These procedures for catalyzing transhydrogenation introduce an element of flexibility into nicotinamide cofactor regeneration and provide another method of controlling the redox behavior of systems of enzymes used for organic synthesis.

Registry No. NAD, 53-84-9; NADPH, 53-57-6; NADH, 58-68-4; NADP, 53-59-8; diaphorase, 37340-89-9; alcohol dehydrogenase, 9031-72-5; glutamic dehydrogenase, 9001-46-1; ribose-5-phosphate, 4300-28-1; L-lactate, 79-33-4; *threo*-D_s(+)-isocitrate, 6061-97-8; 6-pheophogluconic acid, 2464-13-3; pyruvate, 127-17-3; 2-ketoglutarate, 328-50-7.

Reduction of Ferricenium Ion by Horse Heart Ferrocytochrome *c*

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The electron-transfer reactions that can occur between metalloproteins and small transition-metal complexes have been the subject of considerable recent study,[1-4] with horse heart cytochrome *c* being the most extensively investigated protein. In these studies Gray and co-workers[3,5,6] have used the Marcus relationship[7] and Debye–Hückel theory to interpret the second-order cross reaction rate constants for these systems. A major conclusion of these interpretations is that the hydrophobicity and π-bonding character of the ligands of the transition-metal complex are very important in determining the facility with which the protein can transfer electrons to or accept electrons from the complex.

In an effort to test these ideas with a new class of small complexes with hydrophobic π-bonding ligands, we have begun a study of the electron-transfer reactions of ferrocene, ferricenium ion, and their derivatives with metalloproteins. We report here the results of the reduction of ferricenium ion with horse heart ferrocytochrome *c*. The products of this reaction are ferrocene and ferricytochrome *c*.

The rate of the one-electron reduction of ferricenium ion by ferrocytochrome *c* from horse heart (type VI ferricytochrome *c* obtained from Sigma) has been measured with a Durrum Model D-110 stopped-flow spectrophotometer interfaced with a Nicolet Model 1090 digital oscilloscope and an Apple II computer. The reaction, monitored at 550 nm, was run with ferricenium ion and is pseudo-first-order excess over ferrocytochrome *c* in all experiments. Ferricenium hexafluorophosphate was prepared by the oxidation of ferrocene (Alfa Chemical Co.) by using the method of Yang, Chan, and Wahl.[8] Solutions of ferricenium hexafluorophosphate were prepared by dissolving the salt in argon-purged buffer and analyzed by measuring the solution's absorbance at 617 nm with a Cary 14 recording spectrophotometer.

Logarithmic plots of absorbance change vs. time were linear for more than 3 half-lives, establishing a first-order dependence on ferrocytochrome *c*. The reaction was also observed to be first order in ferricenium ion as indicated by data displayed in Figure 1. The second-order rate constant derived from a linear least-squares analysis of the data displayed in Figure 1 is $(6.20 \pm 0.18) \times 10^6$ M^{-1} s^{-1}. All measurements were made at 25.0 °C and μ = 0.50 (phosphate) at pH 7.0 under strictly anaerobic conditions. No hint of rate saturation, as observed[4] for some other protein complex reactions, was observed over the range of concentrations employed here. This is not surprising considering the high ionic strength and limited ferricenium ion concentration range possible in this study. The range of ferricenium ion concentrations was limited by the short reaction half-times at high concentrations and vanishingly small absorbance changes at lower concentrations. It should also be noted that ferrocene, the product of ferricenium ion reduction, is not appreciably soluble in water. However, in our experiments its concentration never exceeded 2.5×10^{-6} M, and at this concentration no precipitation of ferrocene results.

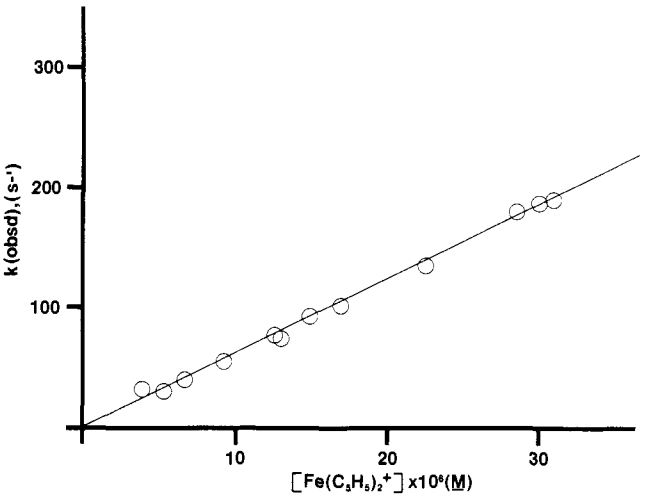

Figure 1. Plot of observed first-order rate constant, k_{obsd}, vs. initial ferricenium ion concentration, $[Fe(C_5H_5)_2^+]$, at 25.0 °C, pH 7.0, and μ = 0.50 (phosphate).

Electron-transfer rates and mechanisms of ferrocene and its derivatives have been of interest, and some information is available regarding their reactivity. Wahl[8] and co-workers have measured the ferrocene–ferricenium ion electron-exchange rate constant directly in NMR studies ($k = (5.7 \pm 1.0) \times 10^6$ M^{-1} s^{-1} in acetonitrile) and Pladziewicz and Espenson[9] have measured rate constants for cross reactions between a number of derivatives of ferrocene and ferricenium ion. Using the Marcus relationship,[7] Pladziewicz and Espenson derive a value of 5.7×10^6 M^{-1} s^{-1} for the ferrocene–ferricenium ion electron-exchange rate constant in 1:1 v/v 1-propanol–water. This rate constant shows little dependence on solvent and ionic strength for the conditions employed in these two studies.

Gray and co-workers[3,5,6] have evaluated protein–metal complex electron-transfer reactions by correcting the second-order cross reaction rate constants and the small complex exchange reaction rate constants for nonspecific electrostatic effects by using Debye–Hückel theory to adjust these rate constants to infinite ionic strength. They then use the Marcus relationship[7] (eq 1) to

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

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

calculate an exchange rate constant (k_{11}) for the protein at infinite ionic strength. Algebraic rearrangement of eq 2 gives[11] which

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

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can be used to calculate the exchange rate constants. In these discussions k_{11} is the protein-exchange rate constant, k_{22} is the metal complex exchange rate constant, k_{12} is the protein–metal complex cross reaction rate constant, K_{12} is the cross reaction equilibrium constant, and Z in the collision frequency.

With this analysis they find the calculated electrostatics-corrected protein-exchange rate constants to depend on the hydrophobicity and π-bonding character of the metal complex ligands. In some cases the protein self-exchange rate constants, corrected for electrostatics and thermodynamics, that are calculated from the second-order cross reaction rate constants between proteins and small complexes are found to differ by as much as a factor of 10^6.[3a] These dramatic differences are attributed to the ability of complexes with hydrophobic and/or π-bonding ligands to penetrate the protein superstructure at the time of electron transfer to a greater degree than can those complexes with hydrophilic ligands.

Using an estimate of 3.8 Å (the calculation is not very sensitive to this estimate) for the radius of ferricenium ion and a value of 16.6 Å for the protein, we calculate[5] a second-order rate constant for ferricenium–ferrocytochrome *c* electron transfer, corrected to infinite ionic strength (k_{12}^{∞}), of $(6.9 \pm 0.2) \times 10^6$ M^{-1} s^{-1}. Using eq 2, the ionic strength corrected k_{12} value, Wahl's value for the ferrocene–ferricenium ion exchange rate constant, a value of 0.260 V[10] for the cytochrome *c* reduction potential, and a value 0.513 V[11] for the ferricenium ion reduction potential, we calculate an electrostatics-corrected cytochrome *c* exchange rate constant, k_{11}^{∞}, of 1.0×10^3 M^{-1} s^{-1}. This is in close agreement with k_{11}^{∞} values derived from other cross reaction rate constants for cytochrome *c* with complexes with hydrophobic ligands.[3b] For example k_{11}^{∞} derived from the $Co(phen)_3^{3+}$–ferrocytochrome *c* reaction is 7.1×10^2 M^{-1} s^{-1}. It should also be noted that these derived protein electron exchange rate constants are very close to the values of k_{11} (10^3–10^4 M^{-1} s^{-1}) measured by Gupta[12] for cytochrome *c*.

We believe that our result supports Gray's conclusion[3b] regarding the importance of hydrophobic π-conducting ligands in electron transfers between proteins and small metal complexes by extending to simple organometallic compounds the treatment heretofore applied only to octahedral coordination compounds. It will be important to see whether this treatment holds in general as additional proteins and organometallic compounds are studied. To this end we are continuing our work on the reactions of cytochrome *c* and other electron-transfer proteins with ferrocene, ferricenium ion, and their derivatives.

Acknowledgment. Research Corp. is gratefully acknowledged for their support of this work.

Registry No. Ferricenium ion, 12125-80-3; ferrocene, 102-54-5; ferricenium hexafluorophosphate, 11077-24-0; ferrocytochrome *c*, 9007-43-6; ferricytochrome *c*, 9007-43-6.

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Hemoprotein Destruction. Iron–Nitrogen Shift of a Phenyl Group in a Porphyrin Complex

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The reaction of hemoglobin with phenylhydrazine results in hemoglobin denaturation and erythrocyte lysis.[1] *N*-phenylprotoporphyrin IX, presumably derived from *N*-phenylheme, is isolated as a major product of the reaction by extraction of phenylhydrazine-treated hemoglobin with acidic methanol.[2,3] The mechanism by which *N*-phenylheme is formed, however, and its relationship to the hemolytic events have not yet been defined. Recent studies have provided spectroscopic evidence for the unexpected existence of a globin-stabilized intermediate that is converted to *N*-phenylprotoporphyrin IX by aerobic treatment with acidic methanol but that on denaturation of the protein with aqueous base in the presence of dithionite reverts to the parent heme.[4,5] We have proposed a structure for the intermediate in which a phenyl group is directly bound to the heme iron[4] but have not been able to support the suggestion with a directly relevant chemical precedent. We now report (a) synthesis and characterization of the iron–phenyl complex of a model porphyrin, (b) facile regeneration of the ferric porphyrin from the complex, and (c) migration of the phenyl group from iron to nitrogen on aerobic treatment with acidic methanol.

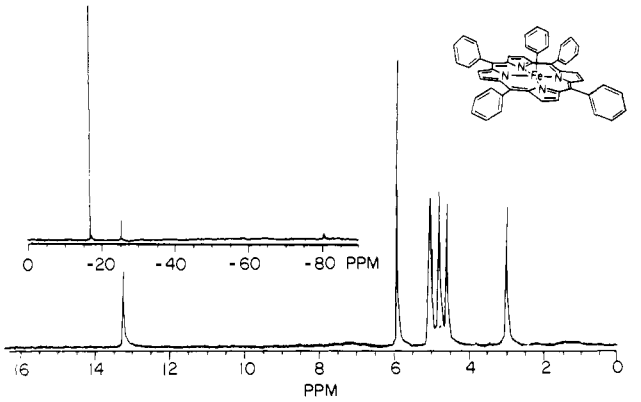

Figure 1. 360-MHz NMR spectrum of PhFeTPP. The spectrum of 10 mM PhFeTPP in deuterochloroform containing 2 mM BHT was taken at 22 °C. The region from 0 to −80 ppm is shown in the inset in reduced scale. BHT peaks at approximately 6.8–7.3 and 1.0–2.2 ppm have been deleted from the spectrum. The structure of PhFeTPP is given.

Phenylmagnesium bromide (0.2 mmol of a 3 M ether solution) was added under argon to a stirred solution of Fe(TPP)Cl[5] (0.14 mmol) in 25 mL of dry, O_2-free tetrahydrofuran (THF). Butylated hydroxytoluene (BHT, 2,6-di-*tert*-butyl-4-methylphenol, 10 mg) was added after 10 min, and the solvent was removed under vacuum. Chromatography of the residue on a 2.5 × 30.0 cm column of basic alumina eluted with 18% THF in hexane containing 0.025% BHT,[6] solvent removal from the deep red product fraction, trituration with hexane, filtration, and drying under vacuum provided the iron–phenyl complex (PhFeTPP) in 53% yield.[7] Signal assignments[7] for the protons due to the parent porphyrin in the 360-MHz NMR spectrum of PhFeTPP (Figure 1) rest on the observation that irradiation of the protons at 5.031 ppm results in collapse of the triplet at 5.893 ppm and sharpening of the peak at 4.555 ppm, while irradiation at 4.765 ppm causes collapse of the 5.893-ppm triplet and sharpening of the peak at 2.955 ppm. These decoupling results and the expectation that

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(5) Heme is used to denote iron protoporphyrin IX regardless of the iron oxidation state. Iron tetraphenylporphyrin (FeTPP) was used as the chloride salt (Fe(TPP)Cl).
(6) The complex rapidly decomposes in the absence of BHT.
(7) PhFeTPP: λ_{max} (ϵ_M) (THF) 419 (108 000), 526 nm (10 130); ^{1}H NMR (360 MHz) (number of protons, peak width, assignment) δ 13.222 (2 H, 16.8 Hz, Fe–Ph *m*-H), −80.67 (2 H, 127.0 Hz, Fe–Ph, *o*-H), −25.517 (1 H, 16.3 Hz, Fe-Ph *p*-H), −17.174 (8 H, 13.4 Hz, pyrrole H), 2.955 (4 H, 20.7 Hz, *meso*-Ph *o*-H, top side), 4.555 (4 H, 22.0 Hz, *meso*-Ph *o*-H, bottom side), 4.765 (4 H, 22.0 Hz, *meso*-Ph *m*-H, top side), 5.031 (4 H, 21.0 Hz, *meso*-Ph *m*-H, bottom side), 5.893 (4 H, t, J = 5.5 Hz, *meso*-Ph *p*-H).

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