Synthesis and Characterization of Low-Spin Bis(amino ester) Iron(III) Porphyrin Complexes

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Introduction

The autoreduction of ferric porphyrins in the presence of aliphatic amines is a well-known reaction.[1−4] Thus bis(aliphatic amine) iron(III) porphyrins have only been observed at low temperature or in high dilution.[5−9] Mixed complexes with cyanide and aliphatic amine ligands[5] and ammonia-ligated iron(III) complex are the two notable exceptions.[6] Amine oxidations are important in the metabolism of both naturally occurring amines and xenobiotics.[10] The prevailing view is that the P450s generally catalyze N-dealkylation of amines when possible and that the microsomal flavin-containing monooxygenases catalyzes N-oxygenation.[11] A plant cytochrome P-450 has also been found to catalyze the N-hydroxylation of L-tyrosine to *N*-hydroxytyrosine.[12] We recently reported the synthesis and characterization of monodentate imine complexes of Ru(II) porphyrins from oxidation of α-racemic amino esters by dioxoruthenium(VI) picket-fence complexes bearing optically active α-methoxy-α(trifluoromethyl) phenylacetyl residues on both sides of the porphyrin plane ($\alpha\beta\alpha\beta$ isomer).[13] In this paper, we describe the complexation of amino esters to iron(III) porphyrins. So far as we are able to determine, they are the first examples of low-spin amino ester-ligated iron porphyrin complexes which have been isolated and characterized. These complexes are relevant to the recent report of the three-dimensional structure of the membrane-embedded cytochrome *f* from turnip chloroplast which has an unprecedented axial heme iron ligand: the amino terminus of the polypeptide chain.[14]

Experimental Section

General Information. All reactions were carried out in dried solvents in Schlenk tubes under an Ar atmosphere. Solvents were distilled from appropriate drying agents and stored under argon. ^{1}H NMR spectra were recorded on a Bruker AC 300P and 200 DPX spectrometers in $CDCl_3$. Tetramethylsilane was used as internal reference. The temperature are given within 1 K. EPR spectra were recorded on a Varian spectrometer at 5 K in frozen CH_2Cl_2. The *g* values were measured with respect to that of diphenylpicrylhydrazyl ($g = 2.0036 + 0.00003$). Visible spectra were measured on a Uvikon 941 spectrometer in CH_2Cl_2. Elemental analyses were performed by the Service Central of Analyses (CNRS) at Vernaison, France.

Caution. *We have not observed detonation of iron porphyrin perchlorates under our conditions, but care is urged.*

Reagents. The following iron porphyrins[15] were prepared by literature methods: Fe (TPP)ClO_4,[16a] Fe(T(*p*-T)P]ClO_4,[16b] Fe(TPP)CF_3SO_3,[16a] [Fe(T(*o*-T)P]CF_3SO_3,[16a] and [Fe(T(*p*-T)P]CF_3SO_3.[16b] All the amino esters are commercially available in their hydrochloride salts. The salts were dissolved in NaOH solution (2 N). The solution was stirred at room temperature for 15 min. The amino esters were extracted with ether and dried under vacuum. For valine methyl ester, it was not possible to remove completely traces of water without progressive degradation of the ligand.

Syntheses. [(NH$_2$CH(CO$_2$CH$_3$)(CH$_2$CH(CH$_3$)$_2$))$_2$Fe(TPP)]CF$_3$SO$_3$, 1. A mixture of [Fe(TPP)]CF_3SO_3 (100 mg, 0.122 mmol) and 140 μL (0.98 mmol) of L-leucine methyl ester in 10 mL of dichloromethane was stirred for 10 min at room temperature to give a red solution. A 40 mL amount of pentane was added and the solution was set aside for 2 days for crystallization at 0 °C. The purple solid was collected on a medium frit and washed with hexane (0.085 g, 63%). Anal. Calcd for $C_{59}H_{58}N_6O_7F_3SFe$: C, 63.95; H, 5.28; N, 7.58. Found: C, 64.64; H, 5.21; N, 7.74. ^{1}H NMR ($CDCl_3$, 293 K): δ 265 (very broad, 2H, NH_2), 23.0 (broad, 2H, CH_β), 11.66 (broad, 2H, CH_β), 11.56 (broad, 2H, CH_γ), 5.96 (t, 4H, *p-meso*-Ph), 5.73 (m, 8H, *m-meso*-Ph), 3.98 (m, 8H, *o-meso*-Ph), −22.0 (s, 8H, pyrrole) (CH_α, $CH_{3\delta}$, and OCH_3, undetected at this temperature). UV−vis (CH_2Cl_2): λ_{max}/nm 414 (ϵ 13 dm^3 mmol^{-1} cm^{-1}), 543 (ϵ 6), 574 (ϵ 5).

For the preparation of the tetraphenyl and *ortho*- and *para*-tolyl valine derivatives, the same procedure can be used with the corresponding perchlorate or triflate analogues in dichloromethane and L-valine methyl ester. However for valine derivatives, a partial contamination (10%) with the thermodynamically stable μ-oxo dimer[17] due to traces of water in the ligand was observed. This may explain why correct analyses were not found with this ligand for **2** and **3**.

[(NH$_2$CH(CO$_2$CH$_3$)(CH(CH$_3$)$_2$))$_2$Fe(T(*p*-T)P)]CF$_3$SO$_3$, 2. Yield: 62%. UV−vis (CH_2Cl_2): λ_{max}/nm 415, 543, 574.

[(NH$_2$CH(CO$_2$CH$_3$)(CH(CH$_3$)$_2$))$_2$Fe(T(*p*-T)P)]ClO$_4$, 3. Yield: 54%. UV−vis (CH_2Cl_2): λ_{max}/nm 415, 541, 571.

The two other compounds, [(NH$_2$CH(CO$_2$CH$_3$)(CH(CH$_3$)$_2$))$_2$Fe(TPP)]CF_3SO_3 (**4**) (UV−vis: λ_{max}/nm 416, 542, 571) and [(NH$_2$CH(CO$_2$CH$_3$)(CH(CH$_3$)$_2$))$_2$Fe(T(*o*-T)P)]CF_3SO_3 (**5**) (UV−vis: λ_{max}/nm 415, 542, 572), were prepared in situ, directly in the NMR tube, in $CDCl_3$ solution.

Results and Discussion

Two major difficulties may be encountered in preparing amino ester ferric complexes of porphyrins. First, the autoreduction of the ferric state may occur, as was previously reported

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(15) Abbreviations used: (TPP)H_2, tetraphenylporphyrin; [T(*o*-T)P]H_2, tetrakis(*o*-tolyl)porphyrin; [T(*p*-T)P]H_2, tetrakis(*p*-tolyl)porphyrin.
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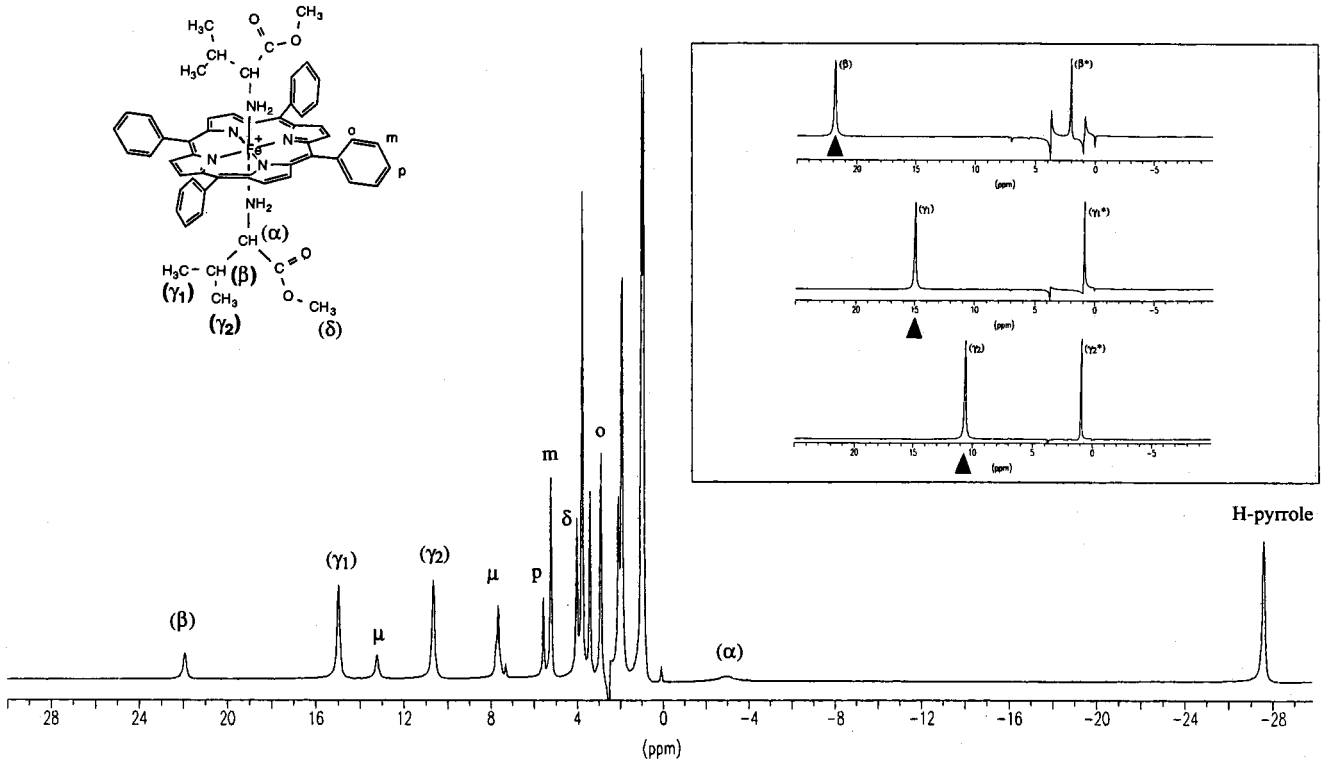

Figure 1. ^{1}H NMR spectrum of $[(NH_2CH(CO_2CH_3)(CH(CH_3)_2)_2Fe(TPP)]CF_3SO_3$ (**4**) recorded at 263 K in $CDCl_3$: (*) free ligand; (L) ligated valine methyl ester; (μ) μ-oxo.[17,20] Inset: Spectra showing saturation tranfer experiments from the ligand methyl group of the complex to the corresponding methyl group of the free ligand.

with phosphine,[3] phosphite,[18] and aliphatic amine[1−4] ligands. Second, the amino ester is probably not a strong coordinating ligand for the ferric state due to a decrease of basicity of the ligand in comparison to alkylamines, thus making iron(III) complexation more difficult. Using perchlorate or triflate, two weak axial ligands,[16] as an intermediate, permit us to solve this problem. Addition under argon of 8 equiv of L-leucine methyl ester to $Fe(TPP)CF_3SO_3$[16b] in CH_2Cl_2 affords the hexacoordinated complex $[(NH_2CH(CO_2CH_3)(CH_2CH(CH_3)_2))_2Fe(TPP)]CF_3SO_3$ (**1**, 63% yield). Complex **1** exhibits a UV−visible spectrum with a Soret band at 414 nm. For the preparation of the other ferriporphyrin derivatives with L-valine methyl ester, $[(NH_2CH(CO_2CH_3)(CH(CH_3)_2))_2Fe(T(p\text{-}T)P)]CF_3SO_3$ (**2**) and $[(NH_2CH(CO_2CH_3)(CH(CH_3)_2))_2Fe(T(p{-}T)P)]ClO_4$ (**3**), a similar procedure can be used.

The ^{1}H NMR spectrum of $[(NH_2CH(CO_2CH_3)(CH(CH_3)_2))_2Fe(TPP)]CF_3SO_3$ (**4**) in the presence of 8 equiv of ligand is shown in Figure 1, and the chemical shifts are listed in Table 1. The shifts of the axial ligand are totally independent of excess ligand at 293 K. Hence axial ligand exchange is slow on the NMR time scale, as judged by the presence of both coordinated and free ligand signals at this temperature. However, saturation transfert experiments[19] between free and ligated amino esters can be conducted in order to assign the amino ester axial ligands (Figure 1). Preirradiation at the frequency of the methyl resonance of the ligated amino ester, at a power level efficient to abolish the resonance in the spectrum, caused a decrease of the intensity of of the corresponding methyl resonance of the free ligand. Thus slow ligand exchange between free and complexed ligands was evident from saturation transfer experiments. Measurement of the relative intensities and relative line widths confirms the assignment.[5,6] The chemical shifts of the

Table 1. Observed Shifts and Separation of the Isotropic Shift into Contact and Dipolar Contributions in $[(NH_2CH(CO_2CH_3)(CH(CH_3)_2)_2Fe(TPP)]CF_3SO_3$ (**4**) in $CDCl_3$ at 293 K

proton type	δ^a	$\delta_{iso}{}^b$	$\delta_{dip}{}^c$	δ_{con}
o−H	3.75	−4.27	−4.27	∼0
m−H	5.59	−2.04	−2.04	∼0
p−H	5.87	−1.76	−1.76	∼0
p-$CH_3{}^d$	1.38	−1.26	−1.26	∼0
H-pyrrole	−23.01	−31.46	−8.32[e]	−23.14

[a] Chemical shifts in ppm. [b] Isotropic shift with the diamagnetic (CN-t-Bu)$_2$Fe(TPP) complex as reference.[24] [c] Based on relative geometric factors ($3\cos^2\theta - 1)/r^3$. [d] *p*-CH_3 shift in $[(NH_2CH(CO_2CH_3)(CH(CH_3)_2))_2Fe(T(p\text{-}T)P)]CF_3SO_3$ (**2**). [e] Using the *o*-H dipolar shift and the relative geometric factor.

axial ligands for the valine complex **4** were at −4.0 (CH_α), 19.1 (CH_β), 11.8 ($CH_{3\gamma}$), and 8.7 ($CH_{3\gamma}$) ppm with line widths of ∼500, 71, 51, and 46 Hz, respectively. Surprisingly the CH_α of the axial ligands appears as a broad signal at high field. In contrast, the CH_α resonances of primary aliphatic amines bound to ferric porphyrins were observed at low field (14−18 ppm).[5] The ^{1}H NMR spectrum for **4** exhibits the expected separate resonances for each diastereotopic methyl group ($\Delta\delta = 3.1$ ppm). This difference is much larger than in the free ligand ($\Delta\delta = 0.06$ ppm). The ^{1}H NMR spectrum for **1** is similar to that of **4**. However, the difference between the two diastereotopic protons of the $CH_{2\beta}$ group is now 11.3 ppm. Unfortunately, separation of dipolar and contact shifts for the axial ligands is quite difficult because the distances between these protons and the metal are not known. A broad resonance for the coordinated NH_2 group is detected at 265 ppm both for **4** and **1**. This peak is assigned on the basis of the chemical shift of coordinated ammonia (240.6 ppm) in bis(ammonia)-ligated low-spin iron(III) porphyrin derivative, previously reported by Goff and Kim.[6] The peaks for the phenyl protons of the porphyrin ring are assigned completely by methyl substitution

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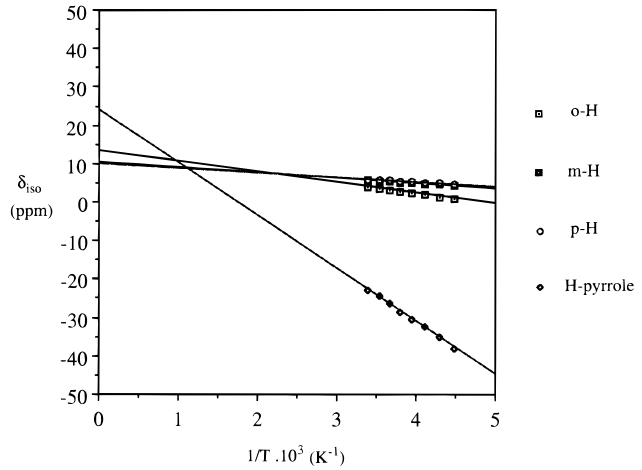

Figure 2. Curie plot of the isotropic shifts vs reciprocal temperature of $[(NH_2CH(CO_2CH_3)(CH(CH_3)_2)_2Fe(TPP)]CF_3SO_3$ (**4**) in $CDCl_3$.

and in combination with proton decoupled experiments. Signals for $[Fe(TPP)]_2O$ are assigned on the basis of previous results.[17,20]

The spectrum of $[(NH_2CH(CO_2CH_3)(CH(CH_3)_2))_2Fe(TPP)]CF_3SO_3$ (**4**) shows expected behavior in that the pyrrole proton signal is found in a highfield position at −23.0 ppm (293 K). It does not contrast with the pyrrole proton of that of $[(NH_3)_2Fe(TPP)]ClO_4$ ($\delta = -20.9$ ppm, 298 K)[6] and provides an essential proof for a similar electronic structure in these derivatives. Evans' magnetic measurements[21] were made for 7.0 mM $CDCl_3$ solutions of $[(NH_2CH(CO_2CH_3)(CH_2CH(CH_3)_2))_2Fe(TPP)]CF_3SO_3$ (**1**) employing Me_4Si as the reference (293 K). The solution magnetic moment ($\mu = 1.9\ \mu_B$) is compatible with the low-spin state $S = {}^1/_2$.

Analysis of the curve in the Curie plot was also made for the $[(NH_2CH(CO_2CH_3)(CH(CH_3)_2))_2Fe(TPP)]CF_3SO_3$ complex (**4**). The temperature dependences of the isotropic shifts of the protons in $CDCl_3$ are shown in Figure 2. The isotropic shifts vary linearly with $1/T$, and the pyrrole protons show a Curie behavior, but the extrapoled lines do not pass through the origin at $1/T = 0$.[22]

To characterize the iron bis(amino ester) structure, analysis of the chemical shift was made according to the method of La Mar.[23] The isotropic shifts were calculated by using $Fe(TPP)(CNt\text{-}Bu)_2$ and related para-substituted diamagnetic complexes as references.[24] The plot δ_{iso} vs $(3\cos^2\theta - 1)/r^3$ for all *meso*-aryl positions for $[(NH_2CH(CO_2CH_3)(CH(CH_3)_2))_2Fe(TPP)]CF_3SO_3$ (and methyl substituent) is given in Figure 3. It is clearly observed that the fit of the isotropic shifts to the geometric factor gives a good correlation. Thus the aryl proton shifts for amino ester complex can be attributed primarily to the dipolar term, i.e., $\delta_{iso} \sim \delta_{dip}$ and therefore $\delta_{con} \sim 0$. It is interesting to note that the mechanism of spin transfer appears here to give rise to conclusions very similar to those observed for low-spin bis(phosphine) ferric complexes of synthetic porphyrins.[25] In this latter case, the phenyl proton shifts of $Fe(TPP)(PMe_3)_2ClO_4$ were found to be essentially dipolar in origin, with a weak contact contribution in the para position. The pyrrole-H dipolar shift

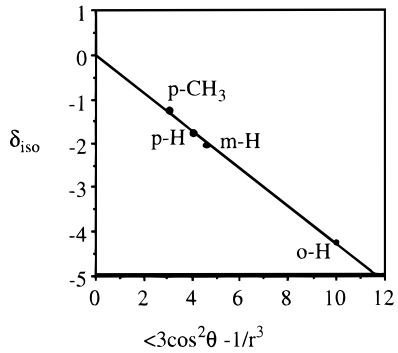

Figure 3. Graph of isotropic shifts at 293 K versus the geometric factor $(3\cos^2\theta - 1)/r^3$ for aryl protons in $[(NH_2CH(CO_2CH_3)(CH(CH_3)_2))_2Fe(TPP)]CF_3SO_3$ (**4**).

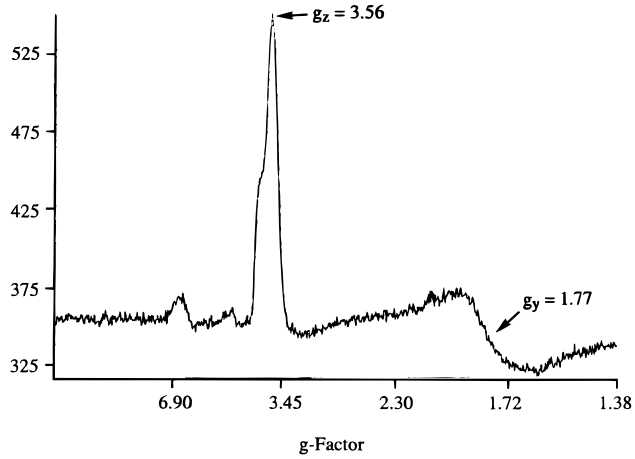

Figure 4. EPR spectrum of $[(NH_2CH(CO_2CH_3)(CH_2CH(CH_3)_2))_2Fe(TPP)]CF_3SO_3$ (**1**) in a CH_2Cl_2 glass, recorded at 5 K.

is determined using the *o*-dipolar shift from Figure 3 and the known ratio of their geometric factors.[26] Thus, using the pyrrole isotropic shift, the contact shift is also sizable by difference. The relatively large contact shift fo pyrrole protons ($\Delta\delta = -23.14$ ppm for **4** and $\Delta\delta = -22.57$ ppm for **1**) favors the interpretation that large spin density is placed on pyrrole carbons and accounts for the observed highfield shift. As previously reported by La Mar[27] and by Walker and Simonis,[26] this pattern of isotropic shifts observed and the Curie behavior of the pyrrole protons are indicative of a classic $(d_{xy})^2\ (d_{xz},d_{yz})^3$ ground state.

It has been recognized that the EPR *g* values of low-spin ferriporphyrins provide valuable information about the orbital of the unpaired electron.[26,28−31] These studies have allowed the identification of the axial ligation in a heme protein by comparison between model complexes and protein of known axial ligation. For low-spin ferric porphyrin complexes, EPR spectroscopy gives generally three *g* values, with $g \sim 1.9$, $g \sim$

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2.2, and $g \sim 2.5$. However much larger g values are now quite common.[29−32] The EPR spectrum of $[(NH_2CH(CO_2CH_3)(CH_2CH(CH_3)_2))_2Fe(TPP)]CF_3SO_3$ (**1**), recorded at 5 K, has a large asymmetric g_z signal at 3.56 and a small signal g_y at 1.77. The signal at g_x is not observed (Figure 4). Similar spectrum was observed for **4** ($g_z = 3.57$, $g_y = 1.76$, and g_x not being observed). Thus both complexes showed highly anisotropic spectra. This is the expected situation for axial ligands that have no planes of symmetry or π-orbitals, as suggested previously.[26] Actually similar results have been reported for $[(NH_3)_2Fe(TPP)]ClO_4$ ($g_{max} = 3.75$)[6] and for ferric octaethylporphyrin bis(*n*-butylamine) complex ($g_{max} = 3.69$).[8] Strong g_{max} spectra are also observed in highly anisotropic low-spin heme proteins[30] and for the axially symmetric bis-ligated low-spin iron(III) porphyrins[31,32] such as those with pyridine and imidazole complexes when the two axial ligands are bound in two perpendicular planes.[31]

In summary, we have shown that ferric porphyrins bearing axial amino ester ligands are quite stable in solution and can be isolated in the solid state. These complexes are models for cytochrome *f*, in which a tyrosine is bound to the iron via the α-amino group of the amino-terminal residue of the protein.[14] The electronic properties of these new derivatives are also similar to those of known bis(amine) complexes.[6,8,32] This may explain why the ϵ-amino group of the conserved Lys 145 of cytochrome *f* was first proposed as the sixth iron ligand.[30]

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