water content are also likely candidates. The use of single or multiple isotopic labels will improve selectivity and sensitivity by providing more spins in a particular location and by increasing the probabilities for spin exchange between nearby spins.

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High-Affinity Binding of Quinine to Iron(III) Porphyrins: Novel Formation of Alkoxide Complexes from Alcohols and Amines

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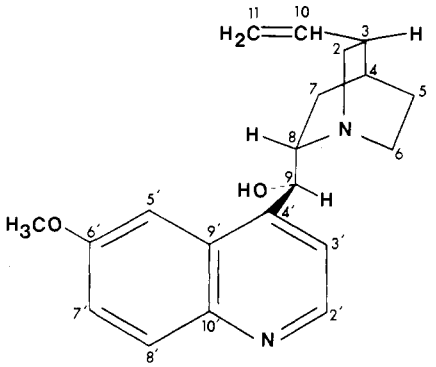
Abstract: The antimalarial drug quinine is shown to have a high affinity for binding to iron(III) porphyrin derivatives in nonaqueous solution. Various spectroscopic measurements are consistent with coordination through a deprotonated benzylic alcohol residue rather than through quinoline or quinuclidine nitrogen atoms. Deprotonation of the alcohol is facilitated by the basic quinuclidine moiety. Addition of simple alcohols and amines to iron(III) porphyrins in aprotic solvents likewise yields alkoxide complexes rather than mixed alcohol–amine complexes. Optical spectra for the quinine adducts resemble those of previously characterized alkoxide iron(III) porphyrin complexes. Proton NMR spectra show hyperfine-shifted porphyrin signals that are distinctive for alkoxide complexes. Hyperfine-shifted quinine resonances also provide compelling evidence for adduct formation. The stoichiometry for iron(III) coordination is 1:1, and the resulting complex is in the high-spin $S = {}^5/_2$ state on the basis of NMR hyperfine-shift patterns, magnetic measurements, and EPR spectra. Quinine binding to μ-oxo dimeric iron(III) porphyrins was not detected. It is thus demonstrated that quinine interactions with heme-containing malarial pigments in the red blood cell are unlikely to involve coordination of drug nitrogen residues or ligation to a μ-oxo dimeric iron(III) porphyrin.

Introduction

The antimalarial drug quinine reportedly exhibits high-affinity binding to iron(III) porphyrin species in both aqueous and nonaqueous solution.[1,2] Aside from the possibility of only π–π stacking interactions between quinoline and porphyrin rings,[3] three basic residues are available for coordination as is apparent in the quinine structure shown below. Suggestions of quinuclidine



nitrogen coordination to iron(III) porphyrins contained in "wet" benzene solution[2] has been challenged[4] on the basis of comparative optical spectra. A 1:1 iron(III) protoporphyrin–quinine aggregated complex is formed at neutral pH in aqueous solution and this adduct is optically active in the Soret wavelength region (negative circular dichroism band) by virtue of coordination of the chiral quinine ligand.[5] The molecular basis for quinine–iron(III) porphyrin interactions has not been unambiguously demonstrated by previous workers for either aqueous or nonaqueous solutions.

A variety of physical measurements reported here for nonaqueous solutions have served to define the quinine binding site in aprotic media. Proton and carbon-13 NMR spectroscopic methods are emphasized, and magnetic, electron paramagnetic resonance, optical spectral, and electrochemical techniques are utilized in a complementary fashion. The vast literature relating molecular and electronic iron porphyrin structures with results for the various physical methods has made possible detailed characterization of the quinine–iron(III) porphyrin interaction. A novel example of iron(III) porphyrin–alkoxide coordination induced by an adjacent amine residue is recognized here for the first time. On the basis of comparative spectroscopic measurements the previously reported mixed "alcohol–amine" iron(III) porphyrin complexes[6] are more reasonably described as alkoxide complexes. In general, alkoxide iron(III) porphyrin complexes can be generated in aprotic media through addition of an alcohol and small quantities of an aliphatic amine. The relevance of quinine alkoxide binding to cellular antimalarial drug receptors cannot be directly evaluated from the nonaqueous model study, but this newly discovered mode of interaction must now be considered among the various possibilities.

Experimental Section

Quinine (Sigma Chemical Co.) was vacuum dried at 125 °C overnight prior to transfer under a nitrogen atmosphere to the drybox. Quinuclidine (Sigma) was recrystallized from *n*-hexane in the drybox. Quinoline

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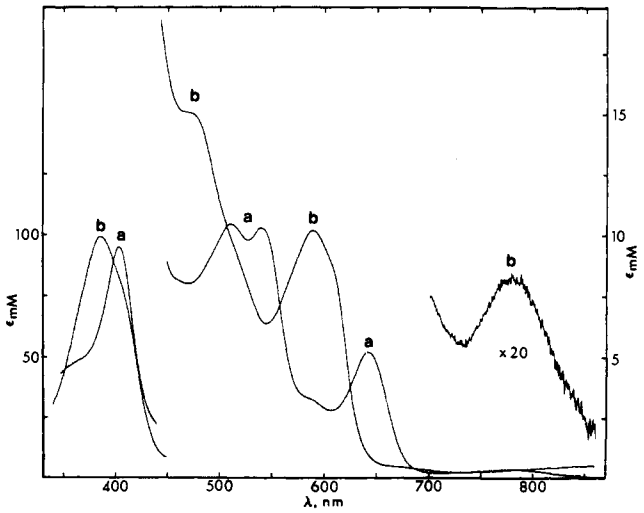

Figure 1. Optical spectra of (PPDME)Fe^{III} complexes, $CHCl_3$ solvent, iron porphyrin 9.3×10^{-5} M, 25 °C, below 450 nm a 0.1-cm cell was employed. (a) (PPDME)FeCl; (b) (PPDME)Fe–quinine complex with total quinine concentration of 0.01 M.

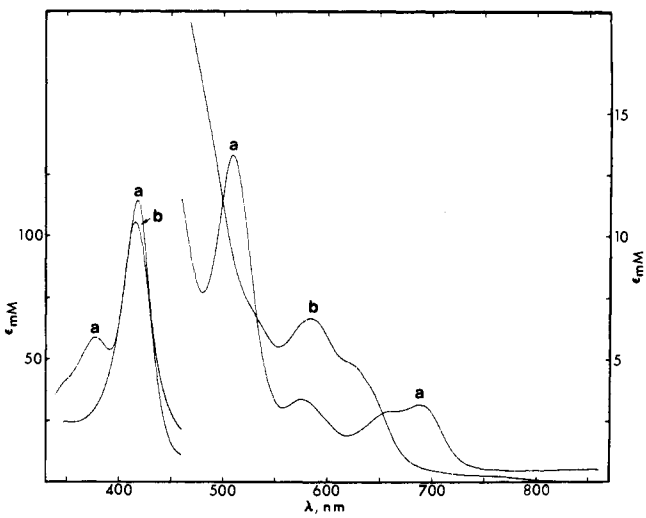

Figure 2. Optical spectra of (TPP)Fe^{III} complexes, $CHCl_3$ solvent, iron porphyrin 9.4×10^{-5} M, 25 °C, below 460 nm a 0.1-cm cell was employed. (a) (TPP)FeCl; (b) (TPP)Fe–quinine complex with total quinine concentration of 0.01 M.

was distilled from potassium hydroxide. Chloroform-d_1 (Merck) was stirred over anhydrous K_2CO_3 for 16 h, distilled from P_2O_5 in a nitrogen atmosphere, and stored in the drybox over activated molecular sieves. The chloride and trifluoromethanesulfonate adducts of (tetraphenylporphyrinato)iron(III) ((TPP)FeCl and (TPP)Fe(SO_3CF_3)) were prepared by the procedures reported earlier.[7] Chloroiron(III) protoporphyrin IX dimethyl ester ((PPDME)FeCl) was obtained by esterification of hemin (Aldrich) with subsequent HCl cleavage of the μ-oxo dimer ((PPDME)Fe)$_2$O.[8] The $SO_3CF_3^-$ complex was prepared by stirring a CH_2Cl_2 solution of the corresponding μ-oxo dimer with 1 M aqueous HSO_3CF_3 for 16 h. Crystalline product was obtained by reducing the volume of the (separated) CH_2Cl_2 solution with simultaneous addition of *n*-hexane. The methine-d_4 chloroiron(III) octaethylporphyrin compound was prepared from the metal-free porphyrin which had been fused with toluenesulfonic-d_1 acid.[9] The integrity of iron porphyrin products was verified by proton NMR and optical spectroscopy.

Solutions used for spectroscopic studies were prepared under a nitrogen atmosphere to suppress competing basic hydrolytic reactions. Manipulations were generally performed in a Vacuum Atmospheres inert atmosphere glovebox. Nuclear magnetic resonance spectra were recorded on multinuclear JEOL FX-90Q and Bruker WM-360 FTNMR spectrometers. Downfield shifts are given a positive sign, and chemical shift values are referenced to $(CH_3)_4Si$. The Evans NMR method[10] was used to measure solution magnetic susceptibilities. A capillary tube (Wilmad) contained the solvent, $(CH_3)_4Si$ reference substance, and the same concentration of quinine as was placed in the 5-mm NMR tube. A diamagnetic correction for the porphyrin was based on the value reported for the TPP ligand[11] and appropriate Pascal constants.

Electron paramagnetic resonance spectra were recorded at −180 °C in a 60:40 toluene–methylene chloride glass using a Varian E-104A X-band instrument. Iron(III) porphyrin concentrations were 1–2 mM. Optical spectra were recorded in $CHCl_3$ solution by using 1- or 10-mm cells in a Cary 219 spectrophotometer.

Cyclic voltammetry was performed using methylene chloride solvent, a platinum bead working electrode, and 0.1 M tetrabutylammonium perchlorate supporting electrolyte. Iron porphyrin concentrations were 1.5 mM and quinine adducts were prepared by adding 3 equiv of the substance. Solutions were deoxygenated by a CH_2Cl_2-saturated nitrogen stream prior to CV scans. Potentials with respect to the Ag/0.10 M $AgNO_3$/acetonitrile reference electrode were adjusted to the usual SCE reference by an empirically determined 0.38-V factor. Measurements were made with a Princeton Applied Research Model 173 potentiostat driven by the Model 175 Universal Programmer. Scan rates were varied from 20 to 500 mV/s.

Table I. Comparative Optical Spectra for Iron(III) Porphyrin Complexes

	λ_{max} (ε, mM)
(PPDME)Fe^{III} complexes	
quinine[a]	386 (98.9), 470 (15.1), 590 (10.2), 780 (0.41)
Cl^- [a]	360 (47.3), 404 (95.1), 510 (10.4), 530 (10.3), 642 (5.2)
μ-oxo dimer[b]	357 (75.2), 397 (115), 573 (14.1), 599 (11.8)
bis(imidazole)[c]	535, 560
mono(imidazole)[d]	403 (102), 498 (11.25), 640 (3.23)
OCH_3^- [e]	394, 576, 763
quinine[f]	410, 495, 608
(TPP)Fe^{III} complexes	
quinine[a]	416 (106), 585 (6.7), 625 (4.6), 760 (0.27)
Cl^- [a]	378 (58.7), 418 (114), 510 (13.3), 576 (3.4), 656 (2.9), 690 (3.1)
μ-oxo dimer[g]	408 (106), 571 (10.7), 612 (4.8)
bis(imidazole)[h]	417 (154), 459 (20.8), 549 (9.04), 580 (5.51), 636 (1.30)
OCH_3^- [i]	417 (105), 575 (8.3), 617 (5.0), 770 (0.3)
OH^- [j]	416 (100), 580 (7.5), 633 (4.4)

[a] Results from this study, $CHCl_3$ solvent, the quinine complexes were generated by addition of quinine to a concentration of 0.01 M. [b] Reference 8 benzene solvent, ε values are on a per iron porphyrin basis. [c] Reference 12, Me_2SO solvent, Soret band position and ε values not reported. [d] Reference 13, horseradish peroxidase in neutral aqueous solution. [e] Reference 14, $CHCl_3$ solvent, ε values not reported. [f] Reference 2, "wet" benzene solvent, ε values not reported. [g] Reference 15, benzene solvent, ε values are on a per iron porphyrin basis. [h] Reference 16, $CHCl_3$ solvent. [i] Reference 17, 1,2-dichloroethane solvent. [j] Reference 18, for iron(III) tetramesitylporphyrin.

Results and Discussion

Optical Spectra. Addition of quinine to chloroform solutions of chloroiron(III) porphyrins results in dramatic visible-region spectral changes. Spectra of (PPDMe)Fe^{III} and (TPP)Fe^{III} adducts are shown in Figures 1 and 2. Well-defined isosbestic points were not observed for titration experiments perhaps due to for-

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mation of minor amounts of μ-oxo dimer (vide infra) or due to secondary association of quinine with the iron porphyrin complex. An indication of the site of quinine coordination may be gained from comparison of spectra for other iron(III) porphyrin complexes listed in Table I. Possible nitrogen coordination is evaluated on the basis of spectra obtained from bis(imidazole) complexes and from horseradish peroxidase (which is known to have one axial histidine ligand). No resemblance is apparent for the spectra of these species and those of the (PPDME)Fe(III) and (TPP)Fe(III) quinine adducts. Further evidence disfavoring coordination of quinine nitrogen atoms comes from insignificant spectral changes associated with addition of quinuclidine and quinoline to chloroiron(III) porphyrin complexes.

Binding of oxygen-derived anionic ligands yields spectra that resemble those of the quinine complexes. Common spectral features are apparent for the (PPDME)Fe(III) μ-oxo dimer and the OCH_3^- complex. It should be noted that the normally very broad, weak long-wavelength band is distinctive at 763 and 780 nm in the OCH_3^- and quinine complexes, respectively. Table I reveals an even closer resemblance for the optical spectra of quinine, OCH_3^-, and OH^- adducts of (TPP)Fe(III). A distinctive long-wavelength band is once again apparent. The considerable perturbation of the high-spin iron(III) porphyrin spectrum by OCH_3^- has been discussed in terms of the increased ligand field and increased charge transfer by this highly basic residue.[17] The hydroxide ion appears to bring the same spectral perturbations. By analogy the optical spectra imply coordination of quinine through the deprotonated alcohol residue.

The iron(III) protoporphyrin–quinine spectrum previously reported[2] for a benzene extract of a neutral aqueous solution has features in common with the spectrum in dry chloroform (Table I), but differences in the spectra suggest involvement of water or important solvent dependence. It should be noted that no spectral changes were apparent when quinine was added to $CHCl_3$ solutions of the μ-oxo dimeric iron(III) porphyrins.

Spin States of the Quinine Adduct. Magnetic and EPR measurements confirm the high-spin iron(III) state for quinine complexes suggested by optical spectral correlations. A magnetic susceptibility measurement for (PPDME)FeCl in $CDCl_3$ solution containing excess quinine revealed a moment of 6.0 ± 0.1 μ_B. This result matches the expected $S = {}^5/_2$ spin-only value of 5.92 μ_B. Corresponding EPR spectra of both (PPDME)Fe(SO_3CF_3) and (TPP)Fe(SO_3CF_3) quinine complexes were obtained. The $SO_3CF_3^-$ derivatives were employed as these spin-admixed $S = {}^5/_2, {}^3/_2$ species have a strong $g \simeq 4$ signal, which is readily distinguished from the $g \cong 6$ high-spin signal. Strong $g = 5.7$ and weak $g = 2.0$ signals observed for the quinine complexes are typical for high-spin iron(III) porphyrins. Appearance of a higher field shoulder on the $g = 5.7$ component was dependent on solvent (glass) composition and the quantity of quinine present. The shoulder may be due to secondary association of excess quinine with the iron porphyrin complex or a consequence of poor glass formation. The $g = 2$ component of quinine complexes is enhanced in intensity relative to the parent chloro complexes. This is also the case for previously reported hydroxoiron(III) porphyrin complexes[18] and is likely to be observed for alkoxy complexes in general.

Electrochemical Measurements. Cyclic voltammetric measurements were attempted in an effort to provide information about possible redox chemistry for the iron porphyrin–quinine complexes. In the presence of excess quinine required to fully ligate the iron porphyrin, the oxidative scan was highly irreversible and large currents indicated quinine oxidation. Reductive scans of the (TPP)Fe–quinine complex showed broad waves in the −0.67- to −0.87-V region which were highly irreversible. A reversible wave at −1.03 V matches that previously reported for the Fe(II) → Fe(I) step.[19] This reduction has been characterized as one involving four-coordinate iron porphyrin species. Thus, the reductive chemistry is consistent with loss of quinine as an iron(II) ligand on the basis of irreversible Fe(III) → Fe(II) waves and an unperturbed Fe(II) → Fe(I) potential. Reduction waves for the (PPDME)Fe–quinine complex were highly irreversible.

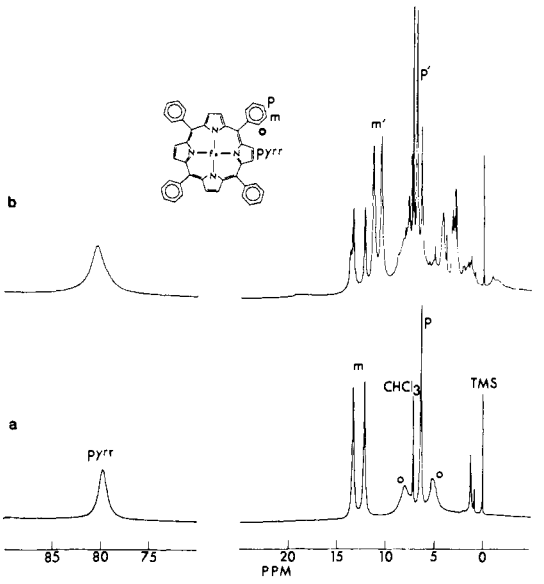

Figure 3. Proton NMR spectra of (TPP)Fe^{III} complexes at 360 MHz, $CDCl_3$ solvent, 25 °C. (a) (TPP)FeCl, 30 mM; signals in the 0.8–1.6 ppm region are due to water in the $CDCl_3$ solvent and heptane of crystallization; (b) (TPP)FeCl, 30 mM, quinine, 30 mM.

Proton NMR Spectroscopy. Effects on the proton NMR spectrum for quinine binding to (TPP)FeCl are described in Figure 3. Proton NMR spectra of high-spin iron(III) porphyrins have been well described.[20,21] Only one facet of the assignments in Figure 3a requires comment; it should be noted that *m*- and *o*-phenyl signals are split into doublets as a consequence of an out-of-plane iron atom and slow rotation of orthogonal phenyl groups. Stepwise addition of quinine reveals corresponding appearance of a new set of *m*-phenyl signals at 11.25 and 10.48 ppm, with retention of a far downfield pyrrole proton signal characteristic of a high-spin iron(III) complex. The spectrum for a $CDCl_3$ solution containing equivalent amounts of (TPP)FeCl and quinine is shown in Figure 3b. Formation of the new (TPP)Fe(III) complex is also apparent when benzene is employed as a solvent. Integration of *m*-phenyl peaks in Figure 3b reveals that 61% of the iron porphyrin has been converted to the quinine-bound form. This implies a 1:1 stoichiometry, as less than 50% of the iron porphyrin would be quinine ligated if bis-coordination were required. Addition of excess quinine causes disappearance of the *m*-phenyl signals of the chloro complex with conversion to those associated with the quinine complex. A small amount of μ-oxo dimer (pyrrole proton signal at 13.45 ppm) is also formed due to persistent traces of water. The (TPP)Fe(SO_3CF_3) complex is converted completely to the quinine complex by 1 equiv of quinine (taking into account measurable formation of μ-oxo dimer). This means that chloride ion effectively competes with quinine, whereas the very weak $SO_3CF_3^-$ ligand is readily displaced. The equilibrium

$$\text{(TPP)FeCl} + \text{quinine} \rightleftarrows \text{(TPP)Fe–quinine}^+ + \text{Cl}^-$$

is thus defined by an equilibrium quotient of 4.1 for the solution conditions described in Figure 3b.

Observation of separate *m*-phenyl signals in Figure 3b for the chloro and quinine complexes indicates slow exchange on the NMR time scale between porphyrin complexes. Certain quinine signals are shifted well outside the usual diamagnetic region upon coordination; broad signals are seen slightly upfield of $(CH_3)_4Si$ and at approximately 18 ppm downfield. These as yet unassigned axial ligand signals represent adduct formation in the slow-exchange limit, as is evident in the lack of dependence on chemical

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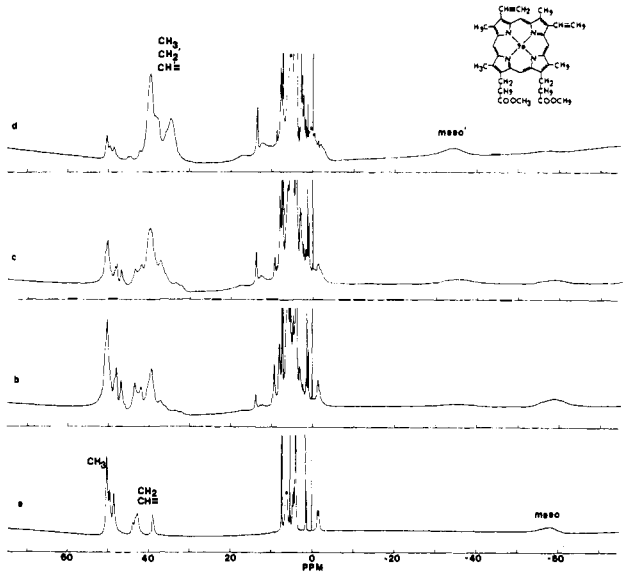

Figure 4. Proton NMR spectra of (PPDME)$Fe^{III}Cl$ at 360 MHz with sequential addition of quinine, $CDCl_3$ solvent, 25 °C, iron porphyrin concentration 30 mM. (a) No quinine added; (b) 10 mM quinine; (c) 20 mM quinine; (d) 30 mM quinine.

shift values for varying ratios of quinine and iron(III) porphyrin. The 18 ppm signal also follows approximate Curie law behavior, indicating that the chemical shift value is not perturbed by a dynamical term. The magnitude of shift, slow exchange, and line broadening for the 18 ppm signal can only be explained by formation of a covalent iron–quinine linkage.

Retention of a split *m*-phenyl signal in the quinine complex provides evidence against symmetrical bis ligation (coordination of two Me_2SO ligands serves to collapse the *m*-phenyl doublet). Simultaneous chloride and quinine binding appears unlikely on the basis of the apparently competitive binding of this ion and the fact that both (TPP)FeCl and (TPP)$Fe(SO_3CF_3)$ yield quinine complexes with identical proton NMR spectra. An attempt to detect the ^{35}Cl NMR signal of the quinine complex was unsuccessful even though the signal for a chloroform solution of Bu_4NCl at the same concentration was readily observable. Sharp ^{35}Cl NMR signals are expected only when the atom is in a rigorously cubic environment. Either coordination to the iron center or (more likely) ion pairing/hydrogen bonding at a protonated quinuclidine residue may explain our inability to detect the ^{35}Cl resonance.

The pyrrole proton signal of (TPP)Fe^{III} is barely shifted upon coordination of quinine. This is not especially surprising in view of the relative insensitivity of this resonance to the nature of the anionic axial ligand.[20] However, an appreciable line-width change is noted for the pyrrole proton signal upon quinine coordination. Respective line widths at half-height are 250 and 700 Hz for chloro and quinine adducts. This observation is entirely consistent with coordination of a moderately strong field alkoxy residue from quinine. The ligand-field strength of axial anionic iron(III) porphyrin ligands is known to modulate NMR line widths through control of the magnitude of the zero-field splitting parameter.[22,23] Relatively stronger field ligands also give a small upfield bias to the *m*-phenyl proton signals as a consequence of reduced spin density at the meso carbon position.[24]

Quinine binding is not restricted to the synthetic (TPP)Fe^{III} species. Proton NMR spectra associated with sequential addition of quinine to (PPDME)FeCl are shown in Figure 4. Striking changes occur in both the far-downfield and far-upfield regions. The fact that conversion to the quinine complex is nearly complete at a 1:1 iron porphyrin–quinine ratio provides further support for this stoichiometry. The ring methyl, methylene, and vinyl signals originally found in the 40–50 ppm region for the chloro complex appear as a group of broad, overlapping signals near 40 ppm in the quinine complex. Separate broad upfield meso proton signals are likewise observed at −58 and −35 ppm for the chloro and quinine complexes, respectively. Diminished hyperfine NMR shifts for ring substituents and for the meso proton of the quinine complex are consistent with coordination of an anionic ligand of increased ligand field strength.[25] In this regard the ordering of absolute hyperfine-shift values for iron(III) deuterohemin dimethyl ester complexes is $Br^- > Cl^- > N_3^- > F^- \sim OPh$; the meso proton signal ranges from −57 to −35 ppm (at 35 °C). The ligand field strength of the putative alkoxide residue of quinine thus appears to be at least as great as that of the fluoride or phenoxide ions.

Coordinated quinine signals are observed outside the diamagnetic region at 17, 13, and −2 ppm much as is the case for the (TPP)Fe^{III} complex. The relatively sharp signal at 13.6 ppm must be assigned to a vinyl proton, as the signal is absent in the spectrum of the (TPP)Fe^{III} and deuterohemin dimethyl ester quinine complexes.

Proton NMR measurements were conducted for additional iron porphyrin species and for the molecular fragments of quinine. No changes were detectable in the high-field proton NMR spectra of $((TPP)Fe)_2O$ solutions to which quinine was added. Likewise, addition of quinoline to a $CDCl_3$ solution of (TPP)FeCl brought no detectable change in the proton NMR spectrum. When "dry" quinuclidine was added to (TPP)FeCl or (PPDME)FeCl the hyperfine proton NMR shift patterns of the porphyrins remained unchanged to temperatures as low as −53 °C. However, when quinuclidine was used as received, a $CDCl_3$ solution 10 mM in (TPP)FeCl and 12 mM in quinuclidine exhibited minor proton NMR resonances at 11.23 and 10.37 ppm in addition to the *m*-phenyl signals at 13.45 and 12.27 ppm for the parent chloro complex. Approximately 30% of the iron porphyrin was converted to the μ-oxo dimer by the "wet" quinuclidine. At lower temperatures the new set of *m*-phenyl signals increased in intensity as signal intensity for the parent chloro complex diminished. Both sets of signals migrated downfield at lower temperatures and thus exhibited apparent Curie law behavior. At −43 °C the intensities of the two sets of doublets were approximately the same. The process was reversible, and upon return to room temperature the 11.23 and 10.37 ppm peaks reverted to minor components. Involvement of water through formation of a coordinated hydroxide ion provides a reasonable explanation for these observations. The sterically hindered quinuclidine base likely stabilizes hydroxide coordination through hydrogen bonding much as is the case for alkoxide coordination in the quinine complex. Hydroxide coordination in sterically hindered iron(III) porphyrins has recently been described.[18] The chemical shift and line-width changes noted here for the "wet" quinuclidine mixtures parallel those previously defined for monomeric hydroxoiron(III) tetraarylporphyrin complexes.[18] Spectral parameters also closely resemble those for the alkoxide complex. Stabilization of hydroxoiron(III) porphyrin complexes by sterically hindred amines may well be a general phenomenon.

Carbon-13 NMR Measurements. The greater dispersion and generally smaller line widths for carbon-13 signals made possible additional binding experiments which support coordination at the quinine alcohol function. In this instance variable amounts of (TPP)FeCl or (PPDME)FeCl were added to a $CDCl_3$ solution 50 mM in quinine. Proton-decoupled carbon-13 spectra are shown in Figure 5. Assignments for the free quinine are taken from a prior report,[26] and the numbering scheme follows that for the structure shown in the Introduction. In principle, exchange rates ranging from the near slow limit to the fast-exchange limit should

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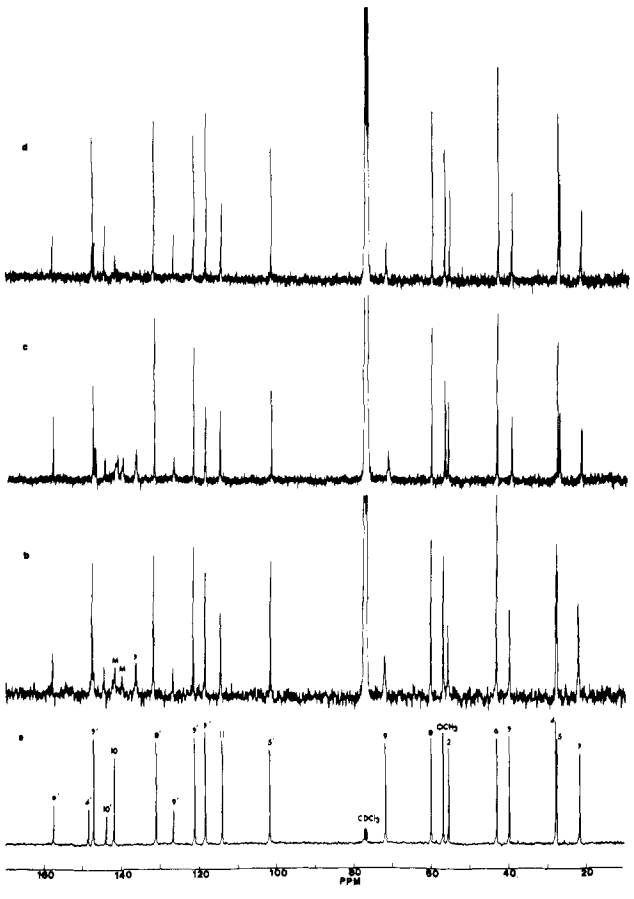

Figure 5. Proton-decoupled carbon-13 NMR spectra at 90 MHz for sequential addition of iron(III) porphyrins to quinine solution, $CDCl_3$ solvent, 37 °C, signals referenced to $(CH_3)_4Si$. (a) ~1 M quinine; (b) 50 mM quinine, 5 mM (TPP)FeCl; signals labeled "M" and "P" represent *m*-phenyl and *p*-phenyl carbon-13 signals from the porphyrin; (c) 50 mM quinine, 15 mM (TPP)FeCl. (d) 50 mM quinine, 5 mM PPDMEFeCl.

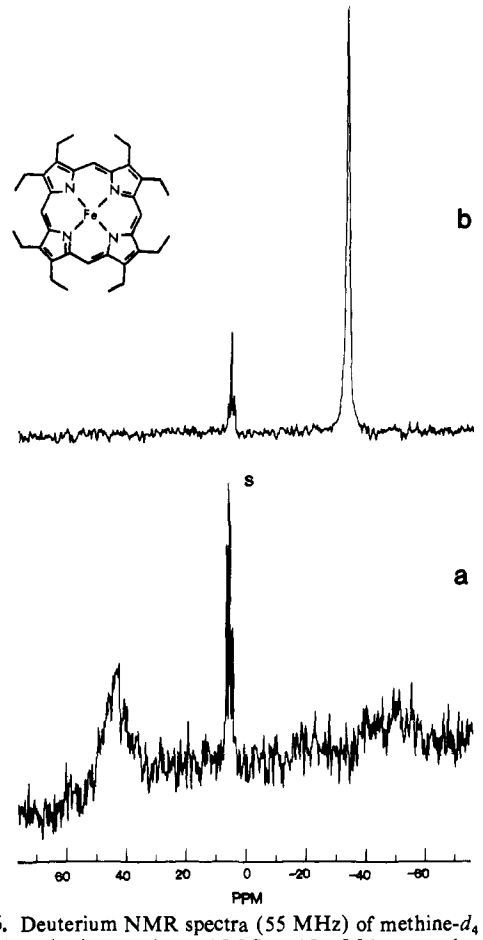

Figure 6. Deuterium NMR spectra (55 MHz) of methine-d_4 iron(III) octaethylporphyrin complexes; 25 °C, ~15 mM iron porphyrin, referenced to $(CD_3)_4Si$ (external), "S" represents solvent peaks. (a) 3:2 methylene chloride–methanol solution; (b) same solution with ~10 equiv of piperidine added.

produce considerable line broadening and/or chemical-shift perturbation for quinine carbon atoms in proximity of the binding site. On this basis coordination by either quinoline or quinuclidine nitrogen atoms may be ruled out. Thus, the 2′- and 10′-carbon signals adjacent to the aromatic nitrogen remain sharp and are shifted less than 1 ppm. Likewise, the 2-, 6-, and 8-carbon signals of the quinuclidine residue are quite unaffected by addition of increasing amounts of iron porphyrin. In contrast, the 9-carbon atom bearing the OH group exhibits a signal that is increasingly broadened by addition of iron porphyrin. The adjacent 4′-carbon resonance also experiences a notable upfield shift. Although quinine ligand exchange is likely to be in the near slow limit for the 9-carbon resonance, a separate coordinated resonance was not detected perhaps due to extreme broadening. Overall the carbon-13 results are supportive of conclusions made from spectroscopic measurements in identifying the benzylic alcohol function as the ligating residue.[27]

General Formation of Iron(III) Porphyrin Alkoxides from Alcohols and Amines. It is demonstrated here that formation of the quinine alkoxide complex is representative of a general reaction between iron(III) porphyrins, alcohols, and amines contained in nonaqueous solvents. Alkoxide formation is a consequence of the strong Lewis acid and oxophilic character of the iron(III) center, as well as the favorable electrostatic result of generating a neutral complex in a low-dielectric medium. The possibility of alkoxide complex formation unfortunately was not recognized for an earlier study in which case the new species were interpreted to be six-coordinate mixed alcohol–amine iron(III) porphyrin complexes.[6] The previous study was concerned largely with NMR spectral changes resulting from addition of small quantities of various aliphatic amines to iron(III) porphyrin solutions containing methanol or ethanol. Several observations from the earlier work in retrospect are consistent with small amounts of amines serving as Brønsted bases (for deprotonation of the alcohol) rather than as ligands.[28] These observations include (i) porphyrin proton chemical shift values that are quite independent of the amine, (ii) absence of hyperfine-shifted signals for "coordinated" amine residues, (iii) a requirement for smaller amounts of more basic amines, (iv) the expectation that a six-coordinate complex would have a far downfield methine proton signal rather than the experimentally observed upfield resonance,[21,24,29,30] and (v), perhaps most importantly, that methine and porphyrin ring methylene chemical shift values are equivalent to those observed for quinine complexes and for the phenoxide complex.[25]

Optical and NMR measurements are reported here that further confirm formation of alkoxide complexes rather than mixed alcohol–amine derivatives. For methylene chloride solutions of 1×10^{-4} M chloroiron(III) porphyrin complexes approximately 6 equiv of methanol and 10 equiv of piperidine were added. Major visible-region optical bands recorded for three iron(III) porphyrin

(27) The vinylic CH= (carbon 10) line width is also quite sensitive to the presence of iron(III) porphyrin compounds. Examination of molecular models reveals that conformations are available that could place the vinyl function of coordinated quinine close to the porphyrin periphery. An equally reasonable explanation is found in possible secondary association of excess quinine with coordinated quinine such that the free vinyl residue is oriented within proximity of the metal center.

(28) At higher concentrations various amines undoubtedly serve as effective ligands yielding the low-spin, bis-ligated iron(III) porphyrin adducts.

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species are as follows: iron(III) tetraphenylporphyrin, 576, 620, 695, 755 nm; iron(III) octaethylporphyrin ((OEP)Fe), 463, 576, 595, 637, 765 nm; and iron(III) protoporphyrin dimethyl ester, 470, 575, 600, 640, 767 nm. These spectra are very similar to those shown in Figures 1 and 2 and listed in Table I for quinine and OCH_3^- complexes. Presence of long-wavelength charge-transfer bands of substantial intensity is most noteworthy. Chemical-shift values of −35 ppm for the methine proton and +33 ppm for ring methylene groups of the "methanol–piperidine" complex of iron(III) octaethylporphyrin[6] are essentially identical with respective signals at −35 and +35 ppm for the quinine iron(III) protoporphyrin dimethyl ester complex. Assignment of the upfield methine signal for an alkoxide complex is made unambiguously in Figure 6b through use of deuterium NMR spectroscopy and in situ generation of methine-d_4–OEPFe(OCH_3).

One additional proton NMR signal reassignment should be made for the presumed dimethanol complex of $OEPFe^+$ reported in ref 6. The methine signal in this derivative reportedly was found in a far-upfield region much as was the case for the parent (OEP)FeCl species.[6] The deuterium NMR spectrum in Figure 6a reveals, to the contrary, that methine-d_4–OEPFeCl contained in a 3:2 mixture of CH_2Cl_2–CH_3OH exhibits a broad *downfield* methine signal at 44 ppm. This downfield signal appears for what is believed to be the major solution species: $(OEP)Fe(CH_3OH)_2^+$. A minor, broad upfield signal persists for the small equilibrium concentration of (OEP)FeCl. This upfield signal was erroneously assigned to the dimethanol complex in earlier proton spectra.[6] A downfield methine proton resonance is fully expected for six-coordinate high-spin iron(III) porphyrin complexes.[21,24,29,30] Clarification of the misinterpretations in ref 6 in fact permits the generalization that for all well-defined species (i) five-coordinate high-spin iron(III) porphyrin complexes exhibit a far-upfield methine proton NMR resonance, whereas (ii) the six-coordinate high-spin iron(III) analogues have a far-downfield signal. This correlation is rationalized by carbon-13 NMR studies that reveal significant unpaired π-spin-density delocalization at the methine carbon of five-coordinate complexes.[24] Although exceptions may well be found for the general proton chemical shift correlation, the hypothesis adds credence to description of the quinine complex as a five-coordinate species. The hypothesis may serve as a guide for additional structural identification of iron(III) porphyrin complexes, as well as for description of coordination numbers in hemoproteins.

Possible Relevance to Antimalarial Action of Quinine. The etiology of malaria involves infection of host red blood cells by the parasitic *Plasmodium* species. Cellular invasion is generally associated with metabolism of hemoglobin and deposition of a "malarial pigment" within the parasite. Iron protoporphyrin IX is a major constituent of the malarial pigment, and in an aggregated form this hemoglobin breakdown product apparently serves as a receptor for concentration of antimalarial drugs.[1] Previous suggestions concerning high-affinity nitrogen coordination to monomeric or μ-oxo dimeric iron protoporphyrin units in the malarial pigment are *not* supported by the nonaqueous results described here. Alkoxide coordination is favored for quinine, and moreover the affinity for quinuclidine or quinoline binding to iron(III) porphyrins is very low. It can be argued that alkoxide coordination is highly unfavorable in an aqueous physiological system. However, the cellular environment of the malarial pigment could well be hydrophobic and of very low dielectric. The previously unrecognized alkoxide binding mode must now be considered among possible mechanisms for quinine–malarial pigment interaction.

Conclusions

Quinine binding results provide a novel example of metalloporphyrin coordination chemistry in which a potentially ambidentate ligand is converted to its zwitterionic form as a consequence of the Lewis acidity of the metal center. A useful scheme is thus available for generating iron(III) porphyrin alkoxide complexes from ligands containing alcohol and basic moieties. It has also been demonstrated that earlier formulations of mixed alcohol–amine iron(III) porphyrin complexes are incorrect and that facile alkoxide ligation occurs in these systems as well. Observations made here provide greater appreciation for potential coordination of serine and threonine alkoxide residues in ferrihemoproteins containing these amino acids in the heme pocket. A new hypothesis is offered describing possible quinine alkoxide coordination to the iron(III) protoporphyrin residues of the malarial pigment.

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Registry No. Quinine, 130-95-0.