The most plausible electron acceptor from rusticyanin is a cytochrome c_{552} with a reduction potential of 0.64 V[7]. There is a report[36] that this cytochrome can be isolated, bound to rusticyanin, and that the copper protein is preferentially reduced by iron(II). Using data published by Imai and co-workers at pH 3.5[36] and assuming the reaction to be first order in iron(II) and cytochrome c_{552}(III)–rusticyanin(II) complex concentrations, a second-order rate constant around 16 M^{-1} s^{-1} for reduction of the rusticyanin component can be calculated. This is not significantly different from the value found in the present study and suggests that the presence of bound cytochrome c_{552}(III) has little effect on the reactivity of rusticyanin.

(c) Kinetics of Reduction by Chromium(II). Reduction of RCu^{II} by chromium(II) in sulfate media is much faster than the corresponding reaction with iron(II) and exhibits no limiting kinetic behavior. The second-order rate constant, $(2.5 \pm 0.5) \times 10^4$ M^{-1} s^{-1}, shows little sensitivity to small amounts ($<10^{-3}$ M) of chloride ion, and it may be that the electron transfer from this powerful reductant is less specific than with iron(II).

There are data available[12,13] for the reductions of other small blue copper proteins, azurins, plastocyanins, and stellacyanin by chromium(II) at pH 4.2. As with rusticyanin the reactions are first order in [Cr(II)] and the second-order rate constants are comparable in magnitude, varying between 10^4 and 10^5 M^{-1} s^{-1}. There does not appear to be any close correlation with reduction potentials.

In chromium(II) reductions of a number of electron-transfer proteins including the blue copper plastocyanins[37] and azurins,[38] some of the chromium(III) product has been shown to remain bound to the protein, labeling and blocking key reaction sites.[33] It is of some interest then to discover whether RCu^{II}, reduced in sulfate media by chromium(II) and reoxidized by $IrCl_6^{2-}$, can be reduced by iron(II) or whether chromium(III) is bound to the protein at the iron(II) reaction site.

Reaction of iron(II) with the chromium(II)-treated protein in sulfate media leads to no significant decrease in rate compared with the untreated protein. Indeed, the rates are slightly enhanced, and the rate law (eq 1) is followed with $a = 12 \pm 1$ M^{-1} s^{-1} and $b = 100 \pm 20$ M^{-1}. These experiments provide further evidence for a rate-limiting protein conformational change in the reduction of RCu^{II} by iron(II). It would appear that the conformational change is marginally facilitated by the binding of chromium(III).

(d) Conclusions. The blue copper protein, rusticyanin, has a reduction potential of 0.67 V (vs. NHE) independent of pH in the range 1–3. The reduction of the oxidized form of the protein by iron(II) is limited by a conformational change in sulfate media, but this limiting rate is absent in chloride media. Reduction of the protein by chromium(II) takes place at a rate comparable to the reductions of other small blue copper proteins by this reagent. Binding of chromium(III) to the protein has little effect on the rate of the conformational change.

Acknowledgment. This research is supported by an Atlantic Richfield Foundation Grant of the Research Corp. and by the U.K. SERC (Grant No. GR/C/09241) for which the authors express sincere thanks.

(36) Sugio, T.; Tano, T.; Imai, K. *Agric. Biol. Chem.* **1981**, *45*, 405–412.
(37) Farver, O.; Shahak, Y.; Pecht, I. *Biochemistry* **1982**, *21*, 1885–1890.
(38) Farver, O.; Blatt, Y.; Pecht, I. *Biochemistry* **1982**, *21*, 3556–3561.

Contribution from the Department of Chemistry,
University of Wisconsin, Eau Claire, Wisconsin 54701

Kinetic Study of the Oxidation of Spinach Plastocyanin by Ferrocenium Ion Derivatives

J. R. PLADZIEWICZ,* M. S. BRENNER, D. A. RODEBERG, and M. D. LIKAR

Received May 15, 1984

The oxidation of reduced spinach plastocyanin by four ferrocenium ion derivatives has been studied at pH 7.0 (0.020 M phos) and $\mu = 0.12$ (NaCl, sodium phosphate) at 25 °C. All four reactions are observed to obey a simple second-order rate law that is first order with respect to the concentration of protein and ferrocenium derivative. No rate saturation at high ferrocenium ion concentrations is observed for any of the reactions studied. The second-order rate constants for the four protein oxidations at 25 °C are 0.20 (±0.02) × 10^6, 1.02 (±0.04) × 10^6, 2.10 (±0.12) × 10^6, and 9.4 (±1.0) × 10^6 M^{-1} s^{-1} for 1,1′-dimethylferrocenium, ferrocenium, chloromercuriferrocenium, and phenylferrocenium, respectively. The protein oxidations have been studied as a function of temperature, and the enthalpies and entropies of activation are 6.7 ± 0.4, 5.5 ± 0.3, 5.0 ± 0.4, and 6.2 ± 0.5 kcal/mol and −11.7 ± 1.2, −12.6 ± 0.8, −13.1 ± 1.5, and −5.9 ± 1.7 cal/(mol K), respectively, for 1,1′-dimethylferrocenium, ferrocenium, chloromercuriferrocenium, and phenylferrocenium. Possible mechanisms for electron transfer are discussed, and the observed second-order rate constants are used to derive apparent protein-exchange rate constants by the Marcus equation.

We are interested in the mechanisms by which metalloproteins undergo electron transfer. An important aspect of this is identifying mechanistic features that diverse metalloproteins share in their electron-transfer reactions. Gray and co-workers[1-5] have shown that small octahedral coordination compounds with hydrophobic liands and ligands that allow delocalization of electron density in the complex through π bonding are more facile at electron transfer with metalloproteins than are small compounds without these features. We reasoned from these observations and related facts that ferrocene and its derivatives might be facile one-electron titrants of metalloproteins. Previous work[6,7] from our laboratory has shown this to be true for electron-transfer reactions of horse heart cytochrome *c*. This report describes our studies on the electron-transfer reactions of plastocyanin from spinach chloroplasts with four ferrocenium ion derivatives. Plastocyanin is a protein of molecular weight 10 500 containing one copper atom per molecule. It cycles between the copper I/II oxidation states and serves an electron-transport function in the photosynthetic pathways in a variety of algae and plants. Its electron-transfer reactions, both with other proteins and with inorganic reagents, have been extensively studied.[1,8] The X-ray crystal structure of plastocyanin from *Populus nigra* is known,[9]

(1) Wherland, S.; Gray, H. B. In "Biological Aspects of Inorganic Chemistry"; Addison, A. W., Cullen, W. R., Dolphin, D., James, B. R., Eds.: Wiley: New York, 1977; p 289.
(2) Mauk, A. G.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1982**, *104*, 7654.
(3) Holwerda, R. A.; Knaff, D. B.; Gray, H. B.; Clemmer, J. D.; Crowley, R.; Smith, J. M.; Mauk, A. G. *J. Am. Chem. Soc.* **1980**, *102*, 1142.
(4) Mauk, A. G.; Coyle, C. L.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1979**, *101*, 5054.
(5) Cummins, D.; Gray, H. B. *J. Am. Chem. Soc.* **1977**, *99*, 5158.
(6) Pladziewicz, J. R.; Carney, M. J. *J. Am. Chem. Soc.* **1982**, *104*, 3544.
(7) Carney, M. J.; Lesniak, J. S.; Likar, M. D.; Pladziewicz, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 2565.
(8) Lappin, A. G. In "Metal Ions in Biological Systems-13"; Sigel, H., Ed.; Marcel Decker: New York, 1981; p 15.
(9) Colman, P. M.; Freeman, H. C.; Guss, J. M.; Murata, M.; Norris, V. A.; Ramshaw, J. A. M.; Venkatappa, M. P. *Nature (London)* **1978**, *272*, 319.

0020-1669/85/1324-1450$01.50/0

Table I. Experimental and Calculated Rate Constants for Ferrocenium Ion Derivative–Plastocyanin Reactions

ferrocenium deriv	E°,[a] V	$10^{-6}k_{22}$,[b] M^{-1} s^{-1}	ΔE°,[c] V	$10^{-6}k_{12}$(obsd),[d] M^{-1} s^{-1}	$10^{-6}k_{12}^{\infty}$,[e] M^{-1} s^{-1}	$10^{-4}k_{11}^{\infty}$(calcd),[f] M^{-1} s^{-1}
1,1′-dimethyl	0.30	8.3 ± 0.8	−0.07	0.20 ± 0.02	0.10	4.5
ferrocenium	0.38	5.7 ± 0.1	0.01	1.02 ± 0.04	0.51	6.7
chloromercuri	0.39	5.3 ± 0.5	0.02	2.10 ± 0.12	1.05	21
phenyl	0.44	18.0 ± 2	0.07	9.4 ± 1.0	4.7	18

[a] E° values for the ferrocenium derivatives were obtained from ref 16 for 1-propanol–water and corrected downward by 0.13 V for application in water solutions as suggested in ref 23. [b] Ferrocenium ion derivative self-exchange rate constants were obtained from ref 15 and 16. [c] ΔE° values were obtained with 0.370 V for the plastocyanin potential[8] and the potentials listed above for the ferrocenium derivatives. [d] All measurements were made at pH 7.0 (0.020 M phos), $\mu = 0.12$ (NaCl, sodium phosphate), 25 °C. [e] Second-order cross-reaction rate constants corrected to infinite ionic strength from eq 4 in ref 22, a charge of 10– for PCu^{I}, a charge of 1+ for the ferrocenium ions, and a radius of 15.8 Å for PCu^{I} and 3.2 Å for the ferrocenium derivatives. [f] Calculated plastocyanin self-exchange rate constant at infinite ionic strength from eq 3 and k_{22} and k_{12}^{∞} given above and ΔE^{∞} obtained from the equilibrium constant derived from the rate constants corrected to infinite ionic strength.

and the copper atom is known to be in a highly distorted tetrahedral environment that is not accessible to solvent. The copper is ligated by two sulfur atoms (Cys-84 and Met-92) and two nitrogen atoms (His-37 and -87). Electron transfer between this copper center and some small inorganic complexes is believed to occur at a hydrophobic region on the protein's surface near His-87.[2,10]

While the application of the Marcus theory[11] to many electron-transfer reactions of small coordination complexes has been very successful, its direct application to reactions of small complexes with metalloproteins has been more difficult.[1,8] It has been shown[6,7] that the reactions of ferrocene and its derivatives with cytochrome *c* do correlate well with the Marcus theory, and we are interested in knowing whether this correlation extends to metalloproteins with different metal centers, coordination numbers and geometries, ligating atoms, and metal-to-protein surface distance. The identification of a series of complexes that behave predictably with diverse metalloproteins would be useful in estimating the self-exchange rate constants of metalloproteins for which this information is not available by other means and for manipulating the redox states of metalloproteins in a specific, rapid, and gentle manner.

A characteristic of many metalloprotein electron-transfer reactions is the association of redox partners prior to electron transer.[1,2,8,12] This has been a prominent feature of many reactions of the plastocyanins.[8,12b] Saturation kinetics have been observed for several reactions of plastocyanin including the oxidation of reduced plastocyanin, PCu^{I}, by cobalt complexes and the reduction of oxidized plastocyanin, PCu^{II}, by $Fe(CN)_6^{4-}$.[12c] If association prior to electron transfer occurs for the ferrocenium–metalloprotein reactions studied thus far, such association must be weak since rate saturation has not been observed in our earlier studies,[6,7] nor in this study. This feature makes the ferrocenium derivatives attractive as more general metalloprotein titrants since the studies might not be complicated by strong binding of titrant to protein.

The known binding of $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ to proteins, for example, has raised some questions[12b,c] regarding metalloprotein redox potentials obtained with this popular redox couple. We believe the ferrocenium derivatives and ferrocene-derivatized electrodes[13] may be useful for future studies of this type.

Experimental Section

Laboratory-distilled water was further purified by reverse osmosis (Sybron/Barnstead Nanopure). All chemicals were reagent grade unless otherwise noted. Argon gas, passed through two chromous scrubbing towers to remove traces of molecular oxygen, was used for preparing anaerobic solutions.

Ferrocene (Alfa Inorganics) was purified by sublimation; 1,1′-dimethylferrocene (Alfa Inorganics) was recrystallized from ethanol; chloromercuriferrocene (Research Organic Chemical) was recrystallized from acetone; and phenylferrocene was prepared by the method of Weinmayer.[14] The ferrocenes were converted to the respective ferrocenium hexafluorophosphate salts by the method of Yang et al.[15] or by reacting the ferrocene with excess iron(III).[7] In preparing solutions for kinetic runs the ferrocenium salts were dissolved in the appropriate buffer, filtered, and analyzed for ferrocenium ion concentration by their visible spectra[16a] [ferrocenium, 618 nm (ϵ = 450 M^{-1} cm^{-1}); 1,1′-dimethylferrocenium, 650 nm (ϵ = 332 M^{-1} cm^{-1}); chloromercuriferrocenium, 623 nm (ϵ = 504 M^{-1} cm^{-1}); phenylferrocenium, 750 nm (ϵ = 521 M^{-1} cm^{-1})] and deaerated by bubbling with argon for 30 min. In some instances when the ferrocenium salts were dissolved in buffer, a small amount of light solid formed, causing slight turbidity in the solution. This was found to have no effect on kinetic results as long as the ferrocenium ion solutions were filtered and analyzed immediately prior to kinetic measurements and when only freshly prepared ferrocenium solutions were used. Phenylferrocenium is particularly difficult to work with, and its solutions undergo significant decomposition within 15 min of preparation. It is somewhat more stable in dilute acid and can be prepared therein and brought to the proper pH by mixing with buffer in the stopped-flow apparatus at the time of reaction.

Plastocyanin was prepared from fresh spinach leaves by a modification of the method of Yocum et al.[17] Washed chloroplasts, prepared by this method, were suspended in 0.01 M Tris buffer, pH 8.0 (300 mL per kg of spinach leaves), and four drops of Triton X-100 detergent were added. The suspension was blended for 10 s in a Warning blender. In some preparations no detergent was used and the chloroplast suspension was sonicated for ca. 5 min instead of blending. The homogenized chloroplasts were treated with 2 mg of DNAase and 2 mg of RNAase, and after the mixture was allowed to stand for ca. 30 min, 1.2 mL of 1. M $MgCl_2$ was added. The suspension was centrifuged at 40000*g* for 45 min to remove chloroplast fragments. The supernate contained the plastocyanin that was further purified by ion exchange and gel chromatography by the method of Yocum.[17] The plastocyanin used in kinetic experiments was purifed to an absorbance (*A*) ratio of A_{278}/A_{597} of 1.7 or less for the oxidized protein. Concentrations of protein were determined on the basis of the spectrum[18] (ϵ_{597} = 4.9 × 10^3 M^{-1} cm^{-1}) of the oxidized protein. Protein solutions frozen in reaction buffer suspended in liquid nitrogen were stable for more than 1 month. Protein from different preparations gave indistinguishable kinetic results. PCu^{I} was prepared by reduction of PCu^{II} with sodium ascorbate or sodium dithionite followed by extensive dialysis against reaction buffer.

Kinetic measurements were made at 597 nm with a Durrum Model D-110 stopped-flow spectrophotometer interfaced to a Nicolet Model 1090 digital oscilloscope and an Apple II computer. Typically four traces from one drive syringe loading were treated per experiment. Each trace consisted of ca. 500–1000 digitized voltages and times. These digitized data were treated by a nonlinear least-squares program to fit for the observed pseudo-first-order rate constant. The initial and final absor-

(10) Freeman, H. C. *Coord. Chem.* **1981**, *21*, 29.
(11) (a) Marcus, R. A.; Sutin, N. *Inorg. Chem.* **1975**, *14*, 213. (b) Marcus, R. A. *J. Phys. Chem.* **1963**, *67*, 853; **1965**, *43*, 679.
(12) (a) Sisley, M. J.; Segal, M. G.; Stanley, C. S.; Adzamli, I. K.; Sykes, A. G. *J. Am. Chem. Soc.* **1983**, *105*, 225. (b) Lappin, A. G.; Segal, M. G.; Weatherburn, D. C.; Sykes, A. G. *Ibid.* **1979**, *101*, 2297. (c) Segal, M. G.; Sykes, A. G. *Ibid.* **1978**, *100*, 4585.
(13) Chau, S.; Robbins, J. L.; Wrighton, M. S. *J. Am. Chem. Soc.* **1983**, *105*, 181. Wrighton, M. S. "Abstracts of Papers", 184th National Meeting of the American Chemical Society, Kansas City, MO, Sept 1982; American Chemical Society: Washington, DC, 1982; CHED 55. Cass, A. E. G.; Davis, G.; Francis, G. D.; Hill, H. A. O.; Aston, W. J.; Higgins, I. J.; Plotkin, E. V.; Scott, L. D. L.; Turner, A. P. R. *Anal. Chem.* **1984**, *56*, 667.
(14) Weinmayer, V. *J. Chem. Soc.* **1955**, *77*, 3012.
(15) Yang, E. S.; Chan, M. S.; Wahl, A. C. *J. Phys. Chem.* **1975**, *79*, 2049; *Ibid.* **1980**, *84*, 3094.
(16) (a) Pladziewicz, J. R. Ph.D. Thesis, Iowa State University, Ames, IA, 1971. (b) Pladziewicz, J. R.; Espenson, J. H. *J. Am. Chem. Soc.* **1973**, *95*, 56; *J. Phys. Chem.* **1971**, *75*, 3381.
(17) Yocum, C. F.; Nelson, N.; Racker, E. *Prep. Biochem.* **1975**, *5*, 305.
(18) Katoh, S.; Shiratori, I.; Takamiya, S. *J. Biochem.* **1962**, *51*, 32.

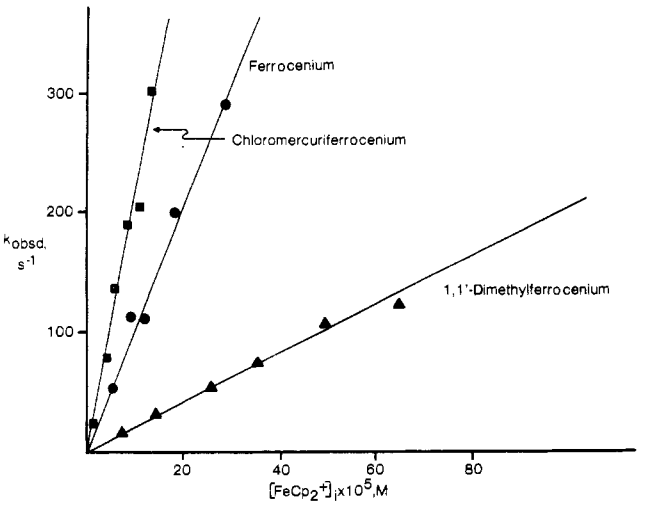

Figure 1. Dependence of the observed pseudo-first-order rate constant, k_{obsd}, on initial ferrocenium ion derivative concentration at 25 °C, pH 7.0 (0.020 M phos), μ = 0.12 (NaCl, sodium phosphate).

bances were fixed in these analyses, and a weighting factor that assumed a constant absolute error in the absorbance measurement was used.

The reaction of 1,1′-dimethylferrocenium with reduced plastocyanin did not always go to completion, and for those runs proceeding to less than 95%, k_{obsd} was obtained from a rigorous, nonlinear least-squares treatment of kinetic data, assuming opposing first- and second-order reactions[19a] using the equation derived by King.[19b] King's equation has the advantage of not being limited to any particular values for initial reactant and product concentrations. The use of this equation requires knowledge of the equilibrium constant that was obtained in these studies by spectrophotometric titration of the reduced protein with 1,1′-dimethylferrocenium at λ = 597 nm. The value obtained at 25 °C, pH 7.0, and μ = 0.12 is 0.035 ± 0.002. This is in agreement with the value based on redox potentials (Table I) given a 0.02-V uncertainty for the potentials listed there.

Results

The oxidation of reduced plastocyanin, PCu^I, by ferrocenium, chloromercuriferrocenium, 1,1′-dimethylferrocenium, and phenylferrocenium was observed spectrophotometrically at 597 nm. At this wavelength one observes an increase in absorbance due to the oxidation of PCu^I to PCu^{II} ($\Delta\epsilon = 4.90 \times 10^3$ M^{-1} cm^{-1}).[18] The ferrocenium ion derivative reduction makes minor contributions to the absorbance change at this wavelength. All four derivatives were found to oxidize PCu^I at pH 7.0 (0.020 M phosphate), μ = 0.12 M (sodium phosphate/NaCl), with a second-order rate law (eq 1).

$$d[PCu^{II}]/dt = k[PCu^I][\text{ferrocenium deriv}] \quad (1)$$

Since all experiments were done with the ferrocenium derivative concentration in 10-fold or greater excess of PCu^I concentration, the first-order dependence of the reaction on PCu^I concentration is established by the linearity (greater than 3 half-lives for all experiments) of the plots of the logarithm of PCu^I concentration vs. time. The first-order dependence of the reaction on the ferrocenium derivative concentration is established by the linearity of the plots of the pseudo-first-order rate constants for protein oxidation, k_{obsd}, vs. initial ferrocenium derivative concentrations, as illustrated in Figure 1. The lines drawn are for a least-squares analysis of the data, assuming zero intercept. Inclusion of an intercept in the fit does not change the fitted slope significantly. Reactions of phenylferrocenium were too rapid to allow a large enough range of phenylferrocenium concentration to be studied to justify plotting in Figure 1. However, for the range of phenylferrocenium concentrations studied ((3.2–5.95) × 10^{-5} M), the reaction was found to be first order with respect to the phenylferrocenium concentration, and as expected, better data were

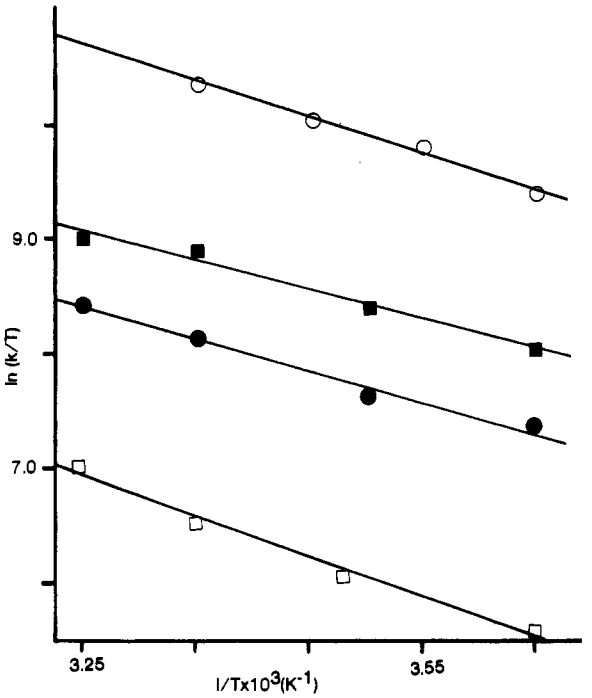

Figure 2. Eyring plots for second-order rate constants for the oxidation of reduced plastocyanin with (○) phenylferrocenium, (■) chloromercuriferrocenium, (●) ferrocenium, and (□) 1,1′-dimethylferrocenium at pH 7.0 (0.020 M phos.), μ = 0.12 (NaCl, sodium phosphate).

obtained at lower temperatures. Second-order rate constants for electron transfer, obtained from the data comprising the plots in Figure 1 and the data for the phenylferrocenium reaction using a least-squares analysis, are given in Table I. The cited uncertainties are 2 standard deviations.

The oxidations of PCu^I were studied as a function of temperature, and the Eyring plots for these reactions are given in Figure 2. The activation enthalpies and entropies obtained from least-squares analysis of these plots for the reactions of phenylferrocenium, chloromercuriferrocenium, ferrocenium, and 1,1′-dimethylferrocenium, respectively, are 6.2 ± 0.5, 5.0 ± 0.4, 5.5 ± 0.3, and 6.7 ± 0.4 kcal/mol and −5.9 ± 1.7, −13.1 ± 1.5, −12.6 ± 0.8, and −11.7 ± 1.2 cal/(mol K).

To fit data for the 1,1′-dimethylferrocenium–PCu^I reaction at the other temperatures, it was necessary to obtain the temperature dependence of the equilibrium constant for this reaction. Spectrophotometric titration of PCu^I with 1,1′-dimethylferrocenium at pH 7.0, μ = 0.12 (NaCl, sodium phosphate), gave values of 0.026 ± 0.005, 0.035 ± 0.002, and 0.050 ± 0.010 at 2.8, 25.0, and 38.2 °C, respectively, and from these a value of $\Delta H°$ of 3.0 ± 0.6 kcal/mol is obtained.

For the 1,1′-dimethylferrocenium–PCu^I reaction at pH 7.0, a few experiments were also done at μ = 0.041 (sodium phosphate) and at μ = 0.25 (NaCl, 0.020 M sodium phosphate). Second-order rate constants for protein oxidation at these ionic strengths are 0.30 (±0.04) × 10^6 and 0.17 (±0.02) × 10^6 M^{-1} s^{-1}, respectively, at 25 °C as compared to 0.20 (±0.02) × 10^6 M^{-1} s^{-1} for μ = 0.12 at this temperature. On the basis of a net charge of 10− for the protein[8] and a charge of 1+ for 1,1′-dimethylferrocenium, the direction of these effects is not surprising.

Discussion

The most direct application of the Marcus theory[11] to outer-sphere electron-transfer reactions is to compare the rate constant calculated from (2) with experimental electron-transfer rate

$$k_{12} = (k_{11}k_{22}K_{12}f)^{1/2}$$
$$\ln f = (\ln K_{12})^2/(4 \ln (k_{11}k_{22}/Z^2)) \quad (2)$$

constants. The use of eq 2 in this manner requires knowledge of the self-exchange rate constants for the protein (k_{11}) and complex (k_{22}) and the reaction's equilibrium constant (K_{12}). Z is the

(19) (a) Adzamli, I. K.; Davies, D. M.; Carmel, S. S.; Sykes, A. G. *J. Am. Chem. Soc.* **1981**, *103*, 5543. (b) King, E. L. *Int. J. Chem. Kinet.* **1982**, *14*, 1285.

collision frequency, in these calculations taken to be 10^{11} M^{-1} s^{-1}. While the k_{22} values for the ferrocenium derivatives[15,16] and equilibrium constants are known, the self-exchange rate constant for plastocyanin is not directly known. NMR studies[20] provide an upper limit estimate of 2×10^4 M^{-1} s^{-1} for k_{11} for plastocyanin. Alternatively, values derived from reactions of other small complexes with plastocyanin or derived from reaction of plastocyanin with other proteins[21] can be used. However, k_{11} values derived in this manner for plastocyanin are found to vary greatly.[1-5,8,21]

An alternate approach is to derive an *apparent* protein self-exchange rate constant, k_{11}, for these reactions from the experimental rate constants by rearranging eq 2 to give eq 3.[6] To

$$\ln k_{11} = (\ln k_{12} - \tfrac{1}{2} \ln K_{12} + \ln Z) - \ln k_{22} - [(\ln Z - \ln k_{12})^2 + \ln K_{12}(\ln Z - \ln k_{12})]^{1/2} \quad (3)$$

account in some degree for the electrostatic effects, the rate constants and equilibrium constants used in eq 3 are first corrected to infinite ionic strength with the Debye–Hückel theory as described by Mauk, Scott, and Gray.[22] The *apparent* protein self-exchange rate constant corrected to infinite ionic strength, k^{∞}(calcd), is then calculated. These values, given in Table I, vary by less than a factor of 5 for the four derivatives studied, have an average value of ca. 1.3×10^5 M^{-1} s^{-1}, and are among the highest such values obtained for plastocyanin. For example, from the reactions of $Fe(EDTA)^{2-}$, $Ru(NH_3)_5py^{3+}$, and $Co(phen)_3^{3+}$ with plastocyanin, values of 7.3×10, 4.9×10^4, and 1.1×10^4 M^{-1} s^{-1} are calculated by Mauk et al.[22] for the *apparent* self-exchange rate constant for plastocyanin at infinite ionic strength. Such high and relatively constant derived protein self-exchange values as those obtained from the ferrocenium cross reactions suggest a similar mechanism for the four derivatives used here and also suggest that the ferroceniums are able to make a close approach to the copper center in PCu^I.[22]

It is interesting to note that no large substituent specific effects are found for the four ferrocenium ions studied and that differences in reactivity can be largely accounted for by differences in the reduction potentials and self-exchange rate constants of the ferrocenium ions. Although for both this study and our previous work[6,7] with cytochrome *c*, chloromercuriferrocenium seems to have a somewhat higher reactivity and 1,1′-dimethylferrocenium a somewhat lower reactivity when compared to the other derivatives. At this time the significance, if any, of these differences is not clear. The absence of large substituent effects suggests a mechanism that is free from requirements for substituent group–protein association or ordering prior to electron transfer. These results support a direct bimolecular collision between the ferrocenium derivatives and plastocyanin, with the hydrophobic region near His-87 being the most obvious collision site on the protein. The relatively high derived *apparent* protein self-exchange values imply a very close approach of ferrocenium iron to the copper in the protein. Similar results were found for the reaction of seven ferrocenium derivatives with cytochrome *c*.[6,7]

The activation enthalpies and entropies for the ferrocenium ion–plastocyanin reactions are similar, with $\Delta H^{\ddagger}$ of 5–6 kcal/mol and $\Delta S^{\ddagger}$ of −6 to −13 cal/(mol K) for each reaction. The activation enthalpies and entropies for these reactions, the ferrocenium ion–cytochrome *c* reactions, and some related ferrocenium–ferrocene reactions are given in Table II. The enthalpies of reaction

Table II. Activation Parameters for Reactions of Ferrocenium Ion Derivatives with Plastocyanin, Cytochrome *c*, and Ferrocene Derivatives

ferrocenium deriv	reductant	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, cal/(mol K)
ferrocenium	plastocyanin[a]	5.5 ± 0.3	−12.6 ± 0.8
	cytochrome *c*[b]	5.0 ± 0.1	−10.6 ± 0.3
	ferrocene[d]	5.6 ± 0.6	−8.9 ± 2.0
	1,1′-dimethylferrocene[c]	3.0 ± 1.0	−15 ± 3
1,1′-dimethyl	plastocyanin[a]	6.7 ± 0.4	−11.7 ± 1.2
	1,1′-dimethylferrocene	5.5 ± 0.2	−8.2 ± 0.7
chloromercuri	plastocyanin[a]	5.0 ± 0.4	−13.1 ± 1.5
	cytochrome *c*[b]	4.3 ± 0.2	−11.6 ± 0.8
n-butyl	cytochrome *c*[b]	5.9 ± 0.5	−9.1 ± 1.7
phenyl	plastocyanin[a]	6.2 ± 0.5	−5.9 ± 1.7
	cytochrome *c*[b]	5.5 ± 0.1	−5.7 ± 0.3
	ferrocene[c]	3.0 ± 0.6	−14.4 ± 2.1

[a] This work. [b] Reference 7. [c] Reference 16b. [d] Recalculated from data in ref 15.

of the plastocyanin reactions do appear to decrease slightly, as is expected from the Marcus theory, as the overall driving force for the reaction increases with the exception of the phenylferrocenium reaction where the increased rate is primarily reflected in the slightly more positive $\Delta S^{\ddagger}$. This modest $\Delta S^{\ddagger}$ effect for phenylferrocenium was also observed in the reactions of ferroceniums with cytochrome *c*.[7]

The similarities of the activation parameters for the ferrocene–ferrocenium ion self-exchange reactions, the ferrocenium–plastocyanin reactions, and the ferrocenium–cytochrome *c* reactions (Table II) is striking. However, a meaningful comparison of the activation parameters for the reactions of ferrocenium with cytochrome *c* and with plastocyanin requires knowledge about the differences, if any, in the thermodynamics of the net reactions involved. Ignoring work terms, the influence of reaction enthalpy on activation enthalpy, for example, is given approximately by eq 4,[11a] where $\Delta H_{12}^{\ddagger}$ is the enthalpy of activation, $\Delta H_{11}^{\ddagger}$ and

$$\Delta H_{12}^{\ddagger} = (\Delta H_{11}^{\ddagger} + \Delta H_{22}^{\ddagger})/2 + \Delta H_{12}^{\circ}/2 \quad (4)$$

$\Delta H_{22}^{\ddagger}$ are the self-exchange activation enthalpies, and ΔH_{12}° is the enthalpy of reaction. ΔH_{12}° for the oxidation of PCu^I by 1,1′-dimethylferrocenium of 3.0 ± 0.6 kcal/mol is available from the temperature dependence of the equilibrium constant determined in this work. From this value and data in Table II one can estimate the apparent $\Delta H_{11}^{\ddagger}$ for this reaction. For the ferrocytochrome *c* reactions with ferroceniums, data on the temperature dependence of the protein potential is available;[24] unfortunately, this information is not available for the ferrocenium ions in water. However, given the large positive ΔH° for cytochrome *c* oxidation and the small observed temperature dependence of the ferrocenium potentials in 1-propanol–water,[16a] it is likely that the contribution from ΔH_{12}° to the $\Delta H_{12}^{\ddagger}$ could be substantially different for the cytochrome *c* reactions than for the plastocyanin reactions.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. We are also grateful to Professor J. B. Howard and G. L. Anderson for advice on the isolation and purification of plastocyanin.

Registry No. 1,1′-Dimethylferrocenium, 12276-63-0; ferrocenium, 12125-80-3; chloromercuriferrocenium, 34742-71-7; phenylferrocenium, 32839-60-4.

(20) Beattie, J. K.; Fensom, D J.; Freeman, H. C.; Woodcock, E.; Hill, H. A. O.; Stokes, A. N. *Biochim. Biophys. Acta* **1975**, *405*, 109.
(21) Wherland, S.; Pecht, I. *Biochemistry* **1978**, *17*, 2585.
(22) Mauk, A. G.; Scott, R. A.; Gray, H. B. *J. Am. Chem. Soc.* **1980**, *102*, 4360.
(23) Carlson, B. W.; Miller, L. L.; Neta, P.; Grodkowski, J. *J. Am. Chem. Soc.* **1984**, *106*, 7233.
(24) Taniguchi, V. T.; Ellis, W. R.; Cammarata, V.; Webb, J.; Anson, F. C.; Gray, H. B. "Electrochemical and Spectrochemical Studies of Biological Redox Components"; Kadish, K. M., Ed.; American Chemical Society, Washington, DC, 1982; Adv. Chem. Ser. No. 201, pp 51–68.