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References and Notes

(1) J. C. Cassatt, W. A. Johnson, L. M. Smith, and R. G. Wilkins, *J. Am. Chem. Soc.*, **94**, 8399 (1972).
(2) J. E. Letter, Jr., and R. B. Jordan, *J. Am. Chem. Soc.*, **97**, 2381 (1975).
(3) K. Kustin, R. F. Pasternack, and E. M. Weinstock, *J. Am. Chem. Soc.*, **88**, 4610 (1966).
(4) J. C. Cassatt and R. G. Wilkins, *J. Am. Chem. Soc.*, **90**, 6045 (1968).
(5) H. A. Flaschka, "EDTA Titrations", Pergamon Press, New York, N.Y., 1964, p 83.
(6) R. G. Wilkins, *Acc. Chem. Res.*, **3**, 408 (1970).
(7) K. Kustin and J. Swinehart, *Prog. Inorg. Chem.*, **13**, 107 (1970).
(8) F. P. Cavasino, E. DiDio, and G. Locanto, *J. Chem. Soc., Dalton Trans.*, 2419 (1973).
(9) R. Fuentes, Jr., L. O. Morgan, and N. A. Matwiyoff, *Inorg. Chem.*, **14**, 1837, 2774 (1975).
(10) M. L. Barr, K. Kustin, and S. T. Liu, *Inorg. Chem.*, **12**, 1486 (1973).
(11) R. K. Boggess and R. B. Martin, *J. Am. Chem. Soc.*, **97**, 3076 (1975).

Kinetic Studies of the Oxidation and Reduction of *Chromatium* High Potential Iron–Sulfur Protein (HiPIP) by Inorganic Complexes. Comparison of the Electron Transfer Reactivities of HiPIP and Horse Heart Cytochrome *c*

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Abstract: Kinetic measurements of the oxidation of reduced *Chromatium* high potential iron-sulfur protein (HiPIP) by $Fe(CN)_6^{3-}$ and $Co(phen)_3^{3+}$ have been made. The rate of reduction of oxidized HiPIP by $Fe(EDTA)^{2-}$ has also been studied. The second-order rate constants are $(2.0 \pm 0.1) \times 10^3$ M^{-1} s^{-1} (25°, μ 0.1 M, pH 7.0 (phosphate)) for HiPIP-$Fe(CN)_6^{3-}$, $(2.8 \pm 0.1) \times 10^3$ M^{-1} s^{-1} (26°, μ 0.1 M, pH 7.0 (phosphate)) for HiPIP-$Co(phen)_3^{3+}$, and $(1.6 \pm 0.1) \times 10^3$ M^{-1} s^{-1} (25°, μ 0.1 M, pH 7.0 (phosphate)) for oxidized HiPIP-$Fe(EDTA)^{2-}$. Activation parameters are: $\Delta H^{\ddagger} = -0.4 \pm 0.1$ kcal/mol, $\Delta S^{\ddagger} = -45 \pm 1$ eu (HiPIP-$Fe(CN)_6^{3-}$); $\Delta H^{\ddagger} = 14.9 \pm 0.7$ kcal/mol, $\Delta S^{\ddagger} = 7 \pm 2$ eu (HiPIP-$Co(phen)_3^{3+}$); $\Delta H^{\ddagger} = 0.8 \pm 0.3$ kcal/mol, $\Delta S^{\ddagger} = -41 \pm 1$ eu (HiPIP-$Fe(EDTA)^{2-}$). The differences between electron transfer kinetic parameters of HiPIP and horse heart cytochrome *c* have been analyzed in terms of relative Marcus theory. The analysis indicates that in both the cytochrome *c* self-exchange and the $Co(phen)_3^{3+}$ cross reaction, electron transfer takes place at the partially exposed heme edge of the protein. The cytochrome *c* k_{11} value based on the $Fe(EDTA)^{2-}$ cross reaction is somewhat smaller than the experimental self-exchange rate constant, which suggests that this redox agent has difficulty in approaching the partially exposed heme edge. The difference in self-exchange rate constants calculated for HiPIP from cross reactions with $Co(phen)_3^{3+}$ and $Fe(EDTA)^{2-}$ is much greater than that for cytochrome *c*. The k_{11} value for HiPIP obtained from the $Co(phen)_3^{3+}$ oxidation is 1×10^4 M^{-1} s^{-1}, whereas that for $Fe(EDTA)^{2-}$ reduction is 1×10^{-2} M^{-1} s^{-1}. It is proposed that HiPIP-$Co(phen)_3^{3+}$ and HiPIP-$Fe(EDTA)^{2-}$ employ different mechanisms of electron transfer. The low reactivity in the latter case suggests that $Fe(EDTA)^{2-}$ is not able to approach the buried [Fe_4S_4*] cluster in HiPIP very closely, and is thus forced to transfer an electron over a relatively large distance ($\geq$3.5 Å).

The kinetics and mechanisms of the electron transfer reactions involving iron-sulfur proteins and simple inorganic complexes have been little studied. In an important recent paper, Bennett and co-workers have reported[1] second-order rate constants and activation parameters for the outer sphere reduction of clostridial rubredoxin, a mononuclear iron-sulfur protein, by $Ru(NH_3)_6^{2+}$. The reduction of rubredoxin by $V(H_2O)_6^{2+}$ and $Cr(H_2O)_6^{2+}$ was also investigated.

We have initiated kinetic studies of the oxidation and reduction of polynuclear iron-sulfur proteins by inorganic complexes that normally employ outer sphere electron transfer pathways. This paper reports rate constants and activation parameters for the oxidation of reduced *Chromatium* high potential iron-sulfur protein (HiPIP) by ferricyanide and by tris(1,10-phenanthroline)cobalt(III), as well as for the reduction of oxidized HiPIP by $Fe(EDTA)^{2-}$. An analysis of the electron transfer reactivities of HiPIP and horse heart cytochrome *c* has been performed based on the relative Marcus theory.[2]

Experimental Section

Reagent grade chemicals were used without further purification. Distilled deionized water was used in making solutions. $K_3Fe(CN)_6$ solutions were prepared from weighed samples; concentrations were checked by absorbance measurements at 420 nm (ϵ 1.0×10^3).[3] $[Co(phen)_3]Cl_3 \cdot 7H_2O$ was prepared from $[Co(NH_3)_5Cl]Cl_2$ (Alfa Inorganics) and 1,10-phenanthroline (phen) by a standard method.[4] The isolated crystals were characterized by spectroscopic measurements in the region 380–220 nm. Concentrations of solutions used for kinetic studies were determined by absorbances at 350 (ϵ 3.7×10^3) and 330 nm (ϵ 4.7×10^3).[5] Solutions of $Fe(EDTA)^{2-}$ were prepared by standard procedures.[6]

HiPIP was extracted from cells of *Chromatium*, strain D (ATCC no. 17899), as described by Bose.[7] Cells were harvested by continuous centrifugation after 4 days of growth, and the protein was purified by variations on published methods.[8,9] The cells were first disrupted by freeze–thaw lysing, with 1% Triton-X added, then centrifuged for 1 h at 10 000 rpm, and cell fragments and mitochondrial particles, which were at the top, were removed. To the resulting yellowish solution, ammonium sulfate was added to 90%

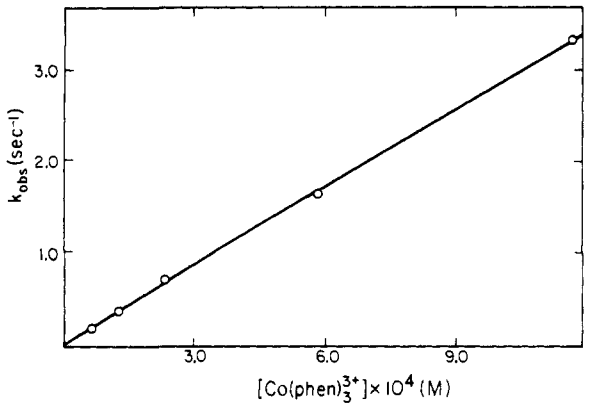

Figure 1. Dependence of k_{obsd} on [Co(phen)$_3^{3+}$] for the oxidation of HiPIP (26°, μ 0.1 M, pH 7.0 (phosphate), [HiPIP] = 7.0 × 10^{-6} M).

of saturation, precipitating most of the material. The precipitate was dissolved in 0.02 M Tris at 4° (pH 8), dialyzed extensively against the same buffer, then absorbed onto a column (2 × 10 cm, DEAE-cellulose Whatman type 52). This column was washed with 0.02 M Tris (pH 8, 40 mM NaCl) and a greenish band was collected, leaving most of the colored material on the column. The greenish fraction was diluted fourfold, absorbed onto a column of DEAE Sephadex A-25 equilibrated with 0.02 M Tris buffer (pH 8), and eluted (0.02 M Tris, pH 8, 0.05 M NaCl). Fractions with an absorbance ratio (272/388 nm) of 2.52 were collected for this work.

Complete reduction of HiPIP was assured by adding a few drops of mercaptoethanol. Solutions for the kinetic measurements were made by dialyzing the protein against the desired buffer. Oxidation of HiPIP was accomplished by passing a concentrated, low ionic strength (μ 0.001 M) solution of protein through a small Bio-Rad Ag 1X-8 column charged to 50% of capacity with Fe(CN)$_6^{3-}$.[8] Negligible amounts of Fe(CN)$_6^{3-}$ were eluted from the column if low ionic strength solutions were used. Enough NaCl was then added to raise the ionic strength to 0.1 M, and the solutions were diluted to the desired concentration with buffer; concentrations were established by spectroscopic measurements at 388 nm (ϵ 1.6 × 10^4)[10] for reduced HiPIP and at 450 nm (ϵ 1.9 × 10^4)[10] for oxidized HiPIP.

Nitrogen was carefully bubbled through all solutions for 45 min prior to kinetic measurements. Traces of glucose oxidase and glucose were added to solutions in certain control experiments to ensure completely anaerobic conditions; no significant differences in the kinetic results were found in these cases.

Most solutions were stored in nitrogen-purged, serum-capped bottles. They were then transferred from the serum bottles to the stopped-flow drive syringes by means of stainless steel needles and Teflon tubing with Hamilton fittings connected to the inlet port. The work with Co(phen)$_3^{3+}$ was done using an all-glass system. The Co(phen)$_3^{3+}$ solution was stored in a serum-capped, round-bottom flask fitted with a nitrogen inlet tube and a glass luer-lock fitting. Solutions were then transferred to the stopped-flow apparatus through Teflon tubing connected to the inlet port.

Kinetic Measurements. Kinetic measurements were made on a Durrum Model D-110 stopped-flow spectrophotometer. The Durrum uses a Kel-F flow system with a 2-cm path length, and has glass drive syringes with O-ring seals. Temperature was controlled with circulating water from a Forma Scientific temperature control unit.

Data Analysis. Absorbance changes as a function of time were displayed on a Tektronix 564 B oscilloscope. The earlier data were recorded by taking pictures of the oscilloscope trace; data points were then obtained by measuring these pictures. Plots of log ($A_t - A_\infty$) vs. t were made to verify first-order kinetic behavior; for each reaction the pseudo-first-order rate constant (k_{obsd}) was obtained from the slope of the line determined by a linear least-squares method. Later data were taken and analyzed by use of an analog input buffer in conjunction with a PDP-10 computer.

Results and Discussion

Absorbance changes show that reduced HiPIP transfers one electron to Fe(CN)$_6^{3-}$ in solution. Solutions of reduced HiPIP mixed with large excesses of Co(phen)$_3^{3+}$ also undergo spectral changes in accord with one-electron oxidation of the protein. These solutions turn pinkish after several hours, indicating that decomposition occurs over that period. However, the slow decomposition does not interfere with the observed electron transfer kinetics. For both oxidation by Co(phen)$_3^{3+}$ and reduction by Fe(EDTA)$^{2-}$, plots of k_{obsd} vs. the concentration of the small molecule reagent reveal a first-order dependence. Figure 1 shows such a plot for the Co(phen)$_3^{3+}$ reaction with reduced HiPIP, and all the kinetic data are set out in Table I. The k_{obsd} values for oxidation of HiPIP by Fe(CN)$_6^{3-}$ deviate slightly from a linear concentration dependence over the range studied (0.1–5.2 mM); however, the small increase in second-order rate constant with [Fe(CN)$_6^{3-}$] could easily be a medium effect, as the oxidant is responsible for a third of the ionic strength at the highest concentration.

Second-order rate constants obtained by least-squares analysis of the kinetic data for oxidation of reduced HiPIP by Fe(CN)$_6^{3-}$ and Co(phen)$_3^{3+}$ are (2.0 ± 0.1) × 10^3 M^{-1} s^{-1} (25°, μ 0.1 M, pH 7.0 (phosphate)) and (2.8 ± 0.1) ×

Table I. Observed Rate Constants for Redox Reactions of HiPIP, μ 0.1 M, pH 7.0 (phosphate)

A. Fe(CN)$_6^{3-}$ oxidation, [HiPIP] = 8.1 × 10^{-6} M, λ 480 nm

10^4 [Fe(CN)$_6^{3-}$] (M)	Temp, °C	k_{obsd} (s^{-1})
1.05	12.0	0.186, 0.185
1.05	17.5	0.187, 0.186
1.04	25.0	0.189, 0.187
1.05	25.0	0.189, 0.185
2.60	25.0	0.50, 0.49
5.20	25.0	1.04, 1.00
10.40	25.0	2.10, 2.10
26.0	25.0	5.70, 5.65
52.0	25.0	12.3, 12.3

B. Co(phen)$_3^{3+}$ oxidation, [HiPIP] = 7.0 × 10^{-6} M, λ 480 nm

10^4 [Co(phen)$_3^{3+}$] (M)	Temp, °C	k_{obsd} (s^{-1})
1.42	12.0	0.13, 0.12
1.42	18.0	0.21, 0.21
1.27	25.5	0.28,[a] 0.27[a]
1.27	25.5	0.32,[b] 0.32[b]
1.27	25.5	0.38,[c] 0.37[c]
1.27	25.5	0.45,[d] 0.46[d]
0.64	26.0	0.20, 0.18
1.27	26.0	0.38, 0.39
1.42	26.0	0.42, 0.40
2.34	26.0	0.73, 0.79
5.85	26.0	1.66, 1.54
11.70	26.0	3.35, 3.28
1.42	33.0	0.85, 0.81

C. Fe(EDTA)$^{2-}$ reduction, [HiPIP] = 8.1 × 10^{-6} M, λ 470 nm

10^4 [Fe(EDTA)$^{2-}$] (M)	Temp, °C	k_{obsd} (s^{-1})
5.0	12.0	0.74, 0.70
5.0	17.0	0.75, 0.76
5.0	24.0	0.79, 0.83
1.0	25.0	0.20, 0.22
1.5	25.0	0.39, 0.36
2.5	25.0	0.44, 0.43
5.0	25.0	0.83, 0.74
7.5	25.0	1.28, 1.21
10.0	25.0	1.71, 1.59
15.0	25.0	2.10, 2.05
20.0	25.0	3.39, 3.30
25.0	25.0	4.29, 4.21

[a] pH 5.2, [HiPIP] = 6.5 × 10^{-6} M. [b] pH 6.0, [HiPIP] = 6.5 × 10^{-6} M. [c] pH 7.0, [HiPIP] = 6.5 × 10^{-6} M. [d] pH 8.0, [HiPIP] = 6.5 × 10^{-6} M.

Table II. Analysis of Electron Transfer Reactions (25°) of HiPIP and Cytochrome *c*

Protein	Reagent	k_{12} (M^{-1} s^{-1})	pH	Buffer	μ(M)	Salt	E_{prot}	E_{rgt}	k_{22} (M^{-1} s^{-1})	k_{11} (M^{-1} s^{-1})[m]	
HiPIP	$Co(phen)_3^{3+}$	2.8 (10^3)[a]	7.0	Phos	0.1	NaCl	350 ± 20[e]	420 ± 20[g]	1.5–15 (10)[j]	1 (10^4)	[0.6–200 (10^3)]
	$Fe(CN)_6^{3-}$	2.0 (10^3)[a]	7.0	Phos	0.1	NaCl	350 ± 20[e]	425 ± 10[h]	5–50 (10^3)[k]	1 (10)	[0.1–20 (10)]
	$Fe(EDTA)^{2-}$	1.6 (10^3)[a]	7.0	Phos	0.1	NaCl	350 ± 20[e]	120 ± 20[i]	1–10 (10^4)[l]	1 (10^{-2})	[0.5–200 (10^{-3})]
Cyt *c*	$Co(phen)_3^{3+}$	1.5 (10^3)[b]	7.0	Phos	0.1	NaCl	260 ± 10[f]	420 ± 20[g]	1.5–15 (10)[j]	1 (10^2)	[0.8–100 (10)]
	$Fe(CN)_6^{3-}$	1.2 (10^7)[c]	7.2	Phos	0.1	NaCl	260 ± 10[f]	425 ± 10[h]	5–50 (10^3)[k]	2 (10^7)	[0.2–10 (10^7)]
	$Fe(EDTA)^{2-}$	2.6 (10^4)[d]	7.0	Phos	0.1	NaCl	260 ± 10[f]	120 ± 20[i]	1–10 (10^4)[l]	10	[0.8–100 (10)]

[a] This work. [b] J. V. McArdle, H. B. Gray, C. Creutz, and N. Sutin, *J. Am. Chem. Soc.*, **96**, 5737 (1974). [c] C. Creutz and N. Sutin, *J. Biol. Chem.*, **249**, 6788 (1974). [d] H. L. Hodges, R. A. Holwerda, and H. B. Gray, *J. Am. Chem. Soc.*, **96**, 3132 (1974). [e] K. Dus, H. de Klerk, K. Sletten, and R. G. Bartsch, *Biochim. Biophys. Acta*, **140**, 291 (1967). [f] R. Margalit and A. Shejter, *Eur. J. Biochem.*, **32**, 492 (1973). [g] E. Paglia and C. Sironi, *Gazz. Chim. Ital.*, **87**, 1125 (1957). [h] I. M. Kolthoff and W. J. Tomsicek, *J. Phys. Chem.*, **39**, 945 (1935). [i] G. Schwarzenbach and J. Heller, *Helv. Chim. Acta*, **34**, 576 (1951). [j] B. R. Baker, F. Basolo, and H. M. Neumann, *J. Phys. Chem.*, **63**, 371 (1959). [k] R. J. Campion, C. F. Deck, P. King, Jr., and A. C. Wahl, *Inorg. Chem.*, **6**, 672 (1967). [l] R. G. Wilkins and R. E. Yelin, *Inorg. Chem.*, **7**, 2667 (1968). [m] The values in brackets are the widest possible range for the given parameters; the quantity outside the brackets was calculated from the best value for each parameter. For cross reaction rate constants, the range used was ±10%.

10^3 M^{-1} s^{-1} (26°, μ 0.1 M, pH 7.0 (phosphate)), respectively. The rate constant given for HiPIP-$Fe(CN)_6^{3-}$ reaction was taken from kinetic results in the oxidant concentration range 0.1–1.0 mM. The pH dependence of the rate of the reaction with $Co(phen)_3^{3+}$ is small in the range 5.2–8. For the reduction of oxidized HiPIP by $Fe(EDTA)^{2-}$, the rate constant obtained is (1.6 ± 0.1) × 10^3 M^{-1} s^{-1} (25°, μ 0.1 M, pH 7.0 (phosphate)). Activation parameters obtained from least-squares analyses of Eyring plots are $\Delta H^{\ddagger}$ = −0.4 ± 0.1 kcal/mol, $\Delta S^{\ddagger}$ = −45 ± 1 eu, for oxidation by $Fe(CN)_6^{3-}$, $\Delta H^{\ddagger}$ = 14.9 ± 0.7 kcal/mol, $\Delta S^{\ddagger}$ = 7 ± 2 eu, for oxidation by $Co(phen)_3^{3+}$, and $\Delta H^{\ddagger}$ = 0.8 ± 0.3 kcal/mol, $\Delta S^{\ddagger}$ = −41 ± 1 eu, for the $Fe(EDTA)^{2-}$ reduction. The Eyring plot for the HiPIP-$Co(phen)_3^{3+}$ reaction is shown in Figure 2.

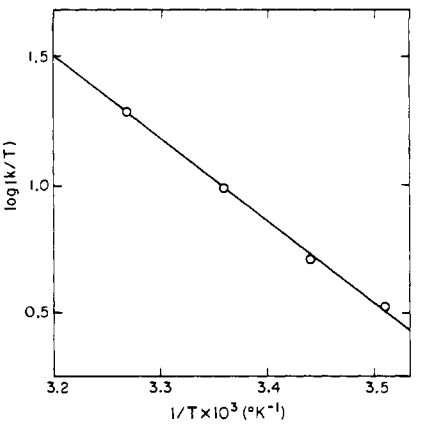

Figure 2. Eyring plot for the reaction of HiPIP and $Co(phen)_3^{3+}$ (μ 0.1 M, pH 7.0 (phosphate), [$Co(phen)_3^{3+}$] = 1.42 × 10^{-4} M, [HiPIP] = 6 × 10^{-6} M).

Several interesting comparisons can be made between the rate constants for electron transfer reactions involving HiPIP and analogous parameters of horse heart cytochrome *c*. The rate for the reduction of ferricytochrome *c* by $Fe(EDTA)^{2-}$ [6] is 2.6 × 10^4 M^{-1} s^{-1} (25°, μ 0.1 M, pH 7.0 (phosphate)), whereas k_{12} values for the oxidation of ferrocytochrome *c* by $Co(phen)_3^{3+}$ [11] and $Fe(CN)_6^{3-}$ [12] are 1.5 × 10^3 M^{-1} s^{-1} (25°, μ 0.1 M, pH 7.0 (phosphate)) and 1.2 × 10^7 M^{-1} s^{-1} (25°, μ 0.1 M, pH 7.2 (phosphate)), respectively. It is noteworthy that the rates for the same reagent with the two proteins are within an order of magnitude of each other, with the exception of the ferricyanide oxidations. The oxidation of ferrocytochrome *c* by $Fe(CN)_6^{3-}$ has been shown[13] to involve precursor complex formation, and is thus a special case, as no evidence for such strong redox ion-to-protein binding has been found for any of the other reactions under consideration.

One observation that may be noted is that the two negatively charged reactants exhibit larger rate constants with cytochrome *c* than with HiPIP. This could mean that the effective charge on the reaction center (or centers) of HiPIP is more negative than the +1.7 estimated[6] for the attack site in the reduction of ferricytochrome *c* by $Fe(EDTA)^{2-}$. It is certainly true that the probability of a negative reaction center is greater for HiPIP than for cytochrome *c*, as the pI values are 3.7[10] and 10,[14] respectively. However, it should be recognized that this question cannot be answered until the ionic strength dependences for the HiPIP reactions are known.

An analysis of the relative reactivities of HiPIP (E = 350 mV)[10] and rubredoxin (E = 57 mV)[15] must take into account the rather large differences in the potentials of these two proteins. Further, any more detailed comparisons of the kinetic parameters for HiPIP and cytochrome *c* (E = 260 mV)[16] are facilitated by compensation for the differences in driving force of the reactions under consideration. One approach is to use the equation of relative Marcus theory,[2] $k_{12} = (k_{11}k_{22}Kf)^{1/2}$, where k_{11} and k_{22} are the electron self-exchange rate constants for the two reactants and k_{12} and K are the rate and equilibrium constants, respectively, for the cross reaction. The coefficient f is assumed to be approximately equal to unity. By using known potentials and the k_{22} for the small molecule, an effective self-exchange rate constant (k_{11}) for the protein may be calculated. If the protein always uses the same activation mechanism in cross reactions, the calculated k_{11} should be a true constant; alternatively, if the electron transfer pathway is different for each small molecule oxidant or reductant, the calculated self-exchange rate could vary substantially.

The exchange rates and the parameters used in the calculation are set out in Table II. Some comments are in order concerning the estimates adopted for the thermodynamic and kinetic parameters. For cytochrome *c*, an extensive study has shown[16] that the potential increases with increasing NaCl concentration at very low ionic strengths, but returns to 260 mV in the region of our kinetic investigations. A range of potentials for $Fe(CN)_6^{3-/4-}$ was adopted because of the observed medium dependence.[17] Potentials for the other reactants have been determined, but their medium dependences are not known. Thus we have assigned rather broad error limits to these potentials.

The self-exchange rate for $Fe(CN)_6^{3-/4-}$ has been carefully studied and shown to be catalyzed strongly and specifically by cations.[18] We have assumed that Na^+ contributes to catalysis to the same extent as does K^+, and that the data

at low cation concentrations may be extrapolated to the conditions used in the present study. The latter assumption is supported to some extent by the agreement between the results at low K^+ concentrations, when extrapolated, and data obtained from NMR experiments that were necessarily done at quite high concentrations.[19] This agreement, of course, may be merely fortuitous. Because of the strong dependence on cations and the imprecision of the extrapolations, the rate for the $Fe(CN)_6^{3-/4-}$ self-exchange should be considered only an order-of-magnitude estimate. For the $Co(phen)_3^{3+/2+}$ self-exchange, the data are poor, as well as indicating a small dependence on anions,[20] and therefore the rate used in this case is also given as a large range. The k_{22} value for $Fe(EDTA)^{2-/-}$ was estimated from the rate of the reaction of $Fe(EDTA)^{2-}$ with the ferric *trans*-cyclohexane-1,2-diamine-*N,N,N',N'*-tetraacetate complex.[21]

The three calculated self-exchange rates for HiPIP span a very wide range (Table II). Taking any one of the k_{11} values, however, it is apparent that the electron transfer reactivity of HiPIP is low relative to rubredoxin. The self-exchange rate for rubredoxin has been estimated[1] to be greater than 10^8 M^{-1} s^{-1}, based on the observed kinetics of reduction of the protein by $Ru(NH_3)_6^{2+}$. There is no reason to believe electronic factors are responsible for this large difference in reactivity, as a relatively nonbonding e orbital of an approximately tetrahedral iron center is involved in electron transfer in both proteins.[1,22] Presumably, the single $[FeS_4]$ unit in rubredoxin is much more accessible sterically to outer sphere redox agents than is the $[Fe_4S_4S_4^*]$ cluster in HiPIP. X-Ray studies on HiPIP would appear to support such a proposal, as the $[Fe_4S_4S_4^*]$ cluster is relatively buried in the interior of the protein.[23] More specifically, examination of models of the HiPIP structure indicates that the closest distance from the protein surface to a cluster edge is about 3.5 Å.[24] In contrast, the $[FeS_4]$ unit in rubredoxin is at the surface of the protein.[25]

The calculated k_{11} for cytochrome *c* based on the $Co(phen)_3^{3+}$ reaction accords well with the experimental self-exchange rate of 300 M^{-1} s^{-1} (25°, μ 0.1 M, pH 7 (Tris), corrected from 40° with the E_a),[26] as noted previously.[11] It is likely, therefore, that electron transfer to $Co(phen)_3^{3+}$ occurs by the same mechanism as the protein self-exchange, and it has been proposed that this pathway involves contact at the partially exposed heme edge of cytochrome *c*.[11] The available evidence suggests that reduction of ferricytochrome *c* by $Fe(EDTA)^{2-}$ also takes place at the partially exposed heme edge.[6] The fair agreement between calculated k_{11} values for cytochrome *c* based on $Co(phen)_3^{3+}$ and $Fe(EDTA)^{2-}$ is somewhat misleading, however, as the latter cross reaction involves redox centers of opposite charge.[6] Correction for charge effects will reduce the estimated k_{11} based on $Fe(EDTA)^{2-}$ (possibly as much as a factor of 10^2),[27] and it is probable that approach to the partially exposed heme edge is somewhat more difficult for this small-molecule redox agent than is the case for $Co(phen)_3^{3+}$.

The calculated self-exchange rate constant for HiPIP from the $Co(phen)_3^{3+}$ oxidation is a factor of 10^6 greater than that calculated from the k_{12} for reduction by $Fe(EDTA)^{2-}$. This difference in electron transfer reactivities is even larger than that estimated for cytochrome *c* with the small molecules under consideration, and is probably related to the fact that the HiPIP redox center is not directly accessible in solution. Indeed, it is apparent that unless a special pathway is available that allows the redox center in HiPIP to come in contact with a reactant ion or molecule, electron transfer will necessarily have to take place through the insulating material between the $[Fe_4S_4S_4^*]$ cluster and the solvent. The anomalously low reactivity of $Fe(EDTA)^{2-}$ with oxidized HiPIP must mean, therefore, that this complex has particularly poor access to the cluster, and is forced to engage in electron transfer over an outer sphere to outer sphere distance of 3.5 Å or more.

The activation parameters observed are certainly not inconsistent with the above proposal that different electron transfer mechanisms are employed in reactions of HiPIP with $Co(phen)_3^{3+}$ and $Fe(EDTA)^{2-}$. First of all, the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values are strikingly different for the two reactions ($Fe(EDTA)^{2-}$: $\Delta H^{\ddagger}$, 0.8 kcal/mol; $\Delta S^{\ddagger}$, −41 eu. $Co(phen)_3^{3+}$: $\Delta H^{\ddagger}$, 15 kcal/mol; $\Delta S^{\ddagger}$, 7 eu). This fact in itself is not necessarily compelling, as it is not possible to analyze in any detail the differences in coulombic and other effects on the activation enthalpies and entropies of the reactions. Comparison of these activation parameters with those for the same reactants with cytochrome *c* ($Fe(EDTA)^{2-}$: $\Delta H^{\ddagger}$, 6 kcal/mol; $\Delta S^{\ddagger}$, −18 eu;[6] $Co(phen)_3^{3+}$: $\Delta H^{\ddagger}$, 11.3 kcal/mol; $\Delta S^{\ddagger}$, −6.2 eu[11]), however, is revealing. The $\Delta(\Delta H^{\ddagger})$ and $\Delta(\Delta S^{\ddagger})$ quantities between the two proteins go in different directions for $Fe(EDTA)^{2-}$ and $Co(phen)_3^{3+}$, and the changes are consistent with mechanisms in which $Co(phen)_3^{3+}$ is able to attack the buried cluster of HiPIP directly, whereas $Fe(EDTA)^{2-}$ cannot do so and must therefore transfer its electron from long range in a process involving minimal protein activation.

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References and Notes

(1) C. A. Jacks, L. E. Bennett, W. N. Raymond, and W. Lovenberg, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 1118 (1974).
(2) R. A. Marcus, *J. Phys. Chem.*, **67**, 853 (1963).
(3) C. K. Jorgensen, *Acta Chem. Scand.*, **10**, 518 (1956).
(4) P. Pfeiffer and B. Werdelman, *Z. Anorg. Allg. Chem.*, **263**, 36 (1950).
(5) T. J. Przystas and N. Sutin, *J. Am. Chem. Soc.*, **95**, 5545 (1973).
(6) H. L. Hodges, R. A. Holwerda, and H. B. Gray, *J. Am. Chem. Soc.*, **96**, 3132 (1974).
(7) S. K. Bose in "Bacterial Photosynthesis", H. Gest, A. San Pietro, and L. P. Vernon, Ed., Antioch Press, Yellow Springs, Ohio, 1963, p 315.
(8) R. G. Bartsch, ref 7, p 315.
(9) R. G. Bartsch, *Methods Enzymol.*, **23**, 644 (1971).
(10) K. Dus, H. de Klerk, K. Sletten, and R. G. Bartsch, *Biochim. Biophys. Acta*, **140**, 291 (1967).
(11) J. V. McArdle, H. B. Gray, C. Creutz, and N. Sutin, *J. Am. Chem. Soc.*, **96**, 5737 (1974).
(12) C. Creutz and N. Sutin, *J. Biol. Chem.*, **249**, 6788 (1974).
(13) (a) E. Stellwagen and R. G. Shulman, *J. Mol. Biol.*, **80**, 559 (1974); (b) E. Stellwagen and R. D. Cass, *J. Biol. Chem.*, **250**, 2095 (1975).
(14) M. D. Kamen, K. M. Dus, T. Flatmark, H. de Klerk in "Electron and Coupled Energy Transfer", Vol. 1, Part A, T. E. King and M. Klingenberg, Ed., Marcel Dekker, New York, N.Y., 1971, p 243.
(15) W. Lovenberg and B. E. Sobel, *Proc. Natl. Acad. Sci. U.S.A.*, **54**, 193 (1965).
(16) R. Margalit and A. Shejter, *Eur. J. Biochem.*, **32**, 492 (1973).
(17) I. M. Kolthoff and W. J. Tomsicek, *J. Phys. Chem.*, **39**, 945 (1935).
(18) R. J. Campion, C. F. Deck, P. King, and A. C. Wahl, *Inorg. Chem.*, **6**, 672 (1967).
(19) M. Shporer, G. Ron, A. Loewenstein, and G. Navon, *Inorg. Chem.*, **4**, 361 (1965).
(20) B. R. Baker, T. Basolo, and H. M. Neumann, *J. Phys. Chem.*, **63**, 371 (1959).
(21) R. G. Wilkins and R. E. Yelin, *Inorg. Chem.*, **7**, 2667 (1968).
(22) R. H. Holm, B. A. Averill, T. Herskovitz, R. B. Frankel, H. B. Gray, O. Siiman, and F. J. Grunthaner, *J. Am. Chem. Soc.*, **96**, 2644 (1974).
(23) (a) C. W. Carter, J. Kraut, S. T. Freer, N. Young, R. A. Alden, and R. G. Bartsch, *J. Biol. Chem.*, **249**, 4212 (1974); (b) C. W. Carter, J. Kraut, S. T. Freer, and R. A. Alden, *ibid.*, **249**, 6339 (1974).
(24) L. E. Bennett, personal communication.
(25) K. D. Watenpaugh, L. C. Sieker, J. R. Herriott, and L. H. Jensen, *Cold Springs Harbor Symp. Quant. Biol.*, **36**, 359 (1971).
(26) R. K. Gupta, *Biochim. Biophys. Acta*, **292**, 291 (1973).
(27) S. Wherland, unpublished calculations.