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 $\times 10^2$ 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.

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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.

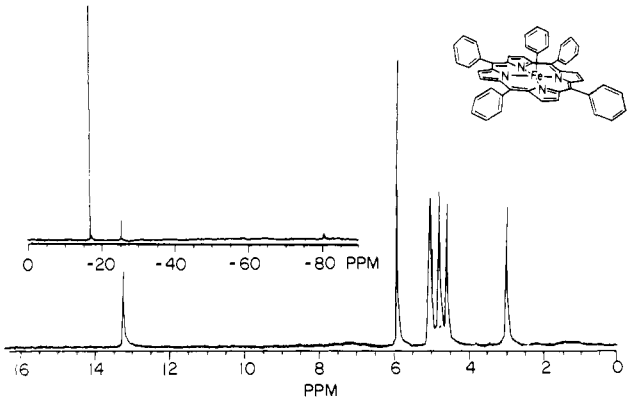

Figure 1. 360-MHz NMR spectrum of PhFeTPP. The spectrum of 10 mM PhFeTPP in deuterochloroform containing 2 mM BHT was taken at 22 °C. The region from 0 to −80 ppm is shown in the inset in reduced scale. BHT peaks at approximately 6.8–7.3 and 1.0–2.2 ppm have been deleted from the spectrum. The structure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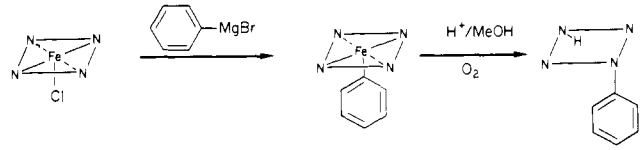
Scheme I



meso-phenyl protons on the same side of the porphyrin ring as the iron–phenyl will appear at higher field than the corresponding protons on the opposite side[8] are only consistent with the indicated assignments. The iron–phenyl proton signals, identified by their absence in the spectrum of the complex prepared with perdeuterated phenylmagnesium bromide, are at strikingly different positions. Whereas the meta protons are shifted to low field (13.22 ppm), the para and ortho protons (the latter exceptionally so) are shifted to high field (−25.52 and −80.67 ppm, respectively). The NMR spectrum indicates that the iron in PhFeTPP is in a low-spin rather than high-spin state because (a) the pyrrole protons appear at −20 ppm rather than +80 ppm and (b) the signal line widths[7] are in the 10–20-Hz rather than 100–300-Hz range.[9] A ferric low-spin state is consistent with that recently attributed to iron–alkyl porphyrin complexes.[10]

Solid PhFeTPP is stable at 25 °C in air but rapidly decomposes in solution in the absence of BHT to FeTPP (isolated and characterized as the chloride salt). The decomposition of PhFeTPP in an NMR tube is accompanied by the appearance of a sharp singlet at 7.25 ppm due (presumably) to benzene formation. No evidence was found in the NMR spectrum for the formation of phenol or biphenyl. If, however, PhFeTPP (0.13 mmol) in 50 mL of THF containing 0.025% BHT is treated overnight with 50 mL of 5% H_2SO_4 in methanol, *N*-PhTPP is obtained in 68% crystalline yield.[11] The high-field positions of the *N*-phenyl protons, noted previously with *N*-phenylprotoporphyrin IX,[2] clearly identify the product. The iron–nitrogen shift requires 1–2 h for completion and depends critically on the presence of oxygen. The yield of *N*-PhTPP is drastically reduced if oxygen is rigorously excluded. The shift is also highly sensitive to the solvent and acid used, although a detailed analysis of these parameters has not yet been completed. The iron–phenyl complex and the subsequent shift to nitrogen are also obtained with protoporphyrin IX, except that side products arise from reaction of the Grignard reagent with the esterified carbonyl side chains unless the Grignard reaction is run at low temperature.[13] The general reaction is given in Scheme I.

Iron–phenyl complexes with porphyrin and nonporphyrin ligands are known.[14] The shift of alkyl groups from cobalt to nitrogen[15] and a similar shift of a vinylidene carbene–iron complex have recently been reported.[16] The present example, however, is the first in which the migration of an aryl group is demonstrated. The stringent parallels in the behavior of the hemoglobin–phenylhydrazine intermediate and the present model system not only substantiate our postulate that the former is an iron–phenyl complex but also provide the clearest demonstration that iron–carbon complexes are formed with hemoproteins.[17]

Registry No. Fe(TPP)Cl, 16456-81-8; PhFeTPP, 70936-44-6; *N*-PhTPP, 81856-91-9; phenyl bromide, 108-86-1.

(8) The peripheral protons on the same side of the porphyrin as the iron–phenyl group are shifted up field by the phenyl group ring current.

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(11) The product was isolated by partitioning the reaction mixture between 100 mL of CH_2Cl_2 and 200 mL of H_2O, separating and washing the organic layer with aqueous $NaHCO_3$, drying (Na_2SO_4), solvent removal, and chromatography on basic alumina (CH_2Cl_2). The bright green porphyrin was crystallized from benzene–petroleum ether (bp 60–110 °C fraction); λ_{max} (ϵ_M) ($CHCl_3$) 444 (385 000), 550 (sh) (17 200), 595 (36 700), 633 (sh) (14 000), 705 nm (8800); λ_{max} of the dication ($CHCl_3$, 2% trifluoroacetic acid) 457 (512 000), 694 nm (87 900); ^{1}H NMR (80 MHz, $CDCl_3$) δ 8.68 (s, 2 H, 5,6-pyrrole H), 8.1–8.4 (m, 16 H, 3,4,7,8-pyrrole H, *meso*-Ph *o*-H), 7.7 (m, 12 H, *meso*-Ph *m*- and *p*-H), 7.4 (s, 2 H, 1,2-pyrrole H), 5.6 (t, 1 H, N–Ph *p*-H), 5.24 (t, 2 H, N–Ph *m*-H), 3.00 (d, 2 H, N–Ph *o*-H); mass spectrometric molecular ion (electron impact, 70 eV) *m/e* 690. The *N*-phenyl proton assignments are based on decoupling experiments, but the porphyrin ring protons have been assigned by analogy to assignments made for *N*-CH_3TPP.[12]

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(13) The dimethyl ester of *N*-phenylprotoporphyrin IX obtained from the rearrangement reaction is identical with the product previously obtained in the reaction of heme with phenylhydrazine.[2] The phenyl–iron complex, in addition to NMR signals attributable to the protoporphyrin IX framework, exhibits signals for the iron–phenyl group at 13.94 (meta protons), −21.64 (para proton), and −77.45 ppm (ortho protons). The positions of these peaks are similar to those of the analogous protons in the TPP system (Figure 1).

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Agreement between Transition-Metal Orbital Populations from X-ray and Polarized Neutron-Scattering Experiments

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We have recently developed formalisms relating transition-metal d-orbital occupancies to the population of multipolar density functions that can be obtained by least-squares refinement of accurate X-ray diffraction data.[1,2] The formalisms have been applied in a number of studies, among which the analysis of the electronic structure of the mineral pyrite (FeS_2)[3] and the transition-metal complex cobalt tetraphenylporphyrin (CoTPP).[4] They generally provide orbital populations that are in agreement with the ordering of the electronic energy levels according to theoretical considerations. Thus, in FeS_2 (local symmetry at the Fe site $\bar{3}m$) the a_g (t_{2g}) orbital which is ligand-field stabilized is fully occupied by 1.98 (12) electrons, while a partial occupancy of 0.66 (15) electrons is obtained for the destabilized e_g' orbital. This partial occupancy of a destabilized orbital in a low-spin complex suggests a covalent metal–ligand interaction commonly described by σ donation. Thus, the results offer the possibility for a quantitative assessment of the importance of covalent interactions.

Further evidence for this interpretation has become available from polarized neutron diffraction measurements that can be interpreted in terms of the *spin* populations of the atomic orbitals by the application of relations between the spin density and spin

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