193 (100), 175 (18), 165 (20), 161 (16), 151 (23), 137 (52), 124 (38), 105 (53), 91 (44).

Anal. ($C_{13}H_{20}O_2$) C, H.

24, ($1R^*,2S^*,4S^*,6S^*,7R^*$)-2-methoxy-1,7-dimethyltricyclo-[$4.4.0.0^{2,9}$]decan-8-one: IR (film) 1710, 1450, 1150, 1014 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.04 (s, 3 H), 1.05 (d, J = 7.0 Hz, 3 H), 1.2-2.7 (m, 11 H), 3.26 (s, 3 H); mass spectrum (70 eV) m/e (rel abundance) 208 (18), 151 (100), 137 (32), 121 (26), 105 (26), 91 (33).

Anal. ($C_{13}H_{20}O_2$) C, H.

Acknowledgment. We thank the National Institutes of Health for their support of this work (Grant R01 AM 10749) and Mrs. Lorraine Guile for her assistance in obtaining mass spectra.

References and Notes

(1) W. Reusch, K. Grimm, J. E. Karoglan, J. Martin, K. P. Subrahamanian, P. S. Venkataramani, and J. D. Yordy, *J. Am. Chem. Soc.*, preceding paper in this issue.
(2) (a) Leading references to these compounds may be found in K. Nakanishi, T. G to, S. Ito, S. Natori, and S. Nozoe, "Natural Products Chemistry", Vol. 1, Academic Press, New York, N.Y., 1974; (b) Pinguisone was reported by V. Benesova, Z. Samek, V. Herout, and F. Sorm, *Collect. Czech. Chem. Commun.*, **34**, 582 (1969), and elucidated by A. Corbella, P. Garibaldi, G. Jommi, F. Orsini, A. DeMarco, and A. Immirzi, *J. Chem. Soc., Perkin Trans. 1*, 1875 (1974).
(3) As noted in our first paper of this series,[4] cyclopropanol **4** is contaminated with a small amount (ca. 10%) of the corresponding trans epimer of **14**. The yields given in eq 4 are corrected for this impurity.
(4) W. Reusch, K. Grimm, J. E. Karoglan, J. Martin, K. P. Subrahamanian, Y-C. Toong, P. S. Venkataramani, J. D. Yordy, and P. Zoutendam, *J. Am. Chem. Soc.*, accompanying paper in this issue.
(5) Obtained as a mixture of epimers.
(6) J. D. Yordy and W. Reusch, *J. Org. Chem.*, **40**, 2086 (1975).
(7) These have included GLC and TLC analysis of reaction mixtures as well as careful examination of the angular methyl signals in the ^1H NMR spectra of the reaction products. Miss J. Huang Shau has recently prepared the cis isomer **21** by an independent route, and it is clear that this compound would have been detected had it been present in greater than 1%.
(8) J. Haywood-Farmer, *Chem. Rev.*, **74**, 315 (1974).
(9) D. A. Lightner and W. A. Beavers, *J. Am. Chem. Soc.*, **93**, 2677 (1971).
(10) (a) P. E. Schueler and Y. E. Rhodes, *J. Org. Chem.*, **39**, 2063 (1974); (b) P. E. Schueler and Y. E. Rhodes, Abstracts, 164th National Meeting of the American Chemical Society, New York, N.Y., August 1972, No. ORGN-129.
(11) (a) P. K. Freeman and D. M. Balls, *Tetrahedron Lett.*, 437 (1967); (b) P. K. Freeman and J. N. Blazevich, *Chem. Commun.*, 1357 (1969); (c) P. K. Freeman, D. M. Balls, and J. N. Blazevich, *J. Am. Chem. Soc.*, **92**, 2051 (1970).
(12) V. F. Kucherov and I. A. Gurvich, *J. Gen. Chem. USSR*, **31**, 731 (1961).

Electron Transfer Reactivity of Spinach Ferredoxin

Jill Rawlings, Scot Wherland, and Harry B. Gray*

Contribution No. 5400 from the Arthur Amos Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, California 91125.
Received August 16, 1976

Abstract: The oxidation of reduced spinach ferredoxin by $Fe(EDTA)^-$, Fe(HEDTA), horse heart ferricytochrome c, and horse metmyoglobin has been investigated. Each reaction follows second-order kinetic behavior (rate = k_{12}[ferredoxin][oxidant]). Rate parameters are: $Fe(EDTA)^-$, $k_{12} = 2.9 \times 10^5\ M^{-1}\ s^{-1}$ (12.5 °C, μ = 0.1 M, pH 7.8), $\Delta H^{\ddagger}$ = 0.7 kcal/mol, $\Delta S^{\ddagger}$ = −31 cal/mol-deg; Fe(HEDTA), $k_{12} = 2.5 \times 10^4\ M^{-1}\ s^{-1}$ (26 °C, μ = 0.1 M, pH 7.8), $\Delta H^{\ddagger}$ = 0.3 kcal/mol, $\Delta S^{\ddagger}$ = −37 cal/mol-deg; ferricytochrome c, $k_{12} = 8.1 \times 10^4\ M^{-1}\ s^{-1}$ (25 °C, μ = 0.1 M, pH 7.0), $\Delta H^{\ddagger}$ = 9.6 kcal/mol, $\Delta S^{\ddagger}$ = −4 cal/mol-deg; metmyoglobin, $k_{12} = 2.3 \times 10^6\ M^{-1}\ s^{-1}$ (25 °C, μ = 0.1 M, pH 7.0), $\Delta H^{\ddagger}$ − 0.9 kcal/mol, $\Delta S^{\ddagger}$ = −32 cal/mol-deg. The electrostatics-corrected self-exchange rate constant calculated for ferredoxin based on $Fe(EDTA)^-$ is $1.7 \times 10^{-3}\ M^{-1}\ s^{-1}$, which indicates an extremely inaccessible protein redox center. Related Marcus-type analysis also suggests that electron transfer from ferredoxin to ferricytochrome c is particularly inefficient. The pH dependences of the ferredoxin–$Fe(EDTA)^-$ and ferredoxin–metmyoglobin reactions, and the ionic strength dependence of the ferredoxin–$Fe(EDTA)^-$ reaction, have been analyzed. At 25.8 °C and pH 7.8, the best fit to the Marcus ionic strength equation yields a charge of −9.4 on ferredoxin. Oxidation of ferredoxin by metmyoglobin fluoride has also been studied. The oxidation apparently is rate limited by fluoride dissociation (k = 0.4 s^{-1}; 25 °C, μ = 0.8 M, pH 7.7).

As part of our continuing study of the reactivity of electron transfer proteins with small molecule probe reagents and with each other, we have studied the reactions of reduced spinach ferredoxin with $Fe(EDTA)^-$, Fe(HEDTA), horse heart ferricytochrome c, and horse metmyoglobin. It is generally accepted that the oxidized form of spinach ferredoxin contains a $(Cys\text{-}S)_2Fe^{III}(S^{2-})_2Fe^{III}(Cys\text{-}S)_2$ redox center.[1,2] Further, electronic spectroscopic studies have indicated that the two Fe(III) sites are nonequivalent, with the more tetrahedral one being involved in the Fe(III)–Fe(II) redox shuttle.[3]

In previous papers[4,5] we have presented a model for interpreting protein electron transfer reactions within the framework of the Marcus outer sphere theory. In this model, the contributions to the kinetic activation energy due to the reagent, general electrostatic influences, and the thermodynamic driving force for the overall reaction, are factored out to leave a quantity that is characteristic of the protein and the particular mechanism by which it engages in electron transfer with the reagent. This quantity is the electrostatics-corrected self-exchange rate constant for the protein, or k_{11}^{corr}. Such a Marcus-type analysis of the available data on the reactions of cytochromes c, blue copper proteins, and HiPIP has led to the definition of a "kinetic accessibility" scale,[5] and to identification of the factors that control small molecule–protein and protein–protein reactivities. The data reported in this paper allow extension of this analysis to spinach ferredoxin.

Experimental Section

The protein was prepared by variations on published procedures.[6,7] Approximately 20 kg of fresh spinach leaves, 10 mL of 1 M Tris base, and 500 "mL" of ice were ground for 5 min in a Waring blender. All subsequent operations were carried out at 4 °C. The extract was squeezed through cheese cloth, and the ionic strength of the solution was made 0.15 M in NaCl. Approximately 500 mL of DEAE-cellulose (Whatman Type 52) was added to the 15 L of filtrate; this was stirred for 2 h, and then allowed to stand for 1.5 h. Most of the filtrate was siphoned off, and the DEAE cellulose was collected by filtration. This was washed with 2 L of 0.15 M NaCl, 0.01 M Tris (pH 7.5), and eluted with 0.15 M Tris (pH 7.5), 0.8 M NaCl. Ammonium sulfate was then added to 90% saturation and the solution was centrifuged

to remove a greenish precipitate. The resultant solution was dialyzed for 12 h against 10 L of 0.1 M Tris (pH 7.5), diluted twofold, and absorbed onto a small (2 × 10 cm) column of DEAE, which was eluted with 0.15 M NaCl, 0.15 M Tris (pH 7.5). The eluent was diluted threefold, absorbed onto a DEAE column (1 × 2 cm), and again eluted with 0.15 M Tris, 0.8 M NaCl. The concentrated solution was then applied to a Bio Gel P 60 column equilibrated with 0.15 M Tris (pH 7.5). The column was washed with 0.15 M Tris (pH 7.5) and 5-mL fractions were collected and analyzed on a Cary 14 spectrophotometer. The ratio of the absorbance at 422 to 275 nm was used as the criterion of purity. Fractions with ratios higher than 0.47 were used.

Horse heart cytochrome *c* (type III), obtained from Sigma, was used without further purification. Horse heart myglobin (type III) was obtained from Nutritional Biochemical Corp. and from Sigma. Solutions of myoglobin were centrifuged to remove an insoluble material, then oxidized with a twofold excess of $K_3Fe(CN)_6$, followed by extensive dialysis. Metmyoglobin fluoride solutions were prepared by dialyzing metmyoglobin against a potassium phosphate buffer solution (μ = 0.05 M), with 0.8 M NaF added. The concentrations of the solutions for kinetics work were determined by absorbances at 550 nm for cytochrome *c* (ϵ 9000),[8] 560 nm for mMb (ϵ 3700),[9] and at 610 nm (ϵ 8700) for mMbF.[10]

Reagent grade chemicals were used without further purification. Distilled deionized water was used in making solutions. Crystals of $Na(FeEDTA)\cdot 3H_2O$[11] and of $Fe(HEDTA)\cdot 1.5H_2O$[12] were prepared as described previously (EDTA = ethylenediaminetetraacetate and HEDTA = *N*-hydroxyethylethylenediaminetriacetate). Standard solutions for kinetics work were prepared from weighed samples and analyzed by iodometry.[13]

The pH of solutions was monitored by using a Brinkmann pH 101 meter with a Brinkmann glass combination electrode. All of these operations were carried out under a nitrogen atmosphere; the nitrogen was passed through two chromous towers before being bubbled gently through all buffer solutions. Hamilton air-tight syringes and standard syringe techniques were used.

Protein solutions for the kinetics measurements were prepared by dialyzing the protein against the desired buffer. The solutions were then diluted to the desired concentration with buffer; concentrations were established by absorbance measurements (ϵ 9400 at 420 nm for ferredoxin[1b]). Separate protein solutions were prepared for measurements at each different temperature. The protein was dialyzed against a Tris buffer chosen such that the pH would be 7.8 at the desired temperature. The ferredoxin solutions were reduced under a nitrogen atmosphere by using 0.1 mL of a standardized $Na_2S_2O_4$ solution. The dithionite solution was standardized by reaction with $K_3Fe(CN)_6$. The amount of $Na_2S_2O_4$ was adjusted such that there was a 20% excess of reducing agent. Typically, however, as judged by the absorbance change, only 80% of the ferredoxin was reduced. Presumably, some of the reduced ferredoxin reacted with trace amounts of oxygen present.

Nitrogen was carefully bubbled through all solutions for 45 min prior to measurement. 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.

Kinetics measurements were made on a Durrum Model D-110 stopped-flow spectrophotometer. 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 dependence; the pseudo-first-order rate constant was obtained from the slope of the line determined by a linear least-squares method. Later data were taken by use of an analogue input buffer in conjunction with a PDP-10 computer.

With several of the fast reactions, when only a small excess of the reagent could be used, the data were also calculated in terms of a second-order process. With second-order conditions, a slight distortion is obtained in the absorbance because of the finite flow deadtime of the system. Alternatively, as has been found by Corbett,[14] a reaction with only a twofold excess of one reagent can be treated as a pseudo-first-order reaction, with an error in the calculated k of less than 2% for 60% conversion. For the fastest reactions, this is well in line with experimental error; thus, most of these data were treated this way.

Table I. Summary of Kinetic Data for the Oxidation of Ferredoxin

Oxidant	k_{12}, $M^{-1} s^{-1}$	Reagent concentration range, mM	$\Delta H^{\ddagger}$, kcal/mol	$\Delta s^{\ddagger}$ cal/mol-deg
$Fe(EDTA)^-$ [a]	2.9×10^5	0.18–0.75	0.7 [b]	−31
Fe(HEDTA) [c]	2.5×10^4	0.20–2.0	0.3 [d]	−37
Ferricytochrome *c* [e]	8.1×10^4	$2.05–6.0 \times 10^{-2}$	9.6 [f]	−4
Metmyoglobin [g]	2.3×10^6	$1.85–5.6 \times 10^{-2}$	−0.9 [h]	−32

[a] μ = 0.1 M, Tris-Cl, pH 7.8, 12.5 °C, [ferredoxin] = 10^{-5} M, 465 nm. [b] Same as *a* and $[Fe(EDTA)^-]$ = 1.9×10^{-4} M; 6.0, 12.5, 19.0, 26.0, 32 °C. [c] μ = 0.1 M, Tris-Cl, pH 7.8, 26 °C, [ferredoxin] = 10^{-5} M, 465 nm. [d] Same as *c* and [Fe(HEDTA)] = 2×10^{-4} M; 12.0, 26.0, 32.0 °C. [e] μ = 0.1 M, phosphate, pH 7.0, 25 °C, [ferredoxin] = 2.5×10^{-6} M, 550 nm. [f] Same as *e* and [ferricytochrome *c*] = 2.05 × 10^{-5} M; 13.0, 18.0, 21.0, 25.0 °C. [g] μ = 0.1 M, phosphate, pH 7.0, 25 °C, [ferredoxin] = 5×10^{-6} M, 560 nm. [h] Same as *g* and [metmyoglobin] = 5.6×10^{-5} M; 12.5, 19.0, 25.0 °C.

Results

The kinetic data on the oxidation of reduced spinach ferredoxin by $Fe(EDTA)^-$, Fe(HEDTA), horse heart cytochrome *c*, and metmyoglobin are summarized in Table I; in all cases the rate law is first order in each reactant. The ferredoxin oxidations by Fe(HEDTA) and $Fe(EDTA)^-$ have similar rate constants, indicating that the extra available coordination site on the HEDTA complex does not facilitate the reaction, as might be expected were electron transfer to proceed by an inner sphere pathway. The pH dependences of the reactions with $Fe(EDTA)^-$ and metmyoglobin are plotted in Figures 1 and 2. The curves are the least-squares best fits to a single pK value. The metmyoglobin data conform to this analysis well, but the $Fe(EDTA)^-$ data are rather poorly fit, indicating that more than one pK is probably involved. The parameters of the curves are given in the figure legends. The ionic strength dependence of the $Fe(EDTA)^-$-ferredoxin reaction is plotted in Figure 3. The lines are the least-squares fits to three functions that have been used to describe ionic strength dependences.[4] Briefly, the transition state theory equation is the full form as given, for example, by Frost and Pearson;[15] the simplified transition state equation, $\ln k = \ln k_0 + 2Z_1Z_2\alpha\sqrt{\mu}$, is also plotted; the third equation is a result derived from the Marcus theory treatment, as presented previously.[4] The parameters of the curves are given in the figure legend. Both the transition state theory and the Marcus theory results are consistent with a large negative charge on ferredoxin, although not as large as predicted from the sequence (Table II).

The oxidation by the metmyoglobin fluoride complex is nearly independent of the heme protein concentration (varying from 0.38 to 0.45 s^{-1} in the metmyoglobin–fluoride concentration range $3.03–15.4 \times 10^{-5}$ M, μ = 0.8 M, Tris-Cl, NaF, pH 7.7, 25 °C, [ferredoxin0 5×10^{-6} M, 610 nm). The rate of formation of the fluoride complex of metmyoglobin was also studied and was found to be second order with a rate constant of 5.6 $M^{-1} s^{-1}$. The k_{obsd} data for all reactions are available.[16]

Discussion

The great variability in electron transfer reactivity that is exhibited by protein–$Fe(EDTA)^{-/2-}$ pairs has been attributed to the hydrophilic nature of the reagent, which does not allow it to penetrate into hydrophobic protein regions. Such penetration often is required in order to achieve good orbital overlap

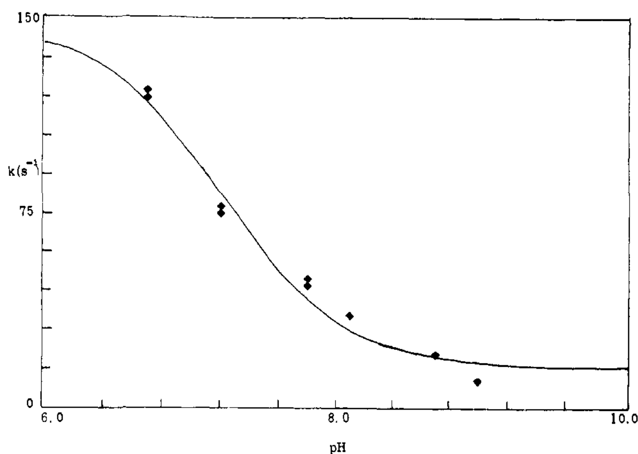

Figure 1. The pH dependence of the reaction of ferredoxin with $Fe(EDTA)^-$ (μ = 0.1 M, Tris-NaCl, 25.5 °C, $[Fe(EDTA)^-]$ = 1.9 × 10^{-4} M). The parameters of the curve are k_1 = 149 ± 11 s^{-1}, k_2 = 15 ± 3 s^{-1}, and the pK is 7.2 ± 0.1.

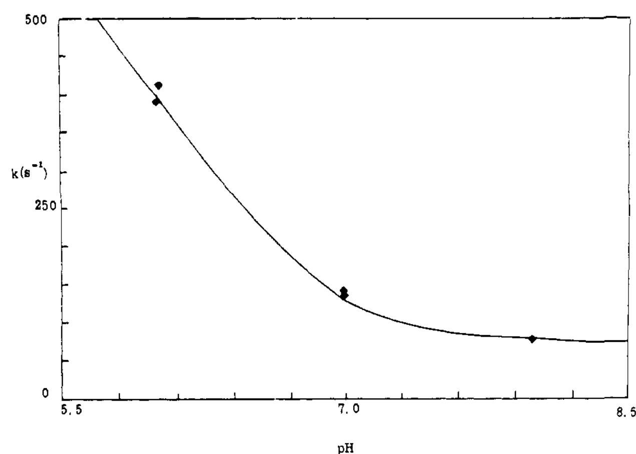

Figure 2. The pH dependence of the reaction of ferredoxin with metmyoglobin (μ = 0.1 M, phosphate, 25 °C, [mMb] = 5.6 × 10^{-5} M). The parameters of the curve are k_1 = 640 ± 60 s^{-1}, k_2 = 70 ± 6 s^{-1}, and pK = 6.1 ± 0.1.

Table II. Protein Properties

	Ferredoxin[a,b]	Horse myoglobin[c,d]
E, mV (25 °C)	−428	50
pH	7.0	6.8
Buffer	Phosphate	Phosphate
μ M	0.1–0.4	0.2
Mol wt, ×10^{-3}	10.482	16.951
r, Å	15.7	18.4
Lysines	4	19
Arginines	1	2
Histidines	1	9 (11)
Glutamates	9	13
Aspartates	11	7
Metal site	−2/−3	1/0
Other charges	0	−2
Total charge	−16.5/−17.5	4.5/3.5
pI		6.99

[a] H. Matsubara and R. M. Sasaki, *J. Biol. Chem.*, **243**, 1732 (1968). [b] N. A. Stombaugh, J. E. Sundquist, R. H. Burris, and W. H. Orme-Johnson, *Biochemistry*, **15**, 2633 (1976). [c] M. Dautrevaux, Y. Boulanger, K. Han, and G. Biserte, *Eur. J. Biochem.*, **11**, 267 (1969). [d] J. C. Cassatt, C. P. Marini, and J. W. Bender, *Biochemistry*, **14**, 5470 (1975).

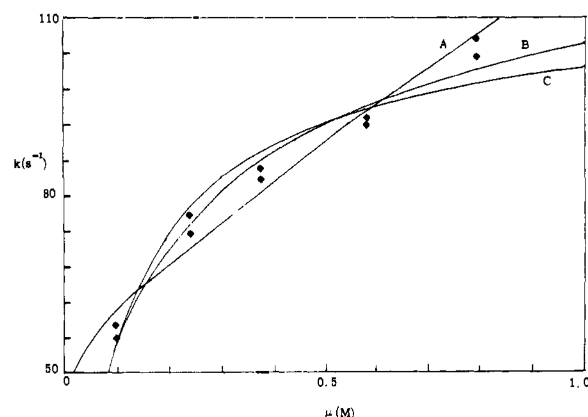

Figure 3. Ionic strength dependence of the $Fe(EDTA)^-$–ferredoxin reaction (25.8 °C, pH 7.8, $[Fe(EDTA)^{2-}]$ 2 × 10^{-4} M). Curve A represents the simplified transition state theory ($Z_{protein}$ = −0.4, $k(\mu = 0)$ = 44.5 s^{-1}. Curve B represents the full transition state theory ($Z_{protein}$ = −8.4, $k(\mu = 0)$ = 6.0 s^{-1}). Curve C is the best fit to the Marcus theory equation ($Z_{protein}$ = −9.4, $k(\mu = \infty)$ = 109 s^{-1}).

between reagent and protein redox centers.[4,5] Because reagent penetration cannot occur without great enthalpic cost, the k_{11}^{corr} value calculated from a $Fe(EDTA)^{-/2-}$ reaction is a good measure of the kinetic accessibility of a protein redox center.[5]

The k_{11}^{corr} $(Fe(EDTA)^-)$ has been calculated for spinach ferredoxin using the formulas and reagent parameters given previously[4] and the protein parameters set out in Table II. The calculated value of 1.7 × 10^{-3} M^{-1} s^{-1} (Table III) is the lowest yet encountered, making spinach ferredoxin the least kinetically accessible protein so far studied. For comparison, *Chromatium vinosum* HiPIP, whose $Fe_4S_4S_4$* center is relatively buried, gives a k_{11}^{corr} $(Fe(EDTA)^{2-})$ of 1.3 × 10^{-2} M^{-1} s^{-1};[5] horse heart cytochrome c, which features a partially exposed heme edge, yields a value of 6.2 M^{-1} s^{-1}.[4] The k_{11}^{corr} $(Fe(EDTA)^{2-})$ for the blue copper protein stellacyanin, which has a kinetically accessible redox center, is 2.3 × 10^5 M^{-1} s^{-1}.[5] A large contribution to the low k_{11}^{corr} for ferredoxin comes from the Coulombic term (w_{11}).

A pattern that has emerged from previous studies is that the less kinetically accessible a protein is to $Fe(EDTA)^{-/2-}$, the

Table III. Marcus Theory Calculations (μ = 0.1 M, 25 °C)

Reactant 1	Reactant 2	w_{12}[a]	w_{21}[a]	w_{11}[a]	w_{22}[a]	ΔG^{*corr} [a,b]	k^{corr}, M^{-1} s^{-1} [b]
Ferredoxin	$Fe(EDTA)^-$	0.731	1.378	2.888	0.493	21.21	1.7 × 10^{-3}
	Ferricytochrome c[c]	−1.197	−0.978	2.888	0.406	9.01	1.5 × 10^6
	Metmyoglobin	−0.601	−0.441	2.888	0.092	13.12	1.5 × 10^3
Metmyoglobin	$Fe(CyDTA)^{2-}$ [d]	−0.163	−0.063	0.092	0.493	19.48	3.2 × 10^{-2}

[a] In kcal/mol. [b] For the protein–small molecule reactions these values are for the protein self-exchange (ΔG_{11}^{*corr} or k_{11}^{corr}); for the protein–protein reactions these are the predicted cross reaction parameters (ΔG_{12}^{*corr} or k_{12}^{corr}). [c] Protein parameters are in ref 4. [d] From the cross reaction data in J. C. Cassatt, C. P. Marini, and J. W. Bender, *Biochemistry*, **14**, 5470 (1975); CyDTA is *trans*-1,2-diaminocyclohexanetetraacetate.

wider will be the variability of its reactivity when k_{11}^{corr} values from different reagents are compared. For example, whereas the k_{11}^{corr} ($Fe(EDTA)^{2-}$) for HiPIP is the low value already quoted, the k_{11}^{corr} ($Co(phen)_3^{3+}$) is 1.4×10^4 M^{-1} s^{-1}, demonstrating the availability to $Co(phen)_3^{3+}$ of a higher reactivity pathway than that employed by $Fe(EDTA)^{2-}$. Evidence for a similar range of reactivity comes from the data on the reactions of ferredoxin with ferricytochrome *c* and metmyoglobin. Protein–protein reactions are more difficult to deal with than protein–small molecule ones, but protein–protein cross reaction rate constants from k_{11}^{corr} ($Fe(EDTA)^{2-}$) are invariably calculated to fall lower than the observed quantities.[5] Put another way, protein–protein reactions previously studied have been found to be more efficient than would be predicted from $Fe(EDTA)^{2-}$ cross reactions. When the calculational method previously used[5] is extended to the ferredoxin–ferricytochrome *c* reaction using k_{11}^{corr} ($Fe(EDTA)^{-/2-}$) values for both proteins, the predicted cross reaction rate constant comes out 1.5×10^6 M^{-1} s^{-1}, which is much larger than the observed value of 8.1×10^4 M^{-1} s^{-1}. This is the first instance of such behavior, and indicates that the highly favorable Coulombic interactions between the oppositely charged proteins do not lead to close approach of the two redox centers, as might have been expected. When k_{11}^{corr} ($Fe(CyDTA)^{2-}$) is calculated for metmyoglobin, and this value is used in turn to calculate the metmyoglobin–ferredoxin cross reaction rate constant, the more typical behavior is observed; that is, the calculated value (1.5×10^3 M^{-1} s^{-1}) is much lower than the corresponding experimental quantity (2.3×10^6 M^{-1} s^{-1}). The Marcus theory calculations are summarized in Table III.

The activation parameters show a similar pattern of a near zero enthalpy and a large, unfavorable entropy in all but the ferredoxin–ferricytochrome *c* reaction. Such a pattern of low enthalpies and large, negative entropies of activation has also been observed in the $Fe(EDTA)^{2-}$ reduction and $Fe(CN)_6^{3-}$ oxidation (but not the $Co(phen)_3^{3+}$ oxidation) of HiPIP, and may be interpreted as indicating a pathway involving long range electron transfer.[17] The metmyoglobin reaction is the first protein–protein reaction to show this pattern of activation parameters. The difference in the activation parameters with ferricytochrome *c* again indicates that this reaction is significantly different from the others. Caution should be exercised in any activation parameter interpretations, however, as compensation behavior may be involved, and the contributions from the reagent exchange and the equilibrium constant of the reaction have not been evaluated.

The last point to be discussed concerns the reaction of the metmyoglobin–fluoride complex. Using a value for the equilibrium constant between metmyoglobin and fluoride of 15 M^{-1},[10] and the measured anation rate constant, a value for the dissociation constant of about 0.3 s^{-1} may be calculated, which is in quite close agreement with the measured oxidation rate constant. This agreement indicates that the rate limiting step in the reduction of metmyoglobin–fluoride by ferredoxin is dissociation of the bound fluoride.

Acknowledgment. This research was supported by the National Science Foundation. S.W. acknowledges an National Science Foundation Graduate Fellowship (1973–1976).

References and Notes

(1) (a) W. R. Dunham, G. Palmer, B. H. Sands, and A. J. Bearden, *Biochim. Biophys. Acta*, **253**, 373 (1971); (b) W. A. Eaton, G. Palmer J. A. Fee, T. Kimura, and W. Lovenberg, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 3015 (1971).
(2) W. O. Gillum, R. B. Frankel, S. Foner, and R. H. Holm, *Inorg. Chem.*, **15**, 1095 (1976).
(3) J. Rawlings, O. Siiman, and H. B. Gray, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 125 (1974).
(4) S. Wherland and H. B. Gray, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2950 (1976).
(5) S. Wherland and H. B. Gray in "Biological Aspects of Inorganic Chemistry", D. Dolphin, Ed., Wiley, New York, N.Y., in press.
(6) D. H. Petering and G. Palmer, *Arch. Biochem. Biophys.*, **141**, 456 (1970).
(7) S. Keresztes-Nagy and E. Margoliash, *J. Biol. Chem.*, **241**, 5955 (1966).
(8) E. Margoliash and N. Frohwirt, *Biochem. J.*, 71, 570 (1959).
(9) P. George, J. Beetlestone, and J. S. Griffith, *Rev. Mod. Phys.*, **36**, 441 (1964).
(10) H. Theorell and A. Ehrenberg, *Acta Chem. Scand.*, **5**, 823 (1951).
(11) H. J. Schugar, A. T. Hubbard, F. C. Anson, and H. B. Gray, *J. Am. Chem. Soc.*, **91**, 71 (1969).
(12) H. J. Schugar, G. R. Rossman, C. G. Barraclough, and H. B. Gray, *J. Am. Chem. Soc.*, **94**, 2683 (1972).
(13) E. H. Swift, *J. Am. Chem. Soc.*, **51**, 1682 (1929).
(14) J. F. Corbett, *J. Chem. Educ.*, **49**, 663 (1972).
(15) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism", 2nd ed, Wiley, New York, N.Y., 1961, p 150.
(16) J. Rawlings, Ph.D. Thesis, California Institute of Technology, 1975.
(17) J. Rawlings, S. Wherland, and H. B. Gray, *J. Am. Chem. Soc.*, **98**, 2177 (1976).