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^{13}C-Nuclear Magnetic Resonance Study of Low-Spin Iron(III) Protoporphyrin IX Dicyano Complex. Individual Hyperfine Shifted ^{13}C Resonance Assignments

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^{1}H-^{13}C COSY spectra of the low-spin iron(III) protoporphyrin IX dicyano complex heve lead to the assignments of the individual hyperfine shifted ^{13}C resonances which permit a direct comparison of the hyperfine shifts between ^{13}C and attached ^{1}H resonances. Parallel and anti-parallel relationships between ^{13}C and attached ^{1}H shifts for the heme methyl and meso resonances, respectively, resulted from the different mechanisms operating on the unpaired spin delocalization have been clearly observed.

NMR has played an important role in characterizing the electronic/molecular structure of paramagnetic hemins and hemoproteins.[1-3] The hyperfine shifted NMR signal arising from interaction of the nucleus of interest with the unpaired electron(s) of the heme iron has been successfully used to investigate the nature of π electron distribution on the heme because of their sensitivity. Although the hyperfine shifted heme peripheral ^{1}H resonances have been utilized in the majority of the work on characterization of heme electronic structure owing to their high resolution,[2-3] the separation of relative contributions for the observed hyperfine shifts in ^{1}H resonances has been found to be difficult and therefore the conclusion drawn from the ^{1}H data remains frequently qualitative.

The usefulness of combined treatment of ^{13}C and ^{1}H hyperfine shift data, with the theory proposed by karplus and Fraenkel,[4] for a quantitative characterization of heme electronic structure has been emphasized in spite of the fact that some complications in analyzing the ^{13}C hyperfine shifts emerge from the ligand-centered dipolar shift due to the anisotropically delocalized unpaired spin density, in addition to the usual metal-centered dipolar shift.[5] Goff has estimated quantitatively the spin density at porphyrin carbon and nitrogen atoms of the low-spin iron(III) bis-imidazole complex of tetraphenylporphyrin from the combined analysis of ^{13}C and ^{1}H data.[6-7] Wüthrich and Baumann have attempted such analysis on iron(III) protoporphyrin IX dicyano complex.[8-9] But without the individual ^{13}C resonance assignment, the nature of π electron distribution of the heme was not characterized in detail from their results. We therefore report herein on the assignment of the individual hyperfine shifted ^{13}C-NMR resonances of the low-spin

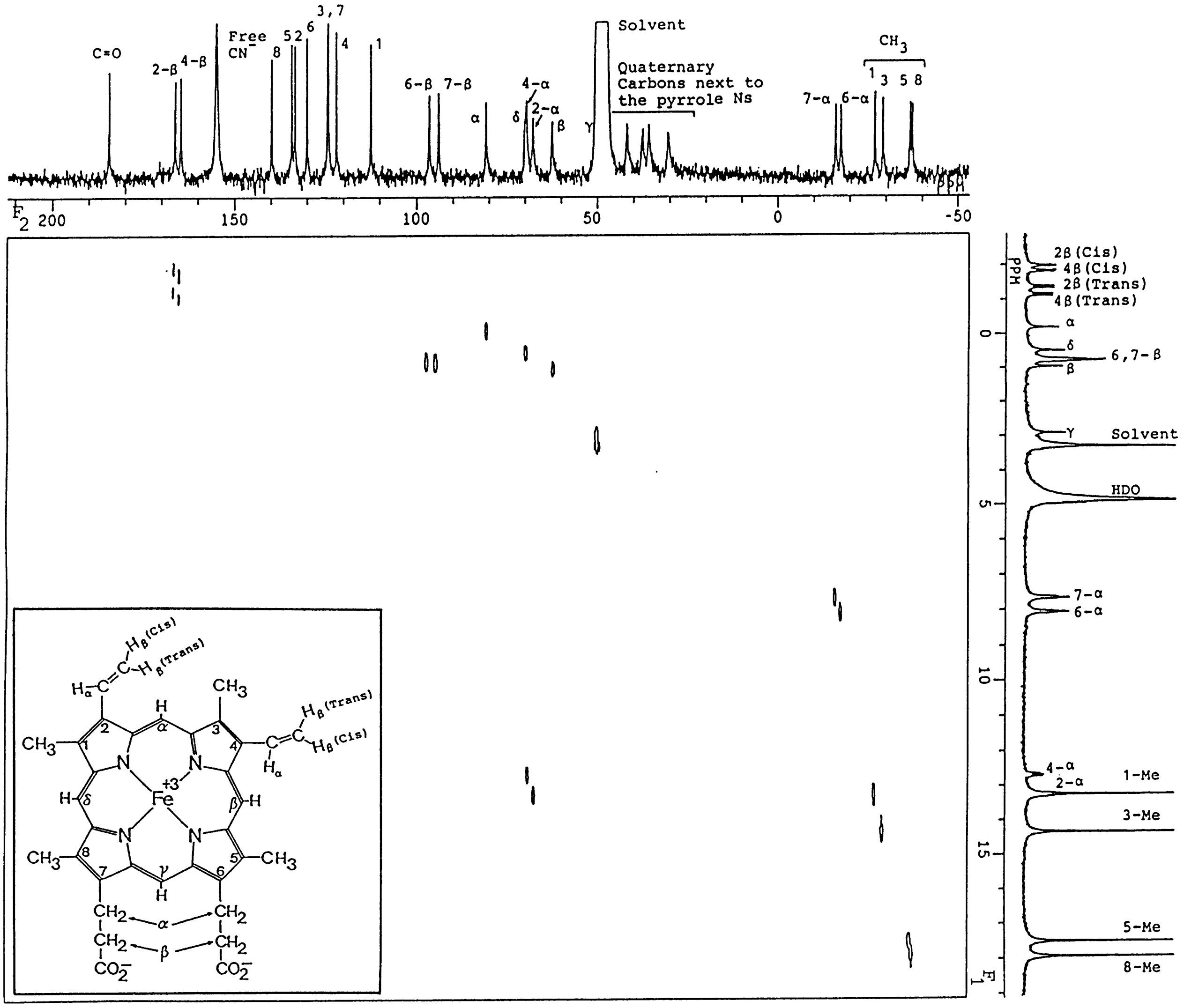

Fig. 1. ^{1}H-^{13}C COSY spectrum of the low-spin iron(III) protoporphyrin IX dicyano complex in CD_3OD at 22 °C. ^{1}H and ^{13}C spectra are illustrated along F_1 and F_2 dimensions, respectively. The assignments of ^{1}H resonances[11] and those of ^{13}C resonances obtained from the present study are shown with the corresponding one-dimensional spectra. The structure and numbering system of the hemin is given in the inset. The ^{1}H-^{13}C COSY spectrum was recorded on a JEOL GX-270 FT-NMR spectrometer equipped with a 10 mm diameter tunable probe using the standard pulse sequence[15] and procedures. The concentration of the sample was about 20 mM. Sixteen protonated carbon resonances are clearly assigned in the ^{1}H-^{13}C COSY spectrum from the known ^{1}H assignments. Meso γ carbon resonance is under the solvent peak. The assignments of the quaternary carbon, C_1 - C_8, and the propionate β carbon resonances were made using the long-range ^{1}H-^{13}C COSY connectivities (results not shown).

iron(III) protoporphyrin IX dicyano complex(1) in CD_3OD using ^1H-^{13}C COSY technique which forms a basis of quantitative characterization of the heme electronic structure using ^{13}C-NMR.

The ^1H-^{13}C COSY spectrum, together with ^1H and ^{13}C spectra, of 1 is illustrated in Fig. 1. One-dimensional spectra are essentially identical to those reported previously. The assignments of the ^1H resonances have been made by means of the isotope labeling[10)] and nuclear Overhauser effect[11)] and are given with the ^1H spectrum. In the ^1H-^{13}C COSY spectrum, the connectivities are established by the ^1H and ^{13}C nuclei whose Larmor frequencies are connected through scalar coupling. As seen in the contour spectrum, sixteen individual protonated carbons of the heme are clearly assigned from the known ^1H resonance assignment. Since the resonances arising from the β protons of the propionate groups at 6 and 7 positions are degenerate, the corresponding carbon resonances were assigned using the long-range ^1H-^{13}C COSY. Resonances of $C_1 - C_8$ have similarly been assigned using the long-range COSY(results not shown). The resonances from the quaternary carbons adjacent to the pyrrole nitrogens around 40 ppm can not be assigned at present because of the experimental difficulty arising from their short relaxation times as reflected by their broad line widths. The present results confirm the partial assignment reported by La Mar et al.[12)] for heme methyl and meso carbon resonances. The spread of $C_1 - C_8$ resonances clearly reflects in-plane asymmetry of the heme electronic structure and those resonances appear to be more sensitive indicators to the asymmetric unpaired spin density distribution within the paramagnetic hemin than the heme methyl proton resonances which have been used in earlier studies.[2)]

With the individual ^{13}C resonance assignments, the observed hyperfine shifts of ^{13}C and attached ^1H nuclei can be directly compared and the combined analysis of ^{13}C and ^1H data provides quantitative interpretation of unpaired spin density distribution within 1. Compared with the observed ^{13}C shifts in the diamagnetic zinc(II) protoporphyrin IX derivative,[13)] together with the results from the similar comparative studies made for ^1H data,[12)] a parallel relationship in the magnitude of the hyperfine shifts between ^{13}C and attached ^1H resonances is observed for the resonances from all the heme peripheral side chains except meso resonances, clearly reflecting predominant π-type unpaired spin density delocalization in low-spin iron(III) complexes.[14)] An anti-parallel relationship is observed for meso ^{13}C and ^1H resonances, suggesting σ-type spin delocalization for those C-H bonds. Although the resonances from the β protons at 6 and 7 positions are degenerate, the corresponding carbon resonances are resolved clearly, demonstrating the sensitivity of ^{13}C-NMR and the difference in the unpaired spin density delocalized to those carbon atoms via hyperconjugation.[9)] From the comparison of the magnitudes of the hyperfine shifts between carbons at 6 and 7, together with the directions of their shifts, it can be concluded that a larger unpaired spin density delocalization to the propionate group at 6 than to that at 7.

The comparison of ^{13}C and ^1H hyperfine shifts enables us to separate relative contributions for the observed hyperfine shift. Quantitative analyses of meso ^{13}C and ^1H shifts and $C_1 - C_8$ hyperfine shifts could provide an improved understanding of spin polarization mechanism at a meso C-H bond and a precise description of unpaired spin delocalization pattern on the heme, respectively. Treatment of both ^{1}H and ^{13}C data combined with their temperature dependence and relaxation studies could facilitate more quantitative characterization of the heme electronic structure. Such studies are in progress in our laboratory.

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