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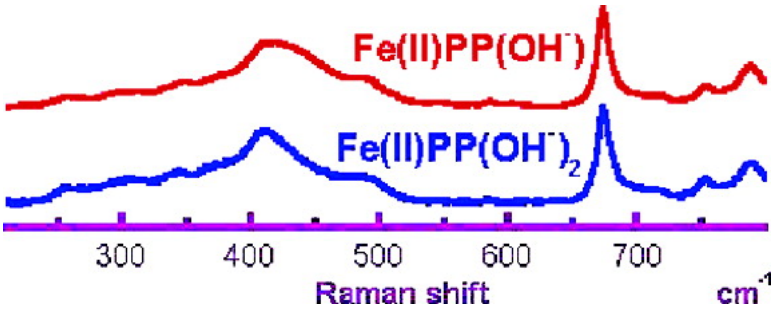

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Spectroscopic Characterization of Hydroxide and Aqua Complexes of Fe(II)-Protoheme, Structural Models for the Axial Coordination of the Atypical Heme of Membrane Cytochrome b_6f Complexes

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Abstract: Electronic absorption and resonance Raman (RR) spectra are reported for hydroxide and aqua complexes of iron(II)-protoporphyrin IX (Fe(II)PP) respectively formed in alkaline and neutral aqueous solutions. These compounds with weak axial ligand(s) represent a biomimetic approach of the unusual coordination of the atypical heme c_i of membrane cytochrome b_6f complexes. Absorption spectra and spectrophotometric titrations show that Fe(II)PP in alkaline aqueous cetyltrimethylammonium bromide (CTABr) binds one hydroxide ion, forming a five-coordinated high-spin (HS) complex. In alkaline aqueous ethanol, we confirm the formation of a dihydroxy complex of Fe(II)PP. In the RR spectra of Fe(II)PP dissolved in neutral aqueous CTABr, a mixture of a four-coordinated intermediate spin form with an HS monoaqua complex (Fe(II)PP(H_2O)) was observed. The spectroscopic information obtained for Fe(II)PP(OH^-), Fe(II)PP(H_2O), and Fe(II)PP(OH^-)$_2$ was compared with that previously reported for the 2-methylimidazole and 2-methylimidazolate complexes of Fe(II)PP, representative of the most common axial ligation in HS heme proteins. This investigation reveals a very remarkable analogy in the spectral properties of, in one hand, the Fe(II)PP(H_2O) and mono-2-methylimidazole complexes and, in the other hand, the Fe(II)PP(OH^-) and mono-2-methylimidazolate complexes. The comparisons of the absorption and RR spectra of Fe(II)PP(OH^-) and Fe(II)PP(OH^-)$_2$ clearly establish that both a redshift of the $\pi-\pi$ electronic transitions and an upshift of the ν_8 RR frequency are spectral parameters indicative of porphyrin doming in HS ferrous complexes. Based upon isotopic substitutions ($^{16}OH^-$,$^{16}OD^-$, and $^{18}OH^-$), stretching modes of the Fe−OH bond(s) of a ferrous porphyrin were assigned for the first time, i.e., at 435 cm^{-1} for Fe(II)PP(OH^-) (ν(Fe(II)-OH^-)) and at 421 cm^{-1} for Fe(II)PP(OH^-)$_2$ (ν_s(Fe(II)−(OH^-)$_2$). The spectroscopic and redox properties of Fe(II)PP(H_2O), Fe(II)PP(OH^-), and heme c_i were discussed and favor a water coordination for the heme c_i iron.

Introduction

Recent crystallographic and spectroscopic investigations of membrane plastoquinone:plastocyanin oxidoreductase or cytochrome b_6f complex (cyt b_6f)[1] have characterized an atypical heme (heme c_i or x) in a protein.[2,3] The axial ligation of this new heme is not clearly established but the X-ray diffraction data suggest coordination by a water molecule or a hydroxide ion.[2] More recently, resonance Raman (RR) spectroscopy has identified a high-spin (HS) species corresponding to the ferrous form of heme c_i.[3] All these observations have renewed the interest to study biomimetic heme model compounds to understand the spectroscopic properties of heme c_i in cyt b_6f. In the present work, we characterized two hydroxide complexes and a monoaqua complex of iron(II)-protoporphyrin IX (Fe(II)PP) using absorption and RR spectroscopies. To distinguish the electronic and steric properties of a water or hydroxide ligand bound to a ferroheme from those of the widespread histidylimidazole ligation found in ferrous myoglobins, hemoglobins, c′-type cytochromes, or peroxidases, the spectroscopic properties of Fe(II)PP complexed with these weak ligands were compared with those of the high-spin (HS) 2-methylimidazole (2MeImH) and 2-methylimidazolate ($2MeIm^-$) complexes of Fe(II)PP.

Experimental Section

Preparations of the Fe(II)PP Complexes. Protohemin chloride (Sigma) (Fe(III)PP(Cl^-)) freshly dissolved in aqueous alkaline solution (10 mM) has been used as a starting material. The neutral and alkaline

(1) Abbreviations used: cyt, cytochrome; CTABr, cetyltrimethylammonium bromide; NaDS.; sodium dodecyl sulfate; TX-100, Triton X-100; EtOH, ethanol; RR, resonance Raman; PP, dianion of protoporphyrin IX; TtbutPP, dianion of α,α,α,α-5,10,15,20-tetrakis(*o*-(*N*-*tert*-butylcarbamoyl)phenyl)-porphyrin; 4c, four-coordinated; 5c, five-coordinated; 6c, six-coordinated; HS, high spin; LS, low spin; IS, intermediate spin; 2MeImH, 2-methylimidazole; $2MeIm^-$, 2-methylimidazolate; *N*-MeIm, 1-methylimidazole; $E_{1/2}$, redox potential.

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10.1021/ja052791g CCC: $30.25

complexes of Fe(II)PP was prepared from Fe(III)PP(Cl^-) by addition of either phosphate buffer (pH 7.0) or hydroxide ion (NaOH or KOH), either in aqueous cetyltrimethylammonium bromide (CTABr) (Sigma) (0.5−2% (w/v)), aqueous sodium dodecyl sulfate (NaDS) (Bio Rad) (0.5% (w/v)), aqueous Triton-X100 (TX-100) (SigmaUltra) (0.5% (w/v)), or aqueous ethanol (EtOH) (Merck, spectroscopic grade) (50% (v/v)). Heme reduction was achieved by anaerobic addition of a small excess of freshly prepared sodium dithionite (Merck) to hemin solutions either under vacuum or equilibrated with wet argon.[4,5] The range of heme concentrations was $(6-10) \times 10^{-6}$ M for the absorption measurements and $(2-10) \times 10^{-4}$ M for the RR experiments.

D_2O (isotopic enrichment: 99.8%), $H_2{}^{18}O$ (isotopic enrichment: 95.1%), and NaOD (isotopic enrichment: 98.5%, 9.8 N in D_2O) were purchased from Euriso-top S. A..

Spectroscopies. The electronic absorption spectra as well as the spectrophotometric titrations were measured using a Cary 5E (Varian) spectrometer. The equilibrium constants for hydroxide binding to Fe(II)PP were determined according to methods previously described.[4a,6] As far as the monohydroxy complex of Fe(II)PP is concerned, some microprecipitation due to the simultaneaous presence of detergent and hydroxide was observed. To minimize the effect of light scattering, the ratios (R) of the absorbances at 440 and 415 nm were determined at a known hydroxide concentration. For the dihydroxy complex, the ratio of the absorbances at 435 and 419 nm was similarly measured as a function of the OH^- concentration. The resonance Raman (RR) spectra were obtained using a Jobin-Yvon U1000 Raman spectrometer equipped with an N_2-cooled charge-coupled device detector (Spectrum one, Jobin-Yvon, France). The samples were excited in the Soret region with the 413.1 nm line of a Kr^+ laser (Coherent Innova), the 441.6 nm line of a He/Cd laser (Liconix), and the 457.9 and 476.5 lines of an Ar^+ laser (Coherent Innova). Improvement of the signal-to-noise ratios of RR spectra was achieved by summations of scans of 30 s. For the determination of the very low-frequency modes (< 200 cm^{-1}), a spectrometer (Jobin-Yvon HG2S) equipped with an EMI photomultiplier was used.[4] The frequencies of the RR bands were determined using a Grams/32 software (Galactic Industries) and methods previously described.[4d] The frequencies of the weakest RR bands were characterized with 1−2 cm^{-1} uncertainties. For the strongest bands, the uncertainty range is decreased to 0.5−1 cm^{-1}.

Results

Electronic Absorption Spectra. Hemin dissolved in aqueous CTABr solution at pH 7 and then reduced by sodium dithionite yields an absorption spectrum in which the α/β bands are detected at 572 and 536 nm (Figure 1A, spectrum (a)). Overlapping bands peaking at 392, 416, 428, and 441 nm are observed in the Soret region. The Fe(II)PP complex formed in aqueous CTABr but in a concentrated hydroxide solution (pH $\geq$ 14) results in a new spectral form with three separate absorption bands at 440 (Soret), 561 (β), and 593 (α) nm (Figure 1A, spectrum (b); Table 1).

The absorption spectrum of hemin reduced by dithionite in aqueous NaDS or in aqueous TX-100 is different from that obtained in aqueous CTABr (Figure 1A (spectrum (a) and B (spectra (a) and (b)). In the Soret region, a broad band with four components (395, 405, 425, and 439 nm) is again observed in aqueous NaDS. However, the relative intensity of the 425 nm component in aqueous NaDS is significantly decreased when compared to the 428 nm contribution observed in aqueous CTABr (Figure 1A (spectrum (a)) and B (spectrum (a)). In the visible region, a 4 nm difference in the peak position of the α band is also detected. The absorption spectrum of hemin reduced in TX-100 exhibits a single Soret band at 426 nm and visible bands at 475, 545, and 564 nm. The observation of a hydroxide form of Fe(II)PP in aqueous NaDS or in aqueous TX-100 was not possible due to a detergent precipitation in concentrated KOH.

The absorption spectrum of dithionite-reduced hemin in neutral aqueous EtOH solution shows a Soret peak at 419 nm and weak α/β bands at 567 and 543 nm (Figure 1C, spectrum (a)). The titration of this complex by hydroxide produces a compound with Soret, β, and α bands at 435, 557, and 591 nm, respectively (Figure 1C, spectrum (b); Table 1). These band maxima are essentially those observed previously for an Fe(II)PP(OH^-)$_2$ complex (435, 558, and 590 nm, respectively).[7]

Spectrophotometric Titrations. The equilibrium measurements made from spectrophotometric titrations of Fe(II)PP by hydroxide in aqueous CTABr solutions fit a binding of one molecule of hydroxide to one molecule of Fe(II)PP (Figure 2 and data not shown). The equilibrium constant (K_D) is 3.4×10^{-1} M in 0.5% aqueous CTABr solution. The OH^- affinity is decreased to 4.5×10^{-1} M when the detergent concentration is increased to 2%.

The titration of Fe(II)PP with OH^- performed in aqueous EtOH confirms the binding of two hydroxide anions to Fe(II)PP.[7] No formation of an intermediate monohydroxy complex was detected, and a K_D of 5×10^{-2} M^2 was measured (Supporting Information S1).

RR Studies of Fe(II)PP in Aqueous Detergent Solution at pH 7−8. When excited at 441.6 nm, the high-frequency regions of RR spectra (1300−1700 cm^{-1}) of the dithionite-reduced hemin dissolved in neutral aqueous CTABr solutions clearly show a mixture of two species (Figure 3). In particular, the relative intensity of the two ν_4 bands observed at 1371 and 1358 cm^{-1} is dependent on the concentration of CTABr (Figure 3, spectra (a) and (b)) but insensitive to the dithionite concentration (spectra not shown). Taking as a reference the RR spectra of the bare Fe(II)PP complex excited at 514.5 or 488 nm,[8] the ν_4 contribution arising from this four-coordinated (4c) intermediate-spin (IS) form is observed at 1371 cm^{-1}. Differences in RR spectra more clearly confirm that hemin reduced by dithionite produces the 4c IS Fe(II)PP form mixed with another ferrous form (Figure 3, spectra (c) and (d)). The 1358 cm^{-1} band and minor bands detected at 1472 and 1525 cm^{-1} are indicative of the formation of a ferrous HS species.[9] This form is RR-active using a blue excitation but RR-inactive when excited in the green region.[8e] In the low-frequency RR spectrum, the HS species displays two weak bands at 408 and 672 (ν_7) cm^{-1} (spectrum not shown).

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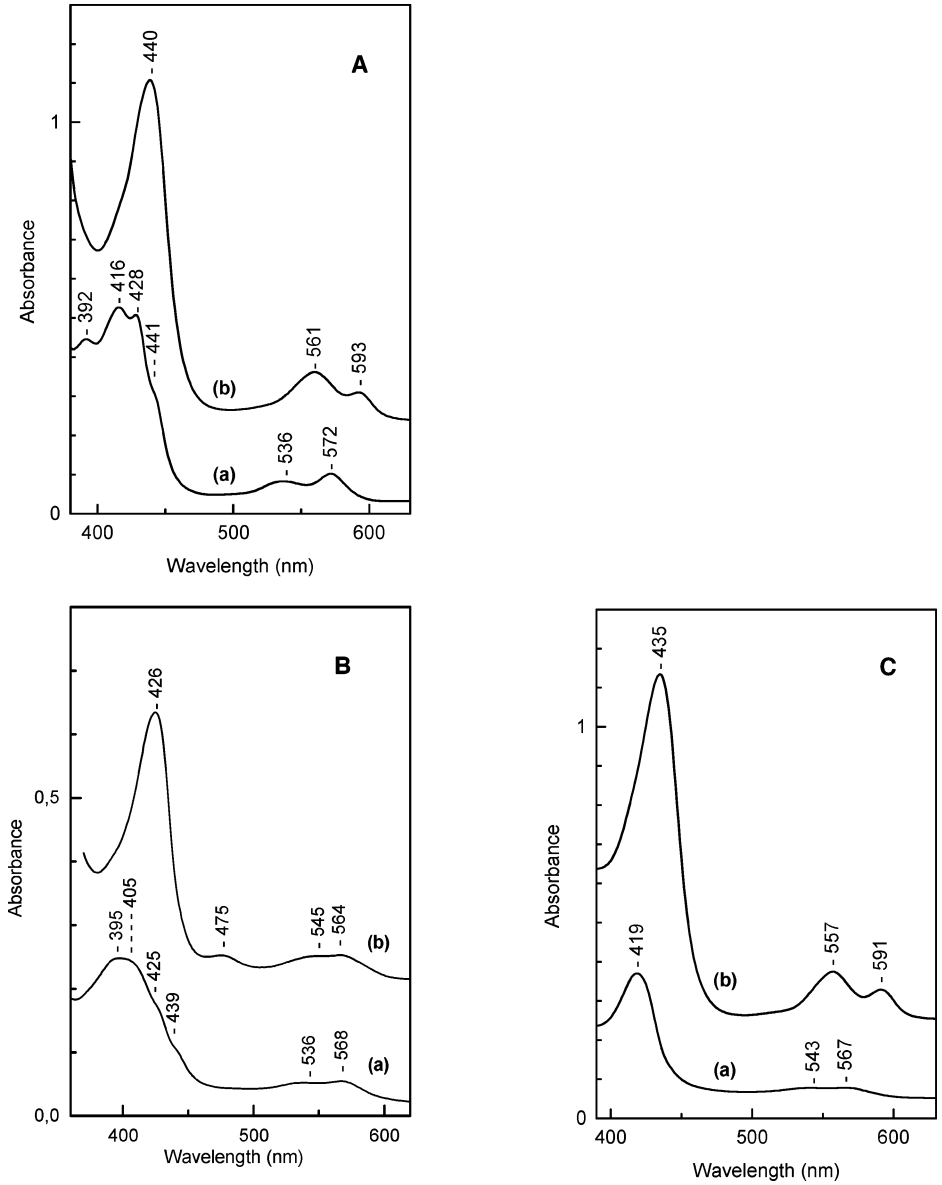

Figure 1. Electronic absorption spectra of dithionite-reduced hemin. (A) in 0.5% aqueous CTABr solution at pH 7.0 (a) and in 3 M NaOH (b); (B) in 0.5% aqueous NaDS at pH 8.0 (a) and in 0.5% aqueous TX-100 at pH 7.0 (b); (C) in 50% ethanol/water at pH 7.0 (a) and in 2.5 M NaOH (b). Heme concentration $= 10^{-5}$ M.

Table 1. Absorption Maxima (nm) of High-Spin Fe(II)PP Complexes

complex	absorption (nm)
Fe(II)PP(H_2O)[a]	428
Fe(II)PP(OH^-)[a]	440, 561, 593
Fe(II)PP(OH^-)$_2$[b]	435, 557, 591
Fe(II)PP(2MeImH)[a]	433, 556
Fe(II)PP(2MeIm$^-$)[a]	441, 560, 592

[a] In 0.5 % aqueous CTABr solution. [b] In a 50 % EtOH/H_2O mixture.

The spectra of hemin reduced in aqueous NaDS at pH 8.0[10] exhibits a Raman signature essentially corresponding to the 4c IS Fe(II)PP, the contribution of the HS species being very minor (Figure 4, spectrum (a)). In the ν_3 region, the observation of a band at 1495 cm^{-1} indicates the formation of a six-coordinated (6c) low-spin (LS) form.

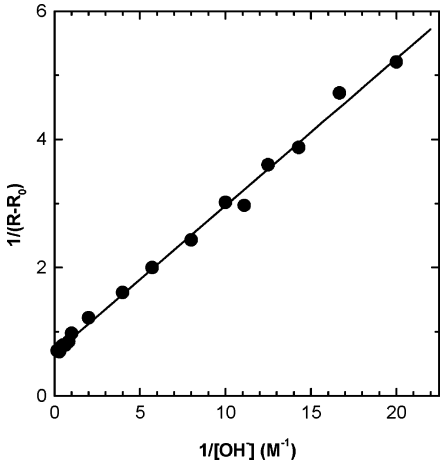

Figure 2. Plot of $1/(R - R_0)$ versus $1/[OH^-]$. R represents the ratio of the absorbances at 440 and 415 nm, R_0 being the R value obtained at pH 7.0. The line corresponds to an affinity constant (K_D) of 3.4×10^{-1} M and fits with a correlation coefficient of 0.995.

(10) The addition of phosphate buffer to aqueous NaDS produces a precipitation of the detergent.

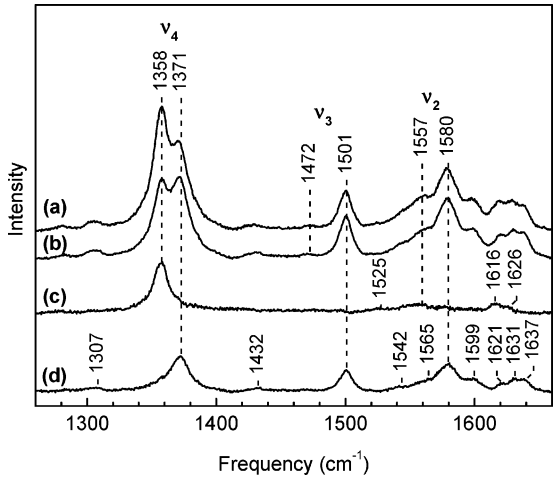

Figure 3. High-frequency regions (1260−1660 cm^{-1}) of RR spectra of dithionite-reduced hemin (a) in 0.5% CTABr aqueous solution, pH 7; (b) in 2% CTABr aqueous solution, pH 7. (c) spectrum (a) minus spectrum (b). (d) spectrum (b) minus 0.6 × spectrum (a). Excitation: 441.6 nm.

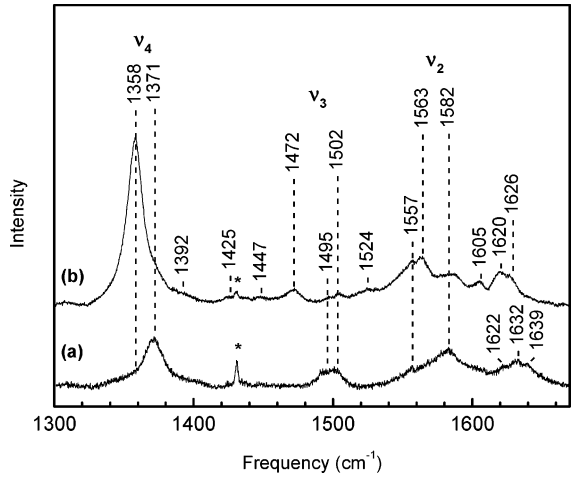

Figure 4. High-frequency regions (1300−1670 cm^{-1}) of RR spectra of dithionite-reduced hemin (a) in 0.5% NaDS aqueous solution, pH 8; (b) in 0.5% TX-100 aqueous solution, pH 7. Excitation: 441.6 nm. The band marked by an asterisk is due to a plasma line.

As far as the RR spectra of hemin reduced in aqueous TX-100 are concerned, the bands observed at 1358, 1472, and 1563 cm^{-1} in the ν_4, ν_3, and ν_2 region, respectively, are clearly associated with the major formation of an HS complex (Figure 4, spectrum (b)). Small contributions of the 4c IS Fe(II)PP form and of a 6c LS complex are however seen in the ν_3 region (1502 and 1496 cm^{-1}, respectively). In the low-frequency regions, major bands corresponding to this HS form are observed at 256, 287, 301, 349 (ν_8), 367, 376, 411, 548, 586, 675 (ν_7), and 716 cm^{-1} (Supporting Information S2). The substitution of $H_2{}^{16}O$ by $H_2{}^{18}O$ produces a clear upshift of two bands. On one hand, a 287 cm^{-1} band is shifted to 295 cm^{-1}. On the other hand, the ν_8 mode is shifted by 2 cm^{-1} from 349 to 351 cm^{-1}. A shoulder on the high-frequency side of the 411 cm^{-1} band is also upshifted upon $H_2{}^{16}O \rightarrow H_2{}^{18}O$ substitution (Supporting Information S2)

RR Studies of Fe(II)PP in Alkaline Aqueous CTABr Solution. The RR spectrum of the Fe(II)PP complex formed in alkaline CTABr solutions exhibits marked differences with respect to that observed for the neutral form of Fe(II)PP (Figures 3 and 5). In the high-frequency regions, a single electron density

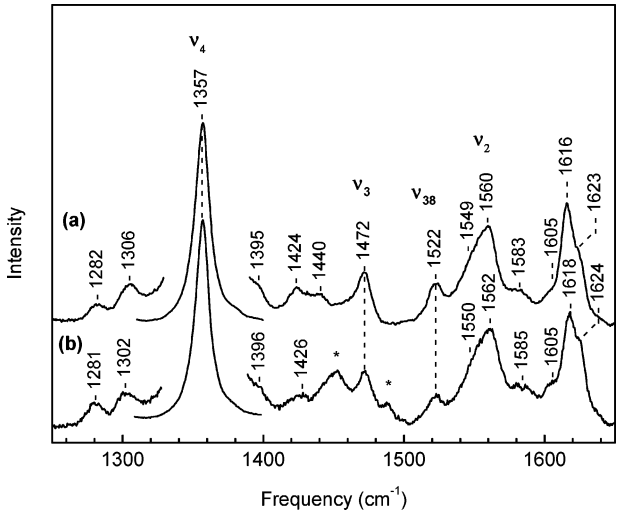

Figure 5. High-frequency regions (1250−1650 cm^{-1}) of RR spectra of dithionite-reduced hemin (a) in 0.5% CTABr aqueous solution and 3 M NaOH; (b) in 50% EtOH/H_2O and 2.5 M NaOH. Excitation: 441.6 nm. The intensity of the ν_4 bands (1357 cm^{-1}) has been divided by a factor of 4. Above spectrum (b), the bands marked by an asterisk are due to free ethanol.

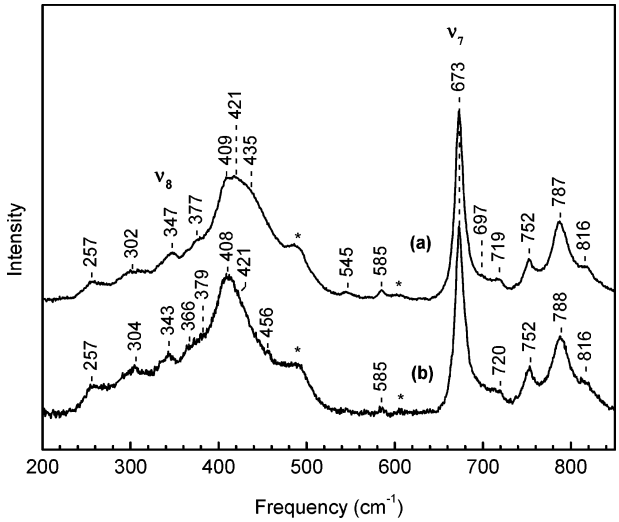

Figure 6. Low-frequency regions (200−850 cm^{-1}) of RR spectra of dithionite-reduced hemin (a) in 0.5% CTABr aqueous solution, 3 M NaOH; (b) in 50% EtOH/H_2O solution, 2.5 M NaOH. Excitation: 441.6 nm. The bands marked by an asterisk are due to the Raman cell.

marker ν_4 appears at 1357 cm^{-1} (Figure 5). The skeletal ν_2, ν_{38}, and ν_3 modes are identified at 1560, 1522, and 1472 cm^{-1}, respectively. Strong contributions of vinyl modes are detected at 1623, 1616 ($\nu(C_a{=}C_b)$), 1424 ($\delta(=C_bH_2)$), and 1306 ($\delta(C_aH=$)) cm^{-1}.[11]

The low-frequency RR spectrum of the monohydroxy complex formed in aqueous CTABr is presented in Figure 6 (spectrum (a)). The ν_7 and ν_8 modes are detected at 673 and 347 cm^{-1}, respectively. On the nonresonant Raman contribution of the cuvette, a very broad feature is observed in the 400−450 cm^{-1} region. Considering its overall width at half-height (54 ± 1 cm^{-1}), this feature appears to be a cluster of overlapping bands. To distinguish its different components, we have measured the RR spectra of the monohydroxy complex with

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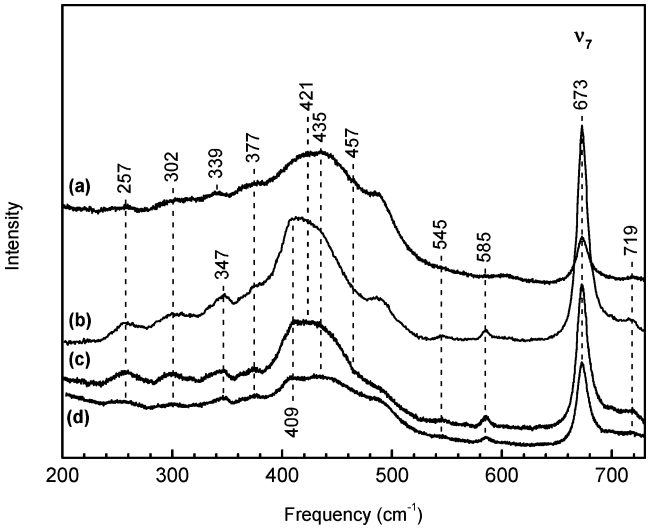

Figure 7. Low-frequency regions (200−730 cm^{-1}) of RR spectra of the monohydroxy complex of Fe(II)PP excited at 413.1 (a), 441.6 (b), 457.9 (c), and 476.5 nm (d).

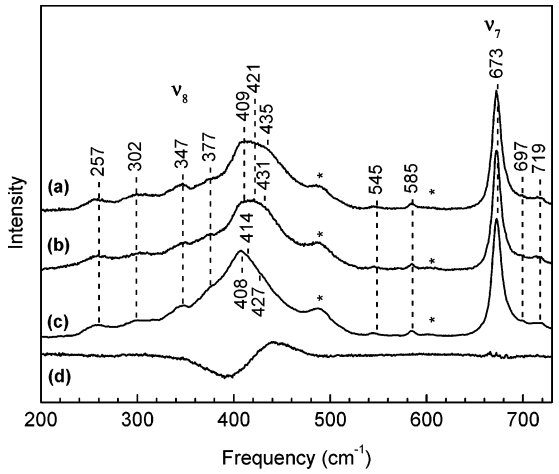

Figure 8. Low-frequency regions (200−730 cm^{-1}) of RR spectra of Fe(II)PP in alkaline CTABr aqueous solution (0.5%). (a) 3 M $Na^{16}OH$ in $H_2{}^{16}O$ (100% $^{16}OH^-$); (b) 3 M NaOD in D_2O (99% OD^-); (c) 3 M $Na^{16}OH$ in $H_2{}^{18}O$ (92.3% $^{18}OH^-$, 7.7% $^{16}OH^-$) (d) Difference spectrum (a)−(c). Spectra (a)−(c) were normalized on the intensity of the ν_7 band. The bands marked by an asterisk are due to the cell.

different Soret excitations. In this way, three main components are thus observed between 400 and 450 cm^{-1}, i.e., at 409, 421, and 435 cm^{-1} (Figure 7). The 409 cm^{-1} band is likely a vinyl deformation mode in analogy with the 409−410 cm^{-1} band observed in the RR spectra of the 2MeImH and $2MeIm^-$ complexes of Fe(II)PP excited at 441.6 nm.[4b] Thus, at least one of the two remaining bands (421 and 435 cm^{-1}) could be a candidate for a vibration involving the axial Fe−OH bond since they are absent in the RR spectra of Fe(II)PP(2MeImH) and Fe(II)PP($2MeIm^-$).[4b]

The ligand deuteration in Fe(II)PP(OH^-) ($^{16}OD^-$ in $D_2{}^{16}O$ versus $^{16}OH^-$ in $H_2{}^{16}O$) produces a small downshift of the high-frequency component of the cluster from 435 (± 1) cm^{-1} to 431 (± 1) cm^{-1} (Figure 8, spectra (a) and (b)). In the difference spectrum ($^{16}OH^-$ minus $^{16}OD^-$), a small S-shaped signal confirms the isotopic sensitivity of the 435 cm^{-1} band (spectrum not shown). When the solvent contains a major concentration of $^{18}OH^-$ and $H_2{}^{18}O$, we observed a more important change in RR spectrum (Figure 8, spectrum (c)). The 435 cm^{-1} band is

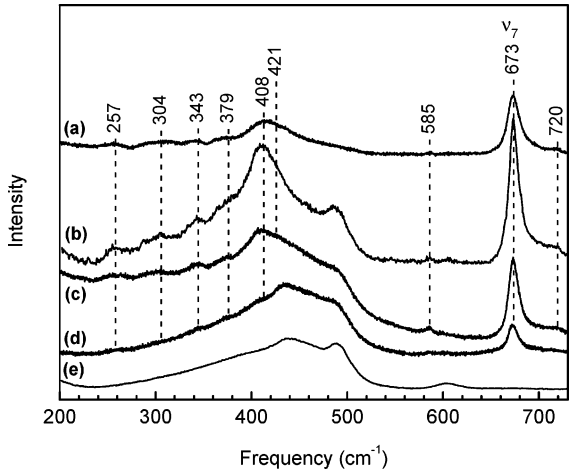

Figure 9. Low-frequency regions (200−730 cm^{-1}) of RR spectra of the dihydroxy complex of Fe(II)PP excited at 413.1 (a), 441.6 (b), 457.9 (c), and 476.5 nm (d). Spectrum (e): nonresonant Raman spectrum of the cell containing a 50% EtOH/H_2O solution, excited at 441.6 nm.

clearly shifted to 414 (± 1) cm^{-1}. This shift is confirmed by the difference spectrum $^{16}OH^-$ minus $^{18}OH^-$ (spectrum (d) in Figure 8).

RR Spectra of Fe(II)PP in Alkaline EtOH/H_2O Solution. The high-frequency regions of RR spectra of the dihydroxy complex of Fe(II)PP formed in 50% EtOH/H_2O show a strong resemblance in frequency as well as in relative intensity with the spectra of the monohydroxy complex (Figures 5 and 6). However, the peripheral modes at 1618 (vinyl $C_a{=}C_b$), 1426 (ν_{42}), and 1302 (vinyl $\delta(C_aH{=})$) cm^{-1} are shifted by 2−4 cm^{-1} (Figure 5). Among the skeletal porphyrin modes, only ν_2 is clearly downshifted by 2 cm^{-1} in changing CTABr by EtOH (Figure 5).

In the low-frequency regions, the frequency of ν_7 remains unaffected (673 cm^{-1}) (Figure 6). On the contrary, the ν_8 mode is downshifted from 347 for the monohydroxy complex to 343 cm^{-1} for the dihydroxy complex. Similarly, a broad band observed at 155 cm^{-1} for the monohydroxy complex is downshifted at 149 cm^{-1} in the RR spectrum of the dihydroxy complex (spectra not shown). Another major spectral difference between the two hydroxide species concerns the 400−450 cm^{-1} regions. Like in the case of the spectra of the Fe(II)PP(OH^-) complex, the broad feature covering this region cannot be considered as a single band. However, its width at half-height is significantly smaller than that observed for the monohydroxy complex (40 ± 1 versus 54 ± 1 cm^{-1}). In fact, the hydroxide complex formed in EtOH/H_2O exhibits two overlapping bands at 408 (± 1) and 421 (± 1) cm^{-1} (Figure 6). These bands exhibits slight changes in relative intensity when the excitation is changed at 413.1 or 457.9 nm (Figure 9, spectra (a)−(c)). The low-frequency bands of Fe(II)PP(OH^-)$_2$ become weak or inactive with the 476.5 nm excitation, a band observed at 439 cm^{-1} being a solvent contribution (Figure 9, spectra (d) and (e)). The ligand deuteration ($^{16}OH^- \rightarrow {}^{16}OD^-$) provokes a small downshift of the 421 cm^{-1} shoulder to 417 (± 1) cm^{-1} (Figure 10, spectra (a) and (b)). This small shift is also identified in the difference spectrum (not shown). The substitution of $^{16}OH^-$ by $^{18}OH^-$ induces a strong shift of the 421 cm^{-1} band under the vinyl deformation mode, resulting in a single broad band peaking at 404 cm^{-1} (Figure 10, spectrum (c)). All the other RR bands are unaffected by the change in mass of the hydroxide ion

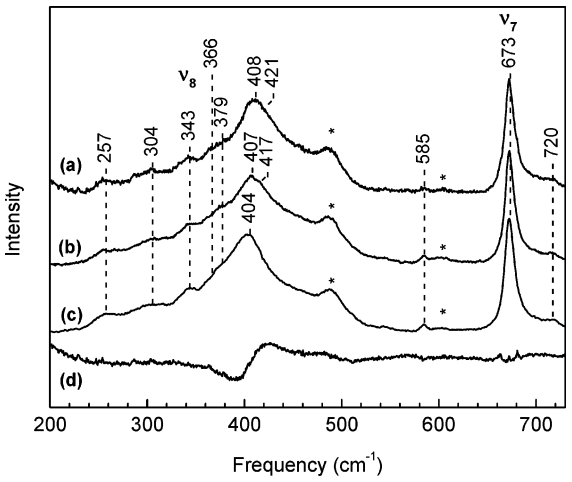

Figure 10. Low-frequency regions (320−480 cm^{-1}) of RR spectra of Fe(II)PP in EtOH/H_2O solution. (a) 2.5 M $Na^{16}OH$ in EtOH/$H_2{}^{16}O$ (100% $^{16}OH^-$); (b) 2.5 M NaOD in EtOH/D_2O (99% OD^-, 1% OH^-); (c) 2.5 M $Na^{16}OH$ in EtOH/$H_2{}^{18}O$ (90.6% $^{18}OH^-$, 9.4% $^{16}OH^-$; (d) difference spectrum (a) − (c)). Spectra (a)−(c) were normalized on the intensity of the ν_7 band. The bands marked by an asterisk are due to the cell.

(Figure 10, spectrum (d)). It is important to note that both the mono- and dihydroxy complexes exhibit a 421 cm^{-1} band. However, the origin of the two bands is clearly different. For the dihydroxy complex, this band is sensitive to $^{16}OH^-/^{16}OD^-$ and $^{16}OH^-/^{18}OH^-$ substitutions. On the contrary, the 421 cm^{-1} band of the monohydroxy complex exhibits no sensitivity to the isotopic changes.

Discussion

Monoaqua Complex of Fe(II)PP. In the 441.6 nm excited RR spectra of ferroprotoheme dissolved in neutral aqueous CTABr, we observed the formation of an HS Fe(II)PP form mixed with the well-identified 4s IS Fe(II)PP complex.[8] An HS/IS spin equilibrium is hardly compatible for a 4c complex. Given the very low concentration of OH^- at pH 7 and the low affinity of Fe(II)PP for OH^-, this HS species cannot be attributed to a residual Fe(II)PP(OH^-) complex. We can also exclude the binding of either Br^- or degradation products of dithionite.[12] Although water is a weak ligand and thus has a very low affinity for Fe(II)-hemes, one can finally consider a water binding when Fe(II)PP is dissolved in neutral aqueous CTABr. The dissociation constant of water to Fe(II)-deuteroheme in wet toluene was estimated to be 10 M.[13] Stabilizing factors however can significantly increase the affinity of H_2O for Fe(II)-porphyrins.[5,14] In this vein, a monoaqua complex of a "picket-fence" Fe(II)-porphyrin (Fe(II)[TtbutPP]) was fully characterized by absorption and RR spectroscopies.[5] The spectral properties of this monoaqua complex strongly resemble those of monoimidazole complexes of the homologue ferrous porphyrin. In the absorption spectra, the Soret and β bands of Fe(II)[TtbutPP](H_2O) were, respectively, observed at 435 and 546 nm, while those of the *N*-methylimidazole (*N*-MeIm) complex peaked at 438 and 544 nm.[5] The RR spectra of the H_2O and *N*-MeIm complexes of Fe(II)[TtbutPP] also show great analogies. The skeletal ν_2 and ν_4 modes were, respectively, observed at 1542 and 1346 cm^{-1} for the monoaqua complex and at 1540 and 1344 cm^{-1} for the mono-*N*-MeIm complex. It is thus clear that the monoaqua and mono-*N*-MeIm complexes of Fe(II)[TtbutPP] adopt very similar electronic structures. In the Fe(II)[TtbutPP](H_2O) complex, the coordinated water molecule was proposed to be H-bonded by at least one well-oriented carbonyl group of a peripheral picket.[5]

The present RR observation of an Fe(II)PP(H_2O) complex formed in neutral aqueous CTABr solution confirms a very similar electronic structure for monoaqua and monoimidazole complexes of a "biological" Fe(II)-porphyrin (Tables 2 and 3). The RR spectra of the Fe(II)PP(H_2O) complex show an HS signature with ν_4, ν_3, and ν_2 bands at 1357, 1472, and 1557 cm^{-1} (Table 2). In the interaction between Fe(II)PP(H_2O) and the CTABr micelle, the positively charged headgroups of the detergent certainly play a role in a relative stabilization of the bound water molecule. In this line, the anionic NaDS detergent has a poor stabilizing effect for the formation of the Fe(II)PP(H_2O) complex. This marked difference between CTABr and NaDS illustrates the influence of the electrostatic interactions on the binding of water to Fe(II)PP.

As far as the hemin reduced in TX-100 is concerned, its absorption and RR spectra exhibit an HS signature very similar to that observed for the Fe(II)PP(H_2O) complex formed in aqueous CTABr. Thus, a water binding to Fe(II)PP could be favored in the neutral TX-100 detergent when compared to the cationic CTABr detergent. However, the hydrophilic part of TX-100 is constituted by a poly(ethylene glycol) chain in which the terminal hydroxyl group has coordinating properties likely very similar to that of H_2O. Two possibilities can therefore be considered. A first one is a direct binding of a water molecule to Fe(II)PP. Alternatively, Fe(II)PP can bind the terminal ethanol group of TX-100, this O-donor ligand interacting with a water molecule via a H-bond. Considering the $H_2{}^{16}O \rightarrow H_2{}^{18}O$ change in aqueous TX-100 that induces clear upshifts of two identified low-frequency bands, the isotopic sensitivity of these bands is likely due to a strong coupling of the axial Fe−ROH stretch (with R = H from water or $-CH_2-CH_2$ from TX-100) with a pyrrole deformation mode (at 287 cm^{-1} in $H_2{}^{16}O$ or 295 cm^{-1} in $H_2{}^{18}O$) and the pyrrole stretching mode (ν_8 at 349 or 351 cm^{-1}).

Finally, the RR observation of an HS aqua complex mixed with the IS 4c Fe(II)PP form is consistent with the multicomponent Soret band observed in the spectra of hemin reduced in CTABr or NaDS. The Soret band of Fe(II)PP(OH^-) in aqueous detergent peaks at 440 nm (vide infra). Considering that the deprotonation of an axial ligand of Fe(II)PP produces a marked redshift of the visible absorption bands,[15a] the Soret maximum of the Fe(II)PP(H_2O) complex is expected to be in the 430 nm region. Comparing the absorption spectra of hemin reduced in aqueous CTABr with those of hemin reduced in aqueous NaDS or TX-100, the 428 nm component can be assigned to the Soret contribution of Fe(II)PP(H_2O) (Table 1).

Monohydroxy Complex of Fe(II)PP. The formation of monohydroxy complexes of Fe(II)-porphyrins has been observed

(12) The addition of KBr up to 1 M does not significantly change the RR spectrum. The presence of a large excess of sodium dithionite (10^3 equiv/heme) has no influence on the RR spectra.
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Table 2. Frequencies (cm^{-1}) of the High-Frequency RR Modes of High-Spin Fe(II)PP Complexes

mode	Fe(II)PP (H_2O) (CTABr)	Fe(II)PP (ROH) (R = H or $-CH_2-CH_2$) (TX-100)	Fe(II)PP (OH^-) (CTABr)	Fe(II)PP (OH^-)$_2$ (EtOH)	Fe(II)PP (2MeImH) (CTABr)	Fe(II)PP (2MeIm$^-$) (CTABr)
$\nu(C_a{=}C_b)$	1626	1626	1623	1624	1624	1624
$\nu(C_a{=}C_b)$	1616	1620	1616	1618	1618	1619
ν_{10}	1603	1605	1605	1605	1604	1605
ν_{37}			1583	1585	1584	1586
ν_2	1557	1563	1560	1562	1560	1559
ν_{11}			1549	1550	1549	1548
ν_{38}	1525	1524	1522	1522	1525	1525
ν_3	1472	1472	1472	1472	1471	1473
$\delta(=C_bH_2)_s$		1447	1440		1445	1443
ν_{28}		1425	1424	1426	1428	1426
ν_{12}		1392	1395	1396	1393	1393
ν_4	1358	1358	1357	1357	1355	1357
$\delta(C_aH{=})$		1305	1305	1302	1307	1307

Table 3. Frequencies (cm^{-1}) of the Low-Frequency RR Modes of High-Spin Fe(II)PP Complexes

mode	Fe(II)PP (H_2O) (CTABr)	Fe(II)PP (ROH) (R = H or $-CH_2-CH_2$) (TX-100)	Fe(II)PP (OH^-) (CTABr)	Fe(II)PP (OH^-)$_2$ (EtOH)	Fe(II)PP (2MeImH) (CTABr)	Fe(II)PP (2MeIm$^-$) (CTABr)
ν_{15}	752	752	752	752	758	755
γ_{11}		716	719	720	718	716
			697		700	699
ν_7	672	675	673	673	675	675
ν_{48}		586	585	585	586	587
γ_{21}		548	545		541	542
$\nu(Fe-OH^-)$			435	421		
			421			
$\delta(C_\beta C_a C_b)$	408	411	409	408	410	409
$\delta(C_\beta C_c C_d)$		376	377	379	380	377
				366		367
ν_8		349	347	343	349	349
γ_7		301	302	304	298	299
ν_9		256	257	257	258	255
$\nu(Fe-N_{ax})$					206	227
			155	149	139	132

but their spectroscopic properties were not fully determined.[15] Our spectrophotometric titrations of Fe(II)PP by hydroxide in aqueous CTABr clearly show the binding of one hydroxide ion to the iron atom. The low affinity of OH^- for Fe(II)PP (ca. 4 $\times$ 10^{-1} M) originates from the null charge of the Fe(II)-porphyrinate system. However, environment effects can strongly increase the affinity of OH^- for the ferrous ion.[15c] The 5c Fe(II)PP(OH^-) complex and the 6c Fe(II)PP(OH^-)(H_2O) complex are thus two plausible monohydroxy complexes formed in aqueous CTABr. This alkaline form shows λ_{max} at 440, 561, and 593 nm. These spectral features are very similar to those of the 2MeIm$^-$ complex of Fe(II)PP in aqueous CTABr (441, 560, and 592 nm) (Table 1). A parallel situation occurs in the family of the Fe(II)-tetraphenylporphyrins with a close similarity in absorption spectra for the OH^- and 2MeIm$^-$ complexes.[15c,16] As far as the RR spectra are concerned, the frequencies of the skeletal ν_2 (1560 cm^{-1}), ν_3 (1472 cm^{-1}), ν_4 (1357 cm^{-1}), ν_{10} (1605 cm^{-1}), and ν_{38} (1522 cm^{-1}) modes confirm the HS state of the monohydroxy complex of Fe(II)PP (Table 2). It is of importance to note that all these frequencies are also very close to those determined for the 2MeImH and 2MeIm$^-$ complexes of Fe(II)PP (Table 2). In the low-frequency regions of RR spectra, the ν_8 mode is observed at 347 cm^{-1} (Table 3). This RR frequency is again close to that determined for the ν_8 mode of various 5c imidazole and imidazolate complexes of Fe(II)-PP (345−349 cm^{-1}).[4a,b] Analysis of the absorption and RR spectra and the fact that the aqueous detergents stabilize the 5c ligation states via an interfacial effect[4a,b] are thus most consistent with the formation of a 5c complex in aqueous CTABr solution, i.e., the formation of Fe(II)PP(OH^-).[17]

To our knowledge, no axial Fe(II)−OH^- mode has been characterized for an Fe(II)-porphyrin complex. On the contrary, a number of ν(Fe(III)−OH) modes was assigned in the 450−575 cm^{-1} region of RR spectra of ferric heme proteins and model compounds.[18] Bands recognizable at 257, 300, 347, 366, and 377 cm^{-1} in the RR spectra of Fe(II)PP(OH^-) was already seen in the Soret-excited RR spectra of Fe(II)PP(2MeImH) and

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(17) (a) A Fe(II)-tetraphorphyrin(OH^-)(H_2O) complex has been studied by X-ray crystallography.[18b] The Fe(II) ion was found to sit above the porphyrin plane towards the OH^- ligand with an Fe−OH distance of 2.18 Å. Considering the Fe−OH_2 distance (2.95 Å), there is no evidence for a water coordination to the Fe(II) ion. The coordination properties of water are expected to be similar to those of alcohol. The coordinating Fe−O distances are in the 2.15−2.35 Å range.[18c] Finally, the negative OH^- ligand is expected to destabilize the binding of a weaker ligand (i.e., water) in the trans position favouring the formation of a 5c complex.[11a] (b) Fleischer, E. B.; Miller, C. K.; Webb, L. E. *J. Am. Chem. Soc.* **1964**, *86*, 2342−2347. (c) Barkigia, K. M.; Palacio, M.; Sun, Y.; Nogues, M.; Renner, M. W.; Varret, F.; Battioni, P.; Mansuy, D.; Fajer, J. *Inorg. Chem.* **2002**, *41*, 5647−5649.

Fe(II)PP(2MeIm$^-$).[4a,b] The ligand deuteration in Fe(II)PP(OH$^-$) produces a downshift of 4 cm^{-1} for a specific band at 435 cm^{-1}. When the mass effect is localized on the oxygen atom of the ligand (^{16}OH$^-$ versus ^{18}OH$^-$), we observe a downshift of this band by ca. 21 cm^{-1}. Assuming an isolated Fe−OH harmonic oscillator with Fe and OH as oscillating units and masses of 56, 17, 18, and 19 amu for Fe, ^{16}OH, ^{16}OD, and ^{18}OH, respectively, the theoretical shifts are −9.4 cm^{-1} for the ^{16}OH → ^{16}OD substitution and −17.9 cm^{-1} for the ^{16}OH → ^{18}OH substitution.[19] The 435 cm^{-1} band is thus assigned to a mode involving a stretching of the axial bond (ν(Fe(II)−OH$^-$)) likely coupled with a stretching of the Fe(II)−N(pyrrole) bonds. It is interesting to note that the experimental sensitivity to ligand deuteration of the 435 cm^{-1} band is weak (4 cm^{-1}) when compared to the theoretical shift (9.4 cm^{-1}). This effect is consistent with a bent geometry for the Fe(II)−O−H grouping. Such a conformation in fact minors the mass effect of the terminal proton or deuteron.[19] The frequency of the ν(Fe(III)−OH) mode of a 5c HS ferriporphyrin was determined at 541 cm^{-1}.[18d] The 435 cm^{-1} frequency reflects a weaker Fe−OH bond in Fe(II)PP(OH$^-$) than that in the ferric complex. The strong Fe(III)−OH bond observed for the ferric derivative is likely related to its ionic character.

Dihydroxy Complex of Fe(II)PP. In aqueous ethanol solution, the spectrophotometric titration of Fe(II)PP with OH$^-$ confirms the formation of a dihydroxy complex, i.e., Fe(II)PP(OH$^-$)$_2$.[7] The characterization of the Fe(II)PP(OH$^-$) and Fe(II)PP(OH$^-$)$_2$ complexes thus offers the possibility to compare the spectroscopic properties of two ferrous HS complexes with identical steric and electronic effects provided by the ligand but differing in coordination geometry.

We have no X-ray data on mono- and bis-(OH$^-$) complexes of Fe(II)-porphyrins. However, the structures of several 5c and 6c HS Fe(II)-porphyrin complexes have been determined and can be used as a reference for each compound. On one hand, the single OH$^-$ binding to Fe(II)PP is expected to produce a porphyrin with an out-of-plane displacement of the iron atom. The most typical examples of this coordination geometry concern the Fe(II)- porphyrin complexes axially coordinated by an imidazole ring or an electronegative oxygen donor.[16c,20] In these 5c HS complexes, the Fe−N(pyrrole) bond lengths are in the 2.09−2.11 Å range and the distance between the porphyrin center and the N(pyrrole) atoms is 2.03−2.05 Å.[16c,20] On the other hand, the porphyrin structure of the Fe(II)PP(OH$^-$)$_2$ complex is expected to be similar to that of bis-alcohol or bis-tetrahydrofuran complexes.[17c,21] In these 6c HS complexes, the porphyrin is nearly planar with an expanded core corresponding to Fe−N(pyrrole) distances of 2.06−2.09 Å.[21]

The wavelengths of the absorption maxima of Fe(II)PP(OH$^-$)$_2$ (435, 557, and 591 nm) are close to those of bis-alkoxide and bis-phenoxide complexes of ester derivatives of Fe(II)PP (427−436, 558−565, and 593−595 nm).[15a,22] These features are in agreement with an HS state for the ferrous ion. The positions of the absorption bands of the HS Fe(II)-porphyrins are sensitive to the nature of the axial ligand(s) and the porphyrin substituents as well as the structure and the environment of the Fe(II)-porphyrin complex. In particular, the negatively charged ligands produce red-shifted absorption spectra.[15a,16] The data obtained for Fe(II)PP(OH$^-$) and Fe(II)PP(OH$^-$)$_2$ confirm this trend (Table 1). Nevertheless, the redshifts (2−5 nm) of the α, β, and Soret bands of Fe(II)PP(OH$^-$) relative to those of Fe(II)PP(OH$^-$)$_2$ (Table 1) can be interpreted in the frame of an out-of-plane distortion of the porphyrin macrocycle for the monohydroxy complex. This distortion is likely a moderate doming similar to that observed for crystallized 5c HS ferrous complexes.[16c,20] Redshifts of the absorption bands were recently established for ruffled and saddled 6c LS Fe(II)-porphyrin complexes.[23] This study shows that a redshift of the absorption bands is also operative when a planar HS Fe(II)-porphyrin becomes domed.

The high-frequency region of RR spectra are essentially identical for Fe(II)PP(OH$^-$) and Fe(II)PP(OH$^-$)$_2$. In passing from the mono- to the dihydroxy complex, small shifts (2−4 cm^{-1}) are however detected for the skeletal ν_2 mode as well as for several vinyl modes. The frequency shifts of the vinyl modes may have two origins. In the one hand, the different environments for the complex formation could induce different polar effects and/or steric effets on the peripheral groups when the porphyrin interacts with either the water and ethanol molecules or the detergent micelle. On the other hand, the different conformations of the porphyrin macrocycle (domed versus planar) could change the electronic and kinematic coupling of the vinyl groups with the adjacent pyrrole rings.

As far as the frequency shift of ν_2 is concerned, it could be assigned to the different conformations adopted by the macrocycle in Fe(II)PP(OH$^-$) and Fe(II)PP(OH$^-$)$_2$. For 6c LS Fe(II)-porphyrins complexes, the ν_2 frequency was found to be sensitive to porphyrin ruffling but insensitive to porphyrin saddling.[23] The downshifted ν_2 frequency observed for Fe(II)PP(OH$^-$) could be associated with the expected porphyrin doming induced by the binding of a single hydroxide ligand. Considering that the same type of pyrrole movement occurs in the saddled and domed conformations,[24] a conclusion

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(24) (a) The saddled and domed conformations produce pyrrole tilts about the $C_\alpha-C_\alpha$ axes. For these two types of porphyrin conformation, only the direction of the tilts differs. The pyrrole rings of the saddled porphyrins are displaced, alternatively, above and below the mean plane of the core. Upon doming, the direction of the tilt is the same for the four pyrroles. The sensitivities of the ν_2, ν_3, ν_{10} frequencies upon porphyrin ruffling were related to the twisting of the methine bridges.[21b,c] Doming and saddling change the Fe−N(pyrrole) bond lengths without any twisting of the $C_\alpha-C_m-C_\alpha$ bonds. (b) Shelnutt, J. A.; Medforth, C. J.; Berber, M. D.; Barkigia, K.; Smith, K. M. *J. Am. Chem. Soc.* **1991**, *113*, 4077−4087. (c) Jentzen, W.; Simpson, M. C.; Hobbs, J. D.; Song, X.; Ema, T.; Nelson, N. Y.; Medforth, C. J.; Smith, K. M.; Veyrat, M.; Mazzanti, M.; Ramasseul, R.; Marchon, J.-C.; Takeuchi, T.; Goddard, W. A., III; Shelnutt, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 11085−11097.

that the frequency of ν_2 could be sensitive to porphyrin doming seems however unlikely. In this line, it is important to consider that the frequency of ν_2 is strongly influenced by the peripheral vinyl groups.[25] Therefore, the downshifted ν_2 frequency of Fe(II)PP(OH^-) can be alternatively attributed to either a steric effect or an electronic perturbation on the vinyl groups and not to a direct effect of the porphyrin doming. We favor this latter interpretation taking into account the behavior of other skeletal modes. In particular, the ν_3 mode that is vinyl-insensitive exhibits no frequency shift in the RR spectra of the two hydroxide complexes (Table 2). In the RR spectra of Fe-heme complexes, this skeletal mode is affected to different extents by the porphyrin distortions.[26]

In the low-frequency regions of RR spectra, the ν_8 mode is downshifted from 347 to 343 cm^{-1} when the coordination is changed from 6 to 5 (Table 3). This mode involves ν(Fe−N(pyrrole)) and $\nu(C_\alpha-C_m)$.[11b,27] For 6c LS complexes of ferroporphyrins, the ν_8 frequency was found to be upshifted by the ruffling as well as by the saddling of the porphyrin macrocycle.[23] For the HS complexes presently investigated, the small vinyl influence on ν_8 strongly supports that its frequency is upshifted upon change in porphyrin conformation (Table 3). In other terms, the increased frequency of the in-plane ν_8 mode does not follow the increase in length of the Fe−N(pyrrole) bonds following the porphyrin doming but is related to the accompanying decrease in core size.[16c,20] In conclusion, both a redshift of the absorption bands and an upshift of the ν_8 RR mode are spectral parameters corresponding to a porphyrin doming in HS complexes. A similar behavior was observed upon porphyrin saddling in 6c LS complexes.[23]

To our knowledge, no axial heme mode involving the Fe(II)−$(OH^-)_2$ bonds was assigned. The experimental shifts observed for a ligand specific band at 421 cm^{-1} (4 cm^{-1} for the $^{16}OH^- \rightarrow {}^{16}OD^-$ substitution and 17 cm^{-1} for the $^{16}OH^- \rightarrow {}^{18}OH^-$ substitution) allow its assignment as a mode of the axial bonds. Assuming a linear three-body oscillator for the HO−Fe−OH grouping, the expected shifts are −11.9 cm^{-1} upon ligand deuteration and −22.8 cm^{-1} upon $^{16}OH^- \rightarrow {}^{18}OH^-$ substitution. Upon $^{16}OH^- \rightarrow {}^{16}OD^-$ substitution, we observe a small experimental shift of the 421 cm^{-1} band. Like in the case of the monohydroxy complex, this effect is again associated with the bent structure of the Fe−O−H and Fe−O−D bonds. All these observations are thus in agreement with the assignment of the 421 cm^{-1} band to a symmetric stretching mode of the axial Fe−OH bonds (ν_s(Fe(II)−$(OH^-)_2$). In the ferric series, a ν_s(Fe(III)−$(OH^-)_2$) mode was characterized at 447 cm^{-1} for an HS dihydroxy complex of a water-soluble Fe(III)-porphyrin.[18e] According to a mechanism similar to that already seen for the monohydroxy complexes, the strongest Fe−OH^- bonds observed for the dihydroxy ferric compound likely originate from an electrostatic interaction between the OH^- anion and the charged iron atom.

Heme c_i of Cytochrome b_6f. The comparisons of the crystallographic structures of cyt bc_1 and cyt b_6f complexes have recently revealed the presence of an extra-heme in cyt b_6f.[2] The detection of this additional heme c_i had no precedence since it was not predicted using conventional biochemical and spectroscopic methods. In particular, heme c_i is not directly observable in the absorption spectra of cyt b_6f due to the superimpositions of the strong electronic transitions of the LS hemes (f, b_L and b_H), chlorophyll-*a*, and β-carotene.

Heme c_i is located in the Q_i site, close to heme b_H. This location blocks any contact between the substrate and heme b_H. Heme c_i is therefore an obligate intermediate in the linear electron transfers between photosystem II and photosystem I as well as in the cyclic electron transfers around photosystem I. This role implies a redox potential for heme c_i close to, or slightly higher than, that of heme b_H (-70 ± 25 mV).[28] The peripheral composition of heme c_i is not fully determined, but it was classified as a c-type on the basis of its thioether link with a conserved cysteine residue.[2a,3] Its most unusual property however concerns its axial ligation since no amino acid is able to axially bind the iron atom.[2a] In the fifth ligand position, an electronic density was assigned to either a water molecule or a hydroxide ion but does not establish a true coordination of this exogenous molecule. The proximity of one propionate group of heme b_H appears to stabilize through H-bonding this trapped or bound OH^- or H_2O molecule.

An RR investigation of a monomeric form of *Chlamydomonas reinhardtii* cyt b_6f has recently identified weak contributions of heme c_i.[3] Among strong RR bands arising from hemes f, b_H, and b_L, the ν_4, ν_3, and ν_2 frequencies of reduced heme c_i were determined at 1354, 1471, and 1576 cm^{-1}, respectively. This set of frequencies was found close to those previously determined for various HS cyt c′ and is thus consistent with an HS state for the ferrous heme c_i.[3,30] The absence of water or hydroxide coordination to reduced heme c_i would produce a spectral signature corresponding to a 4c IS complex.

In vivo, a carrier G with a redox potential ($E_{1/2}$) of about −30 mV has been identified by absorption spectroscopy as a c′-type cytochrome exchanging electrons with heme b_H.[30a,b] Given their similarities in functional and spectroscopic properties, heme c_i can be tentatively associated with carrier G.[3,30] The classical c′-type cytochromes constitute a unique class of cytochromes that exhibit an HS 5c state for its ferrous state. They have redox potentials varying from −10 to +100 mV.[31] Its heme is axially ligated by a H-bonded imidazole ring.[29,3é] The comparison of the RR frequencies observed for reduced heme c_i with those presently determined for H_2O and OH^- complexes of Fe(II)PP unambiguously establishes that heme c_i is coordinated by a water or hydroxide molecule. Unfortunately, the RR frequency of the skeletal porphyrin modes cannot distinguish the H_2O and OH^- ligations. Only, the characterization of the absorption spectrum of reduced heme c_i and/or the

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frequency of the Fe-ligand stretching mode would be clearly informative. From this study on model compounds, one can however conclude that a coordination change from imidazole (or imidazolate) to H_2O (or OH^-) does not drastically modify the electronic structure of the Fe(II)-heme. A parallel situation occurs for homologue ferric complexes.[18f] Consequently, an axial substitution of a H-bonded imidazole ring (i.e., the case of a typical cyt c′)[29] by a H-bonded H_2O molecule (as one of the two possibilities of axial ligation for heme c_i) is expected to produce small effects on the oxidation potential of heme. For a given Fe-porphyrin complex in a given solvent, the redox potential ($E_{1/2}$) of the metal atom is influenced by the axial coordination and particularly by the electronegativity of the ligand(s).[15c,d,33,34] For example, deprotonation at a heme-bound imidazole is known to produce a decrease in $E_{1/2}$ of 700 mV.[33a] The monohydroxy complexes of Fe-"basket handle" porphyrins also exhibit very negative potentials.[15c] This large effect is directly related to the preferential stabilization of the oxidized heme through a charge compensation between the Fe(III)-heme which carries one formal positive charge and the anionic ligand. As the basicity of the axial ligand is increased, the ferric state is stabilized relative to the ferrous state and consequently a more negative $E_{1/2}$ is observed. A similar charge effect on the redox potential can be operative when water and hydroxide complexes of Fe(II)- and Fe(III)-porphyrins are considered.[15c,34,35] The axial deprotonation of the Fe(III)- and Fe(II)-porphyrin(H_2O) complexes obviously increases the ligand basicity. The OH^- ligand is a stronger σ-donor species than H_2O, and consequently, the ferric state is stabilized relative to the ferrous state. A largely more negative $E_{1/2}$ is thus expected for the Fe(III)/Fe(II)PP-(OH^-) redox couple when compared to the Fe(III)/Fe(II)PP-(H_2O) couple in the same environment. Moreover, the cyt b_6f complex uses a so-called Q-cycle to couple electron transfer with proton translocation. In this transmembrane complex, heme c_i is on the stromal face of the Q_i site where proton uptake and reduction of plastoquinone occur. The coupling of ligand deprotonation with a decreased redox potential in an Fe-porphyrin(H_2O)/(OH^-) complex like heme c_i could therefore originate a redox-dependent proton pump very analogous to that proposed for the Fe-porphyrin(imidazole)/(imidazolate) systems.[4b] In addition to the similar properties of the Fe-porphyrin(H_2O)/(OH^-) and Fe-porphyrin(imidazole)/(imidazolate) couples, the low affinities of the H_2O and OH^- ligand for the Fe(II)-porphyrins offer an interesting flexibility in terms of ligand exchange. In this line, heme c_i is a likely binding site for a physiological ligand like plastoquinone. To conclude about the two possibilities of axial ligation for heme c_i, a coordination of the iron atom of heme c_i by a water molecule appears to be the most plausible on the basis of a redox potential close to that of heme b_H. The $E_{1/2}$ value for the aqua complex of a water soluble Fe(III)-/Fe(II)-porphyrin was estimated to be −230 mV.[34a] This value can be positively shifted by 200−300 mV taking into account the degree of hydrophobicity of the heme cavity in proteins.[36] A OH^- binding to heme c_i would produce a too negative $E_{1/2}$ value.

Supporting Information Available: Plot of $1/(R - R_0)$ vs $1/[OH^-]^2$ and low frequency regions (220−730 cm^{-1}) of RR spectra of Fe(II)PP in 0.5% TX-100 aqueous solution at pH 7.0. This material is available free of charge via the Internet at http://pubs.acs.org.

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