Resonance Raman Investigation of Nitric Oxide Bonding in Iron Porphyrins: Detection of the Fe–NO Stretching Vibration

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With Soret-excited resonance Raman spectroscopy (RRS), we have detected the $\nu(Fe^{II}–NO)$ and $\nu(Fe^{III}–NO)$ stretching vibrations for several iron porphyrins, including octaethylporphyrin (OEP), tetraphenylporphyrin (TPP), heme-5, SP-13, SP-14, and SP-15. This result enables us to make the first comparison of the $\nu(Fe^{II}–NO)$ stretching frequencies in iron porphyrins ($\sim$527 cm^{-1}) and hemoproteins ($\sim$554 cm^{-1}), which is of interest if the protein's effect on the Fe–NO bond is to be assessed. Solvent effects on the $Fe^{II}–NO$ bond strength were significant; for Fe^{II}(OEP)(pyridine)(NO), the $\nu(Fe^{II}–NO)$ stretching frequency was observed 5 cm^{-1} higher in CH_2Cl_2 (527 cm^{-1}) than in benzene (522 cm^{-1}). The electronic trans effect was investigated by preparing complexes with *N*-methylimidazole, pyridine, and tetrahydrofuran as proximal ligands, and steric effects were assessed by comparing hindered (1,2-dimethylimidazole) and unhindered (*N*-methylimidazole) axial bases. Interestingly, the $Fe^{II}–NO$ bond strength proved insensitive to alterations in the trans base. These results are contrasted with previous $Fe^{II}–CO$, $Fe^{II}–O_2$, and $Fe^{III}–CN$ studies, in which the iron–ligand bond strength was found to be dramatically affected by properties of the proximal ligand.

Introduction

Nitrosyl complexes have been extensively investigated by X-ray diffraction,[1-4] infrared,[4,5] electron spin resonance (EPR),[6-10] visible absorption,[11-13] extended X-ray absorption fine structure (EXAFS),[14] and resonance Raman spectroscopy (RRS).[15-23] It is known that nitric oxide (NO) binds to both ferric and ferrous hemes with an unusually high affinity.[24] With RRS, the Fe–NO bond can be readily monitored in terms of its stretching mode. The first detection of $\nu(Fe^{II}–NO)$ in hemoproteins (554 cm^{-1}, ferrous HbA–NO) was reported in 1977 by Chottard and Mansuy.[15] Somewhat later, Tsubaki and Yu[22] used RRS to study $\nu(Fe^{II}–NO)$ (551 cm^{-1}) and ν(N–O) ($\sim$1623 cm^{-1}) in nitrosylhemoglobin A (HbA) and myoglobin. For ferric hemoproteins, Benko and Yu[25] were the first to observe $\nu(Fe^{III}–NO)$ and δ-(Fe^{III}–N–O) in nitrosyl complexes of HbA and horseradish peroxidase. Recently, Choi et al.[26] have reported the resonance Raman detection of $\nu(Fe^{II}–NO)$ for the five-coordinate Fe^{II}(TPP)-(NO). However, no infrared or resonance Raman detection of $\nu(Fe^{II}–NO)$ and $\nu(Fe^{III}–NO)$ has been reported for the six-coordinate Fe^{II}(porphyrin)(base)(NO) in the literature. Such information is essential for assessing the protein's influence on the Fe–NO moiety, since the protein systems are also six-coordinate (with histidine as the proximal base).

In this paper, we describe Soret-excited RRS studies of nitric oxide complexes of ferric and ferrous porphyrins. With nitric oxide isotope substitution, the $\nu(Fe^{III}–NO)$ and $\nu(Fe^{II}–NO)$ stretching modes have been clearly identified at $\sim$602 and $\sim$524 cm^{-1}, respectively. The protein's influence on the $\nu(Fe^{II}–NO)$ stretching frequency ($\sim$30 cm^{-1}) is indeed significant. We have also investigated the effects of the solvent and trans base on the $Fe^{II}–NO$ bond. Trans bases of different strengths (*N*-methylimidazole, pyridine, tetrahydrofuran) were studied, and sterically hindered (1,2-dimethylimidazole) and unhindered (*N*-methylimidazole) bases were also compared. We were surprised to find that the $Fe^{II}–NO$ bond is insensitive to the nature of the trans ligand. Solvent effects on the $\nu(Fe^{II}–NO)$ stretching frequency were investigated by comparing complexes of Fe^{II}(OEP)(pyridine)(NO) in benzene, tetrahydrofuran, carbon tetrachloride, chloroform, and methylene chloride. The $\nu(Fe^{II}–NO)$ stretching frequency was generally observed to increase for solvents of higher dipole moment.

Experimental Section

Iron(III) octaethylporphyrin (OEP) chloride was obtained from Strem Chemicals (Newburyport, MA), while iron(III) *meso*-tetraphenylporphyrin (TPP) bromide was purchased from Midcentury (Posen, IL). Both porphyrins were used without further purification. Heme 5, as well

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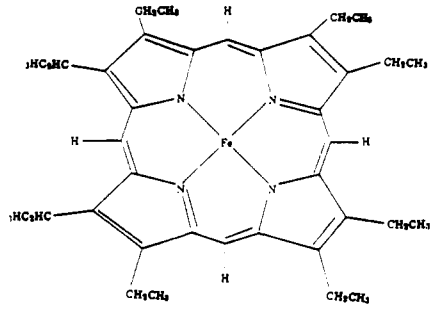
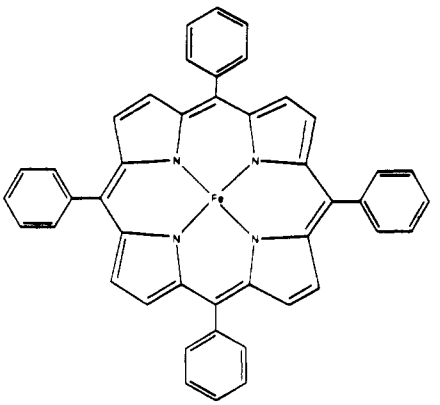


Iron Octaethylporphyrin



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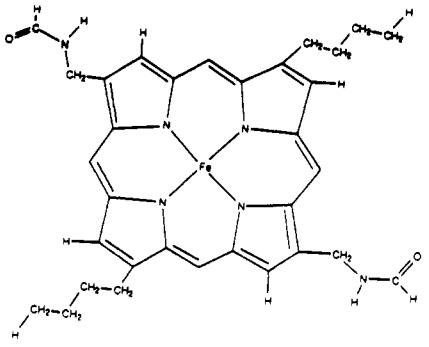
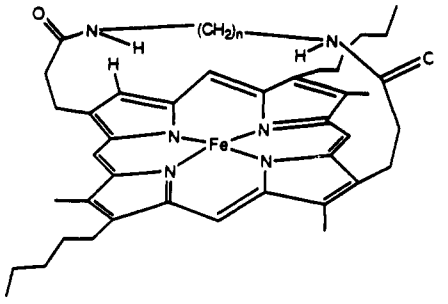


Heme 5



FeSP-13 n=5
FeSP-14 n=6
FeSP-15 n=7

Figure 1. Chemical structures of iron porphyrins.

as the strapped hemes (FeSP-13, FeSP-14, FeSP-15), was synthesized according to literature methods.[27] The NO complexes of these ferric porphyrins (200 μM) were prepared with tetrahydrofuran (THF, 0.10 M), *N*-methylimidazole (*N*-MeIm, 0.15 M), 1,2-dimethylimidazole (1,2-DiMeIm, 0.15 M), and pyridine (0.15 M) as proximal bases in CH_2Cl_2 solution. For preparation of the ferrous complex, methanol (0.1 M) was added to the porphyrin/CH_2Cl_2/base solution prior to the addition of the NO gas; methanol is known to act as a catalyst in the autoreduction of ferric porphyrins by NO.[20] The sample solution was placed in a quartz Raman cell, which was immediately sealed and evacuated. Nitric oxide (from Aldrich, Milwaukee, WI) was then introduced at a pressure of ~1 atm. The isotope-substituted complexes were prepared similarly with $^{15}N^{16}O$ (Bio-Rad Laboratories, Richmond, CA), and $^{15}N^{18}O$ (ICON Services, Summit, NJ).

All reagents were purified prior to use. Methylene chloride was stirred over concentrated sulfuric acid, neutralized with a 10% $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, and distilled from phosphorus pentoxide. Pyridine and *N*-MeIm were distilled from KOH, while 1,2-DiMeIm and methanol were distilled from Na and Mg, respectively. Reagent grade tetrahydrofuran was used as received from Fisher Scientific (Fair Lawn, NJ).

The multichannel laser Raman system has been described previously.[28] The 406.7-nm line of a Spectra-Physics (Mountain View, CA) Model 171 krypton ion laser was employed for excitation. The laser used to obtain each spectrum is listed in the figure captions. To minimize photodissociation and localized heating, the Raman cell was kept spinning throughout the entire data acquisition process. The entrance slit of the monochromator had a width and height of 100 μm and 1 cm, respectively. Fenchone was used to calibrate the spectra; peak positions are considered accurate to ±2 cm^{-1}.

Results and Discussion

Identification of the ν(Fe–NO) Stretching Vibration. We have studied the effects of nitric oxide isotope substitution on the low- (100–700 cm^{-1}) and high (1300–1900 cm^{-1}) frequency regions of Fe^{II}(OEP)(THF)(NO) spectra. (See Figure 1 for structures of all the iron porphyrins studied in this work.) The substitutions reveal one isotope-sensitive line at 527 cm^{-1} for Fe^{II}(OEP)(THF)($^{14}N^{16}O$), which shifts to 519 cm^{-1} ($^{15}N^{16}O$) and to 512 cm^{-1} ($^{15}N^{18}O$) (see Figure 2). It is clear that this line corresponds to the 554-cm^{-1} mode in nitrosyl HbA assigned by others[15] as ν(Fe^{II}–NO). Careful examination of the spectral region from 400 to 500 cm^{-1} reveals no δ(Fe^{II}–N–O) bending mode for any of the porphyrins studied.

Because the autoreduction of Fe^{III}(OEP)(pyridine)(NO) to Fe^{II}(OEP)(pyridine)(NO) is very slow (~48 h), we were able to study the effects of nitric oxide isotope substitution on the low- and high-frequency regions of Fe^{III}(OEP)(pyridine)(NO) spectra. In Figure 3, an isotope-sensitive line appears at 602 cm^{-1} for Fe^{III}(OEP)(pyridine)($^{14}N^{16}O$), which shifts to 596 cm^{-1} for Fe^{III}(OEP)(pyridine)($^{15}N^{16}O$). Other ferric porphyrins studied include Fe^{III}(heme-5)(pyridine)(NO) (602 cm^{-1}), Fe^{III}(SP-14)(pyridine)(NO) (603 cm^{-1}), and Fe^{III}(SP-15)(pyridine)(NO) (601 cm^{-1}). As was the case for the ferrous porphyrins, no bending modes were observed in the spectra. This result is not surprising in view of the linearity of the Fe^{III}–NO system, which is expected on the basis of the total number of electrons (6) in the metal d and ligand π^* orbitals.[29] It is of interest to point out that previous RRS studies of sterically hindered carbonmonoxy strapped hemes[30] indicated that distortion of the Fe^{II}–C–O linkage enhances the intensity of δ(Fe^{II}–C–O) relative to that of ν(Fe^{II}–CO). While the Fe^{III}–NO and Fe^{II}–CO systems are isoelectronic, the Fe^{II}–CO bond length of 1.77 Å observed for Fe^{II}(TPP)(pyridine)-

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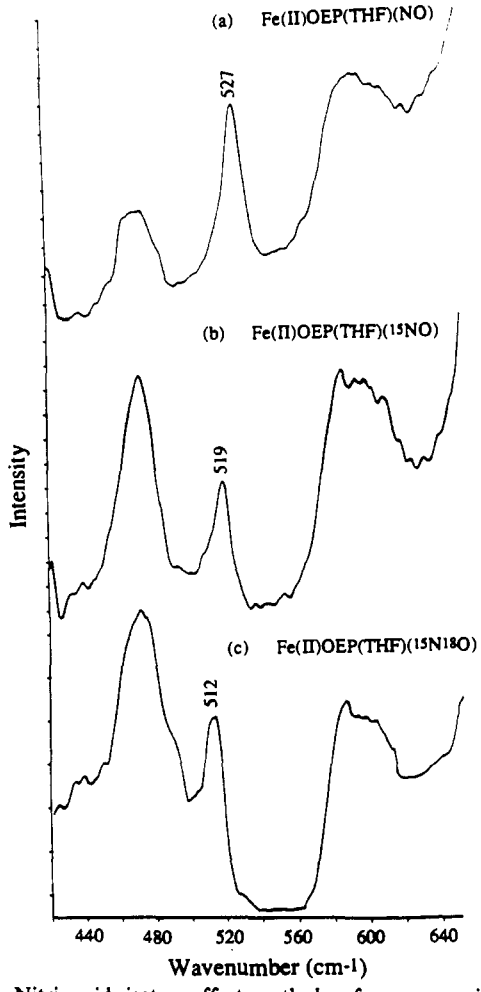

Figure 2. Nitric oxide isotope effects on the low-frequency region (420–650 cm^{-1}) spectra of Fe^{II}(OEP)(THF)(NO). λ_{ex} = 406.7 nm; laser power = 40 mW at the sample. Because only small portions of the spectra are displayed in each figure, it should be mentioned that all features of our RRS data are in good agreement with those previously published for metallooctaethylporphyrins.[60]

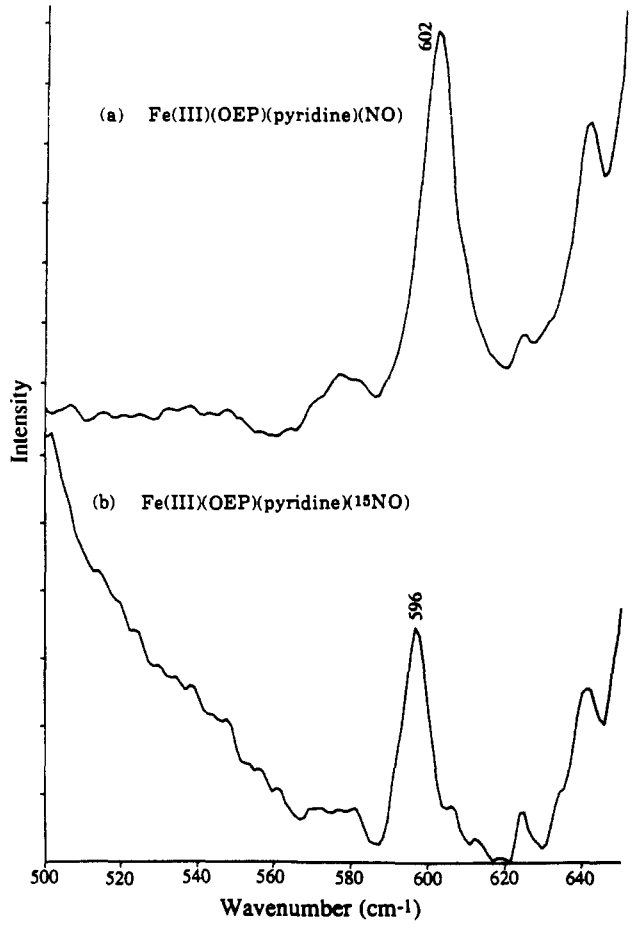

Figure 3. Nitric oxide isotope effects on the low-frequency region (500–650 cm^{-1}) spectra of Fe^{III}(OEP)(pyridine)(NO). λ_{ex} = 406.7 nm; laser power = 40 mW at the sample.

Table I. Position of ν(Fe–NO) for Ferric and Ferrous Porphyrins

ferrous porphyrins	$\nu(Fe^{II}–NO)$ (cm^{-1})
Fe^{II}(OEP)(pyridine)(NO)	527
Fe^{II}(TPP)(pyridine)(NO)	527
Fe^{II}(heme-5)(pyridine)(NO)	528
Fe^{II}(SP-13)(pyridine)(NO)	528
Fe^{II}(SP-14)(pyridine)(NO)	526
Fe^{II}(SP-15)(pyridine)(NO)	524
ferric porphyrins	**$\nu(Fe^{III}–NO)$ (cm^{-1})**
Fe^{III}(OEP)(pyridine)(NO)	602
Fe^{III}(heme-5)(pyridine)(NO)	602
Fe^{III}(SP-14)(pyridine)(NO)	603
Fe^{III}(SP-15)(pyridine)(NO)	601

(CO)[31] is much longer than the Fe^{III}–NO bond investigated in this paper, because the observed $\nu(Fe^{II}–CO)$ stretch (486 cm^{-1})[30] is much lower than $\nu(Fe^{III}–NO)$ of 602 cm^{-1} reported here. It is well-known[29] that the stretching frequency can be correlated with the force constant and bond length. We suggest that the Fe^{III}–NO bond of Fe^{III}(SP-14)(pyridine)(NO) and Fe^{III}(SP-15)-(pyridine)(NO) is short enough to prevent geometric distortion of the Fe^{III}–NO linkage by the strap. Therefore, Fe^{III}–NO maintains its preferred linear configuration, and $\delta(Fe^{III}–N–O)$ is not enhanced.

We have also identified $\nu(Fe^{II}–NO)$ and $\nu(Fe^{III}–NO)$ for several other iron porphyrins, and all of the results are given in Table I. It should be mentioned that unsuccessful attempts to observe $\nu(Fe^{II}–NO)$ in porphyrins were previously reported by Stong et al.[20] Both the excitation wavelength (454.5 nm) and detector (photomultiplier tube) were different from those employed in this study.

Comparison of [Fe^{III}–NO] with the Isoelectronic [Fe^{II}–CO] System. In the absence of distal steric hindrance, both the Fe^{III}–NO and Fe^{II}–CO moieties are known to adopt linear configurations normal to the heme plane. X-ray and neutron scattering crystal structures of carbonmonoxy adducts of hemoproteins[33-42] reveal that nonbonded contacts in the heme pocket force the bound CO out of its preferred linear configuration. Li and Spiro[43] have suggested that these protein-induced distortions result mainly in tilting rather than bending of the Fe^{II}–CO unit. Yu et al.,[30] in studies of sterically hindered carbonmonoxy strapped hemes, observed that distortions in the Fe^{II}–CO linkage resulted in an increase in the $\nu(Fe^{II}–CO)$ stretching frequency. It is of interest to ask whether or not similar distortions occur in NO adducts of ferric hemoproteins.

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The similar $\nu(Fe^{III}–NO)$'s for ferric HRP–NO (604 cm^{-1}), ferric Mb–NO (595 cm^{-1}),[25] and $Fe^{III}(OEP)$(pyridine)(NO) (602 cm^{-1}) indicate that the protein's effect on the $Fe^{III}–NO$ bond is small, compared with the $Fe^{II}–CO$ system. Previously, the $\nu(Fe^{II}–CO)$ stretching frequency was observed 16 cm^{-1} higher for MbCO (512 cm^{-1})[44] than for $Fe^{II}(OEP)$(*N*-methylimidazole)(CO) (496 cm^{-1}).[32] For Mb, the relative stretching frequencies of $\nu(Fe^{III}–NO)$ (595 cm^{-1})[25] and $\nu(Fe^{II}–CO)$ (512 cm^{-1})[44] suggest that the $Fe^{III}–NO$ bond is stronger than the $Fe^{II}–CO$ bond. However, to avoid being misled by mass effects, we calculated the $\nu(Fe^{II}–CO)$ stretching frequency (for Mb–CO) on the basis of corrected mass normalized to the $Fe^{III}–NO$ system. We then obtained a value of 501 cm^{-1} for $\nu(Fe^{II}–CO)$. These frequency numbers (501 cm^{-1} for $Fe^{II}–CO$, 595 cm^{-1} for $Fe^{III}–NO$) do indeed correlate with a force constant that is highest for the $Fe^{III}–NO$ bond. While X-ray data for nitrosyl adducts of ferric hemoproteins are unavailable, a comparison of the stretching frequencies indicates that the $Fe^{III}–NO$ bond is strongest and hence the shortest. Evidently the strength of the $Fe^{III}–NO$ bond renders it less susceptible to protein distortions. It is plausible that this bond is short enough not to sterically interfere with bulky amino acid residues near the binding site.

Protein Effect. The results presented here facilitate the first comparison of the $\nu(Fe^{II}–NO)$ stretching frequencies in model compounds and hemoproteins. For $Fe^{II}(OEP)$(*N*-MeIm)(NO), $\nu(Fe^{II}–NO)$ was detected at 524 cm^{-1}, ca. 30 cm^{-1} lower than that observed for nitrosyl HbA (~554 cm^{-1}).[15] An interesting observation evolves from the comparison of protein effects on $Fe^{II}–NO$ and $Fe^{II}–O_2$. While both of these moieties maintain bent geometries, the protein effect on $Fe^{II}–O_2$[23,49] is much smaller than that on $Fe^{II}–NO$, and the NO binding to Fe^{II}–heme in Hb is ~1000 times stronger than O_2 binding.[24]

To obtain a more definitive assessment of the protein's effect on the $Fe^{II}–NO$ bond, we have carried out resonance Raman studies of $Fe^{II}(PP)$(*N*-MeIm)(NO) (PP = protoporphyrin IX) in aqueous solution at alkaline pH. Because iron protoporphyrin IX is present in Hb and Mb, comparisons of the $\nu(Fe^{II}–NO)$ stretching frequency in Mb–NO and $Fe^{II}(PP)$(*N*-MeIm)(NO) should reflect only the effects of the protein. Unfortunately, slight background fluorescence prevented observation of $\nu(Fe^{II}–NO)$ for the protoporphyrin IX system. But the comparisons we have made between $Fe^{II}(OEP)$(*N*-MeIm)(NO) and Mb–NO and Hb–NO are still useful, because alterations in the heme group have only a slight effect on $\nu(Fe^{II}–NO)$ (see Table I). Furthermore, the $\nu(Fe^{II}–CO)$ stretching frequencies reported for $Fe^{II}(PP)$(*N*-MeIm)(CO) (497 cm^{-1})[45] and $Fe^{II}(OEP)$(*N*-MeIm)(CO) (496 cm^{-1})[32] are very similar.

Trans Effect. Assuming that the $Fe^{II}–N–O$ geometry is not affected by a change in the trans ligand, the $\nu(Fe^{II}–NO)$ stretching frequency may be correlated with the $Fe^{II}–NO$ bond strength (or bond length). In this work, we have examined the influence of trans ligands on the $\nu(Fe^{II}–NO)$ stretching frequency. The ligands studied were *N*-methylimidazole (N-MeIm), pyridine, and tetrahydrofuran (THF). It is also interesting to compare the $\nu(Fe^{II}–NO)$ stretching frequency in complexes with *N*-methylimidazole and 1,2-dimethylimidazole (1,2-DiMeIm) as proximal bases. The methyl group in the 2-position of 1,2-DiMeIm sterically interferes with the porphyrin plane, thereby lengthening the $Fe–N_\epsilon$ bond (N_ϵ represents the N atom of the proximal base). Iron porphyrin complexes with 1,2-DiMeIm provide an excellent model for the tense (or low affinity) state of hemoglobin.[46] Results for $Fe^{II}(OEP)$(*N*-MeIm)(NO), $Fe^{II}(OEP)$(1,2-DiMeIm)(NO), and $Fe^{II}(OEP)$(pyridine)(NO) are shown in Figure 4, while the spectrum of $Fe^{II}(OEP)$(THF)(NO) is displayed in Figure 1. Interestingly, $\nu(Fe^{II}–NO)$ proved insensitive to both electronic and steric trans effects. Kincaid et al.[47] have previously noted similar behavior of $\nu(Fe^{II}–NO)$ of nitrosyl adducts of ferrous cytochrome P450cam. The authors report that the $\nu(Fe^{II}–NO)$ stretching frequency was not altered by proximal cysteine ligation. Apparently, bending of the Fe–N–O linkage substantially increases the energy of the d_{xy}-π orbitals,[47,48] thus rendering the transfer of a lone pair on the axial sulfur energetically unfavorable.

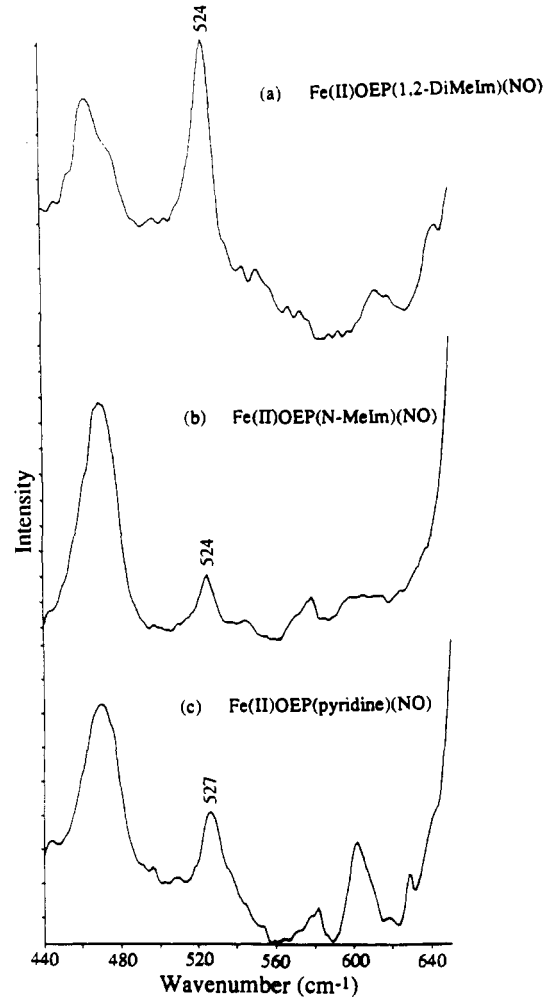

Figure 4. Effects of steric and electronic properties of the trans ligand on $\nu(Fe^{II}–NO)$ in the low-frequency region (440–650 cm^{-1}) spectra of nitrosyliron(II) OEP complexes. The laser power was 40 mW (at the sample) in (a) and (c) and 30 mW in (b). λ = 406.7 nm.

To make certain that we were not studying the bis(nitrosyl) adduct, $Fe^{II}(OEP)(NO)_2$, we prepared this adduct and studied its Raman and absorption spectra. In the absence of any base, $Fe^{II}(OEP)(NO)_2$ can be readily formed under our experimental conditions.[49] The absorption spectrum obtained from this complex was significantly different from that obtained for the $Fe^{II}(OEP)$(base)(NO) complexes (data not shown). Additionally, we observed an isotope-sensitive line at 519 cm^{-1} in the resonance Raman spectrum of $Fe^{II}(OEP)(^{14}N^{16}O)_2$; this line shifts to 514 cm^{-1} upon substitution by $^{15}N^{16}O$. The $\nu(Fe^{II}–NO)$ stretching frequency detected for $Fe^{II}(OEP)(NO)_2$ (519 cm^{-1}) is indeed significantly different from that observed for $Fe^{II}(OEP)$(base)(NO) (~527 cm^{-1}).

Additionally, we have monitored the $\nu(Fe^{II}–NO)$ stretching frequency under conditions which we can be positive that the trans ligand is coordinated. $Fe^{II}(OEP)$(pyridine)(NO) was prepared in neat pyridine, and $\nu(Fe^{II}–NO)$ was detected at 526 cm^{-1} (spectrum not shown). $\nu(Fe^{II}–NO)$ was also observed at

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Table II. Comparison of the Trans Effects for CO, O_2, CN, and NO Complexes of Iron Porphyrins: Results from Resonance Raman Spectroscopy

complex	$\nu(Fe^{II}-CO)$ (cm^{-1})	ref
Fe^{II}(TpivPP)(THF)(CO)	527	32
Fe^{II}(TpivPP)(pyridine)(CO)	486	32
Fe^{II}(TpivPP)(*N*-MeIm)(CO)	489	32
Fe^{II}(TpivPP)(1,2-DiMeIm)(CO)	496	32
complex	**$\nu(Fe^{II}-O_2)$ (cm^{-1})**	**ref**
Fe^{II}(TpivPP)(*N*-MeIm)(O_2)	571	51
Fe^{II}(TpivPP)(1,2-DiMeIm)(O_2)	561	51
complex	**$\nu(Fe^{III}-CN)$ (cm^{-1})**	**ref**
Fe^{III}(SP-15)(*N*-MeIM)(CN)	447	52
Fe^{III}(SP-15)(pyridine)(CN)	456	52
Fe^{III}(SP-15)(1,2-DiMeIm)(CN)	446	52
complex	**$\nu(Fe^{II}-NO)$ (cm^{-1})**	**ref**
Fe^{II}(OEP)(THF)(NO)	527	this work
Fe^{II}(OEP)(*N*-DiMeIm)(NO)	524	this work
Fe^{II}(OEP)(1,2-DiMeIm)(NO)	524	this work
Fe^{II}(OEP)(pyridine)(NO)	527	this work

525 cm^{-1} for both Fe^{II}(OEP)(*N*-methylimidazole)(NO) (in neat *N*-methylimidazole) and Fe^{II}(OEP)(1,2-dimethylimidazole)-(NO) (in neat 1,2-dimethylimidazole). Clearly, these new results unambiguously demonstrate that $\nu(Fe^{II}-NO)$ is insensitive to electronic/steric properties of the trans base.

This lack of an electronic trans effect has been noted previously for the Mn^{II}–NO system. Yu et al.[50] reported almost no difference in the $\nu(Mn^{II}-NO)$ stretching frequencies observed for Mn^{II}-(heme-5)(N-MeIm)(NO) (629 cm^{-1}) and Mn^{II}(heme-5)(piperidine)(NO) (630 cm^{-1}). In contrast, other ligand systems such as Fe^{II}–CO[32], Fe^{II}–O_2,[51] and Fe^{III}–CN,[52] are known to exhibit significant trans effects. In Table II, we compare the sensitivities of the Fe^{II}–CO, Fe^{II}–O_2, Fe^{III}–CN, and Fe^{II}–NO bonds to alterations in the proximal base. Results in Table II indicate that, while the $\nu(Fe^{II}-CO)$ stretching frequency for Fe^{II}(TpivPP)-(pyridine)(CO) is 41 cm^{-1} lower than that of Fe^{II}(TpivPP)(THF)-(CO), the position of $\nu(Fe^{II}-NO)$ is identical (527 cm^{-1}) for Fe^{II}(OEP)(pyridine)(NO) and Fe^{II}(OEP)(THF)(NO). These resonance Raman results are supported by X-ray crystallographic studies, and Table III compares the sensitivities of the Fe^{II}–CO, Fe^{II}–O_2, Fe^{III}–CN, and Fe^{II}–NO bond lengths to the trans ligand identity. Interestingly, the Fe^{II}–NO bond length is very similar for Fe^{II}(TPP)(*N*-MeIm)(NO) (1.74 Å)[3] and Fe^{II}(TPP)(4-methylpiperidine)(NO) (1.72 Å),[4] even though *N*-MeIm and 4-methylpiperidine are bases of different strengths. In contrast, the Fe^{II}–CO bond distance is different in Fe^{II}(deutero)(THF)-(CO) (1.706 Å)[52] and Fe^{II}(TPP)(pyridine)(CO) (1.77 Å).[31]

Similarly, the steric trans effect which is observed for CO, O_2, and CN complexes of iron porphyrins is absent for the NO case. For example, the $\nu(Fe^{II}-O_2)$ stretching frequency for solid Fe^{II}-(TpivPP)(*N*-MeIm)(O_2) appears 10 cm^{-1} higher than that of the sterically hindered Fe^{II}(TpivPP)(1,2-DiMeIm)(O_2) complex,[51] while the $\nu(Fe^{II}-NO)$ stretching frequency is observed at 524 cm^{-1} for both Fe^{II}(OEP)(*N*-MeIm)(NO) and Fe^{II}(OEP)(1,2-DiMeIm)(NO). Results from previous RRS studies of Mn^{II}-(heme-5)(*N*-MeIm)(NO) (629 cm^{-1}) and Mn^{II}(heme-5)(1,2-DiMeIm)(NO) (630 cm^{-1})[50] indicate that the strong Mn^{II}–NO bond of this system is also unaffected by alterations in the steric properties of the proximal base.

Table III. Comparison of the Trans Effect for CO, O_2, CN, and NO Complexes of Iron Porphyrins: Results from X-ray Crystallography

complex	Fe^{II}–CO bond length (Å)	ref
Fe^{II}(deutero)(THF)(CO)	1.706	53
Fe^{II}(TPP)(pyrdine)(CO)	1.77	31
complex	**Fe^{II}–O_2 bond length (Å)**	**ref**
Fe^{II}(TpivPP)(N-MeIm)(O_2)	1.75	54,55
Fe^{II}(TpivPP)(2-MeIm)(O_2)	1.90	56,57
complex	**Fe^{III}–CN bond length (Å)**	**ref**
Fe^{III}(TPP)(pyridine)(CN)	1.908	61
Fe^{III}(TPP)($CN)_2$	1.975	62
complex	**Fe^{II}–NO bond length (Å)**	**ref**
Fe^{II}(TPP)(4-MePip)(NO)[a]	1.72	4
Fe^{II}(TPP)(N-MeIm)(NO)	1.74	3

[a] 4-MePip = 4-methylpiperidine.

Table IV. Solvent Dependence of the $\nu(Fe^{II}-NO)$ Stretching Frequency

solvent	dipole moment (D)	complex	$\nu(Fe^{II}-NO)$ (cm^{-1})
CCl_4	0	Fe^{II}(OEP)(pyridine)(NO)	523
C_6H_6	0	Fe^{II}(OEP)(pyridine)(NO)	522
$CHCl_3$	1.01	Fe^{II}(OEP)(pyridine)(NO)	524
CH_2Cl_2	1.60	Fe^{II}(OEP)(pyridine)(NO)	527
C_4H_8O	1.63	Fe^{II}(OEP)(THF)(NO)	523

These results are in conflict with those from earlier EPR studies of Fe^{II}(protoheme)(base)(NO),[19,57] in which both basicity and steric hindrance of the trans base were found to influence hyperfine structures. This was interpreted as an indication of trans effects on the Fe^{II}–NO bond. Discrepancies between RRS and EPR data have been noted previously in studies of nitrosyl complexes of metalloporphyrins.[19,58,59] It seems that, while EPR reflects only the distribution of the unpaired electron, the resonance Raman vibrational frequencies are a measure of the metal–ligand bond strength to which both s and p bonding contribute. Hence, hyperfine constants in EPR spectra are not necessarily a measure of metal–ligand bond strength.

Solvent Effect. It has been predicted[60] that, in simple "flat porphyrins" such as Fe^{II}(OEP)(base)(NO), the ligand will be exposed to the solvent and show a marked dependence on its physical properties. In this regard, we investigated the effect of the solvent on the $\nu(Fe^{II}-NO)$ stretching frequency, and these results are shown in Table IV. Apparently, nitric oxide does interact with the solvent. Dipole moments of the solvents are as follows in parentheses: CCl_4 (0 D), C_6H_6 (0 D), $CHCl_3$ (1.01 D), CH_2Cl_2 (1.60 D), and C_4H_8O (1.63 D). In general, the $\nu(Fe^{II}-NO)$ stretching frequency for solvents of higher polarity was higher, with THF providing a notable exception to this trend.

Conclusions

We report the first detection of the $\nu(Fe^{II}-NO)$ and $\nu(Fe^{III}-NO)$ stretching vibrations in protein-free porphyrins. This result

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is significant in two different aspects: (1) First, a comparison of the $\nu(Fe^{II}-NO)$ and $\nu(Fe^{III}-NO)$ stretching frequencies in porphyrins and hemoproteins is now feasible. While $\nu(Fe^{II}-NO)$ is observed $\sim 30\ cm^{-1}$ lower in porphyrins than in hemoproteins, the effects of the protein on $\nu(Fe^{III}-NO)$ are small. (2) Second, the detection of $\nu(Fe^{II}-NO)$ in model porphyrins enabled us to study trans effects on this bond. The $Fe^{II}-NO$ bond strength was determined to be independent of the electron-donating and stereochemical properties of the trans base. Clearly, the $Fe^{II}-NO$ bond is unique in this respect, and future work may be directed toward understanding this system more fully.

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