force-field (EFF) calculations on polyamine complexes of Ni(II), and report here calculations aimed at understanding the macrocyclic effect by using the same program, which is that determined by Boyd[10] as modified by Snow.[11] Since there are no low-spin Ni(II) complexes with simple unidentate nitrogen-donor ligands, for example, it is difficult to estimate an 'ideal' low-spin Ni–N bond length. Thus, U was scanned for each complex as a function of ideal, i.e., strain-free initial, M–N, and final adjusted M–N bond lengths from the EFF calculations. All parameters for the EFF were as used previously,[8] except for the varying ideal M–N length.

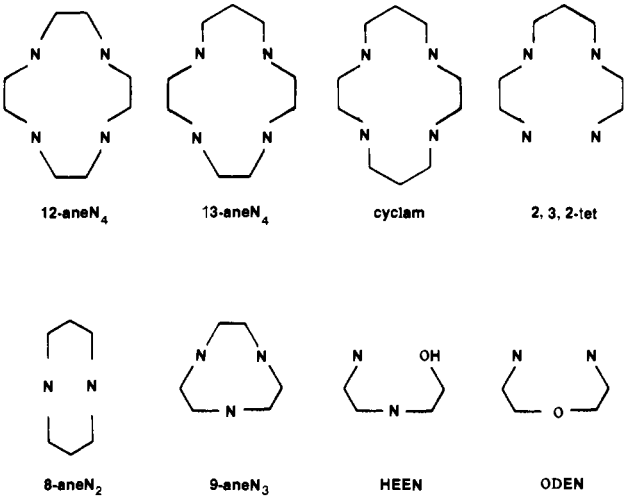

Figure 1. Ligands discussed in this paper.

Before discussing the EFF calculations, we will consider constrictive effects. 8-aneN$_2$ shows[12] in its complexes the properties associated with the macrocyclic effect, enhanced stability as

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