Ferrocene Derivatives as Metalloprotein Redox Probes: Electron-Transfer Reactions of Ferrocene and Ferricenium Ion Derivatives with Cytochrome *c*

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Abstract: The oxidation of ferrocytochrome *c* by six ferricenium ion derivatives has been studied at pH 6.5 (phos) and μ = 0.50 at 25 °C. In addition, the reduction of ferricytochrome *c* by decamethylferrocene at pH 6.5 (0.05 M HEPES), μ = 0.10 (NaCl/HEPES), in 65/35 vol % water/acetonitrile at 25 °C has been studied. The rates of these reactions are found to be first order in protein and in ferrocene or ferricenium ion derivative concentration. The second-order rate constants for the six protein oxidations at 25 °C are 0.93 (± 0.01) × 10^6 M^{-1} s^{-1}, 3.01 (± 0.03) × 10^6 M^{-1} s^{-1}, 6.41 (± 0.11) × 10^6 M s^{-1}, 13.4 (± 0.4) × 10^6 M^{-1} s^{-1}, 14.6 (± 0.3) × 10^6 M^{-1} s^{-1}, and 31.0 (± 1) × 10^6 M^{-1} s^{-1} for 1,1′-dimethylferricenium, *n*-butylferricenium, ferricenium, chloromercuriferricenium, hydroxymethylferricenium, and phenylferricenium, respectively. The second-order rate constant for the protein reduction by decamethylferrocene is 7.8 (± 0.2) × 10^6 M^{-1} s^{-1}. Four of the protein oxidations have been studied as a function of temperature and the enthalpies and entropies of activation for these reactions are 5.0 ± 0.1, 4.3 ± 0.2, 5.9 ± 0.5, and 5.5 ± 0.1 kcal/mol and −10.6 ± 0.3, −11.6 ± 0.8, −9.1 ± 1.7, and −5.7 ± 0.3 cal/(mol K) for ferricenium, chloromercuriferricenium, *n*-butylferricenium, and phenylferricenium, respectively. These results are discussed in terms of current models for protein electron-transfer reactions and the Marcus theory for outer-sphere electron transfer.

Previous studies on the electron-transfer reactions between cytochrome *c* and small inorganic redox reagents suggest that electron transfer occurs at a location near the partly exposed heme edge at cytochrome *c*[1–6] and that inorganic reagents that can effectively access this site transfer electrons more rapidly to or from the protein than do reagents that are excluded from close approach to this site. In particular several small complexes (e.g., $Co(phen)_3^{3+}$) with hydrophobic ligands and metal-to-ligand π-bonding have been found to be especially facile at such electron transfer, while complexes (e.g., $Ru(NH_3)_6^{2+}$) with hydrophilic ligands and metal–ligand bonding that does not promote delocalization of metal electrons onto the ligands are generally much less facile at metalloprotein electron transfer.[5–11] We report herein experiments on a series of ferrocene and ferricenium ion derivatives all of which have hydrophobic ligands and which accomplish delocalization of metal electrons through π-bonding to the aromatic cyclopentadienide ligand. Our results with these complexes support the ideas of Gray and co-workers[5–10] regarding the importance of these features in metalloprotein–small-complex redox reactions.

Another feature of interest in metalloprotein redox reaction is their possible conformity to theory,[2,5–11] in particular to the Marcus theory[12] for outer-sphere electron transfer. An important feature of this theory is the predicted dependence of the cross-reaction rate constant on thermodynamic driving force.[13] Most studies to date have involved modest driving forces typically 0.1–0.25 V or less. A notable recent exception is the work of English et al.[13] on the reaction of the luminescent excited state of $Ru(bpy)_3^{2+}$ ($E°$ of *Ru(II)/Ru(I) = 0.830 V) with reduced blue copper proteins. In addition, Yandell[14] and co-workers have shown a number of reactions of copper complexes with cytochrome *c* to correlate well with thermodynamic driving force over a wide range (ca. 0.5 V) of potentials, although direct comparison with Marcus theory is not possible here since the self-exchange electron-transfer rates of the copper complexes are not independently known. The series of reactions that we report on here are found to conform to the Marcus theory over a significant range of thermodynamic driving force ($\Delta E°'$ = −0.25–+0.31 V) and represent the first extensive series of closely related complexes to behave so predictably over such a thermodynamic range.

The reactions described here also demonstrate the utility of ferrocene and its derivatives as metalloprotein redox titrants. While these complexes have been used on derivatized electrodes,[15] in micelles,[16] and in solution[11,17] for metalloprotein reactions, they have not seen extensive use as protein titrants. Several features that make these complexes well-suited for metalloprotein redox study include their low charge (+1/0), high intrinsic electron-transfer reactivity (self-exchange rate constants of ca. 10^6–10^8 M^{-1} s^{-1}),[18,19] inertness to substitution in both oxidation states, and wide range of redox potentials accessible through derivatization.[20] The low water solubility of the ferrocenes does limit their use to

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Table I. Experimental and Calculated Rate Constants for Ferricenium Ion Derivative-Cytochrome *c* Reactions

ferricenium derivative	$E^{\circ\prime}$,[a] V	$k_{22} \times 10^{-6}$,[a] $M^{-1}\ s^{-1}$	$\Delta E^{\circ\prime}$,[b] V	$k_{12}(\text{obsd}) \times 10^{-6}$,[c] $M^{-1}\ s^{-1}$	$k_{12}(\text{calcd}) \times 10^{-6}$,[d] $M^{-1}\ s^{-1}$	$k_{11}^{\infty}(\text{calcd}) \times 10^{-3}$, $M^{-1}\ s^{-1}$
decamethyl	0.01[b]	38 ± 4	−0.25	0.00047[e]	0.00096–0.0029	0.51
1,1′-dimethyl	0.43	8.3 ± 0.8	0.17	0.93 ± 0.01	2.0–6.3	0.26
n-butyl	0.50	6.5 ± 0.7	0.24	3.01 ± 0.03	5.8–18	0.34
ferricenium	0.51	5.7 ± 0.1	0.25	6.41 ± 0.11	6.4–20	1.3
chloromercuri	0.52	5.3 ± 0.5	0.26	13.4 ± 0.4	7.3–22	4.6
hydroxymethyl	0.52	4.2 ± 0.4	0.26	14.6 ± 0.3	6.5–20	7.0
phenyl	0.57	18.0 ± 2	0.31	31.0 ± 1	28–85	1.5

[a] Ferricenium ion derivative self-exchange rate constants and $E^{\circ\prime}$ values were obtained from ref 18 and 19. [b] $\Delta E^{\circ\prime}$ values were obtained by using 0.260 V for the potential for cytochrome *c*, ref 29, an estimate of 0.01 V for decamethylferricenium based on the observation that each methyl group shifts the potential by ca. 0.05 V for various ferrocene derivatives and based on the differences found for ferrocene and decamethylferrocene potentials in Hepp and Wrighton and Robbins and Smart (Hepp, A. F.; Wrighton, M. S. *J. Am. Chem. Soc.* 1981, *103*, 1238; Robbins, J. L.; Smart, J. C. *Ibid.* 1982, *104*, 1882) and the potentials for ferrocene and its derivatives from ref 19. [c] Except for the decamethylferrocene reaction, all values were measured in aqueous solution, pH 6.5, $\mu = 0.50$ (phos), 25 °C. [d] The ranges in $k_{12(\text{calcd})}$ reflect the use of a value of $1 \times 10^3\ M^{-1}\ s^{-1}$ and $1 \times 10^4\ M^{-1}\ s^{-1}$ for the self-exchange rate constant for cytochrome *c*, respectively, ref 28. [e] The value of k_{12} for decamethylferricenium is derived from the value measured for the oxidation of decamethylferrocene by ferricytochrome *c* in 35/65 (v/v) acetonitrile/water, pH 6.5 (0.05 M HEPES), $\mu = 0.10$ (NaCl/HEPES), and the equilibrium constant for the reaction. No correction for differences in solvent or ionic strength is made for this value since the overall uncertainties are greater than the correction.

very dilute metalloprotein solutions, however.

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, *n*-butylferrocene (Alfa Inorganics) was used as received, chloromercuriferrocene (Research Organic Chemical) was recrystallized from acetone, hydroxymethylferrocene (Fairfield Chemical) was purified by recrystallization from petroleum ether or by sublimation, phenylferrocene was prepared by the method of Weinmayer,[21] and decamethylferrocene (Strem Chemical) was purified by sublimation. The ferrocenes were converted to the respective ferricenium hexafluorophosphate salts by the method of Yang et al.[18] or by reacting the ferrocene with excess iron(III). This was done by dissolving the ferrocene in petroleum ether and preparing an acidic, aqueous solution of ferric nitrate containing a twofold stoichiometric excess of iron(III) and shaking these two solutions in a separatory funnel until the yellow color of the ferrocene was no longer evident in the organic layer. The aqueous layer, containing the ferricenium ion, was then treated with a saturated aqueous solution of sodium hexafluorophosphate. The precipitated ferricenium salt, collected by filtration, was then washed with cold dilute aqueous acid, water, and finally diethyl ether and dried under vacuum. The ferricenium salts can be further purified by recrystallization from water. Ferricenium salts prepared in this way could be weighted and dissolved in dilute aqueous acid to give solutions having spectra in agreement with published values.[20] In preparing solutions for kinetic runs the ferricenium salts were dissolved in the appropriate buffer, filtered, and analyzed for ferricenium ion concentration using their visible spectra (ferricenium, 617 nm, $\epsilon = 410\ M^{-1}\ cm^{-1}$; 1,1′-dimethylferricenium, 650 nm, $\epsilon = 332\ M^{-1}\ cm^{-1}$; *n*-butylferricenium, 625 nm, $\epsilon = 352\ M^{-1}\ cm^{-1}$; chloromercuriferricenium, 623 nm, $\epsilon = 504\ M^{-1}\ cm^{-1}$; hyroxymethylferricenium, 627 nm, $\epsilon = 400\ M^{-1}\ cm^{-1}$; phenylferricenium, 750 nm, $\epsilon = 521\ M^{-1}\ cm^{-1}$; and decamethylferricenium,[22] 778 nm, $\epsilon = 394\ M^{-1}\ cm^{-1}$) and deaerated by bubbling with argon for 30 min. In some instances when the ferricenium salts were dissolved in buffer a small amount of light-colored solid would form causing slight turbidity in the solution. This was found to have no effect on kinetic results as long as the ferricenium ion solutions were filtered and analyzed immediately prior to kinetic measurements and when only freshly prepared ferricenium solutions were used. In experiments with decamethylferrocene as a reducing agent, solutions of decamethylferrocene were prepared by anaerobic dilution of an acetonitrile stock solution. While decamethylferrocene is air-stable in dry acetonitrile, it is readily air-oxidized in the aqueous buffer solutions used in our experiments.

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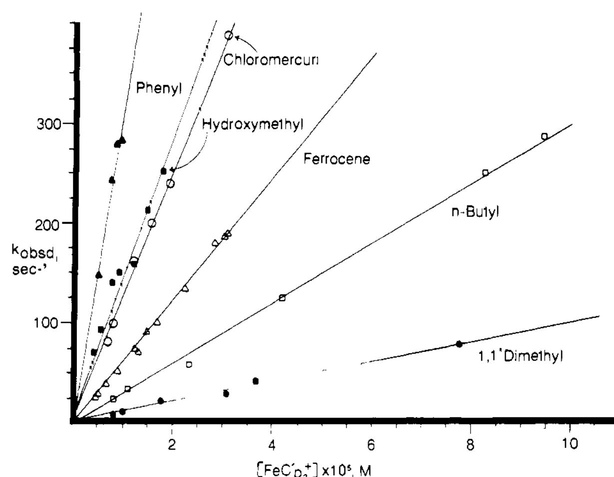

Figure 1. Dependence of the observed pseudo-first-order rate constant, k_{obsd}, on initial ferricenium ion derivative concentration at 25 °C, pH 6.5(phos), $\mu = 0.50$.

Horse heart cytochrome *c* (Sigma Type VI) was used without further purification. Solutions of ferrocytochrome *c* were prepared from ferricytochrome *c* solutions by three methods: (1) reduction under argon with a stoichiometric amount of hexaammineruthenium(II) prepared by Zn–Hg amalgam reduction of the ruthenium(III) chloride salt, (2) reduction by excess sodium dithionite followed by anaerobic gel filtration on G-25 Sephadex or by anaerobic dialysis, and (3) direct reduction for ca. 30 min with Zn–Hg amalgam under an argon atmosphere. Reductions by all three methods gave protein solutions having indistinguishable visible spectra and kinetic behavior. Cytochrome *c* solution concentrations were determined spectrophotometrically on the basis of the change in absorbance ($\Delta\epsilon_{550\,nm} = 18.5 \times 10^3\ M^{-1}\ cm^{-1}$) accompanying the reduction of the protein at 550 nm and the spectrum of the reduced protein.[23]

Kinetic measurements were made 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 using a nonlinear least-squares program to fit for the observed pseudo-first-order rate constant.

Results

I. Oxidations of Ferrocytochrome *c* by Ferricenium Ion Derivatives. The oxidations of ferrocytochrome *c* by ferricenium, 1,1′-dimethylferricenium, *n*-butylferricenium, hydroxymethylferricenium, chloromercuriferricenium, and phenylferricenium were monitored at 550 nm. All reactions on these six systems were carried out with ferricenium ion concentrations at least 10-fold greater than protein concentrations. First-order plots of rate data were linear to greater than 90% completion in all cases.

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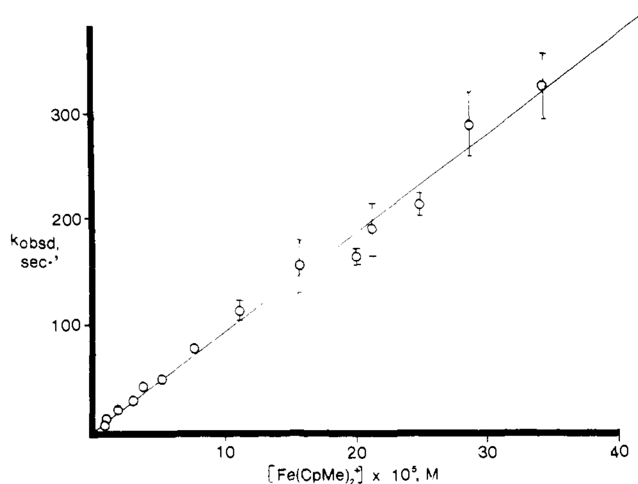

Figure 2. Dependence of the observed pseudo-first-order rate constant, k_{obsd}, on initial 1,1′-dimethylferricenium ion concentration at 25 °C, pH 6.5(phos), μ = 0.50.

Table II. Activation Parameters for Ferricenium Ion–Ferrocytochrome *c* Reactions, pH 6.5, μ = 0.50 (phos)

ferricenium derivative	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, cal/(mol K)
$FeCp_2^+$	5.0 ± 0.1	−10.6 ± 0.3
$FeCpCpHgCl^+$	4.3 ± 0.2	−11.6 ± 0.8
$FeCpCpC_4H_9^+$	5.9 ± 0.5	−9.1 ± 1.7
$FeCpCpPh^+$	5.5 ± 0.1	−5.7 ± 0.3

Plots of observed pseudo-first-order rate constants, k_{obsd}, vs. initial ferricenium ion concentrations are displayed in Figures 1 and 2. The second-order rate constants derived from a least-squares computer analysis of these data assuming zero intercept are given in Table I.

The relatively lower rate of reaction of the 1,1′-dimethylferricenium ion allowed the extension of this study to much higher ferricenium concentration (3.4×10^{-4} M) than was possible for the other derivatives. This was of interest because rate saturation at high inorganic reagent concentration is a possibility and while apparently not yet observed for reactions of cytochrome *c*[24] rate saturation has been observed in reactions of the blue copper proteins[25,26] and iron–sulfur proteins.[27] As can be seen by the linearity of the plot in Figure 2 for all of the 1,1′-dimethylferricenium data, no indication of rate saturation is found here.

Four of the ferricenium ion–ferrocytochrome *c* reactions have been studied as a function of temperature. The Eyring plots for these systems are given in Figure 3. The activation parameters obtained from least-squares fits to these data for ferricenium, phenylferricenium, chloromercuriferricenium, and *n*-butylferricenium are given in Table II.

II. Reduction of Ferricytochrome c by Decamethylferrocene. Since decamethylferricenium is a substantially weaker oxidizing agent than the other ferricenium derivatives used, it was necessary to monitor the reduction of ferricytochrome *c* by decamethylferrocene rather than the analogous protein oxidation reaction. This was done at 550 nm where greater than 99% of the spectral change is due to the protein's change in oxidation state.

Decamethylferrocene is much less soluble than the other ferrocene derivatives studied, and we were unable to study its re-

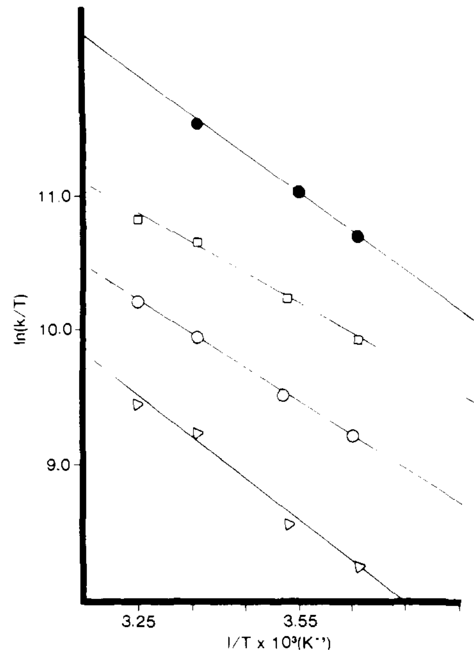

Figure 3. Eyring plots for second-order rate constants for the reaction of ferrocytochrome *c* with four ferricenium ion derivatives at pH 6.5-(phos), μ = 0.50: (●) phenylferricenium; (□) chloromercuriferricenium; (○) ferricenium; and (△) *n*-butylferricenium.

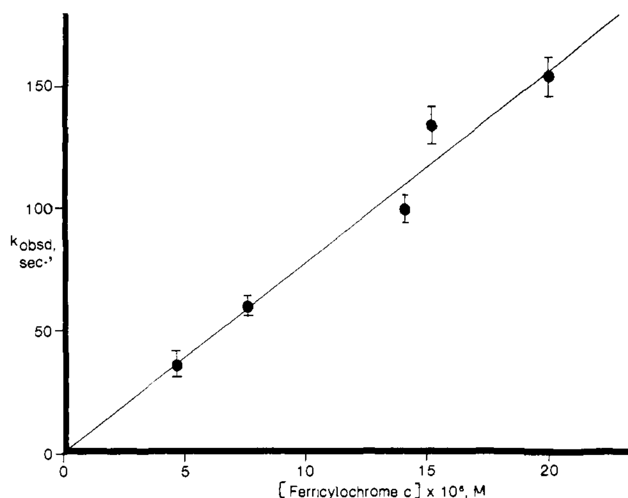

Figure 4. Dependence of the observed pseudo-first-order rate constant, k_{obsd}, on the initial concentration of ferricytochrome *c* for the reaction with decamethylferrocene at 25 °C, pH 6.5 (0.05 M HEPES), μ = 0.10 (NaCl/HEPES), 65/35 vol % water/acetonitrile. The initial concentration of decamethylferrocene was between 0.5×10^{-6} and 1.0×10^{-6} M with protein in 10-fold or greater excess in each experiment.

actions in entirely aqueous medium. Moreover, it is readily air-oxidized in aqueous buffer making it difficult to maintain for kinetic measurements. To circumvent these limitations experiments were done with protein in 10-fold or greater concentration excess of decamethylferrocene and the reactions were done in 65/35 water/acetonitrile by volume at pH 6.5 maintained with 0.05 M HEPES and μ = 0.10 (NaCl/HEPES). The first-order plots of rate data under these conditions were linear to more than 90% reduction completion. The first-order dependence of the observed pseudo-first-order rate constant, k_{obsd}, on initial protein concentration is shown in Figure 4. The second-order rate constant obtained from least-squares analysis of these data is 7.8 (±0.2) × 10^6 M^{-1} s^{-1}.

Since it is of interest to compare these results with the results for the ferricenium derivative reactions in aqueous medium, several experiments were done on the reduction of ferricenium ion with ferrocytochrome *c* in mixed solvents and at different ionic strengths. In 80/20 volume ratio of water/acetonitrile at pH 6.5,

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0.05 M HEPES, and $\mu = 0.10$ (NaCl/HEPES) the reduction of ferricenium by ferroctyochrome c was found to be first order in each reactant with a second-order rate constant of 5.6 (±0.1) × 10^6 M^{-1} s^{-1}. Attemtps to measure the rate of this reaction at 65/35 volume ratio of water/acetonitrile at the same buffer and salt composition were unsuccessful. Under these solvent conditions the reduced protein is greatly more sensitive to air-oxidation and good kinetic data could not be obtained. Additional studies on the ferricenium–ferrocytochrome c reaction in aqueous medium at pH 6.5, 0.05 M HEPES, and $\mu = 0.10$ (NaCl/HEPES) gave kinetic data comparable to that displayed in Figure 1, and analysis of these data gave a second-order rate constant of 4.63 (±0.2) × 10^6 M^{-1} s^{-1}. Hence, the second-order rate constants for ferricenium ion reduction by ferrocytochrome c at 25 °C and pH 6.5 in water (phosphate buffer), $\mu = 0.50$; in water (HEPES buffer, 0.05 M), $\mu = 0.10$ (NaCl/HEPES); and in 80/20 water/acetonitrile (HEPES buffer 0.05 M), $\mu = 0.10$ (NaCl/HEPES) are not seen to vary greatly with solvent, salt, and buffer changes for the limited range of conditions used.

Discussion

The Marcus equation[12] for outer-sphere electron transfer, eq 1, has been used extensively to interpret the redox reactions of

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

metalloproteins with small inorganic complexes.[6] The most direct test of this relationship is to compare the experimental second-order rate constants for electron transfer with the electron-transfer cross-reaction rate constant, k_{12}, calculated from eq 1. The number of protein–small-complex reactions where this can be done is rather limited owing to the lack of experimental values for the self-exchange rate constants for the metalloproteins[6] and in some cases for the small complexes used.[14] The self-exchange rate constants are known for cytochrome c, from NMR studies,[28] and for several ferrocene derivatives, from NMR[18] and other measurements.[19] The experimental and calculated cross-reaction rate constants, the self-exchange rate constants, and the $\Delta E^{\circ\prime}$ values from which the calculated values are obtained are given in Table I. While the self-exchange rate constants and $E^{\circ\prime}$ values for the ferrocene derivatives apply to solvent and salt conditions different from those employed here, Yang, Chan, and Wahl[18] find the exchange rates to be relatively insensitive to changes in solvent and ionic strength and Pladziewicz and Espenson[19,20] find small changes in $E^{\circ\prime}$ values in 1:1 (v/v) water:1-propanol, $\mu = 0.05$ M, $Ba(ClO_4)_2$ vs. 1:1 (v/v) tetrahydrofuran:water, $\mu = 1.00$ ($LiClO_4$). Consequently, we believe the values listed are good estimates of these parameters under our conditions. A value of 0.260 V was used for the protein's reduction potential.[29] Because of uncertainties in the exchange rate constants, $E^{\circ\prime}$ values, and cross-reaction rate constants due to differences in conditions of measurement as well as normal experimental error, differences of less than one order of magnitude between experimental k_{12} values and calculated k_{12} values are probably not significant. In this context the calculated values in Table I agree very well with the experimental values for all seven derivatives studied and over a potential range of ca. 0.56 V. Barring fortuitous cancellation of work terms or reorganizational energies the close agreement of all of the complex–protein reactions with theory supports a common mechanism for electron transfer. Furthermore, it suggests an activated complex with relatively close approach of metal centers and good overlap of electron donating and receiving orbitals.

Since no significant substituent-specific effects, other than those accounted for by the changes in $E^{\circ\prime}$, are observed and since five of the seven derivatives studied have an unsubstituted cyclopentadienide (Cp) ligand, an activated complex with the unsubstituted Cp ligand penetrating the protein's surface end-on near the partly exposed heme edge is attractive. Although if this is correct one might have expected complexes with substituents on both rings, especially decamethylferrocene,[30] to have unusual reactivity. Either the methyl groups are too small to provide a detectable steric factor or the approach of the ferricenium derivatives is with the Cp–Fe–Cp edge of the derivative rather than end-on. The lack of significant substituent effects even for complexes which could hydrogen bond to the protein (hydroxymethylferricenium) or have specific hydrophobic interactions (e.g., phenylferricenium) supports a simple approach of the two reactants.

The relatively large second-order rate constants for the ferricenium–cytochrome c reactions are not surprising. Not only is electron density delocalized in the ferriceniums through metal bonding to the aromatic Cp ligands, but the Cp ligands also present a hydrophobic surface which should be effective at making close approach to the hydrophobic heme pocket on cytochrome c. The delocalization of electron density in these complexes helps to minimize ligand reorganization accompanying electron transfer. This is undoubtedly reflected in the high intrinsic outer-sphere electron-transfer reactivity of these complexes as evidenced by the self-exchange rate constants given in Table I. We expect these complexes to be effective one-electron outer-sphere titrants of other metalloproteins—especially proteins in which the metal is located in a hydrophobic environment.

Gray and co-workers[5-11] have established the importance of hydrophobic π-conducting ligands in electron transfer between proteins and small octahedral coordination compounds. In order to account for differences in size, charge, and experimental conditions for reaction of several complexes with a single protein they first correct the experimental cross-reaction and self-exchange rate constants to infinite ionic strength and then use the Marcus relationship to calculate the protein self-exchange rate constant. With this treatment they find very large differences in apparent protein reactivity with greater apparent protein reactivity when the protein was reacted with small complexes having hydrophobic π-bonded ligands. We were interested in knowing how our organometallic complexes, all of which are π-bonded and hydrophobic, would compare with these more classical coordination compounds. Using the Debye–Hückel equation and Marcus equation as previously described,[4,11] the exchange rate constants and $\Delta E^{\circ\prime}$ values given in Table I, a radius of 16.6 Å for the protein, and a radius of 4.4 Å for decamethylferrocene and 3.2 Å for the other ferrocene derivatives, we derive values for the cytochrome c electrostatics-corrected self-exchange rate constant at infinite ionic strength, k_{11}^{∞}, from each system studied. These values, listed in Table I, are comparable to the values obtained from reactions of cytochrome c with octahedral complexes containing hydrophobic π-bonded ligands (e.g., k_{11}^{∞} derived from the $Co(phen)_3^{3+}$ reaction with cytochrome c is 7.1 × 10^2 M^{-1} s^{-1}) and are much larger than values derived from similar studies with complexes having hydrophilic σ-bonded ligands (e.g., $Ru(NH_3)_6^{2+}$).

The closeness of the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values, Table II, for the four reactions studied as a function of temperature reinforces the argument for a common activation process for the ferricenium ion–cytochrome c reactions. The $\Delta H^{\ddagger}$ values are all within ±1 kcal of 5 kcal/mol, and the $\Delta S^{\ddagger}$ values are all between ca. −6 and −12 eu. The $\Delta S^{\ddagger}$ values are about what is expected for the formation of a simple bimolecular collision complex,[31] and the low $\Delta H^{\ddagger}$ values as well suggest an uncomplicated collision with

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(30) Comparison of the results for decamethylferrocene with the other derivatives is complicated somewhat by the change in solvent composition necessary for the decamethylferrocene–cytochrome c reaction. Of particular concern is the possible change in protein conformation in the acetonitrile–water solvent. We attempted to account for this by measuring the rate of the ferricenium–cytochrome c reaction in several solvent systems as described in the results. Dramatic solvent effects were not found in these experiments. Also, the visible spectra of reduced and oxidized protein were not found to change greatly in going to the acetonitrile–water solvent mixture used. However, the air sensitivity of the reduced protein is markedly enhanced in the acetonitrile–water solvent indicating some changes in protein properties. It is certainly possible that small structural changes could significantly modify reactivity with a small molecule such as O_2 while having little effect on reactivity toward a larger molecule like ferricenium.

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minimal protein structural rearrangement. Moreover, the activation parameters for the cross-reactions between ferrocene and ferricenium ion derivatives[32] are close to the values reported here and to the activation parameters for the ferrocene–ferricenium ion self-exchange reactions[32] suggesting that the ferricenium ion–protein cross-reaction activation processes are modeled well by the ferricenium ion–ferrocene reactions. While this agreement could result from fortuitous cancellations of various factors in the activation process it is worth noting that these cancellations would have to occur across the range of derivatives studied. It is interesting to note that ΔH^{*} apparently does not decrease with increasing $\Delta E^{\circ\prime}$ for these four reactions as might be expected from Marcus theory. This may simply reflect the small range of driving force for these four reactions, and it may require determining ΔH^{*} for a larger range of driving force than was possible in this study for such a relationship to become evident.

Finally, the ferricenium ion derivatives have proven to be a predictable class of cytochrome *c* titrants. They have low charges, are substitution inert, and are facile outer-sphere reagents with a wide range of reduction potentials. Studies[33] with copper proteins and iron–sulfur proteins indicate that these derivatives also react predictably with these metalloproteins.

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Registry No. Cytochrome *c*, 9007-43-6; 1,1′-dimethylferricenium, 12276-63-0; *n*-butylferricenium, 32914-29-7; ferricenium, 12125-80-3; chloromercuriferricenium, 34742-71-7; hydroxymethylferricenium, 34742-72-8; phenylferricenium, 32839-60-4; decamethylferrocene, 12126-50-0.

(32) The activation parameters from ref 19 for the cross-reactions of 1,1′-dimethylferrocene with ferricenium ion and of ferrocene with phenylferricenium ion are ΔH^{*} 3.0 ± kcal/mol and ΔS^{*} −15 ± 3 cal/(mol K) and ΔH^{*} 3.0 ± 0.64 kcal/mol and ΔS^{*} −14.4 ± 2.1 kcal/(mol K), respectively. The activation parameters for the self-exchange reactions recalculated from data in ref 18 are ΔH^{*} 5.5 ± 0.2 kcal/mol and ΔS^{*} −8.2 ± 0.7 cal/(mol K) for 1,1′-dimethylferrocene self-exchange and ΔH^{*} 5.6 ± 0.6 kcal/mol and ΔS^{*} −8.9 cal/(mol K) for the ferrocene self-exchange in acetonitrile.

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Polymer-Bound Substrates: A Method To Distinguish between Homogeneous and Heterogeneous Catalysis

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Abstract: Soluble and cross-linked polymers have been prepared and examined as substrates for hydrogenation reactions in the presence of homogeneous precatalysts, metal sols, and heterogeneous catalysts. These polymeric substrates are hydrogenated in the presence of soluble catalysts, whereas heterogeneous catalysts are ineffective. This phenomenon is proposed as a method to distinguish truly homogeneous from particulate catalysts. Using this test, we have found Crabtree's precatalyst, $[Ir(COD)L_2]PF_6$, to behave as a homogeneous olefin hydrogenation catalyst. The Maitlis precatalyst, $[Rh(C_5Me_5)Cl_2]_2$, acts as a homogeneous catalyst in the hydrogenation of olefins, but behaves like a heterogeneous catalyst in the hydrogenation of polymer-bound benzene derivatives.

The identification of the active metal species in homogeneous catalytic reactions continues to be a difficult and challenging problem. Halpern's elegant study of homogeneous asymmetric hydrogenation[1] illustrates the important point that the catalytically active forms of the metal may be very minor components in the reaction mixture. Other work[2,3] suggests that heterogeneous metal particles may be the true catalysts in some supposedly homogeneous reactions.

Few methods currently exist for distinguishing between homogeneous and heterogeneous catalysts. Physical methods such as light scattering[4] and filtration[3-5] may detect the presence of insoluble material, but they provide no information on the catalytic activity of this material. Differences between the general reactivity patterns of homogeneous and heterogeneous catalysts may serve as useful guidelines.[6,7] However, identification of catalysts based on these patterns is often inconclusive because of the wide differences in reactivity observed within each class of catalysts.

The information available on the chemical reactions of polymers[8-11] suggests that polymers may be useful in distinguishing soluble from particulate catalysts. With appropriate caution,[9] the reactivity of polymers may be compared with that of their low molecular weight analogues. In general, simple homogeneous reactions involving soluble and cross-linked polymers are reported to occur at rates similar to those observed for low molecular weight compounds.[12,13] Rate differences of 5–10-fold are common.[13] However, many heterogeneous reactions of soluble polymers, such as catalytic hydrogenation, are known to be *orders of magnitude* slower than the corresponding reactions of monomers.[9] In fact, higher temperatures and pressures, higher catalyst concentrations, and longer reaction times are typically required to achieve significant reaction of polymeric substrates.[14-16] Although no de-

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