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Evaluation of Iron(III) *N*,*N*'-Ethylenebis((*o*-hydroxyphenyl)glycinate) as a Model for the Iron Binding Site in the Transferrins

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The meso and racemic isomers of ferric *N*,*N*'-ethylenebis((*o*-hydroxyphenyl)glycinate) have been separated and characterized. These complexes have been evaluated as models for the iron binding site in human serum transferrin. Both isomers are found to undergo hydrolysis at high pH with hydrolysis constants, log $K_{MH_{-1}L}$, of 10.3 and 12.8 for the meso and racemic complexes, respectively. These hydrolyzed species, which are probably six-coordinate, are a much closer spectroscopic match to diferric transferrin than the unhydrolyzed forms. The implications of these results with respect to the iron environment in transferrin are discussed.

Introduction

Iron has been recognized as an essential element in human nutrition for several hundred years. To meet the demands of hemoglobin synthesis, the plasma turnover of iron is a large 35 mg/day.[1] This, coupled with the insolubility and toxicity[2] of free iron in the blood, has necessitated a system to regulate and transport this vital element.

In man, human serum transferrin (HST), a glycoprotein of 80 000 molecular weight, which tightly but reversibly binds 2 mol of ferric ion, represents a central component of this system.[3] In addition to its role in transporting iron from sites of absorption and storage to sites of utilization, transferrin may have additional functions in "buffering" metal concentrations in the blood[4] and acting as an antibacterial agent.[5] The clinical ramifications of a thorough understanding of transferrin and iron metabolism in general are numerous.

Although human serum transferrin and the related proteins lactoferrin and conalbumin have been extensively studied, many questions about their structure and function remain. One of the most important of these is the nature, including coordination geometry and stereochemistry, of the metal binding sites. A useful way to study these questions is by an examination of model compounds with physical and chemical properties resembling the known properties of these proteins.

One model that we are actively engaged in evaluating is iron(III) *N*,*N*'-ethylenebis((*o*-hydroxyphenyl)glycinate), FeEHPG, which contains donor groups similar to those implicated in the transferrin metal ion binding site.[1] This compound has been recognized as a potential model of diferric transferrin for a number of years, on the basis of its optical and resonance Raman spectra.[6]

The fact that the iron complex prepared from commercial EHPG is a mixture of the meso isomer and a pair of racemic isomers has been largely unappreicated. Thus, in order to facilitate a more direct comparison with transferrin, we have separated and examined the individual isomers of FeEHPG. In this report we have examined a number of physical properties of these isomers that provide a rigorous test of the similarity of FeEHPG to transferrin. The results of solution chemistry and EPR and visible spectroscopic experiments and their implications for the transferrin metal binding sites are discussed below.

Experimental Section

The ligand (EHPG) was purchased from Sigma Chemical Co. and purified as described by Pecoraro et al.[7] It consists of a 50:50 mixture of the meso and racemic isomers.[8]

meso-Mg[FeEHPG]$_2$ was prepared by stirring 8.0 g of EHPG (0.02 mol), 9.3 g of $MgCO_3$ (0.11 mol), and 8.1 g (0.02 mol) of $Fe(NO_3)_3 \cdot 9H_2O$ overnight in 200 mL of water. The solution turned from brick red to purple-red over the course of a few hours. The solution was then filtered and rotary evaporated to dryness. The crude product could be recrystallized from methanol. The identity of the product as the meso isomer was confirmed by TLC (vide infra), by optical spectroscopy, and by NMR identification of the free ligand after removal of iron. *rac*-Mg[FeEHPG]$_2$ was prepared as described by Bailey et al.,[9,10] except freshly precipitated hydrated ferric oxide was used as the iron source. Its identity was established as above, as well as by X-ray crystallography.[11]

A material consisting of a mixture of meso and racemic isomers was prepared as the sodium salt as described by Spiro.[6] Its composition as a 50:50 mixture of the two isomers was established by TLC.[12] Separation of the meso and racemic isomers was accomplished on silica gel 60-EM precoated glass plates by using the upper layer of 4:1:5 *n*-butyl alcohol/acetic acid/water as a solvent. The *meso*-FeEHPG complex separates as a violet spot with R_f 0.33 and the racemic isomer as a brown spot with R_f 0.29. It was previously reported[13] that these isomers could be separated analytically via paper chromatography and preparatively on cellulose powder with the same solvent. However, in our hands no separation was achieved with either of these procedures.

Optical spectra were recorded on either a Cary 14 or a Perkin-Elmer 552 spectrophotometer, both in solution and in the solid state (KBr pellet).

Extinction coefficients are based on solution iron concentration, as determined by the *o*-phenanthroline method,[14] and are probably accurate to within 5%.

Electron spin resonance spectra were obtained at 77 K with a quartz finger dewar on a Varian E-4 spectrometer operating at 9.2 GHz. Samples were run in 5:1 (v/v) glycerine/water glass or in frozen DMF. Fe(EDTAH) was used as a standard to determine "*g*" values.[15]

The pH measurements for the spectrophotometric titrations were obtained on an Orion Model 501 pH meter with an Orion combination

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(11) Raymond, K. N., personal communication.
(12) References in this work to commercial FeEHPG refer to the 50:50 mixture of isomers as the sodium salt. The pure meso and racemic isomers refer to the magnesium salts.
(13) Ryskiewich, D. P.; Boka, G. *Nature (London)* **1962**, *193*, 472.
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electrode, which was standardized with Fisher pH 7.0 and 10.0 buffers prior to each use. The titrant used was 1 M NaOH. The data from these titrations were plotted via the procedure of Schwarzenbach to yield values of the hydrolysis constant and molar extinction coefficient of the hydrolyzed species.[16]

Relaxivity Measurements. Proton T_1 measurements in water and DMF solutions were carried out on a Bruker 250-MHz spectrometer. Temperature was controlled by heated nitrogen gas. The iron complex concentrations were 5–6 mM, and the pH was varied in H_2O solution from 7.0 to 11.0 by the addition of NaOH. T_1 measurements were performed in coaxial NMR tubes by using the inversion recovery method. CoEHPG was used as a diamagnetic standard.[8]

Results and Discussion

Separation of Isomers. We have separated and characterized the racemic and meso isomers of ferric EHPG. Using 3 equiv of $MgCO_3$ as a base gives preferential crystallization of the racemic complex as reported by Bailey et al.[10] The use of a large excess of the same base affords nearly pure meso isomer. These complexes have distinct EPR, optical, and solution properties and are readily distinguished by these techniques.

A brief report indicating a separation of these isomers has appeared. However, in our hands no separation at all was achieved by using the described techniques, which in any event involved an elaborate and time-consuming chromatographic separation.[13]

In a recent review, Bernauer reports the separation of the optical antipodes of the racemic iron complex along with overall formation constants for the meso ($\log K_{ML} = 33.8$) and racemic ($\log K_{ML} = 35.0$) ligands.[17] Although no experimental data on how these numbers were obtained have ever appeared, our work is in general agreement with the increased stability of the racemic over the meso form of the ligand (vide infra). Bernauer attributes the decreased stability of the meso form to the unfavorability of placing the six-membered phenolate chelate ring in an apical position. This is a requirement of the meso geometry, where the two phenolic groups are fixed in nonequivalent positions. In our studies of the Cu^{2+}, Ga^{3+}, Co^{3+}, and VO^{2+} EHPG complexes, we have isolated only the racemic complexes from the commercial ligand[8,18] (although meso complexes can be prepared by using the pure meso ligand).

Electron Spin Resonance. The EPR spectrum of FeEHPG was recently reported[19] to consist of a broad unstructured, $g \sim 4.3$, signal characteristic of high-spin iron(III) in a rhombically distorted electronic environment. The lack of any splitting was construed as evidence that the geometry around FeEHPG differed from that in diferric transferrin.[20]

We have independently made EPR measurements on the commercial ferric EHPG complexes as well as the pure isomers in a variety of solvents and found, contrary to Ainscough et al.,[19] that the EPR is in fact quite sensitive to the precise geometry around the metal in these chelates and that FeEHPG does resemble transferrin in certain respects.

In frozen aqueous methanol or DMF solutions we do indeed find a simple unstructured $g = 4.3$ signal. Presumably this is due to broadening caused by formation of crystallites and subsequent spin–spin interactions. However, in 5:1 glycerine/H_2O, a well-resolved EPR spectrum is obtained. The spectrum of the commercial complex is seen to be the superposition of the individual meso and racemic isomers (Figure 1). Powder line shapes of EPR spectra of high-spin ferric complexes have been extensively studied by a number of authors.[21] While it is clear that the EPR spectra of the purified isomers of FeEHPG are not close matches to that of transferrin, it is significant that an increasing similarity in the zero-field splitting parameters is observed upon hydrolysis (Table I). A detailed analysis of the EPR and Mössbauer spectra will be reported in a separate publication.[22]

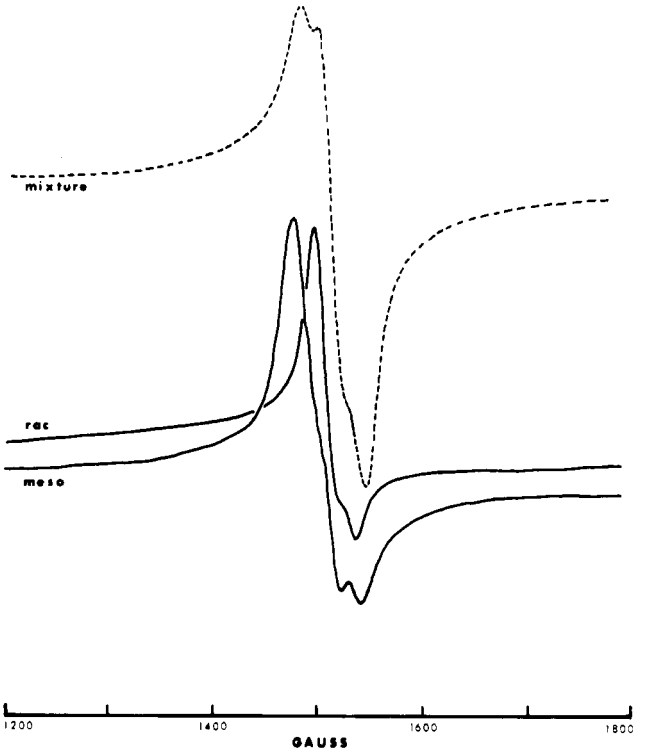

Figure 1. EPR spectra of meso and racemic isomers and the unseparated mixture of isomers of FeEHPG in 5:1 glycerine/water glass at 77 K. Instrument parameters: 9.14 GHz, sweep width 500 G, modulation amplitude 8 G, gain 1×10^5, sweep time 8 min. Iron concentration is 1 mM.

Table I. Zero-Field Splitting Parameters for the FeEHPG Isomers[a]

complex	D, cm^{-1}	λ	complex	D, cm^{-1}	λ
meso-FeEHPG	1.0	0.28	*meso*-FeEHPG	0.7	0.25
rac-FeEHPG	1.2	0.25	(pH 11)		
			transferrin	0.32	0.31

[a] Values determined from Mössbauer spectra; see ref 22.

Optical Spectra. The relatively intense band in the visible region observed for FeEHPG has been assigned as a L → M charge transfer from a $p\pi$ orbital on the phenolate oxygen to a half-filled metal d orbital.[6,23] FeEHPG prepared from the commercial ligand has a $\lambda_{max} = 480$ nm and $\epsilon = 4300$ L/(mol cm) in water. However, a qualitative color change from red to orange was noted upon dissolving FeEHPG in aprotic solvents such as DMF. The spectral shift was associated with an increase in the intensity of the band, so that, in DMF, $\epsilon = 4900$ L/(mol cm). Although charge-transfer bands are expected to be sensitive to solvent changes, there seems little correlation between band maxima and solvent properties such

(16) (a) Harris, W. R.; Carrano, C. J.; Raymond, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 2722. (b) Schwarzenbach, G.; Schwarzenbach, K. *Helv. Chim. Acta* **1963**, *46*, 1390.
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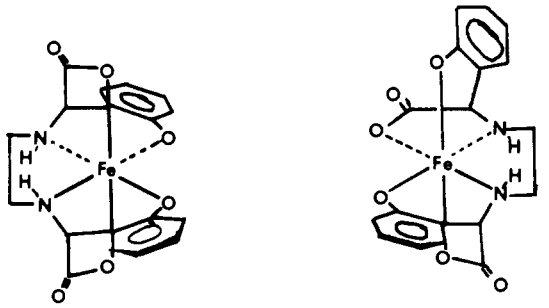
(21) (a) Aasa, R. *J. Chem. Phys.* **1970**, *52*, 3919. (b) Scullane, M. I.; White, L. K.; Chasteen, N. D. *J. Magn. Reson.* **1982**, *47*, 383.
(22) Spartalian, K.; Carrano, C. J. *J. Chem. Phys.* **1983**, *78*, 4811.
(23) Patch, M. G.; Carrano, C. J. *Inorg. Chim. Acta* **1981**, *56*, L71.

Table II. Absorption Maxima of FeEHPG Isomers

complex	λ_{max}, nm (ϵ, L/(mol cm))		
	H_2O	DMF	solid (KBr)
rac-FeEHPG	475 (4450)	458[a]	460
meso-FeEHPG	486 (4105)	463[a]	490

[a] ϵ not determined.



I II

Figure 2. Racemic (I) and meso (II) isomers of FeEHPG. In addition to the isomer shown for I, an additional geometrical isomer is possible, which has both phenolates in the axial positions. The internally compensated meso form has only the one geometric isomer possible.

as dielectric constant. There appear to be two classes of solvents: The first consists of water and the alcohols and is characterized by a peak at ~480 nm. The second class is made up of aprotic solvents such as DMF or Me_2SO with bands at ~460 nm. Within each group there are small shifts that may be due to dielectric properties, but there appears to be a significant change taking place in going from the first group to the second. The band in the aprotic solvents can be shifted back from 460 to 480 nm by the addition of water. The lack of an isosbestic point during this conversion is perhaps not surprising but may indicate that the process is more complicated than a simple two-species equilibrium. The solid-state spectrum, recorded as a KBr pellet, revealed a peak at ~460 nm.

We have repeated many of these measurements on the individual purified isomers, and the results are summarized in Table II. The racemic isomer has a peak at 475 nm in water, as previously reported. The peak shifts to 458 nm in DMF. The band in the solid state is found at 460 nm. The meso isomer displays peaks at 486 nm in H_2O, 463 nm in DMF, and 490 nm in the solid.

There are a number of possibilities that could explain these spectral results. The racemic isomer can exist in either of two geometrical arrangements, with I being the most stable in aqueous solution (Figure 2). It may be that the other geometrical isomer is stabilized in aprotic solvents. However, this is unlikely as the meso isomer, II, can exist in only one geometrical form and yet shows a similar shift on going from H_2O to DMF. An alternative explanation is that the aqueous species is seven-coordinate in solution and upon dissolution in aprotic solvents is converted to a six-coordinate structure.

One of the important structural properties of high-spin iron(III) is its variable coordination. Examples exist where the coordination number of the iron varies between 4 and 8, with seven-coordinate iron being found with a variety of ligands.[24] Solvent-dependent variable coordination has been observed in iron complexes of EDTA for example. In water, methanol, glycerine, or formamide, a seven-coordinate iron complex is obtained.[24] In DMF or Me_2SO a six-coordinate

(24) Garbett, K.; Lang, G.; Williams, R. J. P. *J. Chem. Soc. A* **1971**, 3433.

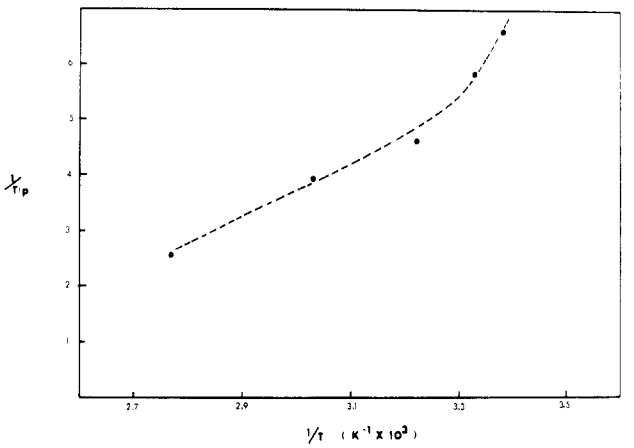


Figure 3. Proton relaxation rates in a 5 mM solution of *meso*-FeEHPG as a function of $1/T$.

complex is observed. Williams et al. have demonstrated that the very weak spin-forbidden d–d bands in these iron complexes may be diagnostic of coordination number. The seven-coordinate species display a single band at ~900 nm. This would be entirely consistent with the observation of a peak assigned as a spin-forbidden d–d band at 900 nm in aqueous FeEHPG,[19] which could represent the seven-coordinate FeEHPG(H_2O). We were unable to consistently observe any near-IR bands in DMF solutions of the complex.

To determine whether solvent (water) was interacting with iron in its primary coordination sphere, we performed a series of relaxivity measurements.

Relaxivity Measurements. Nuclear relaxation in the presence of paramagnetic ions is enhanced due to a paramagnetic contribution that consists of a dipolar interaction (dependent on the ion–nucleus separation) and a scalar or contact interaction (which depends on the spin density at the nucleus in question). This latter term is thought not to be important for many ions, including high-spin ferric ion. The theory of NMR relaxation has been given by a number of authors, and only an extremely abbreviated version is appropriate here.[25-27]

The paramagnetic contribution to the longitudinal relaxation is given by eq 1, where [M] is the concentration of the

$$\frac{1}{T_{1p}} = \frac{[M]}{55.5}\left(\frac{n}{T_{1M} + \tau_m}\right) \quad (1)$$

paramagnetic ion, n the number of water molecules in the first coordination sphere, T_{1M} the longitudinal relaxation time, and τ_m the mean residence time. The dipolar contribution to the value T_{1M} is given by eq 2, where the terms have their usual

$$\frac{1}{T_{1M}} = \frac{2}{15}\left(\frac{S(S+1)\gamma I^2 g^2 \beta^2}{r^6}\right)\frac{3\tau_c}{1 + \omega_I^2\tau_c^2} + \frac{7\tau_c}{1 + \omega_s^2\tau_c^2} \quad (2)$$

meanings. The correlation time, τ_c, an important factor in determining the value of T_{1M}, is in turn determined by whichever is shorter, the electronic spin lattice relaxation time τ_s, the rotational correlation time τ_r, or the exchange rate τ_m, according to

$$1/\tau_c = 1/\tau_s + 1/\tau_r + 1/\tau_m \quad (3)$$

(25) James, T. L. "Nuclear Magnetic Resonance in Biochemistry"; Academic Press: New York, 1975.
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(27) Mildran, A. S.; Granot, J.; Smith, G. M.; Liebman, M. N. In "Methods for Determining Metal Ion Environments in Proteins. 2."; Darnall, D. W., Wilkins, R. G., Eds.; Elsevier: New York, 1980.

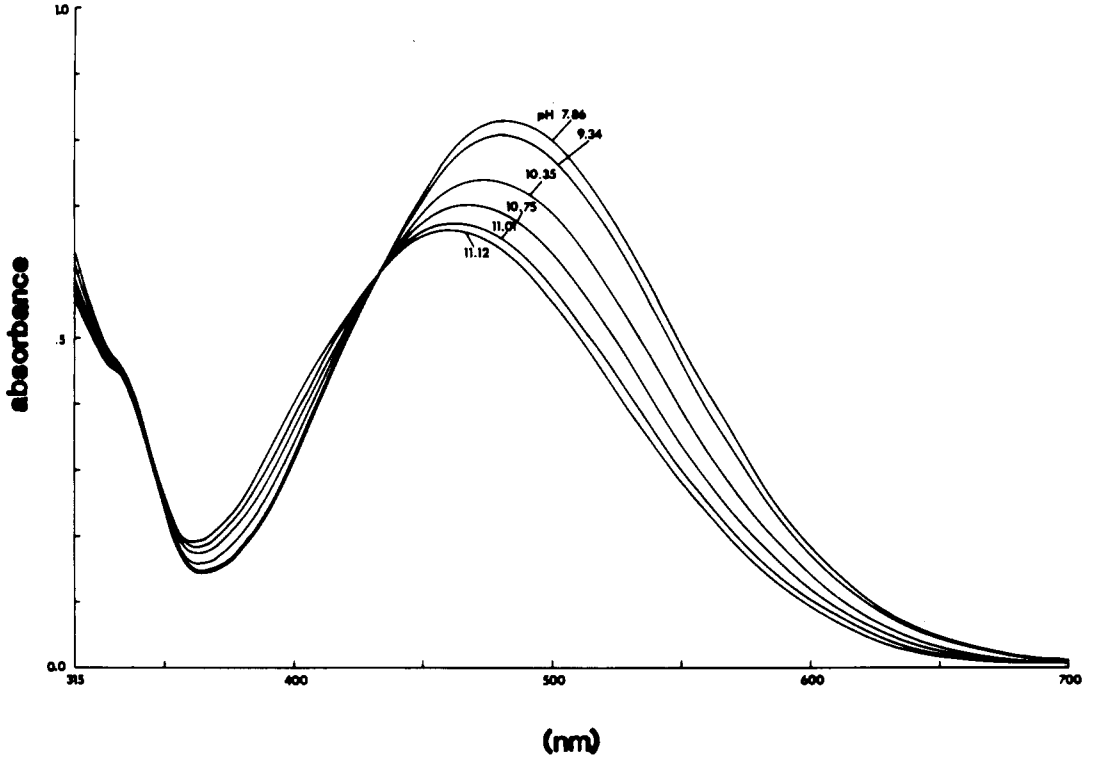

Figure 4. Effect of pH on the optical spectrum of *meso*-FeEHPG. Concentration of FeEHPG is 2.0×10^{-4} M.

T_{1P} can exhibit a frequency dependence at certain values of τ_c and a temperature dependence because both τ_c and τ_m are temperature dependent. If an estimate of τ_c can be determined, then an estimate of n, the number of molecules of H_2O in the first coordination sphere, can be had.[28]

Proton relaxation measurements in both water and DMF solutions of FeEHPG show large effects caused by the paramagnetic center. These effects are essentially unchanged by pH over the range from 7 to 11, but the T_1 is found to increase with temperature (Figure 3).

An analysis of this temperature dependence leads to the unequality[25] that $\tau_m << T_{1M}$ and that $\omega_I^2\tau_c^2 < 1$ and thus $\tau_c < 4 \times 10^{-9}$. This indicates that we are in the fast-exchange limit and can ignore τ_m in eq 1 and 3. A good estimate for τ_r can be had from the Debye–Stokes theory as corrected by the microviscosity model.[25] The effective radius of FeEHPG was calculated from the polarographic data of Schroder[29] by using the appropriate equations;[30] thus, $\tau_r = 2.3 \times 10^{-10}$. Values of τ_s can be estimated for the high-spin ferric ion as $\sim 0.5\text{–}1 \times 10^{-10}$.[31] With use of these values for τ_c and the relaxivity data, the present results are interpreted as there being essentially no primary hydration sphere water or DMF for the racemic isomer. The results, although more ambiguous, are the same for the meso isomer. The data for the meso isomer could be fit with a single water molecule interacting with the iron at a distance of 2.9 Å; however, this is a far longer iron–oxygen bond than is found in other seven-coordinate iron complexes (~2.1 Å).[32] Thus, the most logical interpretation is that the dominant structures in aqueous solution do not possess water molecules in the first coordination sphere,[33] although the possibility of a single bound water and hence a seven-coordinate complex cannot be ruled out.

Given that there is no specific (i.e., seven-coordinate) solvent interaction, what then is the origin of the spectral shifts seen with solvent? Although there is no correlation with simple continuous solvent properties such as refractive index or dielectric constant, there is a correlation between the solvent donor and acceptor abilities (as denoted by the donor and acceptor numbers). The donor–acceptor approach, which has recently been reviewed,[34] has been successful in predicting some physical properties due to solvent interactions. On the basis of these ideas, a strong donor solvent such as DMF or Me_2SO could interact with the hydrogen atom of the ethylenediamine nitrogen. Such an interaction would lead to a lengthening of the N–H bond and a shortening of the Fe–N bond (see Gutman's rules[34]), which would mean a decreasing positive net charge on the iron. Such a decreased positive charge on the iron should shift a LMCT transition to higher energy, as observed. Conversely, interaction of a good acceptor solvent such as H_2O or the alcohols at the carboxylate oxygen would have precisely the opposite effect, leading to a red-shifted LMCT, again in agreement with the results. Such specific solvent interactions with the ligand would also be consistent with the proton relaxation measurements. Data from the crystal structure determination of Bailey et al.[10] show hydrogen bonding of water moleucles to the carboxylate oxygen in the racemic complex. Our solid-state spectra are in agreement

(28) The uncertainties and difficulties in this procedure have been outlined by many authors and should not be underestimated.
(29) Schroder, K. H. *Acta Chem. Scand.* **1964**, *18*, 596.
(30) Meites, L. "Polarographic Techniques"; Wiley: New York, 1965.
(31) These values are estimates and are typical of high-spin ferric ion. The lower values are probably more nearly correct as the lack of an observable ESR signal in solution at room temperature puts an upper limit of about 1×10^{-10} s on the τ_s value, on the assumption that a signal with peak to peak line width of 500 G or less would be observed.
(32) Lund, M. D.; Hoard, J. L. *Inorg. Chem.* **1964**, *3*, 34.
(33) These results are based on τ_s values in the range indicated. If τ_s were to be as small as 10^{-11} s, a possibility, then the data could be fit with a single water molecule at a distance of ~2.1 Å. However, this analysis neglects the effects of secondary (outer-sphere) solvation and the exchangeable amine protons on the proton relaxation rates. An independent study, which considered these effects by comparison of proton relaxation rates among a series of compounds that are known to contain only secondary solvation water molecules and ones known to have a single primary sphere water, also reached results that indicate little or no primary sphere water in both isomers of FeEHPG: Oakes, J., personal communication. In light of these results, it should be realized that eq 2 is no longer strictly correct and the outer-sphere contribution must be considered.
(34) Gutman, V.; Resch, G.; Linert, W. *Coord. Chem. Rev.* **1982**, *43*, 133.

with this. In addition, they would predict little or no interaction of solvent water in the meso complex. However, a crystal structure for this isomer has not been determined. Thus, there could be substantial hydrogen-bonding effects on the position of the charge-transfer band in diferric transferrin, which could take the form of either a blue or red shift depending on the nature of the binding site.

Hydrolysis of FeEHPG. Frost and Martell have previously reported that FeEHPG apparently undergoes a hydrolysis at high pH.[35] In view of the possibility that FeEHPG in H_2O was seven-coordinate with a bound water, we investigated this process in detail.

Figure 4 shows the spectral changes that occur on raising the pH of a solution of *meso*-FeEHPG. The spectrum shifts from a λ_{max} at 485 nm to one near 460 nm with a concomitant loss in intensity. An isosbestic point is maintained at 433 nm, indicating a simple two-species equilibrium. The spectral data from pH 7 to 11 can be treated as a two-component system, as described by eq 4 and 5, where M represents the total iron

$$M = [MH_{-1}L] + [ML] \quad (4)$$

$$A = \epsilon_{MH_{-1}L}[MH_{-1}L] + \epsilon_{ML}[ML] \quad (5)$$

concentration, A is the measured absorbance, and $[MH_{-1}L]$ and [ML] are the concentrations of hydrolyzed and unhydrolyzed species, respectively. These can be rearranged and combined with the equilibrium expression

$$K_{MH_{-1}L} = [ML]/[MH_{-1}L][H] \quad (6)$$

to give the equation

$$\epsilon_{obsd} = \epsilon_{MH_{-1}L} + (\epsilon_{ML} - \epsilon_{obsd})K_{MH_{-1}L}[H] \quad (7)$$

where $\epsilon_{obsd} = A/M$. Plots of ϵ_{obsd} vs. $(\epsilon_{ML} - \epsilon_{obsd})[H]$ were linear at 486 nm and yield values for the hydrolysis constant, log $K_{MH_{-1}L}$, of 10.3 (2) and an ϵ for the hydrolyzed species of 2720 L/(mol cm).

The racemic isomer was decidedly more resistant to hydrolysis, no hydrolysis being evident until nearly pH 12. At the high pH values needed for hydrolysis, competing side reactions made the determination of the hydrolysis constant more difficult. Analysis at 475 nm gave values of log $K_{MH_{-1}L}$ = 12.6 (3) and $\epsilon_{MH_{-1}L}$ = 2160 L/(mol cm).

Although the hydrolysis constant is reasonably accurate for the racemic isomer, $\epsilon_{MH_{-1}L}$ may be highly unreliable. A small change in the slope of the Schwarzenbach plot will have little effect on the determination of $K_{MH_{-1}L}$ but will have a pronounced effect on the value of ϵ, which must be extrapolated. Thus, this value should be used with caution.

There is frequently a direct correlation between the magnitude of the formation constant, K_{ML}, and the corresponding chelate hydrolysis constant.[16a] This is shown for the ferric ion in ref 16. Given the magnitude of the formation constants of the *meso*- and *rac*-FeEHPG of 33.8 and 35.0, respectively, we would anticipate hydrolysis constants of 11.8 and 12.8, in reasonable agreement with the determined values.

Implications for the Transferrin Metal Binding Site. The details of the metal binding site of the transferrins are still being debated. However, from recent work and the results presented here, a clearer picture may be emerging.

UV difference spectra have shown that it is likely that there are only two tyrosines bound to most metals in transferrin.[36] Chemical modification has implicated two histidine nitrogens as well.[37] The anion, bicarbonate/carbonate, is almost certainly directly bound to the metal, and NMR has shown that one water is also in the coordination sphere.[38] Proton-release data indicate three protons released upon iron binding. This has been interpreted as evidence for three tyrosine ligands for iron and a seven-coordinate binding site.[39] However, to explain the proton-release stoichiometry upon metal binding and to be consistent with known Fe^{3+} chemistry and the UV difference data, we have recently speculated that the water bound to Fe^{3+} has been hydrolyzed to yield a hydroxo species.[7] This implies a six-coordinate iron(III) binding site.

Interestingly, while FeEHPG itself has many spectroscopic properties that are similar to transferrin, the hydrolyzed form of the complex is a still closer match. The optical parameters for the hydrolyzed species, λ_{max} = 460 nm and $\epsilon \approx$ 2700 L/(mol cm), are much closer to those of diferric transferrin, λ_{max} = 462 nm and $\epsilon \approx$ 2500 L/(mol cm), than those of the unhydrolyzed complex, $\lambda_{max} \approx$ 480 nm and $\epsilon \approx$ 4300 L/(mol cm). Indeed, addition of base to a red solution of FeEHPG leads to the characteristic salmon pink tint so well-known in transferrin chemistry. Further corroboration for the increased similarity between the hydrolyzed species and the protein comes from preliminary Mössbauer spectra, which reveal zero-field splitting parameters more nearly like those of transferrin (Table I). The hydrolyzed species has donor groups similar to the model we have proposed for transferrin itself[7] and is indeed the best overall spectroscopic match yet reported. Unfortunately, unambiguous assignment of the binding site in transferrin as six- or seven-coordinate is still not possible as the data presented here do not clearly distinguish between these possibilities. However, further work on the synthesis of discrete mononuclear iron hydroxo complexes, at physiologically significant pH values, that retain the basic features of the EHPG molecule are in progress and may prove illuminating.

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Registry No. *meso*-Mg[FeEHPG]$_2$, 86561-38-8; *rac*-Mg[FeEHPG]$_2$, 86561-39-9; iron, 7439-89-6.

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