illustrating the dependence of the relaxation parameters on the correlation times utilize curves generated by the computer system.

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References and Notes

(1) (a) P. H. Bolton and D. R. Kearns, *Biochim. Biophys. Acta*, **517**, 329 (1978); (b) P. H. Bolton and D. R. Kearns, *Nucleic Acids Res.*, **5**, 1315 (1978); (c) P. H. Bolton and D. R. Kearns, *J. Am. Chem. Soc.*, **101**, 479 (1979).

(2) (a) S. W. Englander and J. J. Englander, *Proc. Natl. Acad. Sci. U.S.A.*, **53**, 370 (1965); (b) R. R. Gantt, S. W. Englander, and M. V. Simpson, *Biochemistry*, **8**, 475 (1969).

(3) P. H. Bolton and D. R. Kearns in "Biological Magnetic Resonance", L. J. Berliner and J. Reuben, Eds., Plenum Press, New York, 1978, p 91.

(4) (a) P. D. Johnston and A. G. Redfield, *Nucleic Acids Res.*, **5**, 3913 (1978); (b) *ibid.*, **4**, 3599 (1977).

(5) I. D. Campbell, C. M. Dobson, and R. G. Ratcliffe, *J. Magn. Reson.*, **27**, 455 (1977).

(6) V. A. Bloomfield, D. M. Crothers, and I. Tinoco, Jr., "Physical Chemistry of Nucleic Acids", Harper and Row, New York, 1974.

(7) Ph. Wahl, J. Paoletti, and J.-B. LePecq, *Proc. Natl. Acad. Sci. U.S.A.*, **65**, 417 (1970).

(8) C. P. Slichter, "Principles of Magnetic Resonance", Harper and Row, New York, 1963.

(9) (a) T. L. James, G. B. Matson, I. D. Kuntz, R. W. Fisher, and D. H. Buttlaire, *J. Magn. Reson.*, **28**, 417 (1977); (b) T. L. James, G. B. Matson, and I. D. Kuntz, *J. Am. Chem. Soc.*, **100**, 9490 (1978); (c) T. L. James and G. B. Matson, *J. Magn. Reson.*, **33**, 345 (1979).

(10) D. E. Woessner, *J. Chem. Phys.*, **36**, 1 (1962).

(11) D. Doddrell, V. Glushko, and A. Allerhand, *J. Chem. Phys.*, **56**, 3683 (1972).

(12) (a) W. E. Hull and B. D. Sykes, *J. Chem. Phys.*, **63**, 867 (1975); (b) *J. Mol. Biol.*, **98**, 121 (1975).

(13) M. Levitt, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 640 (1978).

(14) H. B. Gray, Jr., and J. E. Hearst, *J. Mol. Biol.*, **35**, 111 (1968).

(15) (a) S. Arnott, *Prog. Biophys. Mol. Biol.*, **21**, 265 (1970); (b) D. B. Davies, *Prog. Nucl. Magn. Reson. Spectrosc.*, **12**, 135 (1978).

(16) P. Davanloo, I. M. Armitage, and D. M. Crothers, *Biopolymers*, **18**, 663 (1979).

(17) (a) M. Leng and G. Felsenfeld, *J. Mol. Biol.*, **15**, 455 (1966); (b) H. Eisenberg and G. Felsenfeld, *ibid.*, **30**, 17 (1967); (c) B. S. Stannard and G. Felsenfeld, *Biopolymers*, **14**, 299 (1975).

(18) K. Akasaka, A. Yamada, and H. Hatano, *Bull. Chem. Soc. Jpn.*, **50**, 2858 (1977).

(19) T. R. Triton and I. M. Armitage, *Nucleic Acids Res.*, **5**, 3855 (1978).

(20) L. P. G. Wakelin and M. J. Waring, *Biochem. J.*, **157**, 721 (1976).

(21) T. L. James, "Nuclear Magnetic Resonance in Biochemistry: Principles and Applications", Academic Press, New York, 1975, pp 152, 161.

(22) After this manuscript was submitted, an article appeared describing the ^{31}P NMR relaxation of 140 base pair long DNA.[23] The correlation time for internal motion of the phosphate of DNA was found to be 0.4 ns, which is in agreement with the value obtained here for larger DNA. Both values for long, double-stranded DNA are in contrast to the correlation time determined for deoxyoligonucleotides which was reported to be at least 1 ns[16] for reasons discussed in the text.

(23) L. Klevan, I. M. Armitage, and D. M. Crothers, *Nucleic Acids Res.*, **6**, 1607 (1979).

(24) We have recently examined rat-liver ribosomes (P. Bolton, G. Clawson, and T. L. James, submitted) and find that the RNA in intact ribosomes exhibits local motion which is restricted by about an order of magnitude relative to free RNA.

Solution Characterization of "Intermediate-Spin" Iron(III) Porphyrins by NMR Spectroscopy

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Abstract: ^{1}H and ^{13}C NMR spectra of iron(III) porphyrin perchlorate derivatives in noncoordinating solvents are anomalous with respect to the isotropic shift patterns observed for other iron(III) porphyrin species. Both synthetic and natural-derivative iron(III) porphyrins have been examined. Variation of solution conditions rule out chemical equilibria as an explanation for the unusual behavior. The perchlorate ion remains coordinated or tightly ion paired in solution. The measurements support earlier solid-state work which suggests a quantum mechanically admixed $S = {}^{3}\!/_{2}$, $S = {}^{5}\!/_{2}$ spin state formulation. The unperturbed quartet state is of lower energy. Magnetic anisotropy for the $S = {}^{3}\!/_{2}$ state is significant with $\chi_{\perp} > \chi$. The $d_{x^2-y^2}$ orbital is vacant, and the most likely formal ground state for the unperturbed $S = {}^{3}\!/_{2}$ species is $(d_{xy})^2 (d_{z^2})^1 (d_{xz}, d_{yz})^2$. Changes in isotropic shift patterns and magnetic moments with temperature presumably reflect thermal population of Kramer's doublets with varying amounts of $S = {}^{3}\!/_{2}$ and $S = {}^{5}\!/_{2}$ character. Solvent-dependence studies show that aromatic solvents increase the $S = {}^{5}\!/_{2}$ character as compared with chlorinated solvents. Solvent perturbation results may be relevant to the chemistry of cytochrome *c′*, which is also believed to exhibit quantum mechanical spin admixture.

Introduction

Preparation and physical characterization of metalloporphyrins in all possible spin and oxidation states constitutes a major effort among bioinorganic chemists. Biologically relevant iron(II) and iron(III) porphyrin complexes in both high- and low-spin states have received the most attention in this regard.[1] Experimental work[2-4] has also confirmed the theoretical prediction[5] of intermediate-spin $S = 1$ iron(II) porphyrins. Generation of this spin state demands a square-planar environment (no axial ligands) in which a vacant $d_{x^2-y^2}$ orbital is raised in energy and an occupied d_{z^2} orbital is stabilized. Intermediate-spin iron(II) phthalocyanines[6] and macrocyclic complexes[7] are also recognized.

Intermediate-spin $S = {}^{3}\!/_{2}$ character in ferric cytochrome *c′* has been invoked by Maltempo to explain unusual ESR properties and a decrease in magnetic moment at low temperature for this bacterial hemoprotein.[8,9] A *quantum-mechanical* admixture of $S = {}^{3}\!/_{2}$ and $S = {}^{5}\!/_{2}$ states is proposed. This formulation is to be distinguished from a *thermal mixture* of magnetically distinct $S = {}^{3}\!/_{2}$ and $S = {}^{5}\!/_{2}$ states. Quantum-mechanical admixing is presumably the result of a small energy separation between pure spin states. A spin-orbit coupling constant of comparable energy (~300 cm^{-1}) provides a direct mechanism for interaction of the two spin systems. Suggestion of intermediate-spin involvement is not unreasonable in view of literature descriptions of $S = {}^{3}\!/_{2}$ bis(*N*,*N*-dialkyldithiocar-

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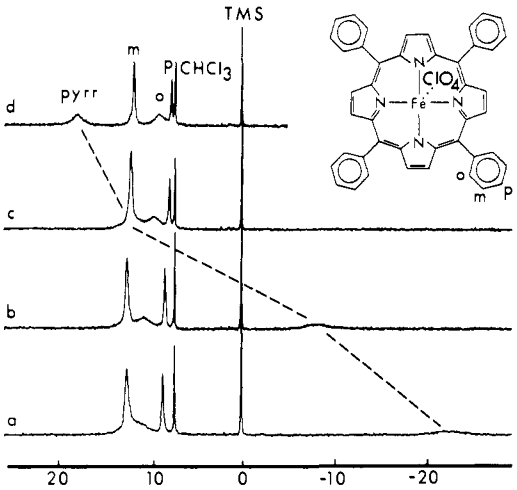

Figure 1. Variable-temperature ^{1}H NMR of FeTPP·ClO$_4$, CDCl$_3$ solvent, 0.01 M: (a) 228; (b) 251.5; (c) 299; (d) 327 K.

bamato)iron(III)[10] and tetraazoiron(III) complexes.[11]

Iron(III) porphyrin perchlorate[12-14] derivatives have recently been prepared and characterized in the solid state. Magnetic moments decrease with temperature, but Mössbauer measurements are consistent with presence of only one magnetic species at all temperatures. Infrared measurements suggest perchlorate coordination in the octaethylporphyrinatoiron(III) perchlorate (FeOEP·ClO$_4$) derivative[12] and a molecular structure determination confirms binding in tetraphenylporphyrinatoiron(III) perchlorate (FeTPP·ClO$_4$).[13,14] Ethanol of solvation induces only small changes in FeOEP·ClO$_4$ magnetic properties,[12] whereas perchlorate ion is displaced from coordination in the diaquo FeTPP·ClO$_4$ adduct.[13] The molecular structure of FeTPP·ClO$_4$ reveals a shortened Fe-pyrrole nitrogen distance, a small Fe-out-of-plane displacement (0.30 Å), and a lengthened Fe-perchlorate oxygen distance (2.03 Å) with respect to high-spin derivatives. On the basis of such structural and magnetic perturbations Reed and co-workers[14] recently proposed an $S = 3/2$, $S = 5/2$ spin admixed formulation for iron(III) porphyrin perchlorate adducts, much as has been formulated for cytochrome *c′*.

Prior to report of the FeTPP·ClO$_4$ molecular structure we had noted anomalous ^{1}H NMR shift behavior for this species generated in situ by addition of perchloric acid to the μ-oxo-bridged dimer. Solid-state characterization of the product[13] stimulated our detailed examination of iron(III) porphyrin perchlorates in solution. Objectives of this study were to discern if a $S = 3/2$, $S = 5/2$ admixture is a viable formulation or simply a "solid-state" effect, and what solution equilibria might be apparent for perchlorate derivatives. Preparation of this manuscript was coincident with appearance of detailed solid-state and preliminary solution characterization of FeTPP·ClO$_4$ by Reed and co-workers.[14]

Nuclear magnetic resonance constitutes perhaps the most powerful spectroscopic technique for solution characterization of metal complexes with short electronic relaxation times. Application to metalloporphyrins has been productive in elucidation of electronic structure, spin delocalization mechanisms, equilibria, dynamical effects, and molecular structure.[15] Of particular concern here is establishing formal d-orbital occupation by comparison of isotropic shift patterns. Correlations have been made between single occupation of the $d_{x^2-y^2}$ orbital and shift direction for pyrrole and ring methyl (or methylene) proton resonances.[3,15] Thus, for first-row transition-metal complexes single occupation of the σ-bonding $d_{x^2-y^2}$ orbital results in predominant unpaired spin density through a σ-type porphyrin MO and downfield shifts for pyrrole and ring methyl protons. Lack of unpaired spin in $d_{x^2-y^2}$ and single occupation of π-bonding d_{xz} and/or d_{yz} places unpaired spin density in a π-type porphyrin MO, resulting in an upfield pyrrole proton shift and a downfield ring methyl shift. These rather straightforward correlations, as well as more subtle observations, have been utilized in solution characterization of iron(III) porphyrin perchlorate derivatives.

Experimental Section

Iron(III) Porphyrin μ-Oxo-Bridged Dimers. Iron(III) tetraphenylporphyrin compounds were prepared as the chloride derivatives or as the μ-oxo-bridged dimers by literature methods.[16,17] Iron(III) octaethylporphyrin and iron(III) etioporphyrin I (FeETIO) were prepared by total pyrrole synthesis,[18] iron insertion, and chromatographic purification.[17] Natural-derivative iron(III) porphyrin dimethyl ester complexes were prepared by standard methods.[19] All iron(III) porphyrins were converted to the μ-oxo-bridged dimer by passage through a deactivated alumina column.[19] Products were characterized by NMR and UV-visible spectroscopy.

Silver Perchlorate Preparation. The perchlorate derivatives of FeTPP, FeTPP(*p*-CH$_3$), and FeTPP(*m*-CH$_3$) were prepared by silver perchlorate reaction with the corresponding chloride analogues in THF solvent.[14,20] The products were not recrystallized from toluene, so as to avoid interfering aromatic signals in the NMR spectrum from toluene of solvation.[14] Two THF molecules of solvation were retained in the unrecrystallized product as judged by NMR integrations. This solvent of crystallization was not removed by direct heating under vacuum at 105 °C. However, THF could be removed if the material was first dissolved in CHCl$_3$ or CH$_2$Cl$_2$, evaporated under a nitrogen stream, and heated under vacuum at 105 °C. *Caution:* We have not observed detonation of iron porphyrin perchlorates under these conditions, but care is urged. In practice, milligram amounts of sample were placed in NMR tubes and a metal, heated vacuum desiccator was employed. Unless otherwise noted, THF of crystallization was removed from FeTPP·ClO$_4$ prior to spectral examination. The diaquo derivative of FeTPP·ClO$_4$ was prepared by recrystallization from THF containing aqueous perchloric acid.[13]

Perchloric Acid Preparation. Cleavage of the μ-oxo-bridged dimer by shaking with aqueous perchloric acid affords FeOEP·ClO$_4$.[12] This general technique was applied to generation of both synthetic and natural-porphyrin derivatives. As prepared by Dolphin et al.,[12] the FeOEP·ClO$_4$ contains ethanol of crystallization, which may be removed under vacuum at 60 °C. In our hands the product homogeneity (as judged by NMR) was improved by avoiding the ethanol crystallization step and direct vacuum drying of the evaporated CH$_2$Cl$_2$ solution at 105 °C. As noted above, preparations were restricted to the milligram scale and heating was performed in a metal vacuum desiccator. Materials prepared by acid cleavage were identical with those prepared by silver perchlorate reaction.

Solvents were generally distilled and stored over molecular sieves, as traces of water (in the absence of acid) will convert the perchlorate derivative to μ-oxo-bridged dimer. Likewise, HCl in chlorinated solvents will displace the perchlorate ion. Accordingly, chloroform and methylene chloride were shaken with water, dried, distilled, and stored over activated 3A molecular sieves. Deuterated chloroform was also treated in this manner.

Nuclear magnetic resonance spectra were recorded in the pulsed Fourier transform mode using a Bruker HX-90E instrument. Proton and carbon-13 spectra were observed at 90 and 22.6 MHz using sweep widths up to 12 and 20 kHz, respectively. Temperature calibration was by the Van Geet thermometer.[21] Chemical shifts are reported with respect to internal Me$_4$Si. Downfield shifts are given a positive sign.

Results

Variable-Temperature Measurements. The ^{1}H NMR spectrum of FeTPP·ClO$_4$ in CDCl$_3$ is anomalous when compared with those of known high-spin five-coordinate,[22] high-spin six-coordinate,[23] or low-spin[24] iron(III) porphyrin complexes. Figure 1c illustrates the room temperature spectral result where phenyl and pyrrole proton resonances are observed in the 6-13-ppm region. Pyrrole signals in the 70-80- and −15-ppm region would be apparent for respective high-spin[22,23] and low-spin[24] species. Variable-temperature spectra

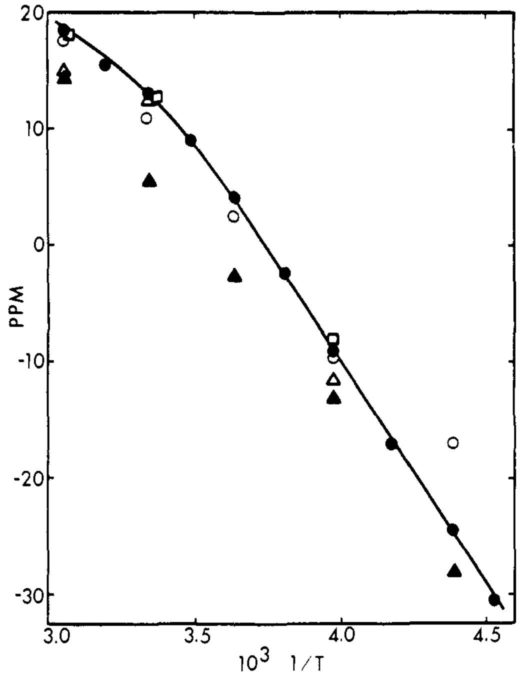

Figure 2. Pyrrole proton resonance values for $FeTPP\cdot ClO_4$ at variable solution conditions, $CDCl_3$ solvent, 0.01 M: (●) pure $CDCl_3$; (○) 0.02 M THF; (▲) $CDCl_3$ saturated with 1 M $HClO_4$ in D_2O; (△) 0.1 M Bu_4NClO_4; (□) 0.001 M $FeTPP\cdot ClO_4$ in pure $CDCl_3$. The curve is drawn only to illustrate deviation from linearity.

of $FeTPP\cdot ClO_4$ in Figure 1 show unexpected behavior in that the pyrrole proton signal is found in a downfield position at near ambient temperature (Figures 1c and 1d) and in an upfield position at low temperature (Figures 1a and 1b). Unequivocal assignment of the pyrrole and phenyl resonances was made by examination of *m*-methyl, *p*-methyl, and perdeuterated phenyl derivatives. The curve in Figure 2 summarizes variable-temperature dependence of the $FeTPP\cdot ClO_4$ pyrrole proton resonance. This complex temperature dependence is paralleled in part for phenyl proton resonances, as may be seen in Figure 3. A magnetically simple molecule is expected to follow Curie-law behavior in that a plot of δ vs. $1/T$ is linear with an intercept equal to the resonance in an analogous diamagnetic complex. The meta-proton resonance clearly shows nonlinear behavior. Although plots for ortho and para signals are reasonably linear, intercepts of 2.9 and 5.4 ppm are to be contrasted with values of 8.0 and 7.7 ppm, respectively, for diamagnetic nickel(II) complexes.[3]

Synthetic iron(III) porphyrin perchlorates with alkylpyrrole side chains also exhibited, in part, anomalous variable-temperature behavior. Both $FeOEP\cdot ClO_4$ and $FeETIO\cdot ClO_4$ were examined. The ^1H NMR spectrum of $FeETIO\cdot ClO_4$ in Figure 4a may be compared with that of the chloride analogue in Figure 4d. The downfield ring methyl shift and larger methylene group splitting for $FeETIO\cdot ClO_4$ should be noted. Methylene proton resonances are very broad at room temperature, but collapse into a singlet with addition of potential ligands (Figures 4c and 4d). Iron inversion through the porphyrin plane may serve to explain such behavior. This observation, as well as the possibility of hindered ethyl group rotation at low temperature,[22] accounts for Curie-plot intercepts of approximately 20 ppm. The largest Curie deviation occurs, however, for the meso-proton resonance (Figure 5). At room temperature the signal is upfield from Me_4Si (−11.4 ppm), and as temperature is lowered the resonance moves toward the Me_4Si reference. This shift is opposite that expected for normal Curie behavior, suggesting magnetic complexity as was observed for $FeTPP\cdot ClO_4$. Natural-derivative iron porphyrin

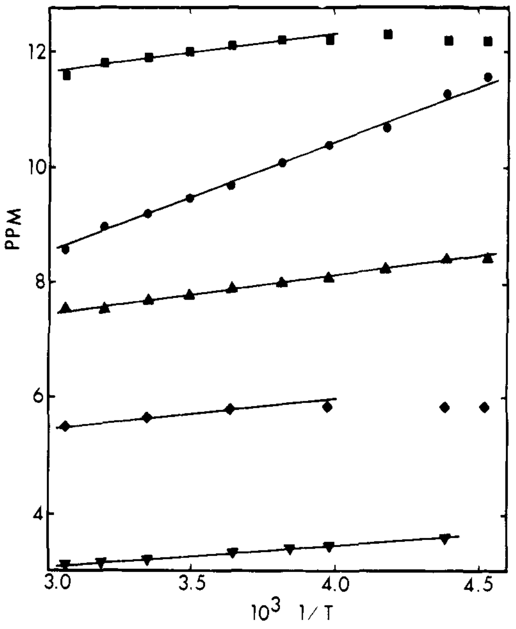

Figure 3. Curie plot for phenyl proton resonances, $CDCl_3$ solvent, 0.01 M: (■) *m*-H; (●) *o*-H; (▲) *p*-H of $FeTPP\cdot ClO_4$; (♦) *p*-CH_3 of $FeTPP(p\text{-}CH_3)\cdot ClO_4$; (▼) *m*-$CH_3$ of $FeTPP(m\text{-}CH_3)\cdot ClO_4$. Lines are drawn only to illustrate deviation from linearity.

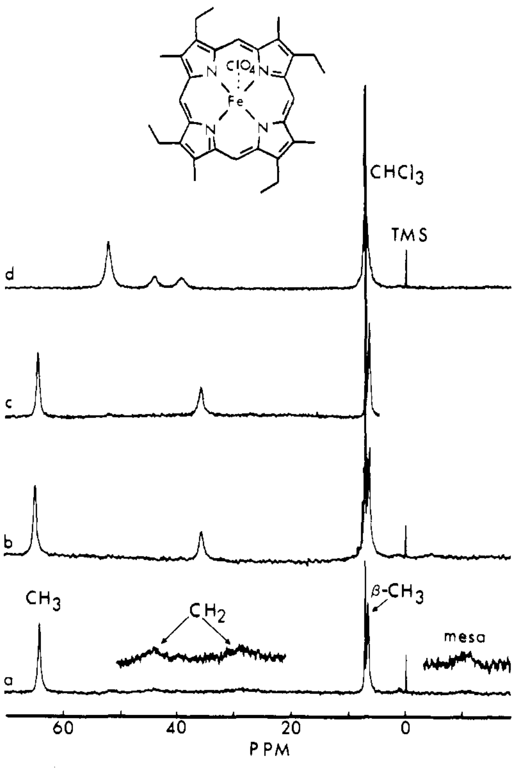

Figure 4. ^1H NMR spectra of $FeETIO\text{-}X$, $CDCl_3$ solvent, 0.005 M, 299 K: (a) $FeETIO\cdot ClO_4$ in pure $CDCl_3$; (b) $FeETIO\cdot ClO_4$ in $CDCl_3$ saturated with 1 M $HClO_4$ in D_2O; (c) $FeETIO\cdot ClO_4$ with 0.1 M Bu_4NClO_4; (d) $FeETIO\cdot Cl$.

perchlorates and $FeOEP\cdot ClO_4$ exhibited the same meso-proton behavior.

Magnetic vs. Chemical Equilibria. Experiments were carried out in an attempt to explain the anomalous shift patterns in terms of chemical equilibria. Results for $FeTPP\cdot ClO_4$ in chloroform solvent are summarized in Figure 2 and representative spectra are shown in Figure 6. Equilibria involving perchlorate coordination–dissociation are ruled out by addition

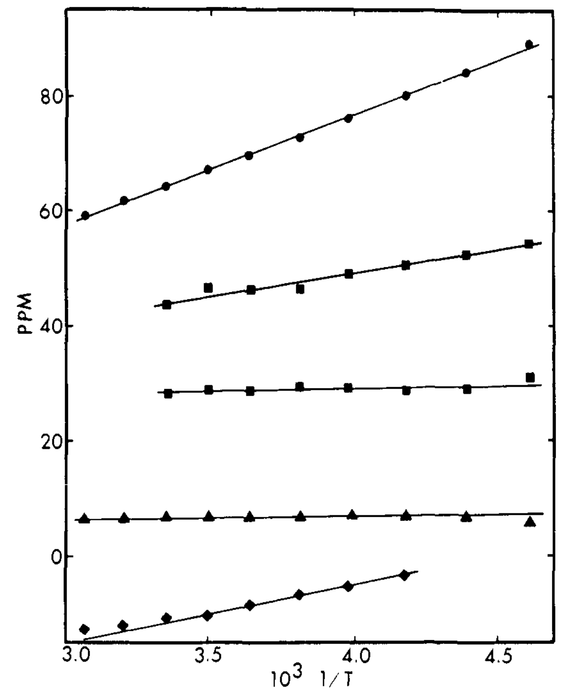

Figure 5. Curie plot for proton resonances of FeETIO·ClO_4, $CDCl_3$ solvent, 0.005 M: (●) ring methyl; (■) methylene; (▲) methyl proton of ethyl group; (◆) meso proton. Lines illustrate deviations from linearity.

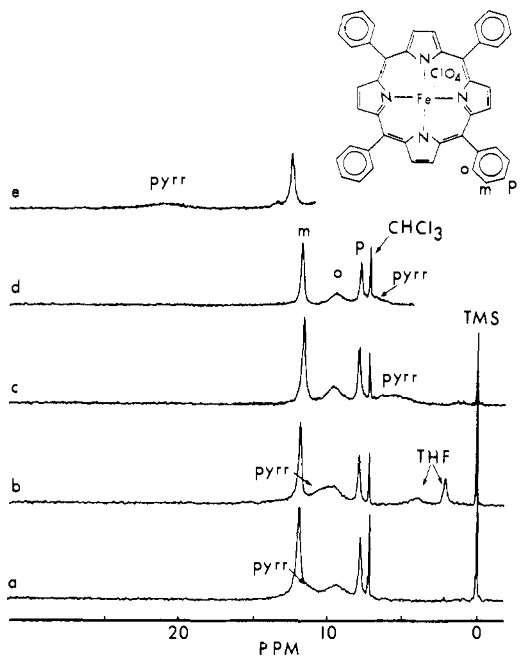

Figure 6. ^1H NMR spectra of FeTPP·ClO_4 for variable solution conditions. $CDCl_3$ solvent, 0.01 M FeTPP·ClO_4, 299 K: (a) pure $CDCl_3$; (b) 0.02 M THF; (c) $CDCl_3$ saturated with 1 M $HClO_4$ in D_2O; (d) 0.1 M Bu_4NClO_4; (e) 0.25 M trinitrobenzene.

of excess perchlorate ion as the tetra-*n*-butylammonium salt (Figure 6d) and by examination of solutions at variable concentrations. Although small spectral deviations are observed in Figure 2 when solutions contain excess perchlorate ion, no gross effect is observed. Identical results for FeTPP·ClO_4 solutions at 1 and 10 mM further refute perchlorate equilibria, as dissociation should be favored at the lower concentration. Lack of concentration dependence also eliminates dimer formation as explanation for anomalous variable-temperature results.

Possible equilibria involving water or other ligands in trace amounts were also evaluated. Saturation of a FeTPP·ClO_4 chloroform solution with 1.0 M aqueous perchloric acid produced a significant upfield bias in pyrrole resonances as compared with a dry chloroform solution. A representative spectrum is included in Figure 6c and variable-temperature data are shown in Figure 2.

As isolated from the FeTPP·Cl-$AgClO_4$ reaction the product contains two THF molecules of solvation. Examination of this material (before usual vacuum removal of THF) revealed THF resonances which were broadened and slightly shifted at room temperature (Figure 6b). This suggests rapid THF ligand exchange, but an equilibrium constant such that only a small fraction of THF molecules are bound. At low temperature THF peaks were broadened and shifted beyond observability, indicating direct interaction with the iron center by a larger mole fraction. Perturbation of pyrrole proton resonances (Figure 2) remains rather small except at the lowest temperature investigated. The same effects were noted for the previously isolated diaquo derivative $[FeTPP(H_2O)_2]ClO_4$·2THF, suggesting dissociation of water ligands in chloroform solvent.

Addition of aqueous perchloric acid or THF to iron porphyrin perchlorate solutions induces little change in resonance positions, but does produce significant line narrowing. An even more dramatic example is noted for FeETIO·ClO_4 in Figure 4b, where previously split methylene resonances are collapsed to a single, relatively sharp line. Figure 4c also indicates that excess perchlorate ion will produce methylene signal collapse.

Addition of a large excess (10% v/v) of dimethyl-d_6 sulfoxide to FeTPP·ClO_4 yielded a spectrum with the pyrrole proton resonance at 63 ppm consistent with bis ligation by Me_2SO as previously observed for the FeTPP·I derivative.[23]

Solvent Dependence. ^1H NMR spectra of FeTPP·ClO_4 were examined utilizing $CDCl_3$, CD_2Cl_2, C_6D_6, and toluene-d_8 solvents. Pyrrole resonance values were considerably different for chlorinated as compared with aromatic solvents. The anomalous temperature effect is noted for both solvent types, but the pyrrole signal was consistently further downfield for the aromatic solvents. Figure 7 summarizes temperature dependence for the pyrrole resonance. Phenyl-proton shifts for the various solvents were essentially equivalent.

Possible importance of π-donor-acceptor interactions in determining the pyrrole proton resonance position is illustrated in Figure 6e. Trinitrobenzene was titrated into a 0.01 M $CDCl_3$ solution of FeTPP·ClO_4. At 0.25 M the trinitrobenzene clearly induced a downfield shift in the pyrrole resonance. This observation is not surprising in view of the electronic structural perturbations previously recorded for aromatic group interaction with cobalt(II) porphyrins.[25,26]

Natural Derivative Iron Porphyrins. Perchlorate complexes of natural-derivative iron porphyrins are readily prepared by aqueous perchloric acid cleavage of the corresponding μ-oxo-bridged dimers. ^1H NMR spectra resemble very closely those for FeETIO·ClO_4 and FeOEP·ClO_4 with the meso-proton signals shifted upfield and all others in a downfield position. The spread of ring methyl resonances, shown in Figure 8 for three derivatives, appears to be a function of 2,4-group inductive power as previously observed for other iron porphyrin complexes.[27] Line widths are considerably smaller than for corresponding $S = 5/2$ chloride derivatives.[28] Very low solubility precluded extensive examination of natural-derivative iron porphyrin perchlorates.

Other Ligands. Cleavage of $(FeTPP)_2O$ by several acids was carried out in attempts to generate other intermediate-spin derivatives. Use of 1 equiv of aqueous HNO_3, HPF_6, and either 1 or 2 (proton) equiv of H_2SO_4 yielded species formulated as FeTPP·NO_3, FeTPP·PF_6, FeTPP·HSO_4, and FeTPP·$(SO_4)_{1/2}$, respectively. Spectra resemble those for high-spin

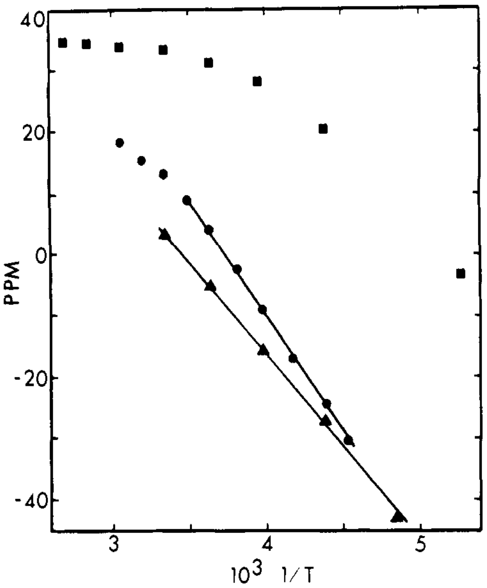

Figure 7. Solvent dependence for pyrrole proton resonance of FeTPP·ClO$_4$: (■) toluene-d_8; (●) CDCl$_3$; (▲) CD$_2$Cl$_2$. Lines are drawn only to illustrate deviations from linearity.

derivatives,[22] except that lines are unusually sharp and the pyrrole proton signal is observed at 73 ppm (26 °C) rather than at 79 ppm. Approximate Curie-law behavior is observed for these species. The meta- and ortho-proton signals are split, indicating an out-of-plane iron structure. Addition of Bu$_4$N-ClO$_4$ at 0.1 M to the FeTPP·(SO$_4$)$_{1/2}$ solution resulted in no spectral change. This indicates that perchlorate does not effectively compete with sulfate as a ligand or as an ion pair.

Attempts to prepare a BF$_4^-$ derivative by addition of aqueous HBF$_4$ to (FeTPP)$_2$O resulted in a mixture of species.[29,30] Work with this acid is complicated by inevitable contamination with fluoride ion and hydrolysis to BF$_3$OH$^-$. The major product exhibited a pyrrole proton peak at 80 ppm with a line width considerably smaller than that for the fluoride derivative. Interestingly, for one species of the product mixture, a resonance was detected upfield from Me$_4$Si (−11 ppm at 26 °C, −25 ppm at −22 °C) which diminished in intensity at lower temperature. The tetrafluoroborate ion does not effectively compete with perchlorate, as addition of Bu$_4$NBF$_4$ at 0.05 M resulted in no significant changes in the FeTPP·ClO$_4$ spectrum.

^{13}C NMR Measurements. ^{13}C NMR spectra were obtained for FeTPP·ClO$_4$ at various temperatures in CDCl$_3$ solution. Spectra are considerably different than those for known high-spin and low-spin iron(III) complexes,[31] in that line widths and isotropic shifts are smaller, and splitting is not observed in *o*- and *m*-phenyl resonances. Variable-temperature measurements reveal nonstandard Curie-law behavior such that, with the exception of the quaternary phenyl resonance, peaks shift further downfield as temperature increases. Such a shift pattern is consistent with increased high-spin character (based on spectra of FeTPP·Cl) at elevated temperature and serves to confirm ^{1}H NMR interpretations.

Solution Magnetic Measurements. Evans' magnetic measurements[32] were made for 0.01 M CDCl$_3$ solutions of FeTPP·ClO$_4$ employing Me$_4$Si as the reference. Solvent density changes with temperature were taken into account.[33] The previously measured susceptibility of mesoporphyrin and appropriate atomic constants were utilized to calculate the diamagnetic correction.[34] Solution magnetic moments for FeTPP·ClO$_4$ are listed in Table I. Corresponding solid-state measurements for FeTPP·ClO$_4$[13] and FeOEP·ClO$_4$[12] are also listed.

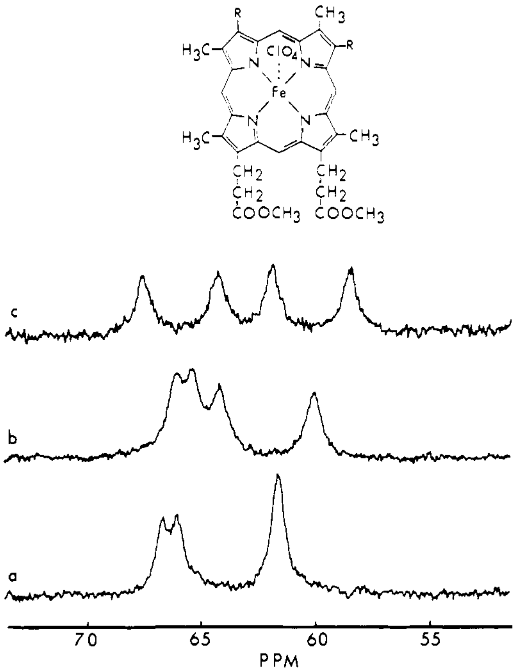

Figure 8. Ring methyl proton resonances for natural-derivative iron porphyrins, CDCl$_3$ solvent, 299 K: (a) iron(III) mesoporphyrin dimethyl ester perchlorate (R = -CH$_2$CH$_3$); (b) iron(III) protoporphyrin dimethyl ester perchlorate (R = -CH=CH$_2$); (c) iron(III) deuteroporphyrin dimethyl ester perchlorate (R = H).

Table I. Magnetic Susceptibilities

species	*T*, K	μ_{eff}
FeTPP·ClO$_4$, soln[a]	327	4.9
	313	4.8
	299	4.8
	275	4.7
	252	4.6
	232	4.6
	215	4.3
FeTPP·ClO$_4$, solid[b]	298	5.0
	40	4.1
FeOEP·ClO$_4$, solid[c]	295	4.8
	191	4.4
	127	4.2
	84	4.1

[a] CDCl$_3$ solvent, 0.01 M, solvent density corrected for temperature. [b] Reference 13. [c] Reference 12.

Discussion

Spin State Admixing. Observation of FeTPP·ClO$_4$ pyrrole proton resonances downfield at high temperature and upfield at low temperature could conceivably be explained by either chemical equilibria or magnetic complexity. However, lack of sizable NMR spectral change suggests no perturbation of equilibria upon addition of species reasonably involved (perchlorate ion, water, or THF). Small isotropic changes are indeed apparent (and expected) for water-saturated CDCl$_3$ solutions and THF-containing solutions as a consequence of ligation or perchlorate solvation. An equilibrium process which cannot be easily distinguished is one involving coordinated and tightly ion paired perchlorate ion. However, this possibility is disfavored by correspondence with solid-state magnetic measurements and the solution infrared spectrum as discussed below.

Solution results are fundamentally compatible with solid-state findings for which a $S = 3/2$, $S = 5/2$ quantum mechanically admixed spin state is invoked.[12,13] Magnetic moments are comparable (Table I) for FeTPP·ClO$_4$ in the solid state and in CDCl$_3$ solution. More importantly, the trends in temperature dependence of magnetic moments are essentially parallel. For quantum-mechanical admixing, the relative contribution of each unperturbed spin state to the five resulting Kramer's doublets is determined by the ratio of a crystal field parameter and the spin-orbit coupling constant. Assuming that the unperturbed quartet state is lower in energy than the sextet, the two lowest energy Kramer's doublets exhibit relatively more $S = 3/2$ character and the two highest energy doublets relatively more $S = 5/2$ character. The intermediate energy Kramer's doublet has total sextet character. Thus, the theoretical model of Maltempo[9] dictates temperature dependence of $\mu_{\rm eff}$ as a consequence of thermal population of Kramer's doublets, and not as result of an equilibrium between spin states.

Thermal population of Kramer's doublets also serves to explain the unusual NMR isotropic shift patterns. Higher temperatures effectively favor increased $S = 5/2$ character, whereas lower temperature enhance the $S = 3/2$ components. At near ambient temperatures and above, the pyrrole proton signal of FeTPP·ClO$_4$ moves in a downfield direction as expected for an $S = 5/2$ species. Movement of the FeETIO·ClO$_4$ meso proton in an upfield direction with increased temperature follows from the known −40-ppm resonance for $S = 5/2$ derivatives. Downfield ring methylene signals are consistent with a σ-spin delocalization mechanism involving a singly occupied MO of $d_{x^2-y^2}$ origin,[3,15] or π-spin delocalization from singly populated d_{xz} and/or d_{yz} orbitals. These ring functional groups are thus not very sensitive to the relative spin contributions.

Formal assignment of a vacant $d_{x^2-y^2}$ orbital and singly occupied d_{xz} and/or d_{yz} orbitals for the $S = 3/2$ state[9,12-14] is entirely consistent with observed low-temperature NMR shifts. Thus, an upfield pyrrole proton signal and downfield methyl/methylene resonances are suggestive of a π-spin delocalization mechanism involving orbitals (d_{xz}, d_{yz}) of appropriate symmetry for overlap with the porphyrin π system.[3,15] This argument assumes that observed shifts are largely contact in origin (vide infra). A very small upfield shift for the meso proton at low temperature suggests a node in the relevant MO at this position. The highest occupied porphyrin π MO (3e) does indeed exhibit a node at the meso carbon and large π spin density at the pyrrole position.[15] Thus, the low-temperature, predominantly $S = 3/2$ shifts are consistent with unpaired spin transfer via porphyrin → Fe charge transfer. This spin delocalization mechanism indicates a formal ground-state configuration for the $S = 3/2$ species of $(d_{xy})^2\,(d_{z^2})^1\,(d_{xz}, d_{yz})^2\,(d_{x^2-y^2})^0$.[3] The variable spread of natural porphyrin ring methyl resonances (Figure 8) further supports a dominant π-spin delocalization mechanism.[27]

The resonance positions for pyrrole and meso protons seemingly reflect the net contribution of quartet and sextet states. Attempts were made to quantitate variable-temperature shifts with relative population of Kramer's doublets by evaluation of a simple two-term partition function. Fits were unsatisfactory as might be expected considering possible involvement of more than two Kramer's doublets. Inclusion of more terms (and variables) in the partition function[35] seems presumptive in the absence of other detailed physical measurements.

Other formulations should also be considered. Thermal equilibrium (rather than admixture) of $S = 3/2$ and $S = 5/2$ states cannot be distinguished from thermal population of Kramer's doublets by NMR measurements alone. Dynamics of spin equilibrium are likely to be much faster than the NMR time scale, such that resonances for "pure" spin state species would not be resolved. Visible-UV and infrared measurements made by Reed et al.[14] appear to show the presence of only one solution species, although the low resolution of these two techniques makes results less than diagnostic. Evidence which on the contrary supports a spin equilibrium formulation is apparent in two sets of Mössbauer and ESR peaks for frozen toluene solutions of FeTPP·ClO$_4$.[14] One set appears to be for a high-spin species and the other for spin-admixed material. Whether these two sets of signals (which are not entirely reproducible) are representative of bulk liquid solutions or whether they appear as a result of imperfect glass formation and freezing problems has not been discerned.[14]

Spin equilibrium between sextet and doublet states is disfavored on theoretical grounds and is not supported by NMR results. Although the low-temperature proton isotropic shifts resemble those for low-spin iron(III) porphyrins in direction, the magnitudes of shifts are considerably greater for perchlorate species. Signal line widths for perchlorate derivatives are several times greater than those observed for low-spin iron(III). Low-temperature ^{13}C NMR spectra of FeTPP·ClO$_4$ are not at all like those of low-spin bis(imidazole)iron(III).[36] Furthermore, generation of low-spin iron(III) porphyrins normally requires two moderately strong-field ligands, and it is not apparent how the weak perchlorate could provide adequate spin pairing energy.

Examination of other weak anionic ligands has not provided a definitive answer as to why perchlorate ion induces spin-state admixture. Several FeTPP·X derivatives (where X = SO_4^{2-}, HSO_4^-, NO_3^-, PF_6^-) exhibit a pyrrole proton signal at 73 ppm rather than at the usual 79-ppm position. Diminished magnetic moments for the solid PF_6^- derivatives led Reed et al. to postulate partial $S = 3/2$ character for this and other complexes.[14] Our 73-ppm pyrrole peak provides only very tentative solution evidence for this formulation. More extensive examination of various anion species, to include the "pure" $S = 3/2$ FeTPP-tricyanomethanide complex,[37] is suggested by these preliminary observations.

Crystallographic studies of FeTPP·ClO$_4$[13,14] and ZnTPP·ClO$_4$[39] prove that perchlorate ion is coordinated in the solid state. Conclusions regarding the solution structure are necessarily less definitive, but NMR results are consistent with either coordination or tight ion pair formation. Perhaps the strongest support for perchlorate coordination appears in the parallel magnetic moments noted for solid state and solution (Table I). A solution infrared measurement is further consistent with perchlorate coordination.[14]

Magnetic Anisotropy. Detailed analysis of proton isotropic shifts can in favorable cases allow determination of metal ion magnetic anisotropy.[38] The isotropic shift is composed of both contact and dipolar terms, which relate through-bond and through-space electron-nuclear interactions, respectively. The magnitude of the dipolar shift is determined by magnetic anisotropy and the spatial arrangement of the nucleus with respect to the metal center. Thus, for axial symmetry, with downfield shifts taken as positive, the dipolar shift is defined by

$$(\Delta H/H)_{\rm dip} = \frac{1}{3N}(\chi_\parallel - \chi_\perp)\frac{3\cos^2\theta - 1}{r^3} \qquad (1)$$

where r is the length of the metal-nuclear vector and θ is the angle between the z axis and the vector. Direct calculation of molecular susceptibilities in TPP derivatives has been possible for those cases where phenyl proton isotropic shifts are totally dipolar in character.[3,15,24] Phenyl proton and phenyl methyl proton resonances in perchlorate derivatives are shifted in a downfield direction at low temperature (Figures 1 and 3). At 228 K isotropic shift values (using diamagnetic nickel(II) references[3]) follow: o-H, 3.3; m-H, 4.5; p-H, 0.73; m-CH$_3$, 1.04; p-CH$_3$, 3.2 ppm. Respective values of $(3\cos^2\theta - 1)/r^3$ are −3.3, −1.6, −1.5, −1.05, and -1.1×10^{21} cm^{-3}. Thus, the observed isotropic shifts do not parallel the calculated geometric factors. Deviation from axial molecular symmetry could account for the discrepancy, but seems unlikely for FeTPP derivatives in solution. Although $S = 3/2$ character is predominant at 228 K, a fractional $S = 5/2$ contribution is expected to perturb the phenyl isotropic shift values to a small extent. Spin density in the phenyl ring delocalized through a π MO will readily account for nonparallel isotropic shifts and geometric factors. If 2- and 1-ppm contributions are assumed for phenyl and methyl protons (sign of the π contribution alternates around the ring), "corrected" isotropic shifts are 5.3, 2.5, 2.7, 2.0, and 2.2 ppm, respectively. These values do indeed parallel geometric factors, suggesting that they approximate dipolar shifts for the predominantly $S = 3/2$ state. At 228 K based on this assumption, estimates of the downfield dipolar term for other resonances follow: pyrrole H, 11; ring methyl, 7; meso H, 18 ppm. The validity of the approach is certainly not proven, but the overall downfield bias of phenyl resonances does strongly suggest $\chi_{\perp} > \chi_{\parallel}$ for intermediate-spin iron(III) porphyrins.

Solvent Dependence. Dramatic differences are noted in ^{1}H NMR spectra of FeTPP·ClO_4 in chlorinated vs. aromatic solvents (Figure 7). Our results for toluene as a solvent are in complete agreement with the room temperature ^{1}H NMR spectrum reported by Reed et al.[14] The statement by these workers that FeTPP·ClO_4 in solution appears to be predominantly $S = 5/2$ is merely a consequence of their choosing toluene as a solvent. Solvent effects are expected considering the sensitivity of crystal field and spin-orbit coupling parameters in determining relative spin state contributions.[9] Differences in perchlorate solvation or π–π porphyrin–solvent interactions could play a role. Experiments with the π-electron acceptor trinitrobenzene reveal considerable increase in the amount of $S = 5/2$ character. Electronic structural perturbations have also been noted for aromatic group interaction with cobalt(II) porphyrins.[26] A π-acceptor complex involving pyrrole groups could lower the relative energy of the $d_{x^2-y^2}$ orbital and thus favor the $S = 5/2$ state.[26] Alternatively, a π-complexed aromatic solvent molecule as noted for the *m*-xylene solvate of FeTPP·ClO_4[14] could serve as a "pseudoligand" and raise the relative energy of d_{z^2}. Effects of aromatic amino acid interaction with the prosthetic group of cytochrome *c′* should be considered in explaining the relative spin state contributions observed for protein obtained from different sources.[9] Variable-temperature NMR studies should provide a particularly powerful physical method for further elucidation of cytochrome *c′* electronic structure.

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References and Notes

(1) Smith, K. M., Ed. "Porphyrins and Metalloporphyrins"; Elsevier: Amsterdam, 1975.
(2) Collman, J. P.; Hoard, J. L.; Kim, N.; Lang, G.; Reed, C. A. *J. Am. Chem. Soc.* **1975**, *97*, 2676–2681.
(3) Goff, H.; La Mar, G. N.; Reed, C. A. *J. Am. Chem. Soc.* **1977**, *99*, 3641–3646.
(4) Lang, G.; Spartalian, K.; Reed, C. A.; Collman, J. P. *J. Chem. Phys.* **1978**, *69*, 5424–5427.
(5) Zerner, M.; Gouterman, M.; Kobayashi, H. *Theor. Chim. Acta* **1966**, *6*, 363–400.
(6) Barraclough, C. G.; Martin, R. L.; Mitra, S.; Sherwood, R. C. *J. Chem. Phys.* **1970**, *53*, 1643–1648.
(7) Riley, D. P.; Stone, J. A.; Busch, D. H. *J. Am. Chem. Soc.* **1977**, *99*, 767–777.
(8) Maltempo, M. M.; Moss, T. H.; Cusanovich, M. A. *Biochim. Biophys. Acta* **1974**, *342*, 290–305.
(9) Maltempo, M. M. *J. Chem. Phys.* **1974**, *61*, 2540–2547.
(10) (a) Wickman, H. H.; Trozzolo, A. M.; Williams, H. J.; Hull, G. W.; Merritt, R. R. *Phys. Rev.* **1967**, *155*, 563–566. (b) Wickman, H. H.; Trozzolo, A. M. *Inorg. Chem.* **1968**, *7*, 63–68. (c) Epstein, L. M.; Straub, D K. *Ibiu.* **1969**, *8*, 560–562.
(11) Koch, S.; Holm, R. H.; Frankel, R. B. *J. Am. Chem. Soc.* **1975**, *97*, 6714–6723.
(12) Dolphin, D. H.; Sams, J. R.; Tsin, T. B. *Inorg. Chem.* **1977**, *16*, 711–713.
(13) Kastner, M. E.; Scheidt, W. R.; Mashiko, T.; Reed, C. A. *J. Am. Chem. Soc.* **1978**, *100*, 666–667.
(14) Reed, C. A.; Mashiko, T.; Bentley, S. P.; Kastner, M. A.; Scheidt, W. R.; Spartalian, K.; Lang, G. *J. Am. Chem. Soc.* **1979**, *101*, 2948–2958.
(15) La Mar, G. N.; Walker, F. A. In "The Porphyrins", Dolphin, D., Ed.; Academic Press: New York, in press.
(16) Adler, A. D.; Longo, F. R.; Finarelli, J. D.; Goldmacher, J.; Assour, J.; Korsakoff, L. *J. Org. Chem.* **1967**, *32*, 476.
(17) Adler, A. D.; Longo, F. R.; Varadi, V. *Inorg. Synth.* **1976**, *16*, 213–220.
(18) Fuhrhop, J.-H.; Smith, K. M. In "Porphyrins and Metalloporphyrins", Smith, K. M., Ed.; Elsevier: Amsterdam, 1975; pp 765–769.
(19) O'Keeffe, D. H.; Barlow, C. H.; Smythe, G. A.; Fuchsman, W. H.; Moss, T. H.; Lilienthal, H. R.; Caughey, W. S. *Bioinorg. Chem.* **1975**, *5*, 125–147.
(20) Mashiko, T.; Kastner, M. E.; Spartalian, K.; Scheidt, W. R.; Reed, C. A. *J. Am. Chem. Soc.* **1978**, *100*, 6354–6362.
(21) Van Geet, A. L. *Anal. Chem.* **1968**, *40*, 2227–2229.
(22) Walker, F. A.; La Mar, G. N. *Ann. N.Y. Acad. Sci.* **1973**, *206*, 328–348.
(23) Zobrist, M.; La Mar, G. N. *J. Am. Chem. Soc.* **1978**, *100*, 1944–1946.
(24) (a) La Mar, G. N.; Walker, F. A. *J. Am. Chem. Soc.* **1973**, *95*, 1782–1790. (b) Satterlee, J.D.; La Mar, G. N.; Frye, J. S. *Ibid.* **1976**, *98*, 7275–7282.
(25) Hill, H. A. O.; Sadler, P. J.; Williams, R. J. P. *J. Chem. Soc., Dalton Trans.* **1973**, 1663–1667.
(26) (a) Fulton, G. P.; La Mar, G. N. *J. Am. Chem. Soc.* **1976**, *98*, 2119–2124. (b) *Ibid.* **1976**, *98*, 2124–2128.
(27) La Mar, G. N.; Viscio, D. B.; Smith, K. M.; Caughey, W. S.; Smith, M. L. *J. Am. Chem. Soc.* **1978**, *100*, 8085–8092.
(28) (a) Caughey, W. S.; Johnson, L. F. *Chem. Commun.* **1969**, 1362–1363. (b) Kurland, R. J.; Little, R. G.; Davis, D. G.; Ho, C. *Biochemistry* **1971**, *10*, 2237–2246.
(29) Cohen, I. A.; Summerville, D. A.; Su, S. R. *J. Am. Chem. Soc.* **1976**, *98*, 5813–5816.
(30) Snyder, R. V.; La Mar, G. N. *J. Am. Chem. Soc.* **1976**, *98*, 4419–4424.
(31) (a) Goff, H. *Biochim. Biophys. Acta* **1978**, *542*, 348–355. (b) Shimomura, E.; Phillippi, M.; Goff, H., unpublished results.
(32) Evans, D. F. *J. Chem. Soc.* **1959**, 2003–2005.
(33) Washburn, E. W., Ed. "International Critical Tables," Vol. 3; McGraw-Hill: New York, 1928; p 27.
(34) Havermann, R.; Haberditzl, W.; Grzegorzewski, P. *Z. Phys. Chem.* (*Leipzig*) **1961**, *217*, 91–109.
(35) Golding, R. M.; Tennant, W. C.; Bailey, J. P. M.; Hudson, A. *J. Chem. Phys.* **1968**, *48*, 764–771.
(36) Goff, H. *J. Chem. Soc., Chem. Commun.* **1978**, 777–778.
(37) Summerville, D. A.; Cohen, I. A.; Hatano, K.; Scheidt, W. R. *Inorg. Chem.* **1978**, *17*, 2906–2910.
(38) Horrocks, W. D. In "NMR of Paramagnetic Molecules", La Mar, G. N., Horrocks, W. D., Holm, R. H., Eds.; Academic Press: New York, 1973; pp 127–177.
(39) Spaulding, L. D.; Eller, P. G.; Bertrand, J. A.; Felton, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 982–987.