Proton Nuclear Magnetic Resonance Study of Equilibria and Paramagnetisms of Ferriprotoporphyrin IX–Ligand Complexations

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Abstract: The binding of cyanide ion, imidazole, and the mixed ligand to ferriprotoporphyrin IX·Cl (hemin) in Me_2SO-d_6 has been studied by ^1H nuclear magnetic resonance. The results show that the cyanide ligation to hemin is a two-step process, with first the formation of a previously unknown monocyano adduct of hemin and then followed by a dicyanohemin in completion. The binding constants K_1 and K_2 were found to be $1.9 \pm 0.3 \times 10^4$ and $1.1 \pm 0.2 \times 10^3$ M^{-1} at 20 °C, respectively. Binding of imidazole to hemin, however, is a concerted process which only forms diimidazolehemin product with an overall binding constant of about 6×10^4 M^{-2} at 20 °C. The relative stabilities of various hemin–ligand complexes were determined as $K_{CN,Im} \geq K_{diCN} > K_{diIm} \sim K_{monoCN} \gg K_{monoIm}$. The iron(III) of intermediary species, monocyanohemin, was determined as low-spin iron ($S = 1/2$) while monoimidazolehemin has been reported as high-spin iron ($S = 5/2$). The rate of exchange among different hemin species was slow on the NMR time scale, at a rate slower than 160 s^{-1} at 65 °C.

Introduction

Many of the iron porphyrin structures are essential to the functioning of biological systems.[2] For example, protoporphyrin IX iron(II), commonly known as heme, and protoporphyrin IX iron(III) (hemin) serve as prosthetic groups for important classes of proteins and enzymes. The proteins can exhibit different functions and participate in reactions such as reversible oxygen binding for transport (hemoglobins) or storage of oxygen (myoglobins), electron transfer (cytochromes *b* and *c*), and hydrogen peroxide utilization (catalases and peroxidases).

Because of their biological importance, iron porphyrin–ligand complexations have been studied extensively by a number of physical and chemical methods. Nuclear magnetic resonance, in particular, has been a suitable one. In the ^1H NMR spectra of the hemin–ligand complexes, the resonances of the hemin protons exhibit a large hyperfine shift. The extent of the shift varies with the nature of the axial ligands. Knowledge of the ^1H chemical shifts has been useful in revealing details of the electronic structure of iron porphyrins, the nature of the iron–porphyrin and the iron–ligand binding, and the magnetic properties of these hemin–ligand complexes.

In spite of a number of NMR investigations[3-5] on the complexation of ligands, such as imidazole and cyanide, to hemin, there have been few studies on the properties of the intermediary species and the relative stabilities of various hemin–ligand complexes, In an earlier communication,[6] we reported a preliminary study of the equilibrium, kinetics, and paramagnetism in a hemin–cyanide system. We are now reporting details of this study and an extension to hemin–imidazole and mixed ligand systems.

Experimental Section

Materials. Iron(III) protoporphyrin IX was obtained from Nutritional Biochemicals Co. Anal. Calcd: C, 62.43; H, 4.95; N, 8.59; Fe, 8.57. Found: C, 62.37; H, 4.95; N, 8.64: Fe, 8.39. Iron(III) protoporphyrin IX dimethyl ester was synthesized from the free base (Sigma Chemical) according to Falk.[7] The crude product was purified by elution column chromatography, using alumina (acid)–chloroform system. The purified ester was dried under vacuum at room temperature. Anal. Calcd: C, 63.58; H, 5.34; N, 8.24. Found: C, 62.96; H, 5.40; N, 8.24. The visible spectrum of the iron ester in Me_2SO-d_6 is similar to the reported esterfied hemin spectrum.[8] Me_2SO-d_6 (Diaprep, Inc.) was dried over type 4A molecular sieves before use. The water content in Me_2SO-d_6 was determined to be less than 0.05% by the Karl Fischer titration method. Imidazole (Eastman) was recrystallized twice from ethanol. Potassium cyanide (Baker reagent) was used as received. $Na^{13}CN$ (Merck) was 90% enriched ^{13}C. All other chemicals were obtained as the best available grade and were used without further purification.

Measurements. Proton magnetic resonance spectra were taken on a Varian HR-220 spectrometer in the FT mode. Between 200 and 4000 transients were accumulated using 8K data points over a spectrum width of 5000 Hz, with a 45 μs (90°) pulse. A pulse repetition time of 2 s was used to ensure no saturation of the signals. All NMR experiments were carried out at 20 °C unless otherwise indicated. It is known that relative areas under the NMR absorption signals are proportional to relative concentration of magnetic nuclei. The equilibrium calculations were done by using the integrated signal areas obtained with 0.002 M hemin solution containing varying amounts of potassium cyanide or imidazole. In general, the signal areas of at least two spectra were integrated with a planimeter and the results averaged. ^{13}C NMR experiments were carried out on a Varian XL-100 spectrometer equipped with pulse FT accessories. The deuterium signal of Me_2SO-d_6 was used as an internal lock. ^{13}C chemical shift was measured relative to an internal tetramethylsilane (Me_4Si). Between 1000 and 40 000 transients, 8K data points, 5000-Hz spectral width, 45-μs (60°) pulse and 2-s repetition time were used in the ^{13}C experiments.

Magnetic moment measurements were performed as described by Evans.[9] A solution of about 1% Me_4Si in Me_2SO-d_6 was used as solvent for the metalloporphyrin. The frequency of the Me_4Si in this solution was compared to that of Me_4Si in Me_2SO-d_6 contained in a coaxial capillary. Because of the geometry existing in the HR-220 spectrometer in which the axis of the cylindrical sample is parallel to the field,[10] the molar paramagnetic susceptibility was calculated by the modified equation[11]

$$\chi_M = \frac{3\Delta\nu}{4\pi\nu}\frac{1000}{C} + \chi_0 M - \chi_D \qquad (1)$$

where χ_M is the molar paramagnetic susceptibility of hemin, $\Delta\nu$ is the frequency separation of two Me_4Si lines in hertz, ν is the frequency (i.e., 220 MHz) at which the proton resonances are being studied, C is the molar concentration of hemin solution, χ_0 is the mass susceptibility of the solvent, M is the molecular weight of hemin, and χ_D is the diamagnetic susceptibility of hemin ($\chi_D = -600 \times 10^{-6}$ erg).[12] The magnetic moments of iron in various hemin species were calculated by the equation

$$\mu = 2.84\sqrt{\chi_M T} \qquad (2)$$

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Results and Discussion

It is known that four of the coordination positions of iron(III) in hemin are occupied by the four pyrrole nitrogens of the porphyrin ring, and the remaining fifth and sixth coordination sites, which are in the axial position, are capable of binding either solvent molecules (e.g., Me_2SO-d_6 in this case) or ligands such as cyanide and imidazole. The nature of the ligand affects the spin state of iron in hemin. In Me_2SO-d_6 solution, where the fifth and sixth ligands are solvent molecules, the iron is in a high spin state, $S = 5/2$.[3c] Ligands such as cyanide, imidazole, or pyridine, however, promote transition of iron from high-spin to the low-spin state, $S = 1/2$.[3a,c,e] A change in the ^1H NMR spectrum is observed by a high- to low-spin transition of the iron porphyrin.

The ^1H NMR spectra of hemin with various ligands has been presented previously and all lines have been assigned.[3a,c,e] In this equilibrium study, we confine ourselves only to the signals of the four porphyrin methyls and follow their spectral changes as a function of ligand concentration. Separate methyl resonances were observed for each hemin species, and the concentration of each was determined form their relative areas.

Ferriprotoporphyrin IX-Cyanide System. The binding of cyanide to hemin can be described by the following equations:

$$\underset{\mathbf{1}}{\text{hemin}^+} + \text{CN}^- \rightleftarrows \underset{\mathbf{2}}{\text{hemin} \cdot \text{CN}} \tag{3}$$

$$\text{hemin} \cdot \text{CN} + \text{CN}^- \rightleftarrows \underset{\mathbf{3}}{\text{hemin}\,(\text{CN})_2^-} \tag{4}$$

$$K_1 = \frac{[\text{hemin} \cdot \text{CN}]}{[\text{hemin}^+][\text{CN}^-]} \tag{5}$$

$$K_2 = \frac{[\text{hemin} \cdot (\text{CN})_2^-]}{[\text{hemin} \cdot \text{CN}][\text{CN}^-]} \tag{6}$$

The observation of transition from high-spin hemin (**1**) to low-spin dicyanohemin (**3**) with an intermediate formation of monocyanohemin (**2**) in solution is illustrated in Figures 1 and 2. Figure 1 shows the ^1H NMR spectra of the four porphyrin methyls of hemin at various potassium cyanide concentrations. In the absence of cyanide (Figure 1a), only the high-spin hemin exists in the solution. The methyl protons (labeled as a_0) of **1** exhibit a large hyperfine shift to low field at 50–70 ppm relative to Me_4Si. Upon addition of cyanide to the solution the areas under these four methyl resonances decrease and finally disappear with the concomitant appearance of a new group of resonance peaks (labeled as a_M in Figures 1b–f) at 10–20 ppm. These new peaks reach a maximum intensity at a cyanide–hemin ratio of about 1:1 and then decrease thereafter, finally disappearing at about a 2:1 ratio. A second group of peaks (labeled as a_D in Figures 1c–g) were also noted in the same chemical shift region. These peaks first appear at a cyanide hemin ratio of about 1:1, reaching maximum intensity at about a 2:1 ratio, and then remain constant at higher cyanide–hemin ratios. The resonances of a_M and a_D are assigned to the methyl protons of **2** and **3** species, respectively.

A plot of the intensity of the resonances, a_M, as a function of the cyanide to hemin ratio (Figure 2) shows a maximum at about 1:1, i.e., demonstrates the stoichiometry of the complex as 1:1. The second complex, a_D, does not reach its maximum value until the ratio of cyanide–hemin is 2, i.e., a 2:1 complex. These observations suggest that the binding constants K_1 and K_2 are large and the former greater than the latter. Owing to the large values of K_1 and K_2, dilute hemin solutions (0.002 M) with varying potassium cyanide concentrations were used to obtain more accurate values of K_1 and K_2. By using eq 5 and 6, and the concentrations of three hemin species obtained at various cyanide concentrations, K_1 and K_2 were calculated to be $1.9 \pm 0.3 \times 10^4$ and $1.1 \pm 0.2 \times 10^3$ M^{-1} at 20°C, respectively. These values are comparable to those reported by Kaziro et al. ($K_1 \sim 4.8 \times 10^4$ and $K_2 \sim 3.4 \times 10^2$),[13] but in contrast to those reported by Shack and Clark ($K_1 \sim 2.2 \times 10^6$ and $K_2 \sim 0.63$)[14] in a pyridine solution using a spectrophotometric method. Although dimerizaton may occur, we estimated that the dimer concentraton is less than 5%.[15] The effect of dimerization of K_1 and K_2 is therefore within our experimental error.

In a study of the formation of deuteriohemin complexes with cyanide in aqueous medium, Schossler et al.,[16] however, observed only one set of signals for the ring methyl protons in δ between 10 and 20 ppm region. The chemical shifts of the methyl protons changed with cyanide ion concentration. They interpreted this as due to a fast exchange between the hydroxycyano and dicyano complexes.

The observation of signals from all three hemin species in cyanide–Me_2SO-d_6 solution requires that they be in the NMR slow exchange limit region. Based on our data, the rate of exchange is calculated to be slower than 160 s^{-1} at 65 °C. A value of 60 s^{-1} at 30 °C was reported for the TPPFe·Cl-*N*-methylimidazole system.[4]

Since a number of Hückel molecular orbital calculations for the iron porphyrin π-electron system with different ring substituents have been introduced to interpret the NMR shift measurements in terms of the electron spin density distribution in the π-electron system, an identification of individual methyl resonances of the porphyrins is important for the theoretical test. We found that the 1-CH_3 and 3-CH_3 may be differentiated from the 5-CH_3 and 8-CH_3 by the introduction of paramagnetic ion Mn^{2+} into the hemin–ligand solutions. Paramagnetic relaxation induced by Mn^{2+} known to complex with carboxylate ion[17] is inversely proportional to the Mn–H distance. Thus, the two methyl protons which are closer to the carboxylate–Mn^{2+} binding sites (5-CH_3 and 8-CH_3) are expected to relax more readily than the more distant 1-CH_3 and 3-CH_3 groups. Upon addition of a small amount of $MnCl_2$ to the hemin–dicyano complex solution, the line widths of the two methyl resonances in the neighborhood of 15 ppm broadened much more than the two methyls between 10 and 12 ppm. The former two resonances at about 15 ppm hence are assigned to the 5-CH_3 and 8-CH_3, and the latter two resonances at 10–12 ppm to the 1-CH_3 and 3-CH_3. These assignments are in agreement with those of Cavaleiro et al.,[18] who have prepared protoporphyrin IX by selective deuteration. Further addition of $MnCl_2$ to the hemin solution resulted in a total loss of all methyl signals which reappeared, though not completely, after addition of EDTA.

In a close examination of the spectra in Figures 1f–g, it is found that the chemical shift of the four methyl resonances varies with the cyanide concentration. Figure 3 shows the chemical shift dependence of the four hemin methyls as a function of cyanide:hemin ratio. The resonance lines begin to shift to high field (i.e., for 1-CH_3) or to low field (i.e., for 3-CH_3, 5-CH_3, and 8-CH_3) at a cyanide:hemin ratio of about 2 and remain constant at a ratio of about 4 and higher. The shifts found are 1.02, 0.63, 0.95, and 0.99 ppm for 1-CH_3, 3-CH_3, 5-CH_3, and 8-CH_3, respectively. We attribute the changes in shifts to the perturbation of the electronic structure of hemin resulting from the titration of the two COOH's of hemin with potassium cyanide. Stoichiometrically this is correct, since the first two cyanide ions occupy the fifth and sixth coordination positions of iron in the hemin, and the additional two CN^- then react with the two terminal carboxylic acids to convert the -COOH to $-COO^-$. This conclusion is supported by the absence of any significant shift upon either adition of KCN to a hemin dimethyl ester solution or addition of imidazole to a hemin solution. The dimethyl ester derivative does not have a titratable COOH group while imidazole does

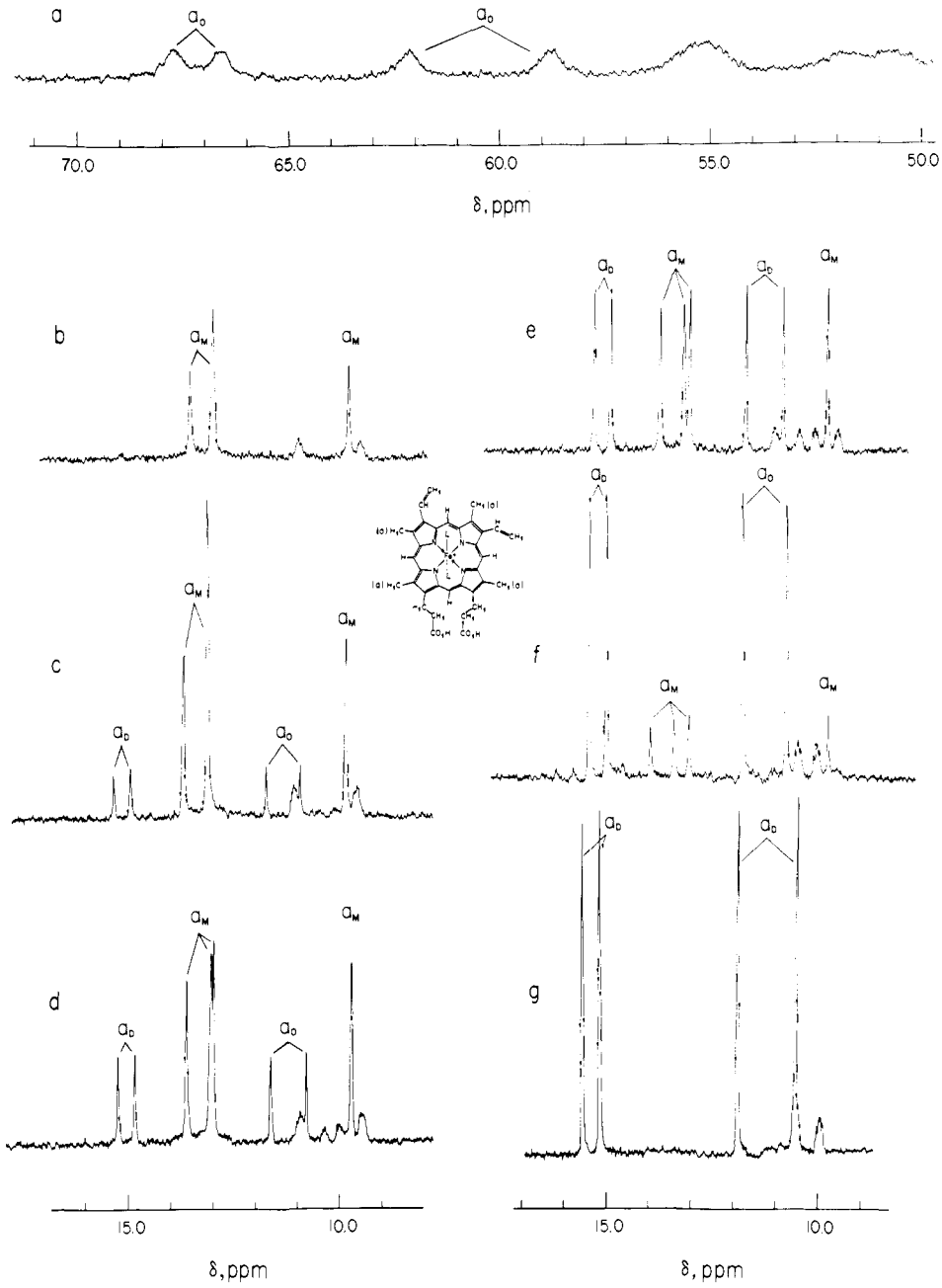


Figure 1. Room temperature (20 °C) 220-MHz ^1H spectra of 0.02 M ferriprotoporphyrin·Cl (hemin) in Me_2SO-d_6 solution at various potassium cyanide concentrations. Only the methyl protons of porphyrin are shown here. The cyanide concentrations are (a) 0.00 M; (b) 0.01 M; (c) 0.02 M; (d) 0.025 M; (e) 0.03 M; (f) 0.04 M; (g) 0.05 M. a_0, a_M, and a_D are assigned as the methyl resonances from the hemin, monocyanohemin, and dicyanohemin species, respectively.

not react with the COOH group.

In an attempt to investigate the ^{13}C resonance of bound cyanide in **2** and **3**, we have measured the ^{13}C NMR spectra of a number of hemin-$Na^{13}CN$ (90% ^{13}C enriched) solutions. For a ratio of $^{13}CN^-$ to hemin less than 2, no ^{13}C NMR signal was observed. This may be due to the fact that the ^{13}C resonance of cyanide in **2** and **3** is either shifted outside our frequency detecting range (we had covered about 800 ppm) or broadened excessively by the paramagnetism of direct bonded iron(III), thus escaping our detection. When the CN^- to hemin ratio was greater than 2, however, a resonance at 113.6 ppm was detected. The chemical shift of this signal remained unchanged until a ratio of about 4, then gradually increased (i.e., downfield shift) finally reaching a constant value of 166.8 ppm at high CN^- to hemin ratio (Figure 4). The large chemical shift dependence of the observed ^{13}C signal on the cyanide concentration cannot be the result of a fast exchange between bound and unbound cyanides, since the rate of exchange is slow in the NMR time scale. It is more likely the result of a change in the equilibrium between fast-exchanging HCN (δ 110.9)[19] and unbound CN^- (δ 168.6)[19] during the addition of $Na^{13}CN$ to the hemin solution. This interpretation is confirmed by a shift of the ^{13}C resonance of a cyanide-hemin (6.6:1) solution from 147.0 to 113.6 ppm when a small amount of 1 N DCl was introduced (Figure 4).

Ferriprotoporphyrin IX–Imidazole System. Addition of increasing amounts of imidazole to hemin solution results in the disappearance of the methyl signals of **1** and the appearance of only one set of new methyl resonances at 10–20 ppm. This contrasts with the hemin–cyanide complexation where two sets of methyl signals were observed in the same chemical shift region. The intensity of these new methyl signals increases and

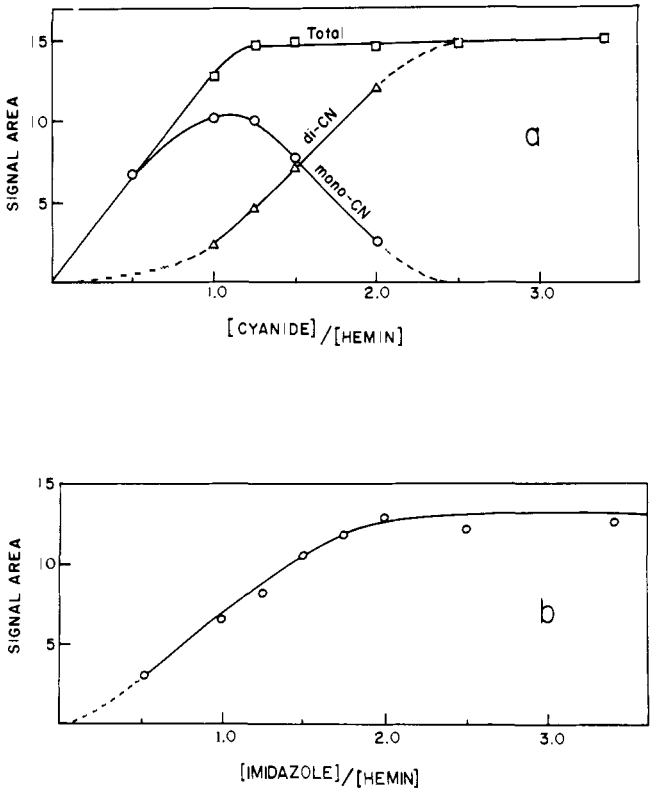


Figure 2. Variation of the methyl resonance signal areas of (a) monocyanohemin (○), dicyanohemin (△), and their sum (□) from a 0.02 M hemin in Me_2SO-d_6 solution as a function of cyanide–hemin ratio; and (b) diimidazolehemin as a function of imidazole–hemin ratio.

reaches a plateau at an imidazole to hemin ratio above 2 (Figure 2b). This determines the stoichiometry of the complex to be 2:1. No spectral evidence could be obtained for the existence of an intermediate species (i.e., monoimidazole adduct). If the assumption is made that the monoimidazole complex does not occur, the binding of imidazole to hemin may be represented as

$$\underset{\mathbf{1}}{\text{hemin}^+} + 2\text{Im} \rightleftarrows \underset{\mathbf{4}}{\text{hemin} \cdot (\text{Im})_2^+} \qquad (7)$$

with the corresponding equilibrium constant K

$$K = \frac{[\text{hemin} \cdot (\text{Im})_2^+]}{[\text{hemin}^+][\text{Im}]^2} \qquad (8)$$

Using the signal areas of the various species in eq 8, a least square of log $[\text{hemin} \cdot (\text{Im})_2^+]/[\text{hemin}^+]$ vs. log [Im] with a slope of 2.1 was obtained which, within experimental error, indicates that two molecules of imidazole are binding to a molecule of hemin in the product and confirms the validity of the assumption. The value of equilibrium constant K obtained from this analysis was $6.3 \pm 0.9 \times 10^4$, comparable to that reported for complexation of imidazole to tetraphenylporphyrin iron(III) chloride ($K \sim 10^5$).[5] Observation of a diimidazole but not a monoimidazole hemin complex suggests that the former is far more stable. This may be because the former is the low-spin iron(III) complex which is known to be much more stable than the high-spin complex, i.e., the latter.

The chemical shift values for the four ring methyl groups (18.5, 18.2, 14.3, and 11.9 ppm) in diimidazolehemin are in agreement with a previous investigation.[3c] It also has been noted that, in contrast to the cyanide–hemin system, when the imidazole to hemin ratio exceeds 2, no apparent changes in chemical shift are observed. Imidazole ($Im + H^+ = ImH^+$, $K = 10^{7.11}$)[20] is a weaker base than CN^- ion ($CN^- + H^+ = HCN$, $K = 10^{8.68}$).[21] Thus the abstraction of protons by imidazole from the two carboxylic acid groups on the hemin is minimal and the chemical environment of the methyl groups remains the same even at increased imidazole concentrations.

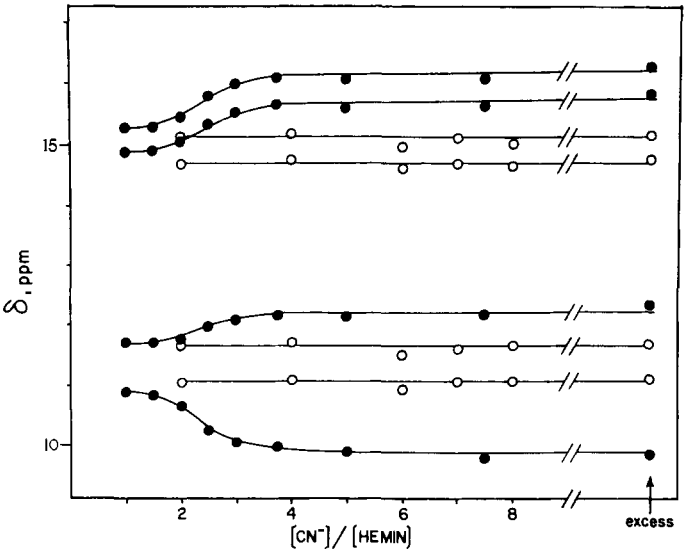


Figure 3. Chemical shift dependence of the four methyls of hemin (●) and hemin dimethyl ester (○) as a function of cyanide–hemin ratio.

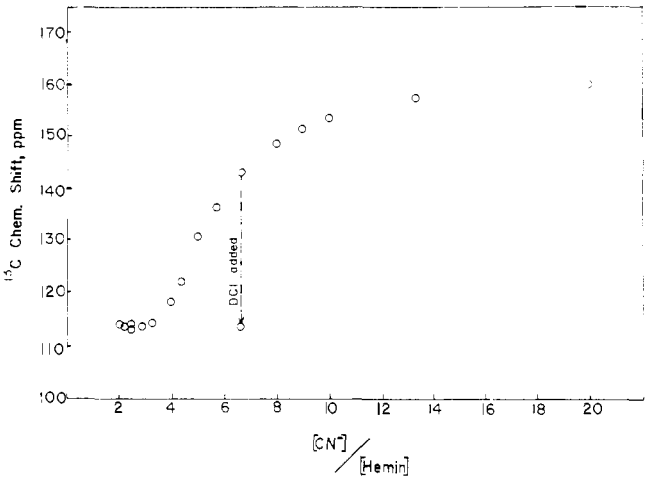
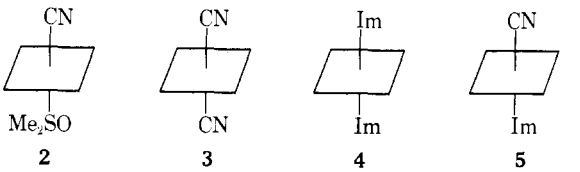


Figure 4. ^{13}C chemical shift dependence of cyanide (90% ^{13}C enriched) as a function of cyanide–hemin ratio.

Ferriprotoporphyrin IX–Cyanide–Imidazole Mixed Ligand System. Recently, La Mar et al.[22] were able to observe NMR signals from mesoprotoporphyrin dimethyl ester in $CDCl_3$ containing cyanide and 1-methylimidazole mixed ligands. The stability of the mixed ligand complex, however, was not determined. We would now like to report the relative stabilities of various hemin complexes in Me_2SO-d_6 containing both cyanide and imidazole ligands. In hemin solutions containing both cyanide and imidazole ligands, the following species have been observed. As a CN^- to hemin ratio of 1.5 to 1, the signal



areas of monocyanohemin (**2**) and dicyanohemin (**3**) are about equal (see Figure 1e). Upon addition of increasing amounts of imidazole to this solution, four new methyl peaks appeared

Table I. Relative Signal Areas for Hemin(LL') Complexes[a]

Ratio of hemin:cyanide:imidazole	Relative signal area: Hemin (CN) 2	Hemin $(CN)_2$ 3	Hemin(CN)(Im) 5
1.0:1.5:0.0	0.52	0.48	0
1.0:1.5:0.65	0.12	0.25	0.63
1.0:1.5:1.5	0	0.17	0.83
1.0:1.5:2.2	0	0.13	0.87
1.0:1.5:3.0	0	0.06	0.94
1.0:2.0:2.0	0	0.20	0.80

[a] The hemin concentration was kept constant at 0.02 M.

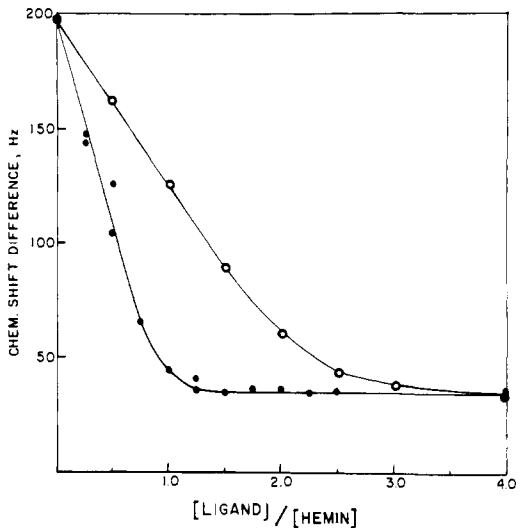


Figure 5. Variation of the chemical shift difference between a 1% Me_4Si in Me_2SO-d_6 (in an internal capillary) and similar solutions containing hemin (0.02 M) and varying amounts of cyanide (●) and imidazole (○). Chemical shifts were measured at 20 °C on a Varian HR-220 spectrometer.

at 16.6, 16.4, 12.2, and 12.0 ppm with concomitant decreases in the signal areas of **2** and **3** (Table I). The signal area of **2** decreased more rapidly than that of **3**, indicating that **3** is more stable than **2**. This is consistent with the calculated binding constants for species **2** ($K_1 = 1.9 \times 10^4$) and **3** ($K_{overall} = K_1K_2 = 2.1 \times 10^7$). Since the chemical shifts of the new peaks differ from those of diimidazolehemin (**4**),[3c] we assign them to four methyl resonances of monocyanomonoimidazole hemin (**5**). This assignment is consistent with the recent report by La Mar et al.[22]

When the imidazole:cyanide:hemin ratio is about 1.5:1.5:1, the signal of **2** was completely lost and the relative areas of species **3** and **5** were 0.17 and 0.83, respectively. Further addition of imidazole, up to an imidazole:cyanide:hemin ratio of 8:1.5:1, resulted in a total loss of signals from **3** and no trace of **4**.

When KCN was introduced to a imidazole-hemin (2:1) solution the signal area of species **4** decreased with the concomitant appearance of species **5**. At an imidazole:cyanide:hemin ratio of about 2:2:1, the relative areas of **5** and **3** were about 0.8 and 0.2, respectively. Based on the results of these experiments, we conclude that the relative stabilities of the hemin complexes are as follow: $K_{CN,Im} \geq K_{diCN} > K_{diIm} \sim K_{monoCN} \gg K_{monoIm}$.

Two factors may contribute to the relatively high stability of monocyanohemin complex over monoimidazole-hemin complex. According to the previously reported ligand field strengths,[23] cyanide is a ligand with stronger ligand field strength than that of imidazole. Evidently, binding of a cyanide ion to hemin causes a substantial lowering of the iron(III) t_{2g} orbitals, with respect to the e_g orbitals, such that the ligand field splitting, Δ, becomes much greater than the spin-pairing energy, P, hence results in a more stable low-spin complex than that of a high-spin imidazole-hemin complex with $\Delta < P$. Another factor could be the mutual electrostatic interactions between the negative charge of cyanide ion and positive charge of iron(III) from hemin.

Magnetic Moment Measurements of Ferriprotoporphyrin IX–Cyanide and Ferriprotoporphyrin IX–Imidazole. The spin states of hemin (**1**) and its complexes, where both the fifth and sixth positions are coordinated by cyanide or imidazole, have been reported.[4a,23] Since the NMR signals from various hemin complexes could be observed, measurement of their magnetic properties, especially the intermediate species, became possible. The spin state of the monocyanohemin was determined from measurements of the paramagnetic susceptibility using the method described by Evans.[9] Figure 5 shows the frequency separation between the signals of 1% Me_4Si in Me_2SO-d_6 (in an internal coaxial capillary) and similar solutions containing hemin (0.02 M) and varying amounts of cyanide. The frequency separation, which is directly related to the bulk paramagnetic susceptibility of the hemin solution, decreases linearly with added cyanide until a 1:1 ratio (cyanide:hemin) is reached and becomes constant thereafter. This demonstrates that both **2** and **3** have the same paramagnetism and, therefore, are in the low-spin state ($S = \frac{1}{2}$). The close resemblance in the proton spectra between **2** and **3** also supports this assignment. Using eq 1 and 2 the effective magnetic moments (μ_{eff}) of iron(III) in **1** and **2** (or **3**) in Me_2SO-d_6 were determined to be 5.0 and 2.1 μ_B, respectively. These values are in good agreement with the reported high-spin ($\mu_{eff} = 5.1$–5.8 μ_B) and low-spin ($\mu_{eff} = 1.7$–2.5 μ_B) values in other ferriporphyrin systems.[4a,24]

Figure 5 shows that the frequency separation decreases linearly with added imidazole until a 2:1 ratio (imidazole:hemin) is reached and becomes nearly constant thereafter. This demonstrates that diimidazolehemin is in the low-spin state, whereas the spin state of monoimidazolehemin may not be determined by this method. Recently, a mono-*N*-methylimidazole adduct of hemin was suggested to be in a high-spin form based on indirect evidence.[4] The only observed high-spin monoimidazolehemin complex is that in myoglobins and hemoglobins where the imidazole group from histidine residue of protein is the fifth ligand.

As expected, the spin state of iron(III) in **5** was determined to be in the low-spin state.

In conclusion, the results of this work clearly show that equilibrium and paramagnetism of ligand (e.g., cyanide and imidazole) binding to hemin in Me_2SO-d_6 can be determined by ^1H NMR. Binding of cyanide to hemin is a two-step process, with first a formation of previously unknown monocyano adduct of hemin and then followed by a dicyanohemin in completion. Binding of imidazole to hemin, however, is a concerted process which only forms diimidazolehemin. The relative stabilities of various hemin-ligand species were determined as $K_{CN,Im} \geq K_{diCN} > K_{diIm} \sim K_{monoCN} \gg K_{monoIm}$. The iron(III) of intermediary species, monocyanohemin, was de-

termined as low-spin iron ($S = 1/2$) while monoimidazolehemin was reported as high-spin iron ($S = 5/2$).

References and Notes

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Catalytic Homogeneous Hydrogenation of Arenes. 6.[1] Reaction Scope for the η^3-$C_3H_5Co[P(OCH_3)_3]_3$ Catalyst

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Abstract: The scope and selectivity of η^3-$C_3H_5Co[P(OCH_3)_3]_3$ as a catalyst precursor for the homogeneous hydrogenation of arenes at room temperature and low pressure (1-3 atm) have been examined for a variety of substituted benzenes and polycyclic aromatic and heterocyclic compounds. Pronounced stereoselectivity for the cis addition of H_2 and the formation of cis ring junctions has been observed. For example, benzene + D_2 gave ≥95% all cis $C_6H_6D_6$, naphthalene + H_2 gave *cis*-decalin with no detectable trans isomer, and anthracene + H_2 gave >95% of the *cis-syn-cis*-perhydroanthracene. The scope of the reaction is reasonably large. Catalytic hydrogenation was demonstrated for benzenes with substitutent groups that include -R, -OR, -COOR, -COR, -CH=CHR, -C≡CR, and $-NR_2$. The catalyst system is subject to steric inhibition by ring substituents on the arene ring. Electron-withdrawing and nucleophilic substituents such as halogen, $-NO_2$, and -CN also inhibit the reaction. The selectivity for arene vs. olefin hydrogenation in competitive systems is discussed. Comparisons are drawn with metal surface catalyzed hydrogenation.

Introduction

A chemoselective and stereoselective hydrogenation of aromatic regions of complex organic molecules remains a major synthetic challenge. Catalytic hydrogenation of aromatic hydrocarbons has long been dominated by metallic heterogeneous systems; nickel, Raney nickel, palladium, platinum, and rhodium are especially active catalysts for these hydrogenation reactions.[2] Hydrogenation of aromatic hydrocarbons with metallic catalysts provides a modest degree of stereoselectivity with an essential cis orientation in the hydrogen addition steps. The stereoselectivity of such catalysts in the hydrogenation of xylenes, mesitylene, and naphthalene is illustrated in Table 1. In chemoselectivity, the metallic catalysts fail in a general fashion simply because the reactivity of the substituent groups toward the metal surface is so high as to engender either substituent group hydrogenation or gross destruction of the substituent group, a more facile process than the hydrogenation of the aromatic system.[3]

In recent years, a number of ostensibly homogeneous reaction mixtures have been described as catalysts for the hydrogenation of aromatic hydrocarbons. Those reported through 1974 were reviewed in a previous paper of this series. Not covered in the earlier review was the report that $(pyridine)_2(dimethylformamide)RhCl_2(BH_4)$ is a catalyst for the hydrogenation of pyridine and quinoline.[4] Recent claims to homogeneous catalytic arene hydrogenations include the following: cobalt and nickel acetylacetonates react with $LiAlH_4$ to give homogeneous black solutions which catalyze the hydrogenation of benzene at 30 °C and 1 atm H_2 in tetrahydrofuran.[5] Klabunde has found that η^6-$CH_3C_6H_5Co(C_6F_5)_2$ and η^6-$CH_3C_6H_5Ni(C_6F_5)_2$ catalyze the hydrogenation of benzene at ~35 atm but these are both short-lived catalysts.[6] Also $[Rh(\eta^5\text{-}C_5Me_5)Cl_2]_2$ has been reported to be an effective catalyst for the hydrogenation of benzene and substituted benzenes at moderate temperatures and pressures.[7a] The latter compound is of interest in that it displays a significantly dif-

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