Scheme I

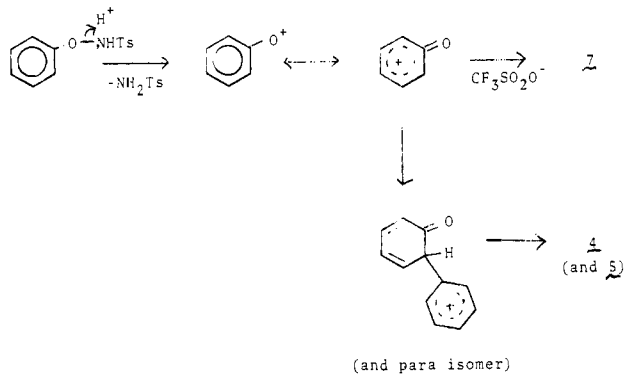
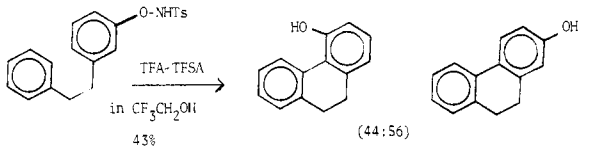


Chart IV



fluoroethanol in the presence of TFA (20 equiv)-TFSA (1 equiv).

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Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Iron(III) Porphyrin–Cyanide Complexes. Location of the Bound Cyanide Ion Resonance

Sir:

Cyanide ion is often chosen as an axial ligand for conversion of high-spin iron(III) porphyrins and hemoproteins to the low-spin form. Aqueous solution studies involving simple iron porphyrins indicate stepwise equilibria to form monocyano and dicyano species. Equilibrium quotients at 25 °C are on the order of 10^5 M^{-1}.[1] Very favorable cyanide ion binding has resulted in use of this ligand for recording nuclear magnetic resonance spectra of low-spin iron(III) porphyrins and hemoproteins.[2] ^1H NMR resonances in dicyano iron(III) porphyrin complexes show solvent dependence[3] and at low temperatures in methanol solution a specific aggregation process has been elucidated.[4] ^1H NMR spectra have been recorded for both monocyano and dicyano complexes in dimethyl sulfoxide solution and ligand exchange was not detectable on the NMR time scale at 65 °C.[5] ^{13}C NMR spectra of dicyano iron(III) porphyrin complexes have been reported, and, because a bound cyanide peak was not observed, it was initially concluded that rapid ligand exchange occurred.[6] During a more extensive ^{13}C NMR study we noted that the free cyanide ion peak was not shifted or broadened in presence of iron(III) porphyrins.[7] Such evidence is clearly indicative of slow cyanide exchange and subsequent use of carbon-13 enriched potassium cyanide has permitted observation of bound cyanide resonances. This report describes initial efforts directed at elucidation of ligand exchange kinetics, solution interactions, and spin delocalization mechanisms. Possible use of carbon-13 enriched cyanide ion as a probe for ferrihemoprotein structure is also under evaluation.

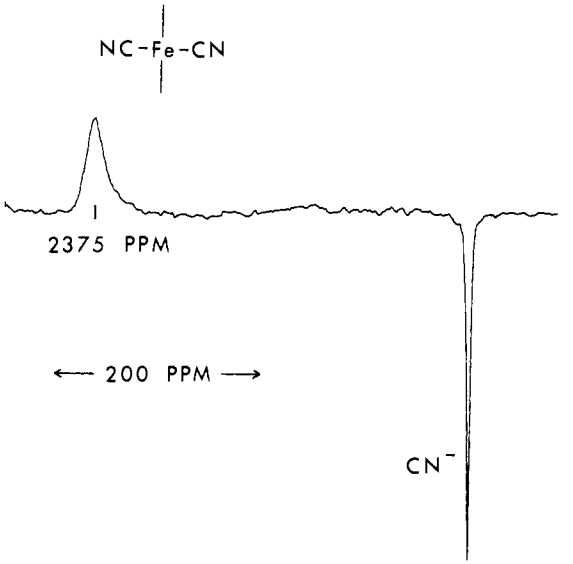
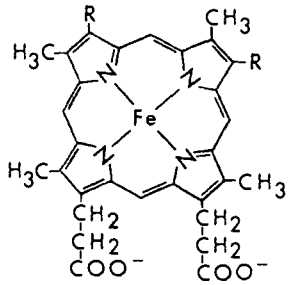


Figure 1. ^{13}C NMR spectrum of iron(III) deuteroporphyrin, 0.01 M, and 90% C-13 potassium cyanide, 0.05 M, in deuterium oxide at 26 °C. A 50-Hz line-broadening factor has been used.

Spectra were recorded at 22.6 MHz using a Bruker HX-90E pulsed Fourier transform spectrometer. Sweep widths of 20 KHz or 40 KHz were employed. From 20 000 to 500 000 transients were accumulated per spectrum at rates up to 15 pulses per second. Resonance positions are referenced to tetramethylsilane using dioxane as a secondary reference for deuterium oxide solutions. Upfield shifts are given positive values. Potassium cyanide enriched to 90 at. % carbon-13 was employed throughout the study. The ^{13}C resonance for potassium hexacyanoferrate(III) at natural abundance in aqueous solution was observed at +3583 ppm (26 °C) in close agreement with earlier broad-line NMR results.[8]

A typical upfield region ^{13}C NMR spectrum of a dicyano iron(III) porphyrin is shown in Figure 1. The resonance for excess free cyanide–hydrogen cyanide is "folded back" and inverted because the signal lies outside the spectral window. Excess potassium cyanide was employed to ensure complete ligation and to solubilize the iron(III) porphyrin via deprotonation of propionic acid groups. Dissolution with sodium deuterioxide and addition of potassium cyanide yielded equivalent results. The porphyrin resonances at natural abundance in carbon-13 are lost in the baseline. The broad, paramagnetically shifted signal corresponds to the bound cyanide species. A potassium cyanide titration of hemin *c* dissolved in pD 12.5, 0.1 M phosphate buffer revealed that the observed signal represents the dicyano complex. A resonance has not yet been located for the monocyano complex, perhaps because of exchange broadening. Line widths for the bound resonances in dicyano complexes are in the range of 360 ± 50 Hz with the exception of the iron(III) 2,4-dibromodeutero-

Table I



Iron species	Porphyrin 2,4-R group	Cyano resonance position[a]
Iron(III) protoporphyrin IX	$-CH=CH_2$	2393[b]
Iron(III) mesoporphyrin IX	$-CH_2CH_3$	2381
Iron(III) deuteroporphyrin IX	-H	2375
Iron(III) 2,4-dibromodeuteroporphyrin IX	-Br	2359
Hemin *c*	$-CH(CH_3)SCH_2-CH(NH_2)COO^-$	2300[c]
Iron(III) 2,4-disulfonate-deuteroporphyrin IX	$-SO_3^-$	2167[c]
Iron(III) *meso*-tetrakis(4-carboxyphenyl)porphine		1968[c]
$K_3Fe(CN)_6$		3583[d]
$K_4Fe(CN)_6$ (diamagnetic)		−177.3[d]
Myoglobin (C_2H_5N*C) (diamagnetic)		−171.3[3]
$Fe(CNCH_2Ph)_4(*CN)_2$ (diamagnetic)		−140.9[f]

[a] Referenced to TMS (upfield shifts are positive); iron(III) porphyrin, 0.01 M; potassium cyanide (90 at. % C-13), 0.05 M; pD 9.5–10.5; deuterium oxide solution, 26 °C. [b] 0.005 m iron(III) porphyrin. [c] 0.03 M sodium deuterioxide. [d] 0.5 M in D_2O. [e] D. Mansuy, J. Y. Lallemand, J. C. Chottard, B. Cendrier, G. Gacon, and H. Wajcman, *Biochem. Biophys. Res. Commun.*, **70**, 595–599 (1976). [f] D. L. Cronin, J. R. Wilkinson, and L. J. Todd, *J. Magn. Reson.* **17**, 353–361 (1975).

porphyrin species for which the line width is 570 Hz. The increased line broadening may reflect an enhanced aggregation tendency for this compound.[9]

A variety of iron(III) porphyrin compounds have been examined as the dicyano complex and results are shown in Table I. ^{13}C resonance values may be compared with other representative carbon-bound iron species listed in the table. Surprisingly little difference is seen in the cyanide resonance positions for iron(III) porphyrin compounds with 2,4 side chains as diverse as vinyl and ethyl groups (the nature of the 2,4 substituents is known to modulate various other physical properties). Negatively charged substituents seemingly affect the bound cyanide resonance to a greater extent than presence of electron-withdrawing groups. Thus, both hemin *c* and iron(III) 2,4-disulfonatedeuteroporphyrin in the dicyano form carry a −5 charge and show significantly smaller isotropic shifts than the −3 charged complexes. Perhaps the proximity of the sulfonate groups to the metal center accounts for the relatively lower isotropic shift observed for iron(III) 2,4-disulfonatedeuteroporphyrin. The synthetic *meso*-tetrakis(4-carboxyphenyl)porphine as the dicyano adduct is also −5 charged and exhibits the smallest bound resonance value observed. However, because of the differing structural type, this compound cannot be directly compared with the natural porphyrin derivatives. Explanation of charge and structural effects on the bound cyanide resonance position must await examination of additional compounds and a complete solvent dependence study.

Solution conditions have been varied in order to discern ionic strength, pD, and concentration dependence. For iron(III) deuteroporphyrin the bound cyanide resonance varied from +2375 ppm in 0.05 ionic strength media to +2395 ppm in 1.0 ionic strength solution (potassium chloride employed). Hemin *c* in deuterium oxide solution exhibited bound resonances differing by <10 ppm for solutions from pD 9.3 to pD 13.3. Although concentration dependence studies are limited by sensitivity and solubility, iron(III) deuteroporphyrin at 10 mM shows a bound peak at +2375 ppm, whereas for 25 mM solution the peak is seen at +2405 pp, ppm. Use of methanol-d_4 rather than deuterium oxide as a solvent results in a 100-ppm increase in the isotropic shift. This solvent dependence has been observed in previous ^1H NMR studies of iron(III) porphyrin dicyano complexes.[3] The explanation given for ^1H NMR work may also serve to explain the ligand ^{13}C resonance pattern in that the strongly hydrogen bonded water molecules lower the ligand field of the bound cyanide ion thus yielding diminished π bonding or a change in magnetic anisotropy.

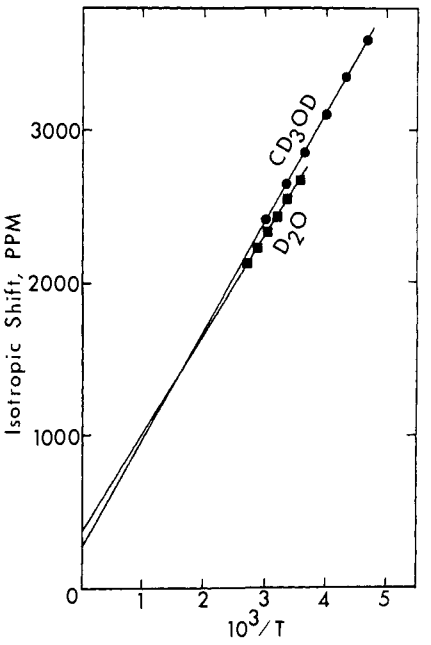


Figure 2. Curie Law plot of bound cyande resonance. Iron(III) deuteroporphyrin, 0.01 M, and 90% C-13 potassium cyanide, 0.05 M.

Results of variable-temperature measurements are given in Figure 2. Over the temperature ranges studied, plots of isotropic shift vs. $1/T$ are linear, but a nonzero intercept is observed. This behavior has also been noted for potassium hexacyanoferrate(III).[8] Detailed calculations of the hexacyanoferrate(III) system are compatible with a dominant spin polarization mechanism for spin delocalization.[8] Deviation from a zero intercept in the Curie Law plot may be explained by spin–orbit coupling which should also produce a nonlinear plot if spectra could be recorded over a wider temperature range.

Cyanide ligand exchange is slow on the ^{13}C NMR time scale even at a temperature of 96 °C. Assuming that a 50-Hz line width contribution from chemical exchange could be detected, the lifetime of bound cyanide ion must be $>6 \times 10^{-3}$ s at 96 °C. If 20 kcal/mol is taken as a reasonable activation energy for ligand exchange, the lifetime for bound cyanide at 25 °C must be on the order of seconds or longer. The lifetime of iron(III) porphyrin bound cyanide in dimethyl sulfoxide solution is reportedly longer than 6×10^{-3} s at 65 °C.[5] These upper rate limits for cyanide exchange are considerably slower than imidazole exchange with iron(III) porphyrins.[10] However, the lifetime for bound cyanide ion in potassium hexacyanoferrate(III) is known to be on the order of days (for the nonphotochemical pathway.[11]

Work is in progress involving mixed cyanide–imidazole iron(III) porphyrin complexes and location of bound cyanide resonances in adducts of the hemopeptides from cytochrome *c*.[12] Potential observation of the cyanide ^{13}C NMR signal in

ferrihemoproteins merits detailed examination of parameters which affect the cyanide resonance position in model iron(III) porphyrin complexes. Thus, carbon-13 enriched cyanide ion may ultimately provide a probe for ferrihemoprotein structure, much as carbon monoxide serves as a powerful probe for ferrohemoproteins.[13]

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Cationization of Organic Molecules in Secondary Ion Mass Spectrometry

Sir:

We show that organic molecules can be ionized from the solid by cationization in secondary ion mass spectrometry (SIMS) and that such ionization shows significant similarities to other recently developed methods of ionizing organic compounds in the solid state. Observed in the SIMS spectra are organometallic ions which are formally due to (i) addition of cations (Ag^+, Pt^+) to the intact organic molecule (M), (ii) fragmentation of the cationized species by loss of simple neutral molecules, and (iii) processes yielding more complex surface-derived entities containing several metal atoms. These novel observations may have importance in the theory of secondary ion emission as well as in the analysis of thermally sensitive organic compounds.

Figure 1 shows the high mass region of the spectrum of *p*-aminobenzoic acid supported on silver.[1] The argentated molecule ($(M + Ag)^+$, *m/e* 244 and 246) is clearly present in good yield. Other ions of interest are *m/e* 226 and 228 (loss of water from the argentated molecule), and *m/e* 199 and 201 (loss of CO_2H). The silver dimer ions (Ag_2^+) are present in high intensity, and they are accompanied by several other sets of ions which appear from the isotopic patterns to include two silver atoms.[3] At lower masses, this spectrum shows *m/e* 150 and 152 $(Ag + 43)^+$, intensity 35 s^{-1} (count rate corresponding to that given in Figure 1); 145 and 147 $(Ag + 38)^+$, 25 s^{-1}; 137 $(M)^+$, 80 s^{-1}; 120 $(M - OH)^+$, 1×10^3 s^{-1}; 92 $(M - CO_2H)^+$, 700 s^{-1}; 77 $(C_6H_5^+)$, 300 s^{-1}; and 65 $(M - CO_2H - HCN)^+$, 1.6 $\times 10^3$ s^{-1}. The base peak in the spectrum at 4×10^4 s^{-1} is K^+

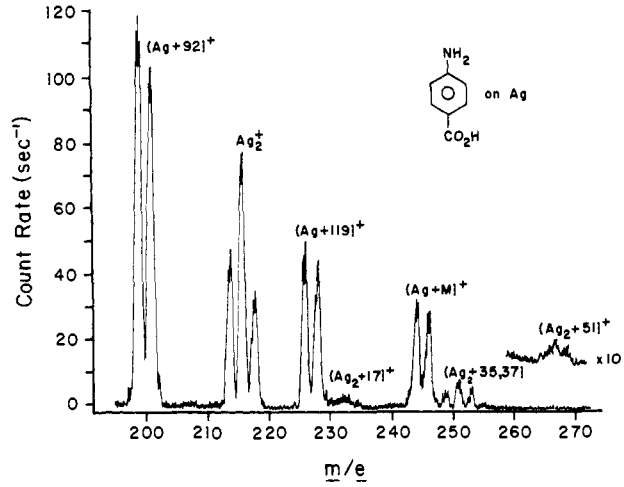

Figure 1. Secondary ion mass spectrum (SIMS) of *p*-aminobenzoic acid on silver. The primary ion current was 3×10^{-9} A cm^{-2} and the scan time ~20 min.

(*m/e* 39), followed by Ag^+ (*m/e* 107 and 109) at 5×10^3 s^{-1}. Other intense organic fragments and other inorganic species appear below *m/e* 65.

The most important feature of these results, metal cationization of complex organic molecules, has not been observed previously in SIMS, although alkali metal addition does occur in some other ionization techniques.[4-8] Only protonation was observed in SIMS studies on organic compounds carried out elsewhere under similar conditions.[9] Our results were unchanged when the primary ion energy, primary ion flux, the solvent from which the organic compound was evaporated (ethanol and water were tried), and the chemical state of the metal (oxidized and reduced) are altered. Some variations in relative ion intensities and sensitivities were, however, observed.

When *p*-aminobenzoic acid was supported on platinum, analogous results were obtained. In particular, ions centered on *m/e* 259 $(Pt + 64)^+$, 287 ± 1 $(Pt + 92)^+$, 314 ± 2 $(Pt + 119)^+$, and 332 ± 2 $(Pt + M)^+$ were detected. The latter three ions, $(M + Pt)^+$, $(M + Pt - H_2O)^+$, and $(M + Pt - CO_2H)^+$, correspond to peaks observed in the spectrum taken on silver. Platinum has a very poor sputter yield and resolution had to be sacrificed to gain the sensitivity required to observe the platinated species. Other platinated ions appeared centered on *m/e* 212 $(Pt + 17)^+$, 222 $(Pt + 27)^+$, 233 $(Pt + 38)^+$, 246 ± 1 $(Pt + 51)^+$, and 271 ± 1 $(Pt + 76)^+$. A series of $(Pt_2 + organic)^+$ ions which corresponded to the Pt_1 series was also observed.

Phenylalanine on silver exhibited results similar to those obtained for *p*-aminobenzoic acid. Its SIMS spectrum included the ions Ag_3^+, Ag_2^+, $(Ag_2 + 35)^+$, $(Ag + M - 46)^+$, and $(Ag + M - 73)^+$. The parent argentated molecule was not detected, perhaps because the benzylic hydrogens promote a rearrangement in which the species can readily eliminate H_2CO_2 ($CO + H_2O$) to give the abundant $(Ag + M - 46)^+$ ion. Benninghoven and coworkers[9] observed $(M + H - 46)^+$, as the major ion for phenylalanine supported on silver; in our experiments Ag is incorporated instead of H in this ion.

A further significant observation closely links these experiments to other ionization methods. When a solution of *p*-aminobenzoic acid and lithium chloride (mole ratio 2:1) in ethanol is evaporated onto silver and bombarded, a spectrum results in which the cationized species shown in Table I are seen. Note that the addition of lithium as well as some argentation occurs. This lithiation is analogous to the alkali metal addition seen in field desorption,[5] plasma desorption,[6] and electrohydrodynamic ionization.[7] The analogy even extends