

Determinants of the FeXO (X = C, N, O) Vibrational Frequencies in Heme Adducts from Experiment and Density Functional Theory

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Abstract: Vibrational spectra of CO, NO, and O₂ adducts of heme proteins contain information on interactions of the heme and its bound ligands with the surrounding protein matrix that may help in elucidating the mechanism of small-molecule activation. Whereas the heme–CO system is well studied and a framework exists for the interpretation of such interactions, heme–NO and –O₂ complexes have not been systematically investigated. Here we examine resonance Raman spectra of all three classes of adducts, combining literature values with new data for Fe^{II}NO porphyrins having both electron-donating and electron-withdrawing substituents. Negative linear correlations are observed for all three adducts between their Fe–XO and X–O stretching frequencies. The slopes of these correlation lines are –0.4 for five-coordinate FeCO and FeNO porphyrins and –0.8 for five-coordinate FeO₂ adducts. Thus, Fe–NO and Fe–O₂ bonds are equally or even more sensitive than Fe–CO bonds to electronic influences that affect metal-to-ligand π back-bonding. However, the responses of the NO and O₂ adducts to trans ligand binding are very different from those for CO complexes. Ligands trans to CO displace the plot to steeper slopes and lower Fe–CO frequencies, reflecting competition of the ligand lone pairs for the σ acceptor orbital, d_{z²}. However, no displacement of the line is observed for six-coordinate FeNO and FeO₂ adducts, but only a shift to higher positions on the line, indicating greater back-bonding. We infer that trans ligand competition for the d_{z²} orbital is not as effective for NO and O₂ as for CO, reflecting the lower energy of the N and O orbitals relative to that of the C orbitals. These results are discussed with the aid of a simple bonding model involving FeXO valence isomers. To examine this model, we applied density functional theory to five- and six-coordinate XO adducts of Fe(II) porphine. Geometries were in good agreement with experiment, as were vibrational frequencies for CO adducts. However, DFT overestimated the Fe–NO bond extension on binding a trans ligand and predicted a decrease in the Fe–NO stretching frequency, whereas an increase is observed. The predicted frequency change was likewise in the wrong direction for Fe–O stretching in six- vs five-coordinate FeO₂ adducts. The results suggest that DFT captures the essential features of back-bonding, but not of the σ competition with the trans ligand, in the cases of NO and O₂.

Introduction

The diatomic molecules CO, NO, and O₂ are ubiquitous in biology, and the receptor molecules that have evolved to bind and activate them are mostly heme proteins.^{1–6} The heme cofactor is able to bind CO, NO, and O₂ reversibly and to provide sites for controlled reactions. The course of these reactions is determined by the disposition of side chains from the surrounding protein residues. Consequently, there is great interest in establishing the nature of the heme protein interactions and their biochemical roles.

Vibrational spectroscopy is a useful probe for these interactions, since they perturb the frequencies of bound ligand

vibrational modes in characteristic ways. The ligand modes can be monitored by infrared and Raman spectroscopy. Raman spectroscopy is particularly useful for heme proteins, because the visible region electronic transitions provide resonance enhancement, not only of the ring modes but also of the bound ligand modes, since the ligands are coupled electronically to the heme.⁷

Vibrational systematics have been worked out for the CO ligand and are well understood.^{7–12} The main variables are (1) the nature of the axial ligand trans to the CO and (2) the polarity of distal residues in the heme pocket and their proximity to the CO. The FeCO bonds are dominated by back-donation of Fe d _{π} electrons into the CO π^* orbitals. Back-bonding decreases the CO bond order while increasing the FeCO bond order. Thus,

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the Fe–CO and C–O stretching frequencies are negatively correlated; $\nu(\text{Fe–CO})$ rises and $\nu(\text{C–O})$ falls as back-bonding increases. Changes in back-bonding result from electron-donating or -withdrawing influences, either of porphyrin chemical substituents or of polar molecules in the vicinity of the bound CO. Thus, solvents which are electron-donating diminish back-bonding, as do electron-donating residues in the heme pocket of heme proteins, whereas solvents or protein residues which are H-bond donors enhance back-bonding. A wide range of $\nu(\text{Fe–CO})$ and $\nu(\text{C–O})$ values are observed for heme proteins and models, but the pairs of data all fall on a single negative linear correlation, provided that the trans axial ligand is held constant.⁹

When the donor strength of the trans axial ligand is increased, then the entire correlation shifts to lower $\nu(\text{Fe–CO})$ values. This is because the trans ligand and CO σ donor orbitals compete for the Fe d_{z^2} bonding orbital. Therefore, stronger trans donors weaken the Fe–CO bond even if the C–O bond order is held constant. Proteins and models with neutral imidazole as trans ligands all fall on the same back-bonding line, while those with imidazolate or thiolate ligands fall on a lower line, and five-coordinate complexes, with no trans ligand, fall on a higher line.^{8,9} It has recently been determined¹² that the slope of the six-coordinate lines is twice that of the five-coordinate line, reflecting the higher rate of change of $\nu(\text{Fe–CO})$, relative to $\nu(\text{C–O})$, when the Fe–CO bond is lengthened by trans ligand binding. The back-bonding pattern has been modeled¹² with density functional theory (DFT) calculations of the FeCO vibrational frequencies for Fe(II) porphine with different substituents and trans ligands. Shifted correlations are observed for the calculated $\nu(\text{Fe–CO})$ and $\nu(\text{C–O})$ frequencies, and the ratios of the slopes are in excellent agreement with experiment.

In this study, we extend the analysis of diatomic ligand vibrations to NO and O₂ adducts of heme. Bonding is more complex for these ligands than for CO, because their extra valence electrons are antibonding with respect to a linear geometry, and they force the ligands to bend.^{13,14} Bound ligand vibrations have been reported for a number of NO and O₂ heme adducts and have been discussed from qualitative perspectives.^{15–24} In addition, kinematic analyses of the bound ligand vibrations have been carried out.^{8,20,21} However, electronic effects on the vibrations have not been examined systematically, either experimentally or theoretically. We report new data and DFT calculations which help to illuminate the systematics; they exhibit both similarities and contrasts with the FeCO pattern.

Table 1. Vibrational Frequencies for Fe^{II}(P)(NO) Adducts

compound ^a	$\nu(\text{Fe–NO})$	$\nu(\text{N–O})$
Five-Coordinate, Fe ^{II} (NO)		
TPP/Bz (CH ₂ Cl ₂)	524 (527)	1678 (1675)
TPP(4-OH)/DMF	530	1663
TPP(4-OCH ₃)/DMF	527	1666
TPP(<i>p</i> -tolyl)/Bz	524	1675
TPP(2,6-di-Cl)/Bz	518	1689
TPP(4-CN)/CH ₂ Cl ₂	523	1686
TPP(2,6-di-F)/Bz	515	1695
TPP(penta-F)/Bz	514	1703
Six-Coordinate, Fe ^{II} (NO)		
Mb ^b	552	1612
P450 ^c	554	1591

^a Abbreviations: TPP, tetraphenylporphyrin; Bz, benzene; DMF, dimethylformamide; Mb, myoglobin; P450, cytochrome P450. ^b Reference 31. ^c Reference 21.

Methods

Preparation of Five-Coordinate Heme(NO) Model Complexes. Fe^{III}(TPP)(Cl) (TPP = tetraphenylporphyrin) was purchased from Aldrich Chemical Co. and used without further purification. The phenyl-substituted Fe^{III}(TPP)(Cl) complexes, 4-OH, 4-CN, 4-OCH₃, 2,6-dichloro, 2,6-difluoro, *p*-tolyl, and pentafluoro, were purchased from MidCentury Chemical Co. and used without further purification. Anhydrous benzene, methylene chloride, and dimethyl formamide were purchased from Aldrich and stored under nitrogen. Fe(II) porphyrin solutions (300 μM) were prepared in a nitrogen drybox and reduced using Zn/Hg. The reduced iron porphyrin solutions were then removed from the drybox in an anaerobic NMR tube sealed with a septum. Nitric oxide gas was generated by reacting sodium nitrite, Na¹⁴NO₂, or its ¹⁵N isotopomer (Cambridge Isotope Laboratories) with sodium ascorbate in an anaerobic aqueous solution. The NO gas was then transferred to the anaerobic Fe(II) porphyrin solutions via gastight syringe. Formation of the Fe(II) and Fe^{II}NO adducts was confirmed by monitoring the heme absorption spectra.

DFT Calculations. Calculations were performed using gradient-corrected DFT with the Becke–Lee–Young–Parr composite exchange-correlation functional (B3-LYP), as implemented in the Gaussian 94 suite of programs.²⁵ Most calculations were carried out with Ahlrich's VDZ,²⁶ and for some adducts we tested the influence of basis set quality. Extension of basis set quality to 6-31G* for C, N, O, H and Ahlrich's VTZ for Fe leads primarily to changes in X–O bond lengths. Because the extended basis set was too expensive for the frequency calculations, we carried out vibrational analysis only with the VDZ basis set.

Results and Discussion

Fe–N and N–O Frequencies for Five-Coordinate Fe Porphyrins. Vibrational data have been reported for a number of heme protein–NO adducts,^{15–19,21–24,27–29} but data for model compounds are sparse. To examine the effects of variable back-bonding, we obtained resonance Raman (RR) spectra for a series of (NO)FeTPP adducts with electron-withdrawing and -donating substituents on the phenyl groups (Table 1). Attempts to characterize six-coordinate adducts were unsuccessful because of the low affinity for trans ligands occasioned by the strong

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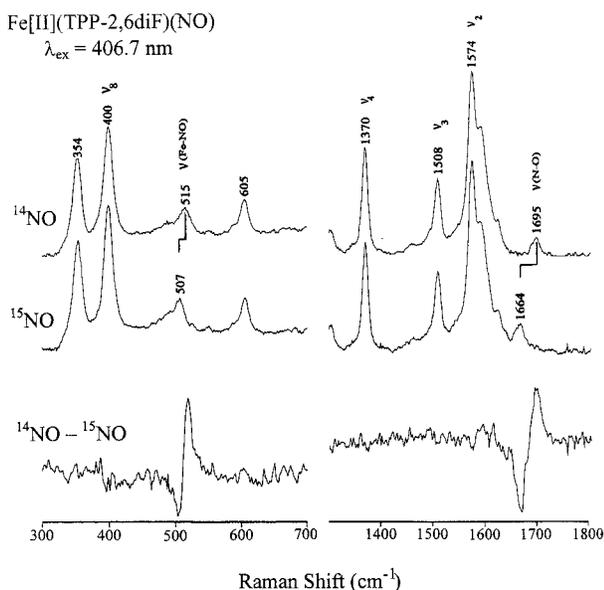


Figure 1. RR spectra (406.7 nm excitation) of $\text{Fe}^{\text{II}}(\text{TPP-2,6-difluoro})\text{(NO)}$ in benzene and the isotope difference spectra in the $\nu(\text{Fe}-\text{NO})$ and $\nu(\text{N}-\text{O})$ regions.

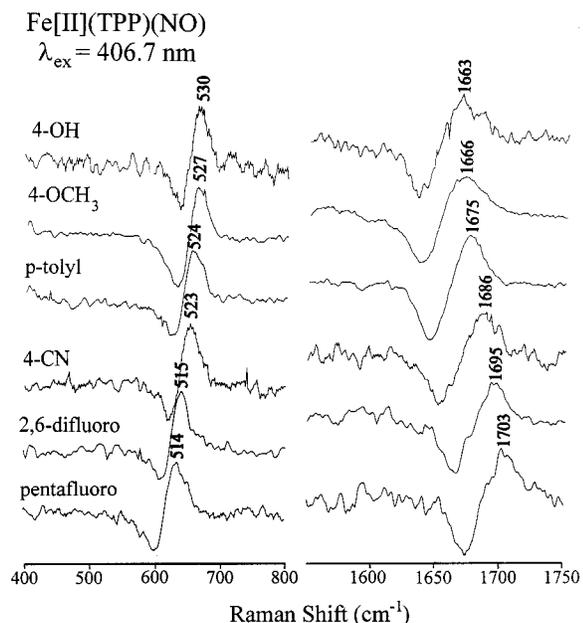


Figure 2. $^{15}/^{14}\text{NO}$ difference RR bands for $\nu(\text{Fe}-\text{NO})$ and $\nu(\text{N}-\text{O})$ for the five-coordinate (TPP)NO adducts in this study (Table 1).

trans-labilizing influence of NO in $\text{Fe}(\text{II})$ adducts.²⁸ However, good quality spectra of the five-coordinate adducts were obtained (Figure 1). Isotopic substitution with ^{15}NO clearly revealed the $\nu(\text{Fe}-\text{NO})$ and $\nu(\text{N}-\text{O})$ bands at ca. 520 and ca. 1680 cm^{-1} , and these shifted systematically to lower and higher frequencies, respectively, as electron-donating substituents were replaced by electron-withdrawing ones (Figure 2).

When plotted against one another, these data describe a negative linear correlation (Table 1, Figure 3), just as the FeCO data do,⁸ although the actual frequencies are quite different for NO and CO. For the TPP-substituted porphyrins, electron-donating groups (OH, CH_3) push the data points higher on the correlation, reflecting greater back-donation, while electron-withdrawing substituents (F, Cl) are low on the line. To our surprise, the slope of the five-coordinate NO correlation, -0.40 , is the same as that of the five-coordinate CO correlation.¹² Thus,

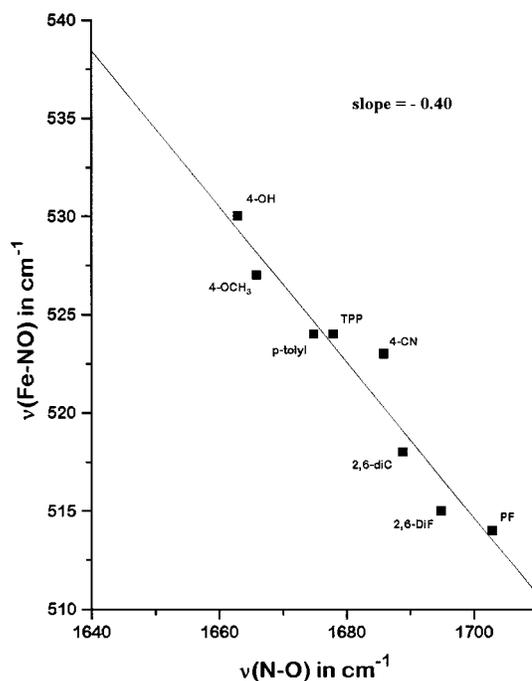


Figure 3. $\nu(\text{Fe}-\text{NO})/\nu(\text{N}-\text{O})$ correlation for the five-coordinate NO adducts in this study (Table 1).

Table 2. Vibrational Frequencies for $\text{Fe}^{\text{II}}(\text{P})(\text{O}_2)$ Adducts

compound ^a	$\nu(\text{Fe}-\text{O}_2)$	$\nu(\text{O}-\text{O})$	ref
Five-Coordinate $\text{Fe}^{\text{II}}(\text{O}_2)$			
TMP	522	1171	33
TMP	516	1188	34
OEP	509	1192	34
TPP	508	1195	34
TPFPP	486	1223	34
PC	488	1207	35
Six-Coordinate, $\text{Fe}^{\text{II}}(\text{O}_2)$			
TPP(pip)	575	1157	36
TPivP(1-MeIm)	568	1159	37-39
TPivP(1,2-Me ₂ Im)	564	1159	39
TPivP($\text{C}_6\text{HF}_4\text{S}^-$)	527	1140	20, 40
TPivP($\text{C}_6\text{F}_5\text{S}^-$)	536	1147	20

^a Abbreviations: TMP, tetramesitylporphyrin; OEP, octaethylporphyrin; TPFPP, tetrakis(pentafluorophenyl)porphyrin; PC, phthalocyanate; TPP, tetraphenylporphyrin; TPivP, picket fence porphyrin; pip, piperidine; MeIm, methylimidazole.

the back-bonding sensitivity to electronic changes at the heme is as high for NO as it is for CO.

From the literature, we were able to compile sufficient data (Table 2) to construct a similar correlation for five-coordinate $\text{Fe}^{\text{II}}(\text{P})\text{O}_2$ adducts (Figure 4). Again, a negative linear correlation is observed, only now the slope is -0.78 , twice as high as that for five-coordinate FeNO or FeCO . Thus, the back-bonding sensitivity for bound O_2 is higher than that for CO.

FeXO Comparison: Trans Ligand Effects. The back-bonding correlations are compared in Figure 5 for CO, NO, and O_2 . The five-coordinate data describe a set of lines which are displaced along the $\nu(\text{X}-\text{O})$ axis, reflecting the greater sensitivity of the X-O bond order to the antibonding influence of the extra valence electrons on NO (one) and O_2 (two), relative to CO. In each case, however, there is compensation of bond order between the $\text{Fe}-\text{XO}$ and X-O bonds as the extent of back-bonding is modulated by electron-donating and -withdrawing effects of porphyrin substituents.

However, the response to trans ligands is very different for the CO adducts, on one hand, and NO and O_2 on the other.

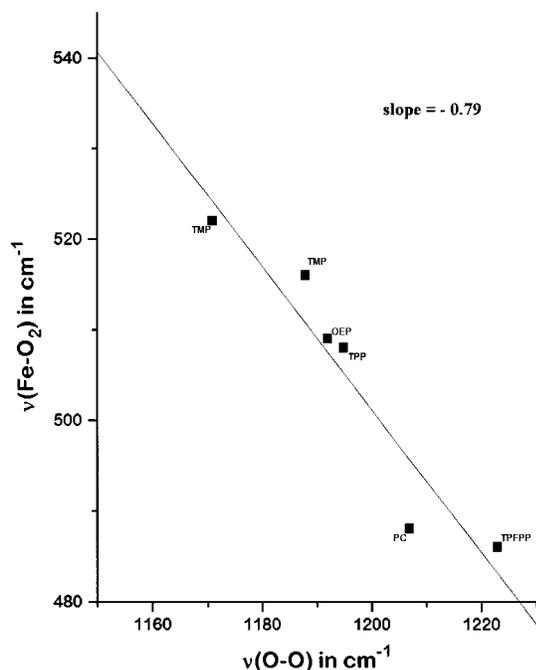


Figure 4. $\nu(\text{Fe}-\text{O}_2)/\nu(\text{O}-\text{O})$ correlation for the indicated five-coordinate O_2 adducts (Table 2).

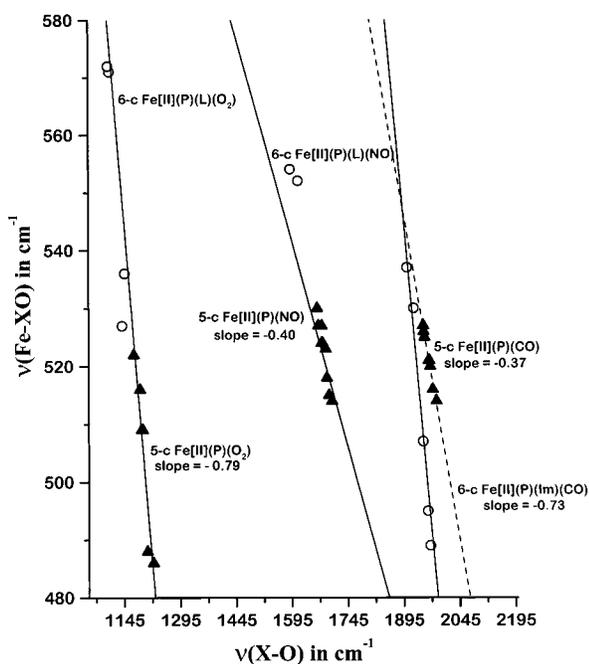


Figure 5. Back-bonding correlations between $\nu(\text{Fe}-\text{XO})$ and $\nu(\text{X}-\text{O})$ of five- (\blacktriangle) and six-coordinate (\circ) XO adducts of Fe(II) porphyrins. For the five-coordinate adducts: CO data from ref 13; NO data from Figure 4; O_2 data from Figure 5. For the six-coordinate NO and O_2 points, see Tables 1 and 2; CO points for adducts with imidazole ligands, see refs 8 and 9.

Both $\nu(\text{Fe}-\text{CO})$ and $\nu(\text{C}-\text{O})$ are diminished when a sixth ligand is added to heme-CO, except when there is a strong distal polarization (e.g., an H bond to the O atom), which greatly increases back-bonding. The six-coordinate data also fall on a negative linear correlation for a given trans ligand, but the slope is increased, as is the spread of the experimental points along the $\nu(\text{Fe}-\text{CO})$ axis. Thus, the trans ligand increases the $\nu(\text{Fe}-\text{CO})$ responsiveness to back-bonding effects. In the case of NO and O_2 adducts, however, addition of a trans ligand produces

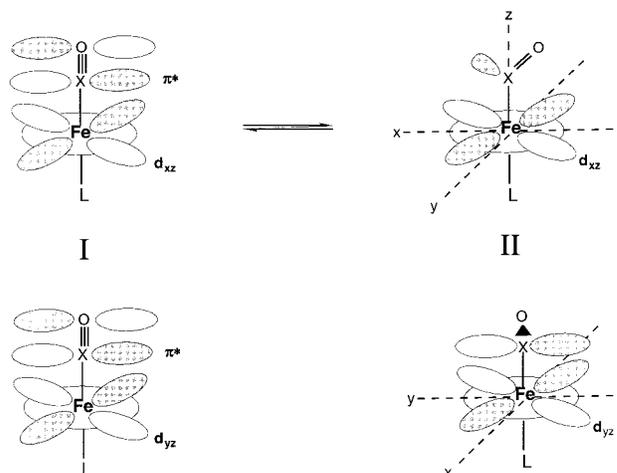


Figure 6. Valence isomer bonding model for XO adducts of Fe(II) porphyrins.

data that fall on the five-coordinate correlations, but at higher positions on the line (open circles, Figure 5). Thus, for the NO and O_2 adducts, a trans ligand enhances back-bonding, but the $\nu(\text{Fe}-\text{XO})$ responsiveness is unaltered.

Bonding in CO, NO, and O_2 Adducts. The bonding of first-row diatomic molecules XY in transition metal complexes has been analyzed many times, generally in the context of molecular orbital theory. The classic paper of Hoffmann et al. discusses the evolution of orbital overlaps and energies as the MXY unit deviates from linearity.¹⁴

These orbitals are complex, and the separate effects of back-bonding and trans ligand bonding are not easy to anticipate. A simple valence isomer model (Figure 6), which is related to the valence-bond approach of Michael et al.,^{13d} is better suited to understanding these effects. The essence of this model is that π^* antibonding electrons are accommodated in a nonbonding lone-pair type orbital, which is directed away from the metal, along the bisector of the FeXO angle. If this orbital is occupied, the angle is bent (isomer II), whereas if it is empty, the angle is linear (isomer I). The structures are isomers, rather than resonance forms, since the nuclei change positions. However, the nuclear positions are averaged on the vibrational time scale, so that a single spectrum is found. NO has one and O_2 has two π^* electrons, producing structures which are increasingly bent; for O_2 the angle is close to the 120° angle expected for the sp^2 hybridization implied by isomer II (Table 3). For NO, the angle is wider, $\sim 140^\circ$ (Table 3), reflecting an isomer I contribution. This contribution also explains the higher $\nu(\text{N}-\text{O})$ than $\nu(\text{O}-\text{O})$, and it accounts for the strong trans effect, which is well known for NO adducts. Isomer II is formally $\text{Fe}^{\text{III}}(\text{NO}^-)$, while isomer I is formally $\text{Fe}^{\text{I}}[\text{NO}^+]$. Isomer I transfers the NO π^* electron to the Fe d_{z^2} orbital, where it is antibonding with respect to the axial ligand σ bonds. The trans ligand loses in this competition, as evidenced by the long Fe-ligand bonds (Table 3).

Back-bonding remains important, even in isomer II, because there is an empty π^* orbital perpendicular to the FeXO bending plane (Figure 6, bottom). The $d_{xz}-\pi^*$ overlap accounts for the negative $\nu(\text{Fe}-\text{XO})/\nu(\text{X}-\text{O})$ correlations for NO and O_2 , as well as for CO. Since there are two $d_{xz}-\pi^*$ overlaps in isomer I and only one in isomer II, the extent of back-bonding might be expected to diminish in the order $\text{CO} > \text{NO} > \text{O}_2$. However, this trend is countered by stabilization of the π^* orbital as a result of the lowering in p orbital energy from C to N to O.

Table 3. Heme Fe^{II}XO Structural Parameters

compound ^a	Fe–XO (Å)	X–O (Å)	Fe–X–O (deg)	Fe–N _p (Å)	δ _{oop} ^c (Å)	Fe–L (Å)	ref
Five-Coordinate CO							
Deut(THF)(CO)	1.706(5)	1.144(5)	177.4(9)	1.980		2.127(4)	41
DFT ^b : Fe(P)	1.727	1.171	180.0	2.013	0.186		this work
Six-Coordinate CO							
TPP(py)(CO)	1.77(2)	1.12(2)	179(2)	2.015	-	2.10(1)	42
DFT ^b : Fe(P)(py)	1.781	1.168	180.0	2.028	0.186	2.102	this work
Five-Coordinate NO							
OEP(NO)	1.722(2),	1.167(3)	144.4 (2),	1.991 (3)	0.29		43
OEP(NO)	1.7307(7)	1.168(11)	142.74(8)	2.016 (1)	0.27		43
TPP(NO)	1.717(7)	1.122 (12)	149.2 (6)	2.001 (3)	0.211 (5)		44
<i>p</i> -tolyl-TPP(NO)	1.717 (7)		149.2 (6)	2.001	0.21		25
2,6-dichloro-TPP(NO)	1.703 (8)		138.8 (9)	2.004	0.31		25
octabromo-TPP(NO)	1.75(6)	1.418 (73)	146.4 (2)	1.986	0.37		25
average	1.723	1.219	145.1	2.002	0.28		
DFT ^b : Fe(P)	1.716	1.198	142.0	2.020	0.15		this work
Six-Coordinate NO							
TPP(1-MeIm)(NO)	1.743(4)	1.14	142.1(6),	2.008(9,4)	0.05	2.180(4)	44, 45
		1.12	138.3(11)				
TPP(4-MePip)(NO)·CHCl ₃	1.721	1.14	138.5(11)	2.328 (10)	0.09	2.328 (10)	46
TPP(4-MePip)(NO)	1.740 (7)	1.11	143.7(6)	2.463(7)	0.11	2.463 (7)	46
average	1.735	1.13	140.7	2.266	0.08	2.324	
DFT ^b : Fe(P)(py)	1.786	1.201	141.6	2.03	0.04	2.066	this work
Five-Coordinate O ₂							
DFT ^b : Fe(P)	1.715	1.314	122.386	2.01	0.22		this work
Six-Coordinate O ₂							
TPivP(2-MeIm)	1.898(7)	1.22(2)	129	1.95(6)	-0.086	2.107(4)	47
TPivP(1-MeIm)	1.75(2)	1.16(8)	131	1.996(4)	0.03	2.07(2)	48
TPivP(1,2-Me ₂ Im)	1.77(3)			1.99(3)		2.29(4)	49
average	1.806	1.19	130	1.979	-0.028	2.156	
DFT ^b : Fe(P)(py)	1.776	1.311	122.9	2.023	-0.019	2.024	this work

^a Abbreviations: P, porphine; py, pyridine; TPP, tetraphenylporphyrin; OEP, octaethylporphyrin; MeIm, methylimidazole; MePip, methylpiperidine; TPivP, picket fence porphyrin; Me₂Im, dimethylimidazole. ^b B3LYP with Ahlrich's VDZ basis set. ^c δ_{oop}, Fe out-of-plane toward nitrogen atom.

Thus, back-bonding is of comparable importance in all three cases.

The slopes of the back-bonding lines express the sensitivity of $\nu(\text{Fe-XO})$ relative to that of $\nu(\text{X-O})$ for a given increment of back-bonding. This sensitivity is expected to increase with increasing Fe–XO bond distance because the rate of change of the $d_{\pi}-\pi^*$ overlap will be greater. It is the change in this overlap that determines the extent of $\nu(\text{Fe-XO})$ variations, whereas $\nu(\text{X-O})$ depends only on the extent of electron transfer from d_{π} to π^* . Consistent with this model, the Fe–XO distance is greater for O₂ (ca. 1.81 Å) than for NO (ca. 1.73 Å) adducts, and the back-bonding slope is twice as high (Table 3, Figure 5).

For CO adducts, the back-bonding slope is twice as high for six- as for five-coordinate adducts, suggesting a substantial lengthening of the Fe–CO bond upon trans ligand binding. This is, indeed, observed (Table 3). Although a strictly five-coordinate crystal structure is not available, one finds a 0.06 Å lengthening of the Fe–CO bond when the structure of (CO)-Fe(TPP)(py), having the strong trans ligand pyridine, is compared with the structure of (CO)Fe(Deut)(THF), having the weak tetrahydrofuran ligand. This lengthening of the Fe–CO bond reflects the competition for the Fe d_{z^2} σ bonding orbital between the σ lone pairs on CO and on the trans ligand. As the trans donor strength increases, the Fe–CO σ bond weakens.

This effect of the trans ligand on $\nu(\text{Fe-XO})$ sensitivity is *not* seen for NO and O₂. As expected, back-bonding is enhanced by the electron donor effect of a trans ligand, resulting in diminished $\nu(\text{X-O})$ and increased $\nu(\text{Fe-XO})$. However, the back-bonding slope is not increased, and the six-coordinate data points fall on the five-coordinate correlations (Figure 5). We

infer that the Fe–XO bond is not significantly lengthened by the trans ligand because the d_{z^2} orbital is monopolized by the NO or O₂ σ lone pairs, which are lower in energy than the lone pair on CO. This inference is supported by the structural data on five- and six-coordinate NO adducts (Table 3). On average, the Fe–NO bond lengthens by only 0.01 Å upon trans ligand binding. Unfortunately, there is no crystal structure for a five-coordinate O₂ adduct.

DFT Analysis. We sought to test these ideas with ab initio calculations using density functional theory. DFT has been shown to give accurate geometries and reliable scaled frequencies for metalloporphyrins and adducts.^{11,12,30,31} For Ni porphine, DFT provided greater accuracy than previous empirical force fields and produced reliable IR and Raman intensities as well.^{30a} In the case of Fe(II) porphine, the proper ground state and geometry were obtained with DFT,^{30b} and for six-coordinate (CO)(ImH)Fe(II) porphine, accurate frequencies were also calculated for Fe–CO and C–O stretching and Fe–C–O bending modes.^{10,11} Moreover, inclusion of electron-donating and -withdrawing groups as porphyrin substituents led to negative back-bonding correlations between $\nu_{\text{Fe-CO}}$ and $\nu_{\text{C-O}}$, and the observed factor of 2 difference in slopes for five- vs six-coordinate FeCO adducts was reproduced.¹² Thus, DFT has

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passed several tests of reliability in exploring electronic effects on bonding in metalloporphyrin adducts.

The present calculations on five- and six-coordinate [XO]-Fe(II) porphine (X = C, N, O) give optimized geometries which are in good agreement with crystal structure determinations on several porphyrin adducts (Table 3). FeCO is linear, while FeNO and FeO₂ are calculated to be bent, with angles of ca. 140° and ca. 125°, as observed. The experimental and calculated Fe—XO and X—O distances are in the 1.7–1.9 and 1.1–1.3 Å ranges. Similar parameters have recently been reported in a DFT analysis of heme—CO and heme—O₂ models by Sigfridsson and Ryde.³²

However, the observed and calculated axial ligand trends are not entirely parallel (Table 3). DFT predicts 0.05–0.07 Å lengthening for all the Fe—XO bonds when imidazole is bound as a trans ligand. This prediction is correct for CO, but not for NO. As noted above, the Fe—NO bond lengthens by only about 0.01 Å upon trans ligand binding. The Fe—NO lengthening is overestimated because the DFT calculation fails to capture the trans labilizing effect of the NO. This can be seen in the near-normal distance, 2.07 Å, calculated for the Fe—N[ImH] in the NO adduct, whereas the observed Fe—N[ligand] distances are much longer, 2.18–2.46 Å. These long trans ligand bonds are a manifestation of the domination by NO of the Fe d_{z²} orbital. This effect is not captured by the DFT calculation, possibly reflecting misestimation of the relative Fe and ligand orbital energies. As a result, the trans ligand is calculated to compete more effectively with NO for the d_{z²} orbital than is actually the case.

DFT-calculated vibrational frequencies for these adducts are compared with experiment in Table 4. The agreement is very

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Table 4. Experimental and Calculated Vibrational Frequencies (cm⁻¹) for XO Adducts of Fe(II) Porphyrins^a

ligand	$\nu(\text{X-O})$		$\nu(\text{Fe-XO})$		$\delta(\text{FeXO})$		ref
	expt	DFT ^a	expt	DFT	expt	DFT	
5-c CO	1975 ^d	1985	531	581	(510, 510)	49, 50	
6-c CO	1970 ^e	1991	496	501	(572, 578) ^b	51, 52	
5-c NO	1669 ^f	1718	522	565	434	this work	
6-c NO	1591 ^g	1687	547	541	444, 450	22	
5-c O ₂	1195 ^h	1159	508	702	349, 347, 397 ^c	32	
6-c O ₂	1157 ⁱ	1176	575	623		34	
	1207 ^j		488		279, 268	33	

^a DFT, Fe(P), and Fe(P)(Py); all calculations reported are from this work. Abbreviations: OEP, octaethylporphyrin; Bz, benzene; ImH, imidazole; cyt, cytochrome; TPP, tetraphenylporphyrin; pip, piperidine; Pc, phthalocyanine. ^b The two degenerate components of the bending mode (E in C_{4v} symmetry) are separated by the orientation of the trans ligand, py. ^c Normal coordinate analysis gives two modes with significant $\delta(\text{FeXO})$ contribution. ^d OEP in Bz. ^e OEP(N-Melm) in Bz. ^f OEP in Bz. ^g Cyt P450. ^h OEP, matrix/ ⁱ TPP(pip) in CH₂Cl₂ [$\nu(\text{O-O})$] and toluene [$\nu(\text{Fe-O}_2)$]. ^j Pc, matrix.

Table 5. Experimental and Calculated Isotope Shifts for Fe^{II}XO Vibrational Frequencies (cm⁻¹)

isotope ^a	$\Delta\nu(\text{Fe-XO})$		$\Delta\delta(\text{Fe-XO})$		$\Delta\nu(\text{X-O})$	
	exptl	calcd	exptl	calcd	exptl	calcd
Five-Coordinate, Fe(P)(CO)						
na- ¹³ C ¹⁶ O	5 ^b	6		15	45 ^b	48
na- ¹² C ¹⁸ O		12		4		43
na- ¹³ C ¹⁸ O	14 ^c	16		17		92
Six-Coordinate, Fe(P)(Im)(CO)						
na- ¹³ C ¹⁶ O	3 ^d	4	14 ^d	16	48 ^d	46
na- ¹² C ¹⁸ O		8	1 ^d	3	48 ^d	45
na- ¹³ C ¹⁸ O	8 ^d	11		18		92
Five-Coordinate, Fe(P)(NO)						
na- ¹⁵ N ¹⁶ O	8 ^e	12		2	31 ^e	33
na- ¹⁴ N ¹⁸ O		5		2		42
na- ¹⁵ N ¹⁸ O	14 ^f	17		4	70 ^f	75
Six-Coordinate, Fe(P)(Im)(NO)						
na- ¹⁵ N ¹⁶ O	5 ^g	13	2 ⁱ	1	27 ^{g,h}	31
na- ¹⁴ N ¹⁸ O	6 ⁱ	1	4 ⁱ	0		41
na- ¹⁵ N ¹⁸ O	16 ^h	14	5 ⁱ	1	68 ^h	74
Five-Coordinate, Fe(P)(O ₂)						
na- ¹⁸ O ¹⁶ O		27		3		39
na- ¹⁶ O ¹⁸ O		2		3		35
na- ¹⁸ O ¹⁸ O	21 ^j	28	4 ^j	5	66 ^j	76
Six-Coordinate, Fe(P)(Im)(O ₂)						
na- ¹⁸ O ¹⁶ O	21 ^k	28	2 ^k	1	31 ^k	39
na- ¹⁶ O ¹⁸ O	1 ^k	1	6 ^k	1	31 ^k	36
na- ¹⁸ O ¹⁸ O	22 ^k	20	8 ^k	3	63 ^k	77

^a na, natural abundance. ^b TPP(Bz), ref 12. ^c TPP(Bz), refs 50, 54. ^d SW Mb, ref 55. ^e This work. ^f sGC, ref 22. ^g Mb, ref 31. ^h Mb, Tomita et al., in press. ⁱ Mb, ref 21. ^j TPP(matrix), ref 33. ^k Pthalocyanato (matrix), ref 34.

good for CO with a trans imidazole ligand. Both $\nu(\text{Fe-CO})$ and $\delta(\text{Fe-C-O})$ are within 5 cm⁻¹ of the experimental values for the OEP complex, while the calculated $\nu(\text{C-O})$ is within 20 cm⁻¹. However, the agreement with experiment is less impressive for the other adducts in Table 4. Thus, for the five-coordinate CO adduct, $\nu(\text{Fe-CO})$ is overestimated by 50 cm⁻¹. The shift from the six-coordinate value is in the right direction, reflecting the observed Fe—CO bond strengthening, but it is double the observed shift. Other DFT calculations on CO adducts show that back-bonding effects associated with electron-donating or -withdrawing porphyrin substituents are also in the right direction, but the slopes of the back-bonding correlations are likewise twice as large as observed.¹² Thus, DFT captures

the essential features of back-bonding to CO and trans ligand competition but predicts too high a sensitivity.

For the NO and O₂ adducts, the direction of the trans ligand effect on $\nu(\text{Fe-XO})$ is incorrectly predicted. The $\nu(\text{Fe-NO})$ frequency is close to the observed value for six-coordinate FeNO but is predicted to increase 25 cm⁻¹ in the five-coordinate adduct, whereas a 25 cm⁻¹ decrease is observed. The $\nu(\text{N-O})$ frequency is overestimated by large amounts, 50 and 95 cm⁻¹, in the five- and six-coordinate complexes. For O₂ adducts, the $\nu(\text{O-O})$ discrepancies are smaller, but the $\nu(\text{Fe-O}_2)$ discrepancies are larger, 38 cm⁻¹ for the six-coordinate adduct and 193 cm⁻¹ for the five-coordinate adduct. A large $\nu(\text{Fe-O}_2)$ downshift is predicted upon binding of the trans ligand, whereas a large upshift is observed instead.

Because the FeNO and FeO₂ units are bent, their vibrational mode compositions are complex. There is mixing of Fe-XO stretching and Fe-X-O bending coordinates, and also of Fe-X-O bending with porphyrin out-of-plane coordinates. Nevertheless, the isotope shifts (Table 5) are satisfactorily reproduced by the calculations, so that the mode compositions are reliable. The mode assigned to $\delta(\text{Fe-X-O})$ is highly mixed, but the mode assigned to $\nu(\text{Fe-XO})$ is ca. 65% Fe-X stretching, according to the calculated potential energy distribution. It is therefore expected to reflect the Fe-XO bond strength. However, the coordinate mixing makes the frequencies highly dependent on the Fe-X-O bond angle, due to kinematic changes. The $\nu(\text{Fe-XO})$ frequency increases as the Fe-X-O

angle deviates from linearity, because the effective mass of the vibrating ligand decreases. This factor may be important in heme proteins, where distal interactions can affect the Fe-X-O angle.³¹

Conclusions

Back-bonding effects dominate the variations in vibrational frequencies for NO and O₂, as well as CO adducts of heme. Although back-bonding is diminished by FeXO bending, this effect is compensated by enhanced back-bonding perpendicular to the bending plane due to the lowered π^* orbital energies in NO and O₂. Adding a trans ligand increases back-donation in all cases, but in addition it increases the slope of the CO back-bonding correlation, reflecting the lengthening of the Fe-C bond. For NO and O₂, the correlation is unshifted, implying insensitivity of the Fe-XO bond length to the trans ligand. This insensitivity is suggested to result from dominance of the d_{z^2} σ orbital overlap by NO and O₂, but not CO. DFT captures the essential features of back-bonding but not of the σ competition with the trans ligand.

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