Inorganic Chemistry
Communication

Vibrational Assignments of Six-Coordinate Ferrous Heme Nitrosyls: New Insight from Nuclear Resonance Vibrational Spectroscopy

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This Communication addresses a long-standing problem: the exact vibrational assignments of the low-energy modes of the Fe−N−O subunit in six-coordinate ferrous heme nitrosyl model complexes. This problem is addressed using nuclear resonance vibrational spectroscopy (NRVS) coupled to $^{15}N^{18}O$ isotope labeling and detailed simulations of the obtained data. Two isotope-sensitive features are identified at 437 and 563 cm^{-1}. Normal coordinate analysis shows that the 437 cm^{-1} mode corresponds to the Fe−NO stretch, whereas the 563 cm^{-1} band is identified with the Fe−N−O bend. The relative NRVS intensities of these features determine the degree of vibrational mixing between the stretch and the bend. The implications of these results are discussed with respect to the *trans* effect of imidazole on the bound NO. In addition, a comparison to myoglobin-NO (Mb-NO) is made to determine the effect of the Mb active site pocket on the bound NO.

Much effort has been spent in the last decades to investigate the binding and activation of small molecules by heme proteins,[1] and it has been found that the proximal ligand plays a key role in these reactions. This issue is also of importance for bacterial nitric oxide (NO) reductase (NorBC). In this case, a ferrous heme nitrosyl has been proposed to be catalytically active in the reduction of NO to N_2O.[2] This is surprising considering that these species are generally stable and quite unreactive.[3] This poses the important question of how a ferrous heme nitrosyl can be activated for catalysis. Previous work in this laboratory on

Scheme 1. Model Complex **2** Investigated Here
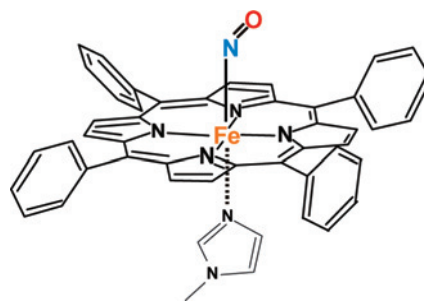
the five-coordinate (5C) model complex [Fe(TPP)(NO)] (**1**; TPP = tetraphenylporphyrin) and related six-coordinate (6C) [Fe(TPP)(MI)(NO)] (**2**; MI = 1-methylimidazole; Scheme 1) has shown that the axial imidazole ligand increases the radical character on the bound NO by weakening the Fe−NO bond,[4] which could play a key role for NorBC catalysis. This is evident from corresponding force constants obtained by normal coordinate analysis (NCA).[4a] However, whereas the vibrational assignments for **1** are well established, there has been a lot of discussion recently about the energies of the Fe−NO stretch, ν(Fe−NO), and the Fe−N−O bend, δ(Fe−N−O), in the 6C model complexes, and how this compares to 6C ferrous myoglobin-NO (Mb-NO).[4−6] In the Mb-NO case, nuclear resonance vibrational spectroscopy (NRVS) has allowed for the identification of ν(Fe−NO) at 443 cm^{-1} and δ(Fe−N−O) at 547 cm^{-1}.[6] In this study, we resolve this important issue and clarify the vibrational assignments of the 6C model complexes. This allows us to (1) determine the effect of the axial imidazole ligand on the electronic structure of the Fe−N−O subunit in comparison to 5C species, and (2) to establish the influence of the Mb binding pocket on the bound NO by comparison with the

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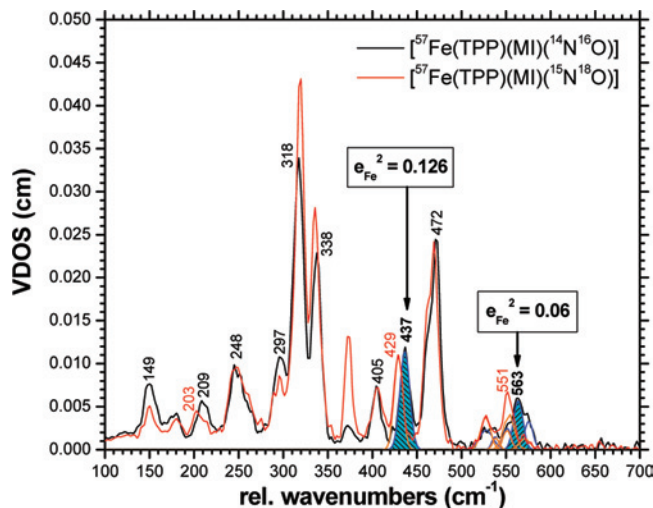
Figure 1. Experimental VDOS data determined from NRVS measurements on [^{57}Fe(TPP)(MI)(^{14}N^{16}O)] (black) and [^{57}Fe(TPP)(MI)(^{15}N^{18}O)] (red) at ~10 K. Experimental e_{Fe}^2 ratios compare well with MbNO (443 cm^{-1}: $e_{Fe}^2 = 0.25$; 547 cm^{-1}: $e_{Fe}^2 = 0.11$).[6]

vibrational data of Mb-NO published before. This is achieved using NRVS on the 6C model complex **2** and the corresponding ^{15}N^{18}O isotope-labeled compound. NRVS is a powerful vibrational method that, because of its different selection rules, allows for the *specific* detection of vibrations that involve motion of the iron center. Hence, NRVS is ideally suited to identify the vibrations of the Fe−N−O subunit in **2**.[6−8] Importantly, the vibrational density of states (VDOS, calculated from the NRVS data) intensity of a mode is directly related to the square of the iron motion, e_{Fe}^2, of this mode.[7,8] Here, we use NCA to calculate polarized VDOS intensities, which allows us to assign the NRVS spectra of **2** for the first time and to accurately determine the magnitude of vibrational mixing between the stretching and bending internal coordinates.

Figure S1 in the Supporting Information shows the NRVS spectra of **2** and of the corresponding ^{15}N^{18}O-labeled complex. Note that both compounds are ^{57}Fe-labeled in order to perform the NRVS measurements. These data identify two isotope-sensitive features at 437 and 563 cm^{-1} for **2** that shift to 429 and 551 cm^{-1}, respectively, upon isotope labeling. Figure 1 shows the VDOS extracted from the NRVS data together with fits to determine the integral intensities of these features. As stated above, the VDOS intensities are directly proportional to the amount of iron motion, e_{Fe}^2, in these modes.[7] The e_{Fe}^2(563 cm^{-1})/e_{Fe}^2(437 cm^{-1}) ratio experimentally determined from Figure 1 is ~0.48 for **2**. This compares well with the e_{Fe}^2(547 cm^{-1})/e_{Fe}^2(443 cm^{-1}) ratio of 0.44 obtained experimentally for Mb-NO.[6] In order to rigorously assign the ν(Fe−NO) and δ(Fe−N−O) vibrations in **2**, we then used our quantum chemistry centered NCA (QCC-NCA), extended with a new module to calculate
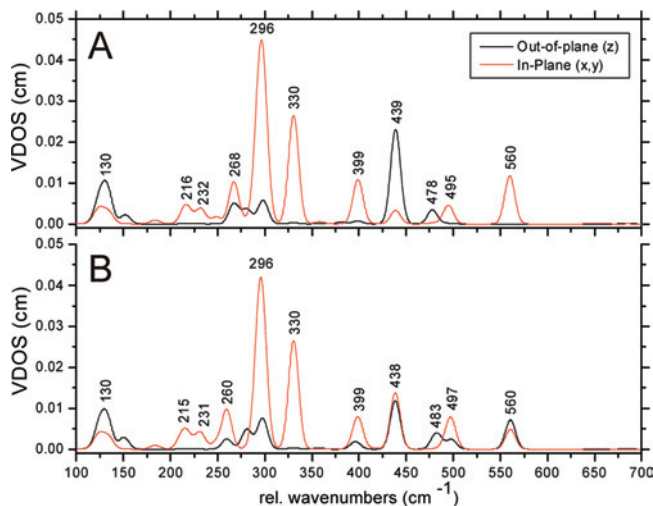
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Figure 2. Calculated polarized VDOS for [^{57}Fe(TPP)(MI)(^{14}N^{16}O)] (in-plane-polarized: red; out-of-plane-polarized: black). A: ν(Fe−NO) = 439 and δ(Fe−N−O) = 560 cm^{-1} (fit A). B: vice versa (fit B, "inverse-fit" in Table 1).

Table 1. Calculated Vibrational Kinetic Energy Distributions of the Fe−N−O Fragment in [^{57}Fe(TPP)(MI)(NO)]

	frequency (cm^{-1})	e_{Fe}^2	$e_{Fe,z}^2$	$e_{Fe,xy}^2$	$e_{Fe,560}^2/e_{Fe,440}^2$
normal^a (fit A)	560	0.157	0.0	0.157	0.45
	439	0.351	0.307	0.044	
inverse-fit (fit B)^b	561	0.159	0.095	0.065	0.47
	438	0.342	0.158	0.184	

^{a} Normal (fit A): ν(Fe−NO) = 439 cm^{-1} and δ(Fe−N−O) = 560 cm^{-1}. ^{b} Inverse-fit (fit B): ν(Fe−NO) = 561 cm^{-1} and δ(Fe−N−O) = 438 cm^{-1}.

Table 2. Comparison of Experimental and QCC-NCA Vibrational Frequencies [cm^{-1}] and of QCC-NCA and Calculated (DFT) Force Constants [mdyn/Å] for [^{57}Fe(TPP)(MI)(NO)]

	exptl^a		QCC-NCA		force constant f	
mode	nai^a	^{15}N^{18}O	nai^a	^{15}N^{18}O	QCC-NCA	calcd^b
ν(N−O)	1630^b	1556^b	1628	1557	11.55	12.22
δ(Fe−N−O)	563	551	560	545	0.799	0.68
ν(Fe−NO)	437	429	439	427	2.38	3.26

^{a} nai = natural abundance isotopes for NO. ^{b} From ref 4a.

(polarized) NRVS intensities, to simulate the spectra. These simulations are based on density functional theory (DFT) calculations on the model [Fe(P)(MI)(NO)] (P = porphine).[4a] Figure 2A shows the calculated NRVS VDOS, where the 437 cm^{-1} feature is fitted to ν(Fe−NO) and the 563 cm^{-1} band to δ(Fe−N−O).[9] Here, the 437 cm^{-1} mode shows out-of-plane polarization (relative to the porphyrin plane), whereas the 563 cm^{-1} feature is exclusively in-plane polarized (cf. Table 1). However, the alternative solution, where ν(Fe−NO) is fitted to the 563 cm^{-1} band and δ(Fe−N−O) to the 437 cm^{-1} feature, is also possible! In fact, both fits lead to very similar isotope shifts (cf. Tables 2 and S3 in the Supporting Information), and as shown in Table 1, the experimental e_{Fe}^2(563 cm^{-1})/e_{Fe}^2(437 cm^{-1}) ratio of ~0.48 is also reproduced by both fits. Which assignment is correct? As shown in Figure 2B, the second solution leads to dramatically different polarizations, where both the 563 cm^{-1} feature and the 437 cm^{-1} band show 1:1 mixed polarizations.

(9) Note that, because of the use of porphine instead of TPP in the NCA simulation, the remaining porphyrin bands are not reproduced well in the fit.
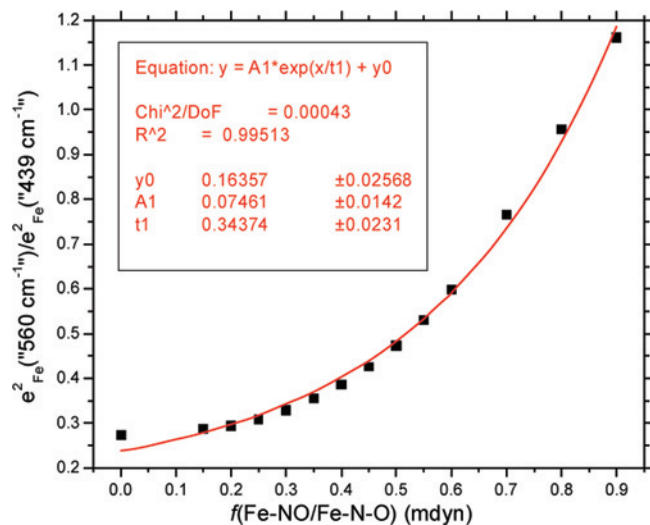
Figure 3. Calculated NRVS VDOS intensities and mode mixing.

Importantly, single-crystal NRVS on **2** by Zeng et al.[6] has shown that the ∼440 cm^{-1} feature is, in fact, out-of-plane-polarized. *Therefore, only fit A is in agreement with the experiment.* Hence, the isotope-labeled NRVS data and detailed simulations presented here in comparison to the NRVS data on Mb-NO in the literature allow us *for the first time to assign the mode at 440 cm^{-1} to ν(Fe−NO) and the band at 563 cm^{-1} to δ(Fe−N−O) in the 6C model complex* **2**. As is evident from Figure 1, there are further changes in the 560−580 cm^{-1} region upon isotope labeling, indicating that δ(Fe−N−O) also shows some mixing with TPP-based vibrations. The inclusion of the full TPP ligand in the vibrational analysis is the topic of an ongoing investigation to further refine this picture.

To obtain reliable force constants *f* for the Fe−NO bond and the Fe−N−O angle, the corresponding interaction force constant *f*(Fe−NO/Fe−N−O) needs to be known. In other words, the vibrational mixing between the stretching and bending coordinates needs to be determined. This is again possible using the e_{Fe}^2(563 cm^{-1})/e_{Fe}^2(437 cm^{-1}) ratio of ∼0.48, because this ratio is, in fact, very sensitive to the magnitude of vibrational mixing, as shown in Figure 3. The exponential relationship in Figure 3 fixes *f*(Fe−NO/Fe−N−O) at ∼0.5 mdyn, leading to the final NCA fit for the NRVS data of **2** shown in Table 2. *The key force constant of the Fe−NO bond is 2.38 mdyn/Å.*

In summary, reliable assignments of ν(Fe−NO) and δ(Fe−N−O) for the 6C model complex **2** have been obtained for the first time in this study. Previously, single-crystal NRVS on **2** had allowed for the determination of the polarizations for the NRVS features of this complex, but because of the lack of isotope labeling, no assignments could have been made.[6] Later, the δ(Fe−N−O) mode in **2** was identified with a band at 530 cm^{-1} from resonance Raman.[4a] In light of our NRVS results, this 530 cm^{-1} band is likely due to the formation of **1** by loss of MI under laser irradiation, as suggested before.[5] Recently, it was also claimed,

again based on Raman spectroscopy, that ν(Fe−NO) in **2** is located at 582 cm^{-1}, and no feature around 440 cm^{-1} was observed.[5] However, our results, combined with the polarized NRVS data of **2** published before, clearly show that this 582 cm^{-1} Raman band cannot correspond to the Fe−NO stretch, because this mode is, in fact, located at ∼440 cm^{-1}. More likely, the 582 cm^{-1} Raman band from ref 5 belongs to δ(Fe−N−O), where the 19 cm^{-1} difference in frequency compared to our NRVS data might be due to the different conditions applied for the measurements (the NRVS data of **2** are taken on microcrystalline solids, whereas ref 5 presents solution Raman data). The exact reasons for this discrepancy between NRVS and Raman data are unclear, especially because the N−O stretching frequency seems quite unresponsive to this change in conditions: ν(N−O) is observed at 1623 cm^{-1} in solution[5] and at 1630 cm^{-1} in a KBr disk[4] and in the pure solid. Alternatively, the 582 cm^{-1} Raman band observed in ref 5 could also belong to an impurity of the corresponding ferric complex. It is striking that the vibrational energy of 582 cm^{-1} is very similar to that of ν(Fe−NO) in the corresponding ferric complex, which was observed at 580 cm^{-1} via NRVS in [Fe(TPP)(MI)-(NO)](BF$_4$).[10] This point needs further study, for example, by performing NRVS measurements on solutions of the complexes.

The obtained force constants for **2** underline the previously described trend that the axial imidazole donor weakens both the Fe−NO and N−O bonds, as is evident from smaller *f*(Fe−NO) and *f*(N−O) force constants in this case compared to **1** (2.98 and 12.53 mdyn/Å, respectively).[4a] This leads to increased radical character on the NO in the 6C case, which might be key for the utilization of this species for catalysis in NorBC. Finally, the strong similarity in the ν(Fe−NO) frequencies in **2** and Mb-NO does not indicate any difference between these species. It is actually the δ(Fe−N−O) bending mode that is more sensitive and shows a 15 cm^{-1} shift, which, however, indicates only a relatively small effect of the Mb binding pocket on the bound NO in contrast to a recent suggestion.[5]

Supporting Information Available: Experimental section, simulated NRVS spectra, IR spectra of **2** before and after NRVS measurements, and QCC-NCA results for the two different fits. This material is available free of charge via the Internet at http://pubs.acs.org.

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