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Raman Evidence for the Formation of a Monoaquo Adduct of Iron(II) Tetrakis((*N-tert*-butylcarbamoyl)phenyl)porphyrin. A Model for Hemoproteins at Low pH

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Electronic absorption and resonance Raman (RR) spectra are reported for various complexes of iron(II) tetrakis((*N-tert*-butylcarbamoyl)phenyl)porphyrin (Fe^{II}(TtButPP)) in wet methylene chloride or toluene. The peculiarity of this ferroporphyrin lies in the chemical structure of meso-phenyl groups which contain reversed secondary amide groups with respect to those included in the "picket-fence" ferroporphyrin (Fe^{II}(TpivPP)). This spectroscopic study shows that Fe^{II}(TtButPP) can bind a water molecule, forming a five-coordinated high-spin complex. The stabilization of this aquo complex is ascribed to the formation of (an) internal H-bond(s) between the coordinated water molecule and one (or two) of the four carbamoyl carbonyl groups of the porphyrin. On the other hand, the RR spectrum of the four-coordinated Fe^{II}(TtButPP) derivative exhibits the ν_2 and ν_4 porphyrin modes at 1561 and 1362 cm^{-1}, respectively. These frequencies, which are significantly lower than those previously detected for Fe^{II}(TpivPP) (1566 and 1370 cm^{-1}), indicate an increase in the degree of porphyrin ruffling. Finally, we show that the frequencies of the ν_1 and ϕ_4 phenyl modes of various complexes of Fe(tetraarylporphyrin) are linearly correlated. The low frequencies observed for the modes of the Fe(TtbutPP) derivatives are in agreement with an electron-withdrawing effect of carbonyl groups of the TtBuPP porphyrin on the phenyl rings as well as on the phenyl–porphyrin bonds. A comparison of RR spectra obtained for the Fe^{II}(TtButPP)(1MeIm) and Fe^{II}(TtButPP)(H_2O) complexes with available RR data on deoxymyoglobin suggests that deoxyhemoproteins at low pH could transiently bind a water molecule after rupture of the normal heme–His ligation.

Introduction

Iron porphyrins are the sites of many biochemical processes. The roles played by the hemoglobins, myoglobins, cytochromes, peroxidases, monooxygenases, and dioxygenases in the higher organisms illustrate how specific interactions between the same prosthetic group (iron protoporphyrin IX) and various proteins are crucial in the determination and the regulation of a particular biological function.

In order to simulate both structure and activity of sites in these hemoproteins, numerous chemical model systems have been synthesized.[2] In the past two decades, several complementary approaches have been used. Porphyrin models have been indeed devised to characterize the proximal effect caused by various strains of the axial ligand on the porphyrin macrocycle.[2a–c] Other models have been designed to test the effects provided by various steric protections on the reactivity of metalloporphyrins.[2d–i] More recently, attempts have been made to introduce discrete modifications of the microenvironment in the vicinity of the reaction center.[2f] In this latter series of compounds, various superstructured porphyrins have been prepared in order to create different environmental situations around the metal ion (local solvation, dipole orientation). The "picket-fence" (TpivPP),[3] "basket-handle", and "picket–handle" iron porphyrins belong to this large class of compounds.[2g–i]

In a current study of biomimetic hemes, a new picket-fence porphyrin, $\alpha,\alpha,\alpha,\alpha$-5,10,15,20-tetrakis(*o*-(*N-tert*-butylcarbamoyl)phenyl)porphyrin (TtButPP), has been recently synthesized.[4] The structural originality of this compound is a change in orientation of the four secondary amide groupings of the pickets, from Ph–NH–CO–R in the classical Collman picket-fence porphyrin (TpivPP)[2g] to Ph–CO–NH–R in the TtbutPP porphyrin. Some electrochemical properties of the Zn and Cu complexes of the TtButPP dianion have been recently described and showed that this new peripheral arrangement destabilizes the negatively charged species, in comparison to the opposite phenomena observed in TpivPP and amide-linked BHP (a-BHP).[5] This effect may rely on the change in orientation of local dipoles due to the reversal of amide groups. Thus, the Fe(TtButPP) derivatives offer a convenient system with which one may determine dipolar effects on the electronic structure of the iron macrocycle and on its axial ligation.

In this work, we have attempted to characterize such effects using resonance Raman (RR) spectroscopy. This vibrational method has been shown to be a powerful technique for assigning both the molecular structure of hemes and their environments in hemoproteins and model compounds.[6] We have obtained the RR spectra of various complexes of Fe^{II}(TtButPP) and in particular have observed the formation of a monoaquo ferrous

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(3) Abbreviations used: TPP = dianion of 5,10,15,20-tetraphenylporphyrin; TpivPP = dianion of $\alpha,\alpha,\alpha,\alpha$-5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrin; TtButPP = dianion of $\alpha,\alpha,\alpha,\alpha$-5,10,15,20-tetrakis(*o*-(*N-tert*-butylcarbamoyl)phenyl)porphyrin; a-BHP = dianion of amide-linked basket-handle porphyrins; 1MeIm = 1-methylimidazole; 2MeImH = 2-methylimidazole; 1,2Me_2Im = 1,2-dimethylimidazole; DCImH = 1,5-dicyclohexylimidazole; 4c = four-coordinated; 5c = five-coordinated; 6c = six-coordinated; HS = high spin; IS = intermediate spin; LS = low spin; Mb = deoxymyoglobin.

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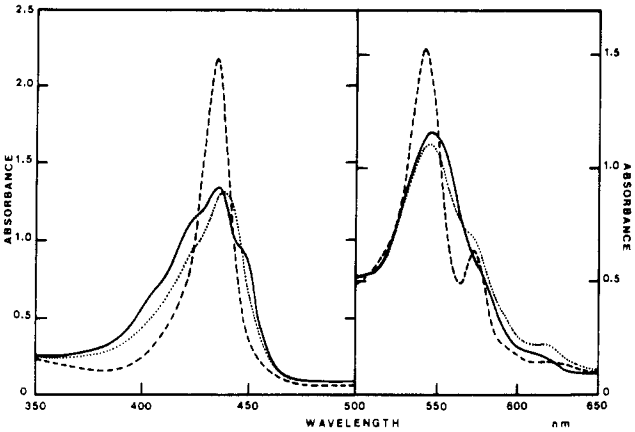

Figure 1. Visible absorption spectrum of Fe^{II}(TtButPP) (0.1 mM) prepared in wet methylene chloride (—), spectrum after addition of 1MeIm (final concentration 0.04 mM) (···), and spectrum after further addition of 1MeIm (final concentration 10 mM) (- - -). The path lengths of optical cuvettes used are 1 and 10 mm for the 350–500- and 500–560-nm regions, respectively.

compounds. These new data characterize a ligation state accessible to ferrohemoproteins at low pH.

Experimental Section

The chloride derivative of iron(III) $\alpha,\alpha,\alpha,\alpha$,-5,10,15,20 tetrakis(*o*-(*N-tert*-butylcarbamoyl)phenyl)porphyrin used in this work was synthesized, purified, and characterized by methods previously described.[4] The preparation and manipulation of ferrous derivatives of TtButPP as well as the absorption and Raman measurements were performed under anaerobic conditions. The chloroferric derivative was reduced under an argon atmosphere using the two-phase method (toluene/water or methylene chloride/water) reported by Momenteau.[7] Various imidazoles (1-methylimidazole, 2-methylimidazole, or 1,5-dicyclohexylimidazole) were anaerobically added using the same experimental conditions. Dilute porphyrin solutions (ca. 0.5–0.7 mM) in methylene chloride were used for the Raman experiments.

RR spectra were recorded at room temperature (20 ± 1 °C) on a Jobin-Yvon Raman spectrometer (HG 2S) with 441.6-nm excitation provided by a Liconix (Model 4050) He–Cd laser. Spectra were collected and averaged with a multichannel analyzer (Tracor Northern TN 1710).[6c–e] Under these conditions, the frequency precision is between 0.5 and 2 cm^{-1}, depending on the band intensity.

The UV–visible absorption spectra were recorded with a Varian DMS-200 spectrophotometer. The equilibrium constants for the binding of the various imidazole ligands to Fe^{II}(TtButPP) were determined at 25 °C by spectrophotometric titrations according to methods previously described.[8]

Results and Discussion

Fe^{II}(TtButPP) in Wet Solvent. In the absence of added ligand, the UV-visible absorption spectrum of the ferrous complex of TtButPP prepared in wet methylene chloride (or wet toluene) exhibits band maxima at 546 and 435 nm with shoulders at 421 and 448 nm (Figure 1). This spectrum is different from that previously obtained for the four-coordinated intermediate-spin (4cIS) iron(II) tetraphenylporphyrin derivative (Fe^{II}(TPP)) prepared under the same solvent conditions.[8] Indeed, this latter compound exhibits a split Soret band with two components peaking at 420 and 445.5 nm. However, it is worth noting that the electronic spectrum of Fe^{II}(TPP) in tetrahydrofuran or in ethanol[8] displays strong analogies with that presented in Figure 1. These spectral comparisons may therefore be consistent with a partial binding of an O-donor ligand (R–O–R type) to Fe^{II}(TtButPP).

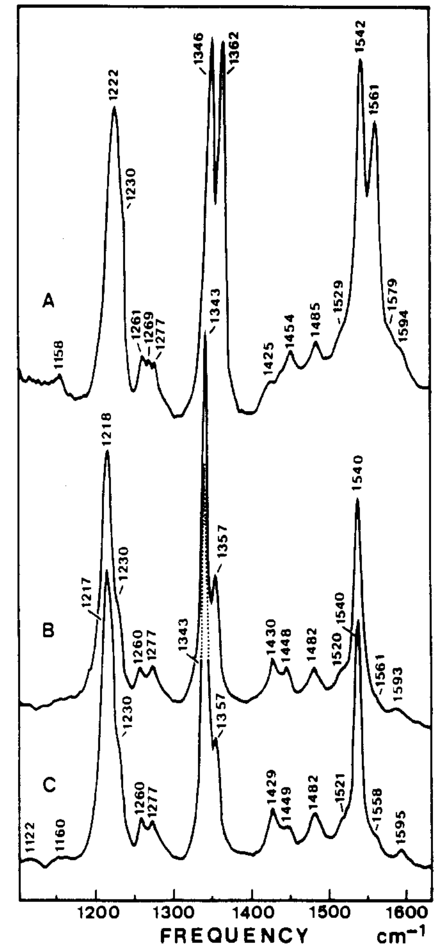

Figure 2. High-frequency regions (1100–1650 cm^{-1}) of RR spectra of various Fe^{II}(TtButPP) derivatives in wet methylene chloride: (A) no addition of ligand; (B) addition of 1,2Me$_2$Im (2 mM); (C) addition of DCImH (4 mM).

The high-frequency regions (1100–1650 cm^{-1}) of the RR spectra of Fe^{II}(TtButPP) in wet methylene chloride clearly support the coexistence of two distinct spectral forms, since the skeletal porphyrin modes ν_4 and ν_2[9] exhibit two components at 1346 and 1362 cm^{-1} and at 1542 and 1561 cm^{-1}, respectively (Figure 2). From the Raman data presently available[10] for various four-, five-, and six-coordinated iron(II) tetraarylporphyrin derivatives, the frequencies at 1362 and 1561 cm^{-1} are indicative of a perturbed 4cIS ferrous heme (Table I), while the second series of frequencies (1346 and 1542 cm^{-1}) is attributable to a ferrous high-spin (HS) heme (Table I).

In order to determine the nature of this HS species, we first checked that no internal or external ligand could bind the metal

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Table I. Selected Resonance Raman Vibrations of Various Iron–Tetraarylporphyrin Complexes (cm^{-1})[a]

	porphyrin modes		phenyl modes	
derivative	ν_2	ν_4	ϕ_4	ν_1
	4cIS Fe(II)			
Fe(TPP)	1565	1370	1603	1240
Fe(TpivPP)	1566	1370	1608	1259
Fe(TtButPP)	1561	1362	1594	1221
	5cHS Fe(II)			
Fe(TPP)(2MeImH)	1538	1342	1598	1229
Fe(TpivPP)(2MeImH)	1540	1344	1606	1252
Fe(TtButPP)(1MeIm)	1540	1344	1592	1218
Fe(TtButPP)(1,2Me$_2$Im)	1541	1344	1594	1218
Fe(TtButPP)(DCHImH)	1540	1343	1595	1217
Fe(TtButPP)(H$_2$O)	1542	1346	1591	1219
	6cLS Fe(II)			
Fe(TPP)(1MeIm)$_2$	1557	1354	1599	1227
Fe(TpivPP)(1MeIm)$_2$	1560	1355	1607	1253
Fe(TtButPP)(1MeIm)$_2$	1556	1354	1591	1215
	5cHS Fe(III)			
Fe(TPP)(Cl)	1555	1366	1605	1240
Fe(TPP)(μ-O)	1553	1359	1599	1237
Fe(TpivPP)(Br)	1556	1366	1609	1259
Fe(TpivPP)(μ-O)	1556	1364	1605	1256
Fe(TtButPP)(Cl)	1553	1360	1596	1223
Fe(TtButPP)(μ-O)	1553	1361	1595	1225

[a] The RR data for the Fe(TPP) and Fe(TpivPP) derivatives are from ref 10b.

ion. Using "Dreiding" molecular models, the binding of an amide (carbonyl or amine) "picket" group to the metal atom via an intra- or an intermolecular interaction can be excluded. This simulation makes clear that the axial ligand(s) of the HS species is (are) provided by the solvent or its dissolved water. Taking into account that methylene chloride and toluene have no coordinating property for ferrous porphyrins,[7,8] water appears to be the most likely candidate for the axial binding of Fe^{II}(TtButPP). In order to ascertain this possibility, we therefore have modified the relative water concentration in the organic solvent. Bubbling dry argon in the solvent phase separated from the aqueous phase produces a decrease in water content in methylene chloride.[8] This treatment induces a change in the relative intensities of both components of ν_2 and ν_4 modes, corresponding to an increased contribution of the 4cIS species (Figure 3A). On the contrary, the relative intensities of these bands are reversed when small quantities of degassed water are readded (Figure 3B). Hence, the contribution of the HS species is directly related to the water concentration dissolved in the solvent. Unfortunately, we were unable to directly obtain the RR spectra of pure forms because of oxidation problems during the time-consuming experiments of solvent drying or of titration by water. Nevertheless, the RR spectra of these limiting forms can be attained by differential methods (Figure 3C,D). The difference spectra allow the observation of a band at 1360 cm^{-1} in Figure 3D. The presence of such a band has been correlated with the formation of a five-coordinated (5c) species.[10h]

It is interesting to add that both the IS and HS species present no photosensitivity. Indeed, a change in the experimental conditions, i.e. using stirred sample solutions, low laser powers (≈5 mW), and a defocused laser beam,[6e] does not affect the RR spectra of Fe^{II}(TtButPP) in wet methylene chloride (data not shown). Finally, when small quantities of air are added to the system, we observed RR spectra not significantly different from that we obtained for the μ–oxo derivatives of Fe^{III}(TtButPP) and Fe^{III}(TPP) (Table I).[10a] Therefore, the RR spectra of the HS form observed in Figures 2 and 3 cannot be associated with the formation of a stable dioxygen derivative of Fe^{II}(TtButPP).

In the low-frequency regions (150–500 cm^{-1}) or RR spectra of Fe^{II}(TtButPP) in wet methylene chloride, we failed to detect any specific band assignable to an Fe–ligand mode (Figure 4, spectrum A).

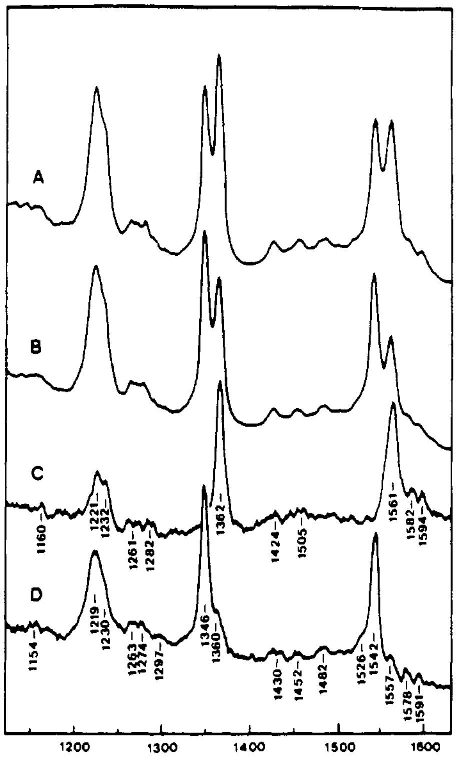

Figure 3. High-frequency regions (1100–1650 cm^{-1}) of RR spectra of Fe^{II}(TtButPP) prepared in wet methylene chloride: (A) spectrum after drying by argon; (B) spectrum after readdition of water (see text); (C) computed spectrum of the intermediate-spin form, ((spectrum A) – 0.92 × (spectrum B)) × 2; (D) computed spectrum of the high-spin form, ((spectrum B) – 0.60 × (spectrum A)) × 2.

The Aquo Complex of Fe^{II}(TtButPP). The RR spectra presented in Figures 2 and 3 show that the formation of the HS Fe(II) species directly depends on the water concentration in the solvent. Taking into account the optical data presented above, this form is therefore associated with an aquo complex of Fe^{II}(TtButPP). This new compound may be either 5c or 6c, although the apparent low affinity of water for Fe^{II}(TtButPP) makes less probable the latter coordination state. A further support to a five-coordination comes from the frequency of the ν_2 porphyrin mode. This skeletal mode is sensitive to the size of the porphyrin core (d),[10f] which is itself sensitive to the coordination number of the iron atom.[11,12] On the basis of the empirical relation $\nu_2 = k(A - d)$ (where k and A are two constants equal to 312.5 cm$^{-1}\cdot$Å^{-1} and 6.98 Å, respectively),[10f] the core dimension is calculated to be 2.030 (±0.003) Å from the 1542-cm^{-1} frequency of the aquo complex of Fe^{II}(TtButPP). This value is consistent with a 5cHS ferrous complex. Indeed, crystal structures of 1-methylimidazole or 2-methylimidazole complexes of various 5cHS iron(II) tetraarylporphyrins show that the iron atom is located out of the porphyrin plane by 0.34–0.55 Å.[11e,f,h] This position gives a core dimension of 2.033–2.043 Å.[6e,11e,f] On the other hand, the metal atom is in the porphyrin plane for the 6cHS

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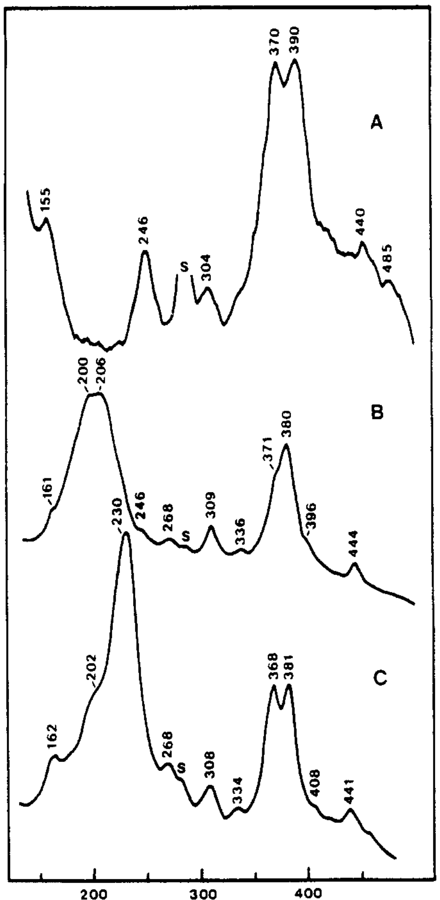

Figure 4. Low-frequency regions (150–500 cm^{-1}) of RR spectra of the Fe^{II}(TtButPP)/Fe^{II}(TtButPP)(H$_2$O) mixture (A), of Fe^{II}(TtButPP)(1,2Me$_2$Im) (ligand concentration 2 mM) (B), and of Fe^{II}(TtButPP)(1MeIm) (C) in wet methylene chloride. Spectrum C is a computed spectrum in which the 6cLS (1MeIm)$_2$ component has been removed (see also caption of Figure 6). S corresponds to a solvent band at 285 cm^{-1}.

bis(tetrahydrofuran) complex of Fe^{II}(TPP).[12] This structure forces an expansion of the macrocycle core with a large *d* distance (2.057 Å).[12] From the ν_2 frequency, the aquo complex of Fe^{II}(TtButPP) is thus best described as a 5cHS complex, i.e. Fe^{II}(TtButPP)(H$_2$O).

Although a partial binding of water has been suspected in the reaction of carbon monoxide with ferrodeuteroheme,[13a] no direct spectroscopic evidence has been provided for such a binding to a ferroporphyrin. Indeed, bare Fe^{II}(TPP) and Fe^{II}(TpivPP) have no affinity for water.[13b,c] Therefore, the binding of water to Fe^{II}(TtButPP) likely occurs inside the cavity formed by the four pickets. Moreover, the chemical nature as well as the stereochemistry of the porphyrin side chains of Fe^{II}(TtButPP) appears to be directly responsible for the water stabilization. Crystallographic data available on different Fe(TpivPP), Fe(a-BHP), and mono "handle"-bis"picket" iron(II) tetraphenylporphyrin derivatives have shown the amide N-H groups of pickets and/or of handles (Ph-NH-CO-R) pointing toward the interior of the cavity generated by the porphyrin superstructures.[11e-h] Dreiding models indicate that the carbamoyl carbonyl groups of Fe(TtButPP) (Ph-CO-NH-R) can assume this orientation. Such a positioning of the carbonyl groups in the Fe-TtButPP derivatives should therefore be particularly adequate for H-bonding interactions with the water protons. Moreover, a molecular Dreiding model for a Fe^{II}(TtButPP)(H$_2$O) complex confirms this possibility of interaction between a (the) water proton(s) and one (or two adjacent) carbonyl group(s) (Figure 5). Although water is a weak-field ligand, H-bonding interactions are also expected to strongly increase its ligand strength.

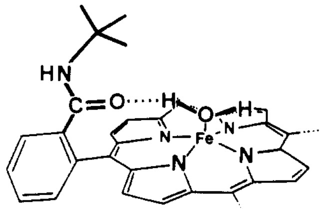

Figure 5. Schematic representation of a water molecule interacting with both the ferrous ion and the carbonyl group of a picket of Fe^{II}(TtButPP).

In conclusion, absorption and resonance Raman spectroscopies favor the HS form of Fe^{II}(TtButPP) in wet solvent as a monoaquo complex with the water oxygen atom bound to the ferrous ion, the stabilization of this water molecule in the cavity being due to H-bonding interactions with one or two picket carbonyl groups (Figure 5).

Four-Coordinated Fe^{II}(TtButPP). Table I shows that the frequencies of the ν_2 and ν_4 modes of Fe^{II}(TtButPP) differ by 4–5 and 8 cm^{-1}, respectively, with respect to those detected for Fe^{II}(TPP) and Fe^{II}(TpivPP).[10b] Similar frequency variations have been observed for the ν_2 and ν_4 modes of four-coordinated β-substituted pyrrole iron(II) porphyrins.[14] The high-frequency modes (1350–1650 cm^{-1}) of metalloporphyrins are both sensitive to the core dimension and the deformation of the porphyrin macrocycle.[10d,f,15] Thus, the decrease in frequency of the ν_2 and ν_4 modes of Fe^{II}(TtButPP) can be interpreted as either an increase in the porphyrin deformation or a decrease in the core size. The comparisons of the ν_2 and ν_4 frequencies of Fe^{II}(TpivPP) and Fe^{II}(TtButPP) (Table I) show a shift of ν_4 which is twice that of ν_2. This difference in frequency shifts cannot account for a major effect on the core dimension since, in this case, the frequency shift of ν_2 is expected to be higher than that of ν_4.[10f] On the other hand, an increase in the degree of porphyrin ruffling is expected to affect more the ν_2 frequency than the ν_4 frequency.[15] Considering the macrocycle of Fe^{II}(TPP), which adopts a ruffled structure in solution as well as in the crystal,[15,16] the frequencies of the skeletal modes of Fe^{II}(TtButPP) therefore should indicate a higher degree of porphyrin ruffling. On the other hand, a porphyrin ruffling may facilitate tilts of phenyl rings toward the porphyrin plane, allowing a partial electron delocalization in the phenyl and porphyrin rings. In this hypothesis, the electron-withdrawing capability of the *o*-carbonylphenyl groups could decrease the electron density at the porphyrin macrocycle and therefore could increase the core size. This partial delocalization may also provide a means of stabilizing positively charged complex in metallo-TtButPP derivatives.[4]

Effects of Base Addition. Absorption spectroscopy has been used recently to characterize a mixed water/imidazole axial ligation in Fe^{II}(a-BHP) porphyrins containing both an imidazole ring inserted in one handle and hydrophobic amino acids in the opposite handle.[17] The spectra of these 6cLS derivatives exhibit a Soret band maximum in the 420–425-nm region.[17] We have therefore looked for a complexation of both a water molecule and an imidazole-type ligand on the iron atom of Fe^{II}(TtButPP).

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Under anaerobic conditions, addition of 1,2-dimethylimidazole ($1,2Me_2Im$) to the Fe^{II}(TtButPP)/Fe^{II}(TtButPP)(H_2O) mixture yields an absorption spectrum characteristic of a pure 5cHS complex (band maxima at 441, 551, 570, and 614 nm) (spectrum not shown). During the spectrophotometric titrations by $1,2Me_2Im$, we found no evidence of a 6cLS complex of Fe^{II}(TtButPP), the data obtained at fixed wavelengths fitting with the binding of one $1,2Me_2Im$ molecule per molecule of Fe^{II}(TtButPP) with an association constant of 2.3×10^4 M^{-1}.[18] The RR spectra exhibit spectral features characteristic of a pure 5cHS complex (Figure 2, Table I) without any detectable presence of LS base/H_2O ligation.

In order to avoid the possible competition between a bound water molecule and an imidazole ligand for the same site inside the cavity formed by the four pickets of Fe^{II}(TtButPP), we have also tested the effect of 1,5-dicyclohexylimidazole (DCImH) addition. The binding of this ligand is indeed expected to be different from the ligation of $1,2Me_2Im$. In fact, Dreiding molecular models indicate strong steric hindrances between the bulky cyclohexyl substituents of DCImH and the porphyrin pickets. Thus, DCImH should be able to preferentially bind Fe^{II}(TtButPP) at the free face of the porphyrin. Moreover, DCImH has no substituent in positions 2 and 4 on its imidazole ring and therefore does not introduce any strain at the heme. The RR spectra obtained after addition of low concentrations of DCHImH (1–4 mM) to the Fe^{II}(TtButPP)/Fe^{II}(TtButPP)(H_2O) mixture indicate the formation of a pure 5cHS complex (Figure 2). Thus, even with this ligand, an Fe^{II}(TtButPP)(H_2O)(base) complex cannot be formed.

Finally, gradual additions of 1-methylimidazole (1MeIm) to Fe^{II}(TtButPP) in wet methylene chloride induce two sequential changes in the absorption spectrum. At low ligand concentrations ($\approx 4 \times 10^{-5}$ M), the Soret band is red-shifted at 438 nm while a single band is observed at 544 nm in the visible region (Figure 1). In Figure 6C, we see an RR spectrum which is very similar to that of Fe^{II}(TtButPP)($1,2Me_2Im$) (Figure 2B). These spectral signatures (absorption and RR) characterize a 5cHS Fe(II) complex. When the 1MeIm concentration is increased up to ca. 10 mM, an essentially 6cLS complex formed with absorption bands peaking at 434, 540, and 572 nm (Figure 1). This spectrum is typical of a $(1MeIm)_2$ complex. Titrations at fixed wavelengths confirm a two-step binding of 1MeIm with apparent constants of 1.3×10^5 and 2.4×10^4 M^{-1}. Finally, the frequencies of the ν_2 and ν_4 RR modes (1556 and 1354 cm^{-1}, respectively) are also in accord with the formation of a bis(base) compound (Figure 6D, Table I).

Thus, using both absorption and RR spectroscopies, we did not detect any formation of a stable Fe^{II}(TtButPP)(base)(H_2O) complex though such a mixed axial coordination state has been observed in the hanging-imidazole complex Fe^{II}(a-BHP).[17] The 5cHS state of Fe^{II}(TtButPP)(H_2O) requires a position of the iron atom out of heme plane which likely optimizes the H-bonding interaction(s) with the carbonyl picket group(s). Upon base binding, the strong field of the imidazole ligand likely repels the iron atom in the porphyrin plane. This movement is expected to both increase the distance between the water protons and the picket carbonyl groups and change their relative orientations. These structural changes could easily remove the water molecule from the iron ion, forming a monoimidazole complex of Fe^{II}(TtButPP). The absence of formation of a mixed base/H_2O complex in the Fe^{II}(TtButPP) derivatives may therefore indicate that the water stabilization necessitates an out of the heme plane position of the iron atom.

The mode involving the Fe–N(1MeIm) stretch has been observed at 225 cm^{-1} in the low-frequency regions of RR spectra of Fe^{II}(TpivPP)(1MeIm).[19] It is located at 230 cm^{-1} in the spectrum of Fe^{II}(TtButPP)(1MeIm) (Figure 4C). A similar upshift is found when the frequencies of the corresponding mode of Fe^{II}(TpivPP)($1,2Me_2Im$) and Fe^{II}(TtButPP)($1,2Me_2Im$) are compared (200 and 206 cm^{-1}, respectively) (see ref 19 and Figure 4B). These systematic frequency shifts (5–6 cm^{-1}) suggest a slight strengthening of the Fe–N(axial imidazole) bond in the Fe^{II}(TtButPP) derivatives, which may have its origin in the change in orientation of the CO–NH dipoles inserted in the pickets.[4a]

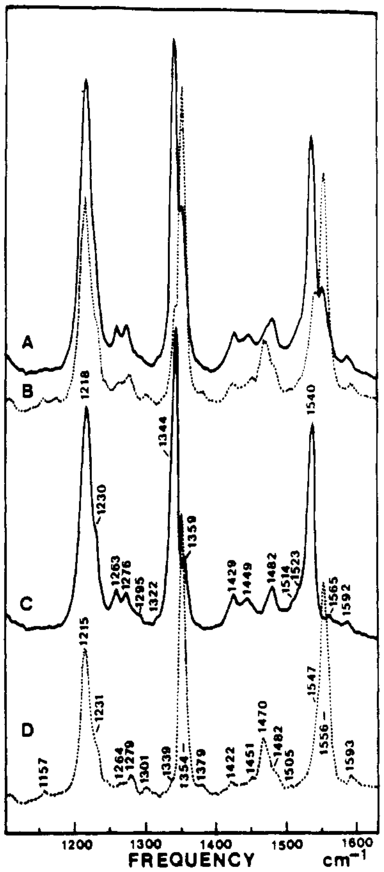

Figure 6. High-frequency regions (1100–1650 cm^{-1}) of RR spectra of Fe^{II}(TtButPP) in wet methylene chloride: (A) spectrum in the presence of 1MeIm (final concentration 1 mM); (B) spectrum after further addition of 1MeIm (final concentration 11 mM); (C) simulated spectrum of Fe^{II}(TtButPP)(1MeIm), (spectrum A) – 0.30 × (spectrum B); (D) simulated spectrum of Fe^{II}(TtButPP)$(1MeIm)_2$, (spectrum B) – 0.23 × (spectrum A).

Phenyl Modes of Tetraarylporphyrins. Several phenyl modes have been identified in the RR spectra of various complexes of tetraarylporphyrins.[10a,b,c] Isotopic experiments and normal-mode calculations have, in particular, assigned modes involving C(methene)–C(phenyl) and C(phenyl)–C(phenyl) stretching ($\nu_{sym}(C_m–C_{ph})$ and $\nu(C_{ph}–C_{ph})$, respectively).[9d,10a,d] The former (ν_1) has been located in the 1230–1238-cm^{-1} region of RR spectra of Fe(TPP) derivatives.[10a,b] For Fe(TpivPP) compounds, its frequency has been observed in higher frequency regions (1250–1260 cm^{-1}1).[10b] As far as the Fe(TtButPP) derivatives are concerned, Table I shows that the ν_1 frequency is decreased in the 1215–1223-cm^{-1} region. A similar situation is observed for the $\nu(C_{ph}–C_{ph})$ mode (ϕ_4), which is seen in the 1598–1605-, 1605–1608-, and 1591–1596-cm^{-1} regions in the RR spectra of Fe(TPP), Fe(TpivPP), and Fe(TtButPP) derivatives, respectively (Table I). Moreover, the plot of the ν_1 and ϕ_4 frequencies for selected 4cIS, 5cHS, and 6cLS Fe(TPP), Fe(TpivPP) and Fe(TtButPP) compounds (Figure 7) clearly displays a linear dependence of these frequencies. Since the Fe(TPP) derivatives exhibit ν_1 and ϕ_4 frequencies which are intermediate of those

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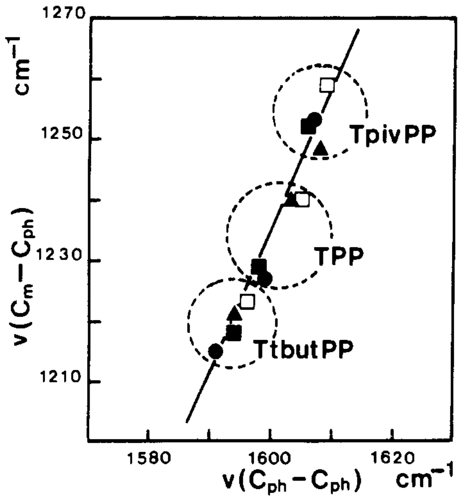

Figure 7. Relationship between the frequencies of the ν_1 ($\nu(C_m–C_{ph})$) and ϕ_4 ($\nu(C_{ph}–C_{ph})$) modes of four series of Fe(TPP), Fe(TpivPP), and Fe(TtButPP) compounds (see text and Table I): open squares, four-coordinated ferrous complexes; full squares, 2MeImH or 1,2Me$_2$Im ferrous derivatives; circles, (1MeIm)$_2$ ferrous derivatives; triangles, chloride or bromide ferric derivatives. The straight line is drawn according the following linear equation: $\nu_1 = (2.333\phi_4) - 2498$ (with ν_1 and ϕ_4 in cm^{-1}).

corresponding to the Fe(TpivPP) and Fe(TtButPP) compounds (Table I), this relation cannot easily account for a mass effect of the different ortho substituents of metallotetraarylporphyrins on the phenyl modes but should rather correspond to an electronic perturbation of the $C_{ph}–C_{ph}$ and $C_m–C_{ph}$ bonds by these substituents. More precisely, the frequency variations of the ν_1 and ϕ_4 modes appear to be predominantly determined by electronic effects transmitted from the ortho substituents to the phenyl–porphyrin bonds through the phenyl rings. For the Fe(TtButPP) derivatives, the decrease in electron density at the phenyl rings is consistent with an electron-withdrawing effect of *o*-carbonyl groups on the phenyl rings according to the following mesomeric formula:

$$\text{Fe–Porph(Ph–CO–NH–R)}_4 \leftrightarrow \text{Fe–Porph(Ph}^+\text{–CO}^-\text{–NH–R)}_4$$

The increased electronegative character of the carbonyl groups in the latter limiting form could also favor the stabilization of a bound water molecule.

Hemoproteins at Low pH. The ferrous deoxyhemoproteins in solution at pH 2–4 present changes in absorption spectra in relation to altered affinities for carbon monoxide.[20] Time-resolved RR spectra of the acidic form of deoxymyoglobin (Mb) were studied by Han et al..[21a] They found that the iron(II) protoheme remains 5cHS and proposed the binding of a weak ligand in place of the normal Fe–His linkage broken by the histidylimidazole protonation. However, a more recent study[21b] suggests a 4c heme in strong interaction with a histidylimidazolium ion.

The RR spectra of Fe^{II}(TtButPP)(H$_2$O), of Fe^{II}(TtButPP)(1MeIm), and of Mb at acidic and neutral pH are qualitatively interesting to compare. On one hand, the high-frequency RR spectra of the two Fe^{II}(TtButPP) derivatives characterize HS complexes (Figures 3 and 6). The same spectral regions of RR spectra of Mb at pH 7.0 or 4.0 give an identical diagnosis.[21a] On the other hand, the low-frequency regions of RR spectra of Fe^{II}(TtButPP)(1MeIm) and of Mb at pH 7 exhibit a strong specific band at 206 and 220 cm^{-1}, respectively, which is characteristic of an Fe–N(axial imidazole) ligation. On the contrary, this band is lacking in the RR spectra of Fe^{II}(TtButPP)(H$_2$O) (Figure 4) as well as in that of Mb at acidic pH.[21] Therefore, the parallel behaviors of RR spectra of the two sets of compounds strongly support the hypothesis made by Han et al.,[21a] i.e. that Mb at low pH could bind a water molecule after rupture of the proximal heme–His bond.

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