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Oxygen-17 Nuclear Magnetic Resonance Study of Water Exchange on Water-Soluble Iron(III) Porphyrins

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Kinetic parameters for water exchange with the Fe(III)-TMpyP and Fe(III)-TPPS monomeric complexes at low pH were measured. For the former, k_1 (per H₂O) at 25 °C is 7.8×10^5 s⁻¹, $\Delta H^\ddagger = 13.8$ kcal mol⁻¹, and $\Delta S^\ddagger = 14.7$ cal mol⁻¹ deg⁻¹; for the latter, k_1 at 25 °C is 1.4×10^7 s⁻¹, $\Delta H^\ddagger = 13.7$ kcal mol⁻¹, and $\Delta S^\ddagger = 20.2$ cal mol⁻¹ deg⁻¹. The exchange rate is enhanced by a factor of 10^4 - 10^5 over simple Fe³⁺(aq) at 25 °C, ΔH^\ddagger decreasing from 18 to 14 kcal mol⁻¹. In complex solutions of pH ≥ 5 the line broadening and shift of the ¹⁷O resonance in H₂O vanishes. The number of coordinated waters could not be determined but appears to be the same for both complexes. NMR shifts due to rapid exchange of water in the outer sphere were observed. Magnetic susceptibility measurements over a 5-75 °C range gave essentially high-spin values (5.9 μ_B) at 5-25 °C, which seemed to fall of slightly with increasing temperature. Difficulty was encountered in explaining theoretically the relaxation of oxygen-17 in water molecules bound to the Fe(III) in the complexes.

Introduction

The water-soluble porphyrins *meso*-tetrakis(*N*-methyl-4-pyridyl)porphine (H₂TMpyP) and *meso*-tetrakis(*p*-sulfonatophenyl)porphine (H₂TPPS) were synthesized by Fleischer¹ and by Hambright and Fleischer² some years ago. They are considered to be model compounds for aqueous porphyrin chemistry and have been considerably studied in the form of various metal complexes. In the aqueous solutions the axial interaction of water with the metal is of fundamental importance in relation to both redox and substitution reactions. We wish to report the results of an oxygen-17 NMR study of the Fe(III) complexes of the above ligands which was undertaken to obtain data on the kinetics of water exchange, in the hope that we might gain information on the aqueous solution structures.

Experimental Section

Materials. The salt Li₃Fe^{III}TPPS·9H₂O was kindly supplied by Dr. E. B. Fleischer. Na₄TPPS·12H₂O was purchased from Strem Chemicals, Inc., and used without further purification. TpyP was also purchased from Strem Chemicals. It was converted to the *p*-toluenesulfonate (tosylate) salt by literature methods.³ The Fe^{III}TMpyP tosylate was prepared from the perchlorate² by dissolving in 1:1 DMF-H₂O and passing the solution through a Bio-Rad AG3X4A column in the tosylate form. The resulting solution was evaporated to dryness at 25 °C under reduced pressure. The solid was washed with 50 mL of AR acetone and dissolved in absolute ethanol. Anhydrous diethyl ether was added, forming a tarry purple precipitate. This was filtered and washed with AR acetone, leaving a mixture of brown and purple solids. After 3 h of drying in a drying pistol at 60 °C, a brown solid remained. Elemental analyses were done by Galbraith Laboratories and were consistent with four waters of hydration. Anal. Calcd: C, 57.14; N, 6.75; H, 4.80; S, 9.65; Fe, 3.36. Found: C, 56.91; N, 6.56; H, 4.74; S, 10.03; Fe, 3.12. The iron analysis was done by using Drabkin's method.⁴ The UV and visible spectra were consistent with those obtained by Pasternak et al.⁵ Proton NMR spectra were consistent with those of Goff and Morgan.⁶ No evidence of decomposition of the solutions was found under the conditions used.

Oxygen-17-enriched water obtained originally from the Mound Laboratory, Monsanto Research Corp., was used at ca. 2-4% ¹⁷O. It was purified by distillations from sulfuric acid and then from sodium hydroxide.

Equipment. NMR spectra for characterization and μ_{eff} determinations were taken on JEOL 100-MHz and Bruker WH-90 FT instruments. The oxygen-17 data were obtained on the wide-line spectrometer developed here.⁷ Measurements of pH were done on a Beckman Research Model pH meter and UV-visible spectra taken on a Cary Model 14 instrument.

Sample Preparations and Measurements. Spherical Pyrex NMR bulbs were used for the ¹⁷O NMR measurements. The FeTMpyP samples were prepared by combining weighed amounts of salt, tosylic acid, and water. The resulting solution was transferred to the sample tube which was sealed by using a torch. The pH was determined on identical samples prepared in ordinary water. The FeTPPS samples were made in similar fashion, but perchloric acid was used for pH adjustments. Blank or reference solutions contained the metal-free ligands under similar conditions except that the blank TPPS solution was used at pH 10 to prevent the precipitation found at low pH. Sample temperatures were controlled and known to ± 0.1 °C by being thermostated in the probe by a circulating silicone oil system. Dispersion signals were observed at frequencies from 13.1 to 5.75 MHz. Line-width measurements at several rf levels were made and extrapolated to zero rf level by using a weighted linear least-squares program. A minimum of four measurements of both widths and shifts were made at each temperature for samples and blank. Percentage errors in line widths and shifts were ca. 2-5% on the average.

Treatment of Data and Results

Magnetic Susceptibility. The Evans method⁸ was employed to obtain μ_{eff} in D₂O, with 2% *tert*-butyl alcohol as reference, over the temperature range 5-75 °C. For Fe^{III}TMpyP at pH 0.6 and 0.034 M, μ_{eff} (in Bohr magnetons) was found to decrease slightly from 6.0 at 5 °C to 5.7 at 75 °C. The trend, while only slight, may be real as NiCl₂ solutions showed no such effect. More study would be needed to decide if a real effect exists. The μ_{eff} values at the lower temperatures are in agreement with the literature.⁹ For Fe^{III}TPPS at 0.034 M and a pH range of 1.5-2.8 μ_{eff} varied from 5.7 at 5 °C to 5.2 at 75 °C, independent of pH in this range. Again further study might be of interest here. The TPPS values found are a bit lower than the literature values, 5.9¹⁰ and 6.0,⁹ but there are enough sources of error in such measurements to cause us to refrain from speculation. The μ_{eff} values are based on the Fe analyses of the compounds.

Oxygen-17 NMR. The methods and equations pioneered by Swift and Connick¹¹ and modified by us¹² were used in these

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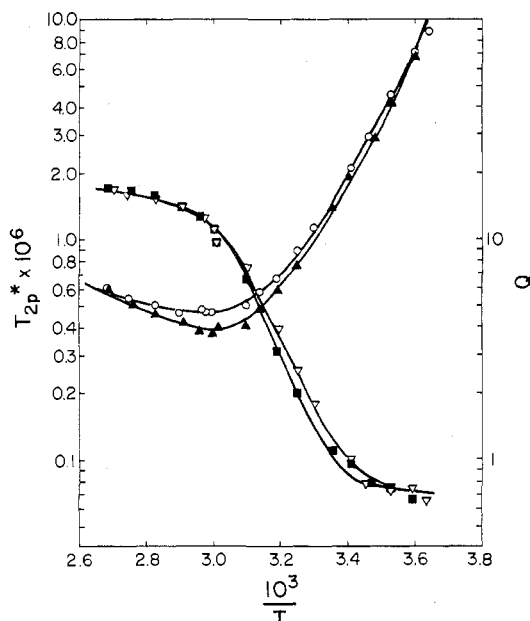


Figure 1. T^*_{2p} and Q for $H_2^{17}O$ in the FeTMpyP system: \circ , T^*_{2p} at 5.748 MHz; \blacktriangle , T^*_{2p} at 11.493 MHz; ∇ , Q at 5.748 MHz; \blacksquare , Q at 11.493 MHz.

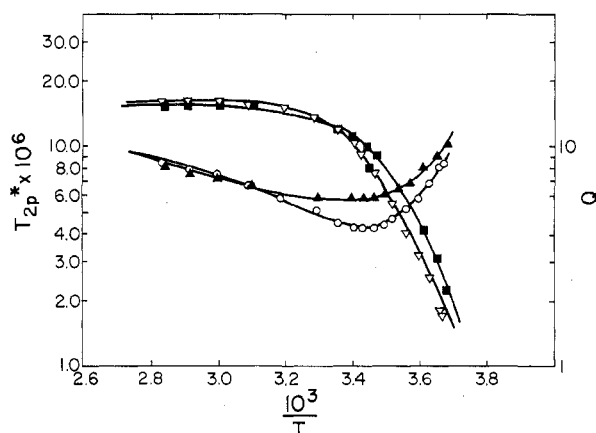


Figure 2. T^*_{2p} and Q for $H_2^{17}O$ in the FeTPPS system: \circ , T^*_{2p} at 5.748 MHz; \blacktriangle , T^*_{2p} at 11.493 MHz; ∇ , Q at 5.748 MHz; \blacksquare , Q at 11.493 MHz.

studies. The quantities T^*_{2p} and Q are defined as $T^*_{2p} \propto m(\text{FeP})/m(\text{H}_2\text{O})\pi(\Delta\nu)$ and $Q \propto m(\text{H}_2\text{O})TS/m(\text{FeP})$, where $m(\text{FeP})$ is the molality of the metalloporphyrin, $m(\text{H}_2\text{O})$ is the molality of the solvent water, $\Delta\nu$ is the increase in line width (in Hz) over the blank, T is the absolute temperature, and S is the relative frequency shift $\Delta\omega/\omega_0$. Representative data are shown by the points for two fields in Figures 1 and 2. The solid lines in these figures are generated from parameters found by using a nonlinear least-squares computer fit.¹³ The expressions used were as follows: $T^*_{2p} = \tau_M[(1/T_{2M} + 1/\tau_M)^2 + \Delta\omega_M^2]/[1/T_{2M}(1/\tau_M + 1/T_{2M}) + \Delta\omega_M^2]$; $Q = T(\Delta\omega_M/\omega_0)[\tau_M^2(1/T_{2M} + 1/\tau_M)^2 + \Delta\omega_M^2]$. For purposes of calculation it was assumed that one water molecule was bound to the iron. Here, τ_M is the mean lifetime for exchange of a bound H_2O , T_{2M} is the relaxation time for oxygen-17 in a bound H_2O , $\Delta\omega_M$ is the difference (in rad s^{-1}) in resonance frequencies for a bound H_2O and the solvent, and T is the Kelvin temperature. The Q values have been corrected by subtraction of an outer-sphere shift contribution. This shift,

Table I. Average Parameters Obtained from Computer Fitting

	FeTMpyP	FeTPPS
$k_1(1/\tau_M)$ at 25 °C, s^{-1}	$(7.8 \pm 0.1) \times 10^5$	$(1.4 \pm 0.01) \times 10^7$
ΔH^\ddagger , kcal mol ⁻¹	13.8 ± 0.1	13.7 ± 0.1
ΔS^\ddagger , cal mol ⁻¹ deg ⁻¹	14.7 ± 0.3	20.2 ± 0.3
Q (outer sphere), K	0.72 ± 0.02	0.75 ± 0.05
T_{2M} at 100 °C, s	$(0.62 \pm 0.01) \times 10^{-6}$	$(0.96 \pm 0.02) \times 10^{-6}$
E_a , kcal mol ⁻¹	-2.8 ± 0.1	-2.2 ± 0.1
A/h , MHz	24.9 ± 0.5	22.6 ± 0.5

as well as the inner-sphere shift, was assumed to obey Curie's law. The temperature dependencies of τ_M and T_{2M} were assumed to follow the usual Eyring kinetic expression. The Q and T^*_{2p} data, weighted by their standard deviations, at all concentrations and at the three fields were fitted together to a single set of parameters. No field dependence of T_{2M} was found, the variations in $\Delta\omega_M^2$ being sufficient to account for the field effects. If the data are fitted separately, at each field the individual fits are "better" (though not significantly so). Averaging the individual fits gives the same values as found when all data are taken together. The solid lines in the figures are calculated from the final parameters. The fits are very satisfactory. The average parameters obtained for pH 1.1, $m(\text{FeTMpyP}) = 0.0352$ and 0.0180 , and for pH 2.9, $m(\text{FeTPPS}) = 0.0298$, are given in Table I. The errors shown are standard deviations given by the computer program. Here $k_1(1/\tau_M)$ at 25 °C is the specific rate constant for exchange of one water molecule, with corresponding ΔH^\ddagger and ΔS^\ddagger values, E_a refers to the temperature dependence of T_{2M} , and A/h is calculated from the limiting shifts at high temperatures (corrected for Q outer sphere) and is the scalar-coupling constant per H_2O molecule obtained by using the Bloembergen equation¹⁴ and by using the spin-only moment for Fe(III) ($5.9 \mu_B$).

Some experiments were done at higher pH. For the TMpyP case, pH 4 gave no change from the lower pH data, but at pH 5.5 the line broadening and shifts were gone. For the TPPS complex, pH 3 caused no changes in the NMR data, but at pH 7 the shift and line broadening vanished. Detailed studies have not been made of these effects. Presumably oligomeric species or hydrolyzed (OH^- complexes) ones are formed at the higher pH.⁵

Discussion

The porphine ligands studied here exert a large labilizing effect in the Fe(III) systems as has been found in Co(III), Cr(III),¹⁵ and Fe(III)¹⁶ substitution studies. The $k_1(25^\circ\text{C})$ for $\text{Fe}(\text{H}_2\text{O})_6^{3+}$ is only 190 s^{-1} , and ΔH^\ddagger is 18 kcal mol^{-1} .¹⁷ The TPPS ligand is somewhat more labilizing than TMpyP (a factor of ca. 20 at room temperature), reflecting perhaps more charge donation to the iron. The relatively small difference appears, however, to be an entropic effect on the face of it. Other parameters are similar for the two systems. Unfortunately we do not as yet have a calibration on the limiting inner-sphere shift per water molecule¹² so we cannot say how many waters are bound at this time. It appears though to be the same number for each system studied here.

The outer-sphere Q of ca. 0.7 can be compared to that of $\text{Fe}^{3+}(\text{aq})$ where it is 2,¹⁷ suggesting fewer outer-sphere water molecules near the Fe center in the porphyrins as might be expected from the ligand structure. For $\text{Cr}^{3+}(\text{aq})$ a value of 1.1 was calculated from data given by Alei.¹⁸ The outer-

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sphere shift is expected to be proportional to the effective magnetic moment, other things being equal.

The apparent variation of μ_{eff} with temperature was incorporated in the data-fitting process and showed no significant change in the kinetic parameters for FeTMpyP, while for FeTPPS about a 30% change in k_1 was found. The data in the latter case do not define k_1 as well, due mainly to the faster rate of exchange which has less influence on the relaxation of H_2^{17}O .¹¹

The T_{2M} results are interesting in that the lack of field dependence and the negative E_a are not consistent with only the Bloembergen-Morgan model¹⁹ for electron-spin relaxation. We considered other possible relaxation mechanisms including

nuclear quadrupole interaction and anisotropic g factors but could not draw any firm conclusions from the data at hand. In general, physically unrealistic parameters were required. This problem does not affect the kinetic conclusions seriously but deserves more study in its own right.

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Magnetic Properties of a (Tetraphenylporphyrin)iron(III) Thiolate: Fe(TPP)(SC₆H₅)(HSC₆H₅)

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Mössbauer, electron paramagnetic resonance, and magnetic susceptibility data for the title complex are reported for the temperature range 1.4–300 K. The crystalline material prepared by standard methods is magnetically complex. There are at least two magnetic sites, each of which shows temperature-dependent spin-state changes over the interval between 300 K and approximately 100 K. At 300 K, the iron(III) is nearly all in the high-spin $S = 5/2$ state. At low temperature, the iron is in lower spin states or possesses a reduced moment owing to dimerization or cooperative magnetic phenomena. The zero field Mössbauer spectra for sample temperatures below 77 K consist of two major patterns. One of these is a simple quadrupole doublet and the other is a multiline, magnetic relaxation broadened spectrum. The later is sharpened by application of a small polarizing magnetic field and at 4.2 K the Mössbauer parameters are $\Delta E_1 = 1.78 \pm 0.01$, $\delta E_1 = 0.53$ and $\Delta E_2 = 2.82$, $\delta E_2 = 0.38$ mm/s. With increasing temperature ($T > 80$ K), the quadrupole spectra decrease in intensity and are replaced by the same or similar high-spin iron(III) patterns. Conversion temperatures for the two low-temperature species are approximately 250 and 200 K, respectively. In addition to the patterns just described, a low-intensity, sample-dependent high-spin iron(III) pattern is observed; this pattern does not show a significant temperature variation. The susceptibility and EPR data are consistent with the gradual transitions observed in the Mössbauer patterns. The effective magnetic moment of the material decreases slowly from $5.7 \mu_B$ at 300 K to $3.7 \mu_B$ at 20 K, with a sharp decrease occurring below 20 K to a value of $2.8 \mu_B$ at 1.5 K. At room temperature, the major EPR absorption is from high-spin iron(III). This absorption decreases with decreasing temperature and is replaced by a low-spin iron(III) pattern. At helium temperatures, the major absorption is from low-spin iron(III). It is characterized by a g tensor with principal components (2.363, 2.240, 1.965). These values are shown to be incompatible with an isolated 2T_2 level and probably reflect a significantly admixed low-spin state. Possible models consistent with these results and with structure data of Strouse and co-workers are discussed. The ligation of the iron in the title complex is similar to that of the active site of cytochrome P-450. Similarities between magnetic properties of the analogue and the enzyme are also considered.

I. Introduction

The subject of this study is an iron(III) porphyrin thiolate that was originally prepared as an analogue for cytochrome P-450.² It is well-known that iron in P-450 is bound to at least one sulfur ligand,³⁻¹¹ as originally suggested by Mason

et al.³ It is likely that the second axial ligand is nitrogen,^{7a,10} although direct structure data to confirm this are not yet available.¹¹ It is possible that the ligation of iron varies during the complex reaction cycle of P-450. The thiolate discussed in this work is most closely related to the oxidized form of cyt P-450, containing iron(III), as shown in Figure 1. The figure gives the reaction cycle of cyt P-450 from *Pseudomonas putida* (denoted P-450_{cam}) as reported by Gunsalus and co-workers.⁴ Several groups have shown that upon substrate (camphor) binding, trivalent iron in P-450_{cam} is at least 70% converted from the low spin to the high spin form.^{5,6} The structural factors involved in this conversion are not understood, and it is desirable to isolate such properties by preparing more easily

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