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Resonance Raman Detection of Iron–Ligand Vibrations in Cyano(pyridine)(octaethylporphinato)iron(III): Effects of Pyridine Basicity on the Fe–CN Bond Strength

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The influence of axial ligand basicity on the bonding of iron(III) in cyano adducts of octaethylporphyrin has been studied by resonance Raman spectroscopy. In a six-coordinate ferric low-spin complex, cyano(pyridine)(octaethylporphinato)iron(III), Fe(OEP)(CN)(py), Raman lines at 449 and 191 cm^{-1} were assigned to the ν(Fe–CN) and ν(Fe–py) stretching modes, respectively. When pyridine was displaced with its derivatives, py-X, where X = 4-cyano, 3-acetyl, 3-methyl, 4-methyl, 3,4-dimethyl, and 4-dimethylamino, the ν(Fe–CN) stretching frequency was found to decrease in the complex with a high pyridine basicity. It was concluded that the stronger the trans pyridine basicity, the weaker the iron–carbon (cyanide) bond. A clear frequency shift was observed in the ν_4 mode, though most of the porphyrin vibrations were insensitive to the ligand substitution. The frequency of the ν_4 mode, which is the C_a–N(pyrrole) breathing vibration of the porphyrin skeleton, was found to increase with an increase in pyridine basicity. This is contrary to what was found in ferrous low-spin hemes as CO complexes. The ν_4 shift in the CN complexes was explained in terms of forward π donation; donation of electrons from the porphyrin π orbital to the d_π vacancy of the low-spin iron(III) weakened the C_a–N(pyrrole) bonds and hence decreased the ν_4 frequency.

Introduction

Heme proteins and enzymes are ubiquitous in nature and participate in many biologically important reactions. To assess the essential factors that control the heme functions, it is necessary to understand the structure of the heme moiety, as well as the protein matrix surrounding the heme. Recently, carbon monoxide (CO) adducts of heme proteins and enzymes, such as hemoglobins,[2] cytochrome P-450s,[3] cytochrome oxidases,[4] and peroxidases,[5] have been studied by resonance Raman spectroscopy. The immediate environments of the heme could be studied by monitoring the ν(Fe–CO) stretching, δ(Fe–C–O) bending, and bound ν(C–O) stretching Raman lines, since these vibrations of bound CO are sensitive to geometrical differences at the distal side of the heme.[3a,6]

Besides the role of distal environment, the role of the proximal ligand of the heme must be crucial in regulating the reactivity of the heme. In hemoglobin, it was proposed that strong intersubunit interactions in the T state might pull the proximal histidine away from the porphyrin plane and stretch the Fe–His bond, which thus controls the cooperative oxygen binding to the heme iron.[7] Such strain in the Fe–His bond of the deoxyhemoglobin was observed in the R–T difference of the ν(Fe–His) stretching frequency.[8] In peroxidases, the modulation of the heme iron reactivity has long been postulated to be caused by removal of the labile proton from the proximal histidine.[9]

To ascertain the influence of the proximal ligand, detection of the iron–ligand stretching Raman lines should bring about the most direct information on the nature of the iron–ligand bond. These Raman lines are in general sensitive to, and hence are good indicators of, the alteration of the iron–ligand bonding. The trans effect of the proximal base on the iron–ligand bond was studied in the ferrous heme–CO complexes,[10] where it was found that the weaker the iron–trans ligand bond, the stronger the iron–carbon (CO) bond. A similar effect was noted even in the iron(IV) oxoporphyrin complexes.[11] In the ferric hemes, however, no sufficient study has been done on the trans effect of the proximal base.

In this paper, we describe the effects of ligand field strength on the iron–ligand vibrations to characterize the nature of iron–ligand bonding by resonance Raman spectroscopy in the six-coordinate ferric low-spin complexes Fe(OEP)(CN)(py-X).[12] The Fe–ligand bonding in the ferric hemes must be different from that found in ferrous hemes in which the π back-bonding is significant. The cyanide anion was used as a ligand because it binds to ferric hemes with high affinity. Since the cyanide anion is assumed to bind essentially perpendicular to the heme plane, as was found in the crystals of the complexes Fe(TPP)(CN)(py)[13] and Fe(OEP)(CN)(py),[14] the changes in Raman frequencies can be attributed exclusively to the trans pyridine bases. The relationship between the ligand basicity and the frequencies will provide a clue to understanding the Fe–CN bonding in the heme proteins studied recently by resonance Raman spectroscopy.[2b,15]

(1) (a) Nagoya City University. (b) University of Tokyo.
(2) (a) Tsubaki, M.; Srivastava, R. B.; Yu, N.-T. *Biochemistry* **1982**, *21*, 1132. (b) Yu, N.-T.; Benko, B.; Kerr, E. A.; Gersonde, K. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 5106. (c) Gersonde, K.; Kerr, E.; Yu, N.-T.; Parish, D. W.; Smith, K. M. *J. Biol. Chem.* **1986**, *261*, 8678.
(3) (a) Uno, T.; Nishimura, Y.; Makino, R.; Iizuka, T.; Ishimura, Y.; Tsuboi, M. *J. Biol. Chem.* **1985**, *260*, 2023. (b) Tsubaki, M.; Ichikawa, Y. *Biochim. Biophys. Acta* **1985**, *827*, 268. (c) Tsubaki, M.; Hiwatashi, A.; Ichikawa, Y. *Biochemistry* **1986**, *25*, 3563.
(4) (a) Argade, P. V.; Ching, Y. C.; Rousseau, D. L. *Science (Washington, D.C.)* **1984**, *225*, 329. (b) Uno, T.; Nishimura, Y.; Tsuboi, M.; Kita, K.; Anraku, Y. *J. Biol. Chem.* **1985**, *260*, 6755.
(5) (a) Evangelista-Kirkup, R.; Smulevich, G.; Spiro, T. G. *Biochemistry* **1986**, *25*, 4420. (b) Smulevich, G.; Evangelista-Kirkup, R.; English, A.; Spiro, T. G. *Biochemistry* **1986**, *25*, 4426. (c) Uno, T.; Nishimura, Y.; Tsuboi, M.; Makino, R.; Iizuka, T.; Ishimura, Y. *J. Biol. Chem.* **1987**, *262*, 4549.
(6) Yu, N.-T.; Kerr, E. A.; Ward, B.; Chang, C. K. *Biochemistry* **1983**, *22*, 4534.
(7) (a) Perutz, M. F. *Nature (London)* **1970**, *228*, 726. (b) Perutz, M. F. *Nature (London)* **1982**, *273*, 495. (c) Shulman, R. G.; Hopfield, J. J.; Ogawa, S. *Q. Rev. Biophys.* **1975**, *8*, 325.
(8) (a) Nagai, K.; Kitagawa, T.; Morimoto, H. *J. Mol. Biol.* **1980**, *136*, 271. (b) Ondrias, M. R.; Rousseau, D. L.; Shelnutt, J. A.; Simon, S. R. *Biochemistry* **1982**, *21*, 3428. (c) Desbois, A.; Lutz, M.; Banerjee, R. *Biochim. Biophys. Acta* **1981**, *671*, 177. (d) Matsukawa, S.; Mawatari, K.; Yoneyama, Y.; Kitagawa, T. *J. Am. Chem. Soc.* **1985**, *107*, 1108.
(9) (a) Morrison, M.; Schonbaum, G. R. *Annu. Rev. Biochem.* **1976**, *45*, 861. (b) Nicholls, P. *Biochim. Biophys. Acta* **1962**, *60*, 217. (c) Blumberg, W. E.; Peisach, J. In *Probes of Structure and Function of Macromolecules and Membranes*; Chance, B., Yonetani, T., Mildvan, A. S., Eds.; Academic: New York, 1971; Vol. II, p 215. (d) Schonbaum, G. R. In *Oxidases and Related Redox Systems*; King, E., Mason, H. S., Morrison, M., Eds.; Pergamon: Oxford, U.K., 1982; p 671. (e) Mincey, T.; Traylor, T. G. *J. Am. Chem. Soc.* **1979**, *101*, 765. (f) Teraoka, J.; Kitagawa, T. *J. Biol. Chem.* **1981**, *256*, 3969. (g) de Ropp, J. S.; Thanabal, V.; La Mar, G. N. *J. Am. Chem. Soc.* **1985**, *107*, 8268.
(10) Kerr, E. A.; Mackin, H. C.; Yu, N.-T. *Biochemistry* **1983**, *22*, 4373.
(11) Schappacher, M.; Chottard, G.; Weiss, R. *J. Chem. Soc., Chem. Commun.* **1986**, 93.
(12) Abbreviations: OEP, octaethylporphyrinato; TPP, tetraphenylporphyrinato; PPDME, protoporphyrin dimethyl esterato; py, pyridine; py-d_5, perdeuteriated pyridine; 4-CNpy, 4-cyanopyridine; 3-Acpy, 3-acetylpyridine; 4-Mepy, 4-methylpyridine; 3-Mepy, 3-methylpyridine; 3,4-Me_2py, 3,4-dimethylpyridine; 4-Me_2Npy, 4-(dimethylamino)-pyridine; py-X, pyridine derivative; 2-MeIm, 2-methylimidazole.
(13) Scheidt, W. R.; Lee, Y. J.; Luangdilok, W.; Haller, K. J.; Anzai, K.; Hatano, K. *Inorg. Chem.* **1983**, *22*, 1516.
(14) Scheidt, W. R.; et al., unpublished results.

0020-1669/88/1327-3215$01.50/0

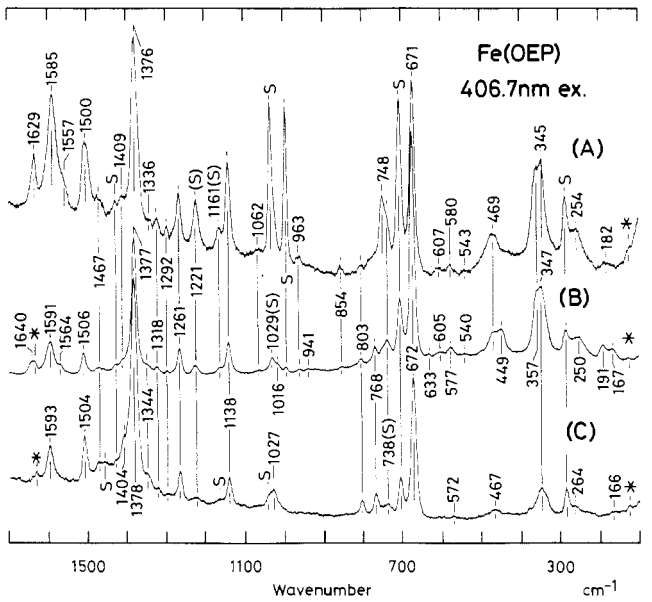

Figure 1. Resonance Raman spectra of the Fe(OEP) moiety with various ligands: (A) Fe(OEP)Cl dissolved in the solution of 25% pyridine in CH_2Cl_2; (B) Fe(OEP)(CN)(py) prepared by dissolution of crystalline Fe(OEP)(CN)(py) in a solution of 4% pyridine in CH_2Cl_2; (C) $[Fe(OEP)(CN)_2]^-$ prepared by dissolution of crystalline Fe(OEP)Cl in a KCN-saturated solution of 50% methanol in CH_2Cl_2. The bands marked with asterisks and S denote those of plasma lines of a krypton laser and of the solvent, respectively. The bands marked with (S) denote that partial contribution to the bands from the solvent may exist. Raman scattering was excited with the 406.7-nm line; Slit width 5 cm^{-1}.

Materials and Methods

Sample Preparation. The Fe(OEP)Cl complex was prepared by the method of Whitlock et al.[16] The crystalline Fe(OEP)(CN)(py) complex was prepared by similar methods in the literature.[13,17] The complex substituted by ^{13}CN, Fe(OEP)(^{13}CN)(py), was prepared by using K^{13}CN (99 atom % ^{13}C, from Wako Chemicals).

A few grains of the crystalline Fe(OEP)(CN)(py) complex were dissolved in methylene chloride solution containing an appropriate amount of pyridine derivatives (py-X), and thus the complexes with py-X, Fe(OEP)(CN)(py-X), were prepared in situ by the substitution reaction of pyridine. The derivatives used were 4-cyanopyridine (4-CNpy), 3-acetylpyridine (3-Acpy), 3-methylpyridine (3-Mepy), 4-methylpyridine (4-Mepy), 3,4-dimethylpyridine (3,4-Me_2py), 4-(dimethylamino)pyridine (4-Me_2Npy) (all from Tokyo Kasei Co.), pyridine (py, from Wako Chemicals Co.), and pyridine-d_5 (py-d_5, 99 atom % ^2H, obtained from CEA). The detailed sample conditions will be given in each figure caption.

The $[Fe(OEP)(CN)_2]^-$ solution was prepared in situ by dissolving Fe(OEP)Cl crystals in a KCN-saturated solution of 50% methanol and 50% methylene chloride, and the resultant solution was filtered. The sample solutions were adjusted to about 100–200 μM in porphyrin concentration by the appropriate addition of the solvent and subjected to the resonance Raman measurements.

Resonance Raman Measurements. Resonance Raman scattering was excited with a 406.7-nm line of a krypton ion laser (Spectra Physics, SP 164-01), which was operated with the laser power of approximately 10 mW at the sample. Resonance Raman spectra were measured by using a Jasco R-800UV spectrometer. Detection was carried out on a Hamamatsu Photonics R-585 photomultiplier or on a Tracor Northern IDARSS detector system as described previously.[5c,18] The spectral data were all transferred to a PDP11/23 DEC minicomputer and processed.

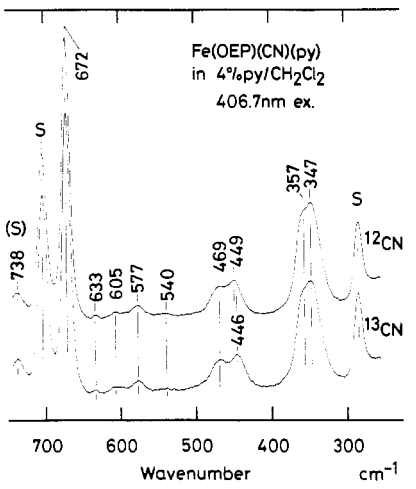

Figure 2. Effect of the substitution by isotope-labeled CN on the resonance Raman spectrum of the Fe(OEP)(CN)(py) complex: top, ^{12}CN; bottom, ^{13}CN. Crystalline Fe(OEP)(^{12}CN)(py) and Fe(OEP)(^{13}CN)(py) were dissolved in a 4% pyridine/CH_2Cl_2 solution. Spectral conditions were the same as in Figure 1.

The Raman cell was spun throughout the measurements to minimize the photoreduction reaction[4b] and to prevent local heating. The frequency scale of resonance Raman spectra was calibrated on the basis of the Raman lines of indene and methylene chloride, the latter being contained in all sample solutions.

Results

Resonance Raman Spectra of Fe(OEP) Complexes. The resonance Raman spectrum of the dicyanide complex, $[Fe(OEP)(CN)_2]^-$, is shown in Figure 1C. The oxidation state, spin state, and coordination number of the heme iron could be elucidated by the frequencies of some marker bands.[19] The ν_2, ν_3, and ν_4 bands[20] were observed at 1593, 1504, and 1378 cm^{-1}, respectively, indicating that $[Fe(OEP)(CN)_2]^-$ is a six-coordinate ferric low-spin complex. The spectral features were consistent with those reported previously.[21]

The resonance Raman spectrum of Fe(OEP)(CN)(py) is shown in Figure 1B. The ν_{10}, ν_2, ν_{11}, ν_3, and ν_4 bands were observed at 1640, 1591, 1564, 1506, and 1377 cm^{-1}, respectively, indicating that Fe(OEP)(CN)(py) is also a six-coordinate ferric low-spin species in solution. The resonance Raman profile of Fe(OEP)(CN)(py) (spectrum B) was very similar to that of $[Fe(OEP)(CN)_2]^-$ (spectrum C). However, the resonance Raman bands at 1640 and 1564 cm^{-1} in spectrum B were not observed in spectrum C. In addition, Raman bands at about 450 and 190 cm^{-1} in the Fe(OEP)(CN)(py) complex (spectrum B) are clearly detected.

The resonance Raman spectrum of the Fe(OEP)Cl complex dissolved in methylene chloride solution containing 25% (v/v) pyridine is also indicated in Figure 1A. If the cyanide were dissociated from the heme moiety in the Fe(OEP)(CN)(py) complex, spectrum B would be the same as spectrum A, but this is not the case. The ν_{10}, ν_2, ν_{11}, and ν_3 frequencies in spectrum A were lower than those observed for two other six-coordinate low-spin complexes and rather resemble those found for ferric horseradish peroxidase[5c,21a] and Fe(PPDME)Cl,[21a] which are five-coordinate species.

Detection of ν(Fe–CN) and ν(Fe–py) Stretching Modes. In Figure 2 are shown the resonance Raman spectra of the Fe(OEP)(CN)(py) complexes upon cyanide isotope substitution in the frequency region 250–750 cm^{-1}. The 449-cm^{-1} line observed

(15) (a) Kerr, E. A.; Yu, N.-T.; Gersonde, K. *FEBS Lett.* **1984**, *178*, 31. (b) Sitter, A. J.; Reczek, C. M.; Terner, J. *Biochim. Biophys. Acta* **1985**, *828*, 229. (c) Henry, E. R.; Rousseau, D. L.; Hopfield, J. J.; Noble, R. W.; Simon, S. R. *Biochemistry* **1985**, *24*, 5907.
(16) Whitlock, H. W., Jr.; Hanauer, R.; Oester, M. Y.; Bower, B. K. *J. Am. Chem. Soc.* **1969**, *91*, 7485.
(17) Scheidt, W. R.; Haller, K. J.; Hatano, K. *J. Am. Chem. Soc.* **1980**, *102*, 3017.
(18) (a) Makino, R.; Uno, T.; Nishimura, Y.; Iizuka, T.; Tsuboi, M.; Ishimura, Y. *J. Biol. Chem.* **1986**, *261*, 8376. (b) Uno, T.; Nishimura, Y.; Tsuboi, M. *Biochemistry* **1984**, *23*, 6802.
(19) (a) Spiro, T. G. In *Iron Porphyrins*; Lever, A. B. P., Gray, H. B., Eds.; Addison-Wesley: Reading, MA, 1983; Part II, p 89. (b) Spiro, T. G. *Adv. Protein Chem.* **1985**, *37*, 111. (c) Asher, S. A. *Methods Enzymol.* **1981**, *76*, 371. (d) Felton, R. H.; Yu, N.-T. In *The Porphyrins*; Dolphin, D., Ed.; Academic: New York, 1978; Vol. III, Part A, p 347.
(20) Abe, M.; Kitagawa, T.; Kyogoku, Y. *J. Chem. Phys.* **1978**, *69*, 4526.
(21) (a) Callahan, P. M.; Babcock, G. T. *Biochemistry* **1981**, *20*, 952. (b) Choi, S.; Spiro, T. G. *J. Am. Chem. Soc.* **1983**, *105*, 3683.

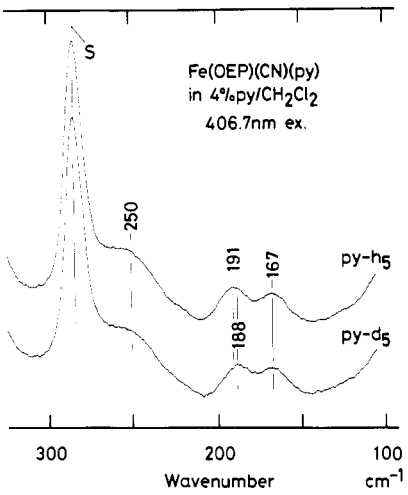

Figure 3. Effect of the substitution by isotope-labeled pyridine on the resonance Raman spectrum of the Fe(OEP)(CN)(py) complex: top, pyridine-h_5; bottom, pyridine-d_5. Crystalline Fe(OEP)(CN)(py) was dissolved in a 4% pyridine/CH_2Cl_2 solution. Spectral conditions were the same as in Figure 1.

for the ^{12}CN adduct (top spectrum in Figure 2) was found to downshift by 3 cm^{-1} for the ^{13}CN adduct (bottom spectrum). Accordingly, the 449-cm^{-1} line in the Fe(OEP)(CN)(py) complex was assigned to the ν(Fe–CN) stretching mode.[22] The ν(Fe–CN) stretching frequency is similar to that observed for hemoglobins,[2b,15a,c] myoglobin,[15b] and heme model compounds.[23]

The ν(Fe–CN) stretching frequency (449 cm^{-1}) found for the Fe(OEP)(CN)(py) complex is 35 cm^{-1} lower than the ν(Fe–CO) stretching frequency (484 cm^{-1}) found for the ferrous CO complex, Fe(TPP)(CO)(py).[24] This is well understood in light of the longer Fe–CN bond (1.934 Å) compared to the Fe–CO bond (1.77 Å), as found in the X-ray crystallographic studies of the ferric CN complex, Fe(OEP)(CN)(py),[14] and of the ferrous CO complex, Fe(TPP)(CO)(py),[25] respectively.

In Figure 3 are shown the resonance Raman spectra of Fe(OEP)(CN)(py) in the frequency region 100–350 cm^{-1}. It is apparent that the 191-cm^{-1} line (top spectrum) downshifts by 3 cm^{-1} upon pyridine perdeuteriation (bottom spectrum). Accordingly, this line was assigned to the ν(Fe–py) stretching mode.

To confirm our assignments for ν(Fe–CN) and ν(Fe–py) stretching modes, we have calculated the isotope shifts of these modes on the basis of a simplified model of the linear three-body oscillator py–Fe–CN. With the stretching force constants of 2.01 and 0.92 mdyn/Å for the Fe–CN and the Fe–py bonds,[26] respectively, the calculation predicted 5- and 3-cm^{-1} downshifts for the ν(Fe–CN) and the ν(Fe–py) stretching modes upon ^{13}CN and py-d_5 substitution, respectively. This result agreed with the observed shifts.

Effect of Pyridine Substitution on the Porphyrin Vibrations. In Figure 4 are shown the resonance Raman spectra of the 4-cyanopyridine and 4-(dimethylamino)pyridine complexes, Fe(OEP)(CN)(4-CNpy) and Fe(OEP)(CN)(4-Me_2Npy). These are the complexes with pyridine bases on the typical extremes of the basicity scale. Most of the Raman frequencies were unchanged upon pyridine ligand substitution. The ν_2 and ν_3 modes were observed at 1591 and 1506 cm^{-1}, respectively, indicating that both were six-coordinate ferric low-spin species. The frequencies are quite similar to those observed in the Fe(OEP)(CN)(py) complex (Figure 1B). However, the frequencies of the ν_4 mode at about 1377 cm^{-1}, the ν(Fe–CN) stretching mode at about 450 cm^{-1}, and the ν(Fe–py) stretching mode at about 190 cm^{-1} were shifted upon pyridine substitution.

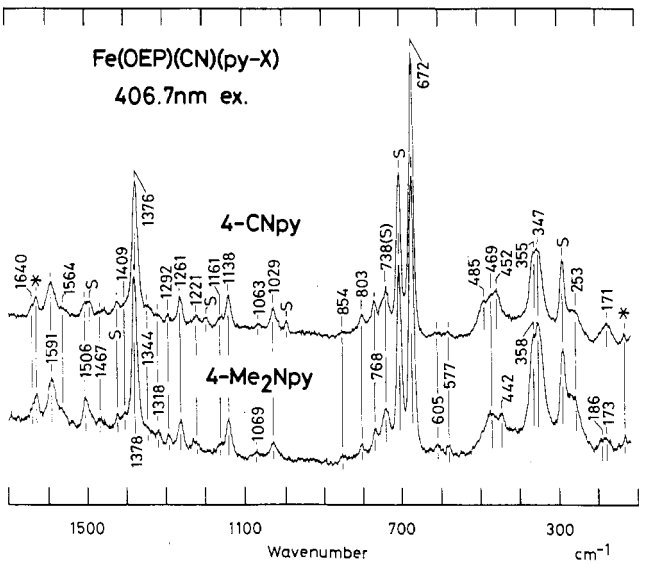

Figure 4. Resonance Raman spectra of the Fe(OEP)(CN)(4-CNpy) (top) and the Fe(OEP)(CN)(4-Me_2Npy) (bottom) complexes. Crystalline Fe(OEP)(CN)(py) was dissolved in CH_2Cl_2 solutions containing 0.52 M 4-CNpy (top) and 0.049 M 4-Me_2Npy (bottom). Spectral conditions were the same as in Figure 1.

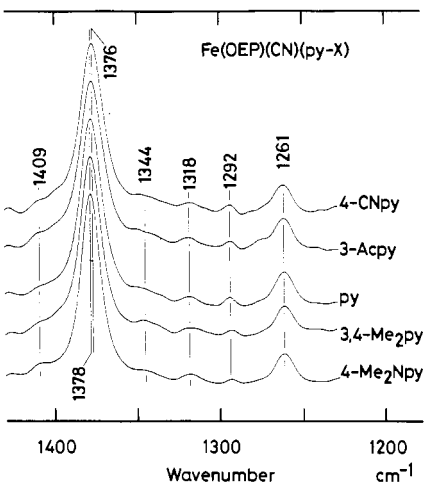

Figure 5. Effect of pyridine substitution on the 1250–1450-cm^{-1} frequency region resonance Raman spectrum of Fe(OEP)(CN)(py-X). Crystalline Fe(OEP)(CN)(py) was dissolved in CH_2Cl_2 solutions containing 0.52 M 4-CNpy, 0.51 M 3-Acpy, 0.50 M py, 0.51 M 3,4-Me_2py, and 0.049 M 4-Me_2Npy. Data acquisition time was 100 s for each spectrum. Other spectral conditions were the same as in Figure 1.

In Figure 5 is shown the effect of pyridine substitution on the spectrum of Fe(OEP)(CN)(py-X) in the frequency region 1250–1450 cm^{-1}. The spectra are aligned in the order of the pyridine basicity. Though most of the Raman features were insensitive to the pyridine substitution, the frequency of the ν_4 mode showed clear dependence on the axial ligand basicity. Namely, the ν_4 line observed at 1376 cm^{-1} in the 4-CNpy adduct gradually increased in frequency and was found at 1378 cm^{-1} for the 4-Me_2Npy adduct. The ν_4 mode is mainly due to the C_a–N(pyrrole) breathing vibration of the porphyrin skeleton.[20] The upshift of

(22) For the Fe–C–N linkage, two vibrational modes that are sensitive to the ^{13}C substitution are expected to occur around 450 cm^{-1}. The δ(Fe–C–N) bending mode occurs around 400 cm^{-1} [2b,15a,c] and is expected to show about 15-cm^{-1} downshift upon ^{13}C substitution. This contrasts with what was actually observed. The ν(Fe–CN) stretching mode is expected to occur at about 450 cm^{-1} and should show about 4-cm^{-1} downshift upon ^{13}C substitution. This agrees with the present observation.

(23) (a) Tanaka, T.; Yu, N.-T.; Chang, C. K. *Biophys. J.* **1984**, *45*, 365a. (b) Tanaka, T.; Yu, N.-T.; Chang, C. K. *Biophys. J.* **1987**, *52*, 801.

(24) Kerr, E. A.; Yu, N.-T.; Bartnicki, D. E.; Mizukami, H. *J. Biol. Chem.* **1985**, *260*, 8360.

(25) Peng, S.; Ibers, J. A. *J. Am. Chem. Soc.* **1976**, *98*, 8032.

(26) The frequency of the ν(Fe–CN) stretch is very close to that observed (179 cm^{-1}) in the bis(pyridine) adduct of ferrous porphyrin: Wright, P. G.; Stein, P.; Burke, J. M.; Spiro, T. G. *J. Am. Chem. Soc.* **1979**, *101*, 3531. These authors used an Fe–N stretching force constant of 1.96 mdyn/Å, which differs from the value we have used (0.92 mdyn/Å). The discrepancy is due to the difference of the model systems used for the calculation; we used a simplified model in which we treated the pyridine molecule as a single dynamical unit with a mass of 79 amu.

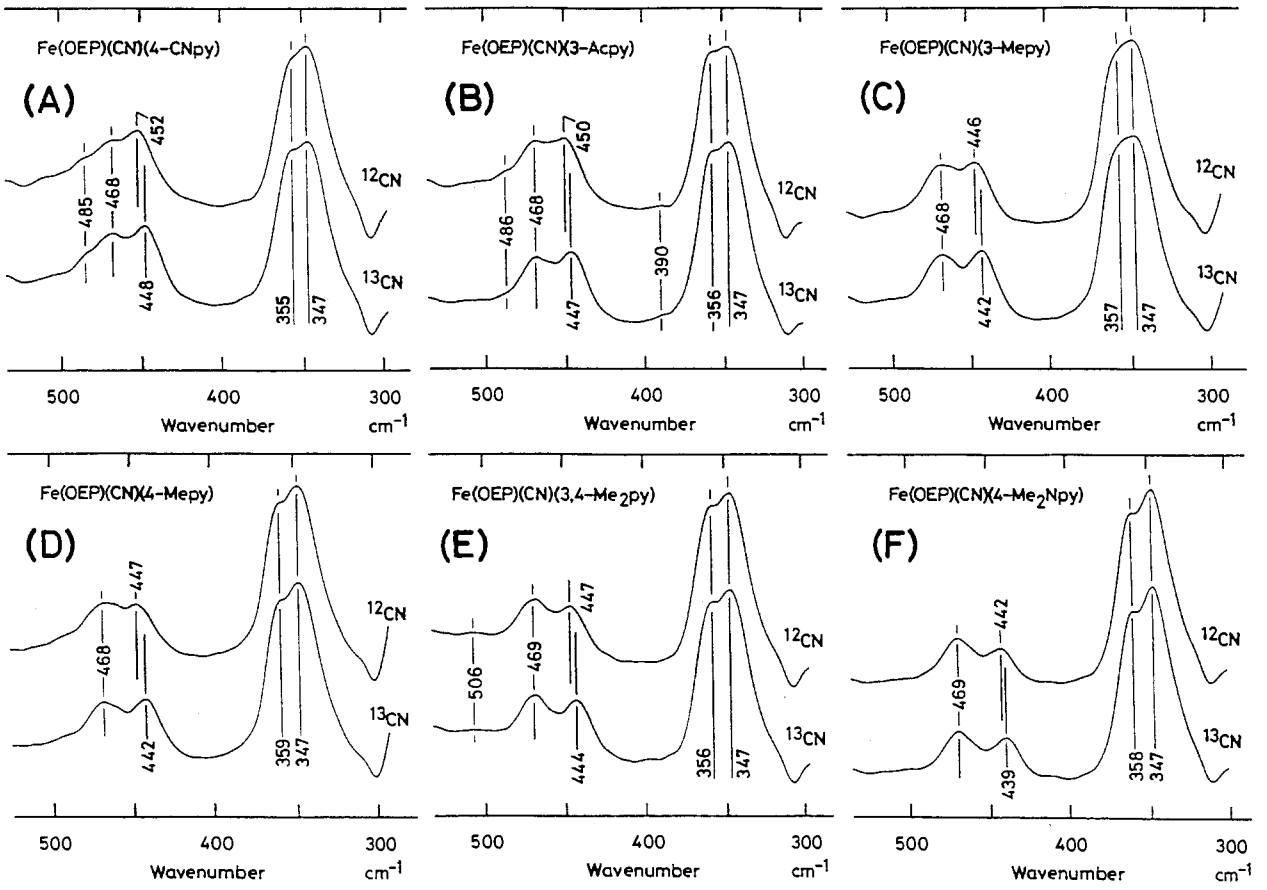

Figure 6. Effect of the substitution by isotope-labeled CN on the resonance Raman spectrum of Fe(OEP)(CN)(py-X). Crystalline Fe(OEP)(CN)(py) was dissolved in CH_2Cl_2 solutions containing 0.52 M 4-CNpy (A), 0.51 M 3-Acpy (B), 0.41 M 3-Mepy (C), 0.41 M 4-Mepy (D), 0.51 M 3,4-Me_2py (E), and 0.049 M 4-Me_2Npy (F). Data acquisition time was 500 s for each spectrum. Other spectral conditions were the same as in Figure 1.

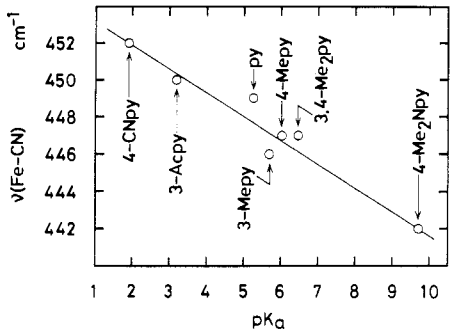

Figure 7. Effect of ligand basicity on the ν(Fe–CN) stretching frequency.

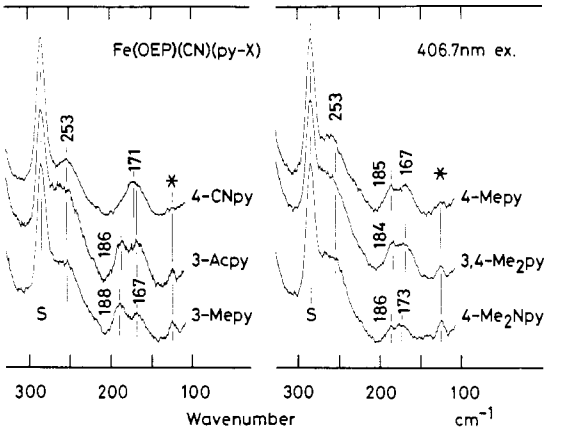

Figure 8. Effect of pyridine substitution on the 100–350-cm^{-1} frequency region resonance Raman spectrum of Fe(OEP)(CN)(py-X). Sample and instrumental conditions were the same as in Figures 6 and 1, respectively.

this mode indicated the increase in the C_a–N bond strength of the porphyrin.

Effect of Ligand Basicity on the Fe–Ligand Vibrations. Figure 6 shows the effect of CN isotope substitution on the spectra of the complexes with 4-CNpy (A), 3-Acpy (B), 3-Mepy (C), 4-Mepy (D), 3,4-Me_2py (E), and 4-Me_2Npy (F). In each spectrum, Raman lines at about 350, 360, and 470 cm^{-1} were insensitive to the CN isotope substitution. Besides these common lines, the Raman lines observed at 452 (A), 450 (B), 446 (C), 447 (D, E), and 442 cm^{-1} (F) were downshifted by about 4 cm^{-1} upon ^{13}CN substitution, respectively. Accordingly, these lines were assigned to the ν(Fe–CN) stretching mode.[27]

The frequency of the ν(Fe–CN) stretching mode showed clear trans ligand dependency; the ν(Fe–CN) stretching frequency was found to be a linear function of the pK_a value of the pyridines, as shown in Figure 7. Since the pK_a value and the ν(Fe–CN) stretch are measures of the ligand basicity and of the Fe–CN bond strength, respectively, it is concluded that the higher the trans ligand basicity, the weaker the Fe–CN bond.

In Figure 8 is shown the effect of pyridine substitution in the frequency region 100–330 cm^{-1}. Resonance Raman bands at about 170 and 250 cm^{-1} are common in each spectrum. A Raman band at 186 (3-Acpy), 188 (3-Mepy), 185 (4-Mepy), 184 (3,4-Me_2py), or at 186 cm^{-1} (4-Me_2Npy) is characteristic of individual pyridine substitution and corresponds to the 191-cm^{-1} band assigned to the ν(Fe–py) stretching mode in the Fe(OEP)(CN)(py) complex (Figure 3). In the spectrum of the Fe(OEP)(CN)(4-CNpy) complex, the ν(Fe–py) stretching band was ambiguous. However, the Raman modes observed in the frequency region 1450–1700 cm^{-1} indicated the formation of six-coordinate species (Figure 4, top). Since the ligation of cyanide was indicated by the detection of the ν(Fe–CN) stretching mode (Figure 6A), 4-CNpy must be ligating at the trans site in this complex. The

(27) The assignment of another weak line at about 485 cm^{-1} is uncertain.

Raman band assignable to the ν(Fe–py) stretching mode should be accidentally overlapping the porphyrin vibration at about 170 cm^{-1}.

Discussion

Core Size of Cyano Complexes. In the resonance Raman spectra of porphyrin complexes, all the skeletal modes above 1450 cm^{-1} depend on porphyrin core size.[28] At Soret excitation, the distinct peaks in this frequency region are mainly attributed to A_{1g} modes,[19a] as are observed in the $[Fe(OEP)(CN)_2]^-$ complex (Figure 1C). In the spectra of Fe(OEP)(CN)(py), Fe(OEP)(CN)(4-CNpy), and Fe(OEP)(CN)(4-Me_2Npy) (Figures 1B and 4), we observed B_{1g} modes at 1640 cm^{-1} (ν_{10}) and 1564 cm^{-1} (ν_{11}), in addition to the A_{1g} modes at 1591 cm^{-1} (ν_2) and 1506 cm^{-1} (ν_3). The insensitivity of these modes to the substitution of the pyridine bases indicates that the structure of porphyrin macrocycle is not affected by the ligand basicity and that the frequency change in the ν(Fe–CN) stretching mode is essentially independent of the porphyrin skeletal structure.

An inverse linear correlation has been found between the porphyrin core size and the skeletal vibrations.[28,29] From the frequencies of the ν_{10}, ν_{11}, and ν_3 modes, Ct–N, the porphyrin center-to-pyrrole nitrogen distance, was calculated to be 1.99 Å for the Fe(OEP)(CN)(py) complex on the basis of the linear correlation. This value coincides with that found by an X-ray crystallographic study (1.98 Å).[14] For porphyrin derivatives with saturated peripheral groups such as the OEP moiety, the ν_2 frequency has been found to be higher than that estimated from the value of the Ct–N distance.[21] By setting the Ct–N distance at 1.99 Å, we calculated the ν_2 frequency to be about 1580 cm^{-1}, which is about 10 cm^{-1} lower than that actually observed (1591 cm^{-1}).

Detection of ν(Fe–py) and ν(Fe–CN) Stretching Raman Lines. We could first locate both of two axial ligand vibrations, ν(Fe–py) and ν(Fe–CN) stretching modes, at 191 and 449 cm^{-1}, respectively, in the Fe(OEP)(CN)(py) complex (Figures 2 and 3). In the cyanide complexes of hemoglobins, resonance Raman bands assignable to the δ(Fe–C–N) bending mode were detected around 400 cm^{-1}.[2b,15a,c] In the Fe(OEP)(CN)(py) complex, however, a Raman band assignable to the δ(Fe–C–N) bending mode was undetected (Figures 2 and 6). It is reminiscent of what was found in the ferrous CO complex; the δ(Fe–C–O) bending mode was not detected in the model system,[10] while in hemoglobin, the δ(Fe–C–O) bending mode was detected.[2a]

In the ferric cyanide complex of an insect hemoglobin, CTT III, both of the ν(Fe–CN) and the ν(Fe–His) stretching modes were recently detected at 454 and 311 cm^{-1} in the ^{54}Fe (CTT III)($^{12}C^{14}N$) complex at pH 9.2.[15a] Though the ν(Fe–CN) stretching frequency was comparable with that found in the Fe(OEP)(CN)(py) complex (449 cm^{-1}), the ν(Fe–His) stretching frequency was considerably higher than the ν(Fe–py) vibration (191 cm^{-1}). It is of considerable interest to assume that the protein surrounding the heme has invoked the appearance of the δ(Fe–C–N) bending mode and the high frequency of the ν(Fe–His) stretching mode.

It was found that the higher the trans ligand basicity, the lower the ν(Fe–CN) stretching frequency (Figure 7). Since the CN ligand is assumed to be sterically unstrained and the Fe–C–N linkage is linear, the lower frequency corresponds to the weaker Fe–CN bond. This may be rationalized in terms of the relative σ- and π-donating character of pyridine ligands. In σ bonding, the donation of electron density from a strong base through the Fe–N(py) bond will weaken the trans σ Fe–C(CN) bond due to the competition from both sides of the same d_{z^2} (Fe) orbitals. Namely, the electron density donated to the iron by the pyridine base will repulse the negatively charged cyanide ion in σ-character. In terms of π-donation, the substantially shortened Fe–N(py) bond in the complex with a stronger base will increase the pyridine-to-iron π-donation, which will increase the strength of the d_π (Fe)–π^* (CN) ligand interactions and hence will weaken the Fe–CN bond.

Correlation between ν_4 Mode Frequency and Ligand Basicity. A similar relationship between the trans ligand and the Fe–ligand bond was found earlier in ferrous CO complexes of "picket fence" porphyrin.[10] In addition to the Fe–CN vibration, we have found that the ν_4 frequency was affected by the pyridine basicity (Figure 5). The C_a–N(pyrrole) breathing mode, ν_4, has long been recognized to be characteristic of the electron density at the heme iron.[30] The ν_4 frequency is decreased with the increase of electron donation from the ligand to the iron in the ferrous low-spin heme complexes. This has been attributed to weakening of the C_a–N bonds caused by the increased π-back-donation from the d_π (Fe) to the π^* (porphyrin) orbitals.[30b,31] What was found in the present ferric system is, however, quite contrary to the ferrous heme situation.

The Fe(OEP)(CN)(py-X) complexes showed that the ν_4 frequency *increased* with the increase of electron donation from the pyridine base to the iron. This is well accounted for in terms of *forward* donation from the porphyrin π orbital to the d_π vacancy of the iron, which is supposed to be significant in six-coordinate ferric low-spin hemes.[19b,32] In the complex with a low pyridine basicity, the stronger forward π-donation leads to the lower electron density of the π orbital of the porphyrin. The electron deficiency in this bonding-type orbital will weaken the porphyrin bonding and hence decrease the ν_4 frequency. In the Fe(OEP)(CN)(py-X) complexes with the more basic pyridine ligand, more electrons are donating from the ligand to the iron, and the resulting increase in the electron density at the iron d_π orbital pushes electrons "backward" to the porphyrin π orbital. This may suppress the degree of forward donation and hence will increase the ν_4 mode frequency. This supposition is congruent with the insensitivity of the core size markers (ν_{10}, ν_2, ν_{11}, and ν_3 modes) in the Fe(OEP)(CN)(py-X) complexes upon the pyridine substitution. These modes are mainly C_b–C_b and C_a–C_m stretching vibrations of the porphyrin,[20] and the forward donation seems to unaffect the C_b–C_b and C_a–C_m bond strengths.[19b]

In conclusion, we found that pyridine ligand basicity considerably affects the Fe–cyanide bonding in the ferric heme–CN system. The trans ligand was also found to be responsible for the electron distribution in the porphyrin ring via the porphyrin *forward* donation. The forward donation may well stabilize the higher valency states of heme iron in peroxidase intermediates.

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(28) Choi, S.; Spiro, T. G.; Langry, K. C.; Smith, K. M.; Budd, D. L.; La Mar, G. N. *J. Am. Chem. Soc.* **1982**, *104*, 4345.
(29) (a) Spaulding, L. D.; Chang, C. C.; Yu, N.-T.; Felton, R. H. *J. Am. Chem. Soc.* **1975**, *97*, 2517. (b) Huong, P. V.; Pommier, J.-C. *C. R. Hebd. Seances Acad. Sci., Ser. C* **1977**, *285*, 519. (c) Scholler, D. M.; Hoffman, B. M. *J. Am. Chem. Soc.* **1979**, *101*, 1655.
(30) (a) Yamamoto, T.; Palmer, G.; Gill, D.; Salmeen, I. T.; Rimai, L. *J. Biol. Chem.* **1973**, *248*, 5211. (b) Spiro, T. G.; Strekas, T. C. *J. Am. Chem. Soc.* **1974**, *96*, 338.
(31) (a) Spiro, T. G.; Burke, M. *J. Am. Chem. Soc.* **1976**, *98*, 5482. (b) Kitagawa, T.; Kyogoku, Y.; Iizuka, T.; Saito, M. I. *J. Am. Chem. Soc.* **1976**, *98*, 5169.
(32) Shulman, R. G.; Glarum, S. H.; Karplus, M. *J. Mol. Biol.* **1971**, *57*, 93.