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Probing Vibrational Anisotropy with Nuclear Resonance Vibrational Spectroscopy**

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One of the important issues of nitrosyl (nitric oxide, NO) iron porphyrinate derivatives (hemes) is to develop a detailed understanding of the molecular basis for selectivity (recognition) between the diatomic ligands NO, CO and O₂. The sensing of these gaseous molecules is predominantly by heme-based proteins,[1] and heme- and heme protein-diatom interactions continues to be an active area of research.[2] In Nature, NO is

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Dedicated to Prof. R. G. Hayes on the occasion of his 74th birthday.

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discriminated from O₂ quite efficiently by a number of systems;[3]–[5]most prominent are those of soluble guanylyl cyclase and the NO sensing protein of *Clostridium botulinum*. Conformational changes in the protein imparted upon ligand binding is a plausible explanation for such differentiation.[6,7] Very low frequency doming modes, sometimes referred to as reactive modes,[8] may facilitate binding and release of diatomic molecules. [9],[10] Infrared and resonance Raman spectroscopy have provided insight into the interplay of structure and function of heme active sites[11] However, these techniques have some inherent limitations, especially in the low frequency regime where mode assignment is hampered by weak signal, spectral congestion and low sensitivity to isotopic substitution.[8] Also, the directionality of iron within particular vibrations, a potential mechanistic indicator, cannot easily be determined. Our recent studies of the nitrosyl (NO) derivatives of iron porphyrinates have emphasized interesting aspects of nitrosyl dynamics of both the five- and six-coordinate NO species,[8],[12]–[16] including Nuclear Resonance Vibrational Spectroscopy (NRVS, sometimes termed Nuclear Inelastic Scattering, NIS) studies.[8],[15]–[19]

The direction of metal ion motion in a metal complex will be influenced by the surroundings, with the most significant interactions expected to be between the metal and its donor atoms. Naively, the principal metal motion directions would then be expected to be along the metal–ligand bonds.[20] We report in this paper the remarkable directional nature of the iron vibrations in [Fe(OEP)(NO)],[21] as determined by an oriented single-crystal NRVS experiment; the observed dynamics are only partially in accord with the above expectations. NRVS is a synchrotron-based vibrational technique with unique site selectivity for iron and provides quantitative information on all vibrational frequencies for which there is iron motion.[22] We have previously used oriented single crystals of heme derivatives[8],[15,16],[23,24] to assign the in-plane and out-of-plane vibrations, a distinction that is well-developed for heme derivatives with the planar equatorial porphyrin ligand. Selectivity between in-plane and out-of-plane modes results because only iron motion in the direction of the incident exciting beam will lead to signal intensity in the NRVS experiment. In contrast with conventional approaches to mode assignment based on isotope substitution, single-crystal NRVS measurements simultaneously characterize the directional character of all vibrational motions of the ⁵⁷Fe probe atom and may distinguish among divergent predictions resulting from quantum chemical calculations. In our previous oriented crystal experiments, we made measurements in which the X-ray beam was either perpendicular to all porphyrin planes in the crystal or parallel to the porphyrin planes; however the in-plane measurement was always made in a single arbitrary general direction in the porphyrin plane.

In a new study, we now explore iron motion in specific directions in the porphyrin plane of [Fe(OEP)(NO)],[25] which provides an additional dimension of spectral analysis for heme derivatives. Single-crystal measurements probed two orthogonal directions in the porphyrin plane: “x”, parallel to the intersection of porphyrin and FeNO planes, and “y”, perpendicular to the FeNO plane. Experimental limitations require that a separate crystal be used for measurements in the “z” direction, perpendicular to the porphyrin plane. The observed VDOS in the three orthogonal directions are displayed in Figure 1. The uniqueness of the observed vibrational spectra were verified by replicate measurements on separately oriented crystals (Figure S1), measurement “nn” with the X-ray beam in-plane and parallel to the N₂–Fe–N₄ direction of the porphyrin plane, and a measurement in a more general direction in the porphyrin plane (Figures S2 and S3). These latter two measurements confirm that the x and y directions can be taken as the fundamental directions. Moreover, the sum of the x, y, and z directional component spectra agree well with the experimental powder (isotropic) spectrum (Figure S4). We also note a very recent theoretical paper that presented a “First-principles calculation of NRVS” that predicted the possibility of observable anisotropy.[27]

Spectra recorded with the porphyrin core perpendicular to the exciting X-ray beam probe Fe motion along z and show a total of five distinct features (black line, Figure 1). Two of these represent iron motion with both in-plane and out-of-plane components (394 and ~ 225 cm^{-1}). The remaining three have essentially pure out-of-plane iron motion. The broad absorption at extremely low frequency ($< \sim 60$ cm^{-1}) is in the region where lattice modes are also expected,[28] but the absorption in the z direction has greater intensity and a shift to slightly higher frequency that is consistent with observation of extremely low frequency modes that are intramolecular in character. Differences between the averaged x and y intensity and z intensity confirms a substantial z direction signal (Figure S5).

DFT calculations[29] predict spectral assignments and kinetic energy distributions that support and enhance the oriented crystal data. The calculations suggest that three modes, predicted at 37 , 41 , and 47 cm^{-1} , contribute to the broad unresolved observed band below 100 cm^{-1} (Figure S5). All three have substantial motions of the peripheral ethyl groups as well as motion of both iron and porphyrin core atoms consistent with that of a doming mode (Figure S6).[34] The 158 cm^{-1} mode (predicted 139 cm^{-1}) is also a doming mode. The relatively high frequency of this mode appears related to the peripheral groups of octaethylporphyrin; the analogous signal observed at 140 cm^{-1} in $[\text{Fe}(\text{OEP})(\text{Cl})]$ is also likely to involve heme doming, although previously misassigned to in-plane vibrations.[20] The very strong signal at 517 cm^{-1} is the Fe–NO stretch; the calculated frequency for this mode is 623 cm^{-1} . The large discrepancy is typical for predicted Fe–NO stretching modes in five-coordinate NO porphyrinate complexes.[8] This band has modest yet interesting shoulders, one on each side of the main peak. These are coincident with the 510 cm^{-1} and 523 cm^{-1} vibrations of Fe observed along the x - and y - directions, respectively (Fig. 1). The shoulders are also apparent in the powder spectrum shown in Figure S4 which also displays the summation of x , y and z contributions. The remarkable agreement between these spectra attests to the capability of NRVs for showing subtle features that might have previously been dismissed as adventitious, and we judge the shoulders as possible unforeseen z components of the predicted 503 and 522 cm^{-1} modes.

Significant differences in the x and y NRVs spectra are observed (Figure 1), although in principle the iron should have equivalent interactions with each porphyrin nitrogen leading to degeneracy (or nearly so) among in-plane NRVs spectra. To facilitate the x and y comparisons, we show in Figure 2 the observed VDOS in the two directions in a “mirror” plot for the 125 – 480 cm^{-1} spectral region. Similar diagrams for the (few) remaining peaks at higher or lower frequencies are given in the SI (Figures S7 and S8). Although the two directional spectra have similarities, there are obvious differences in both peak intensities and frequencies. The most striking difference is the 394 cm^{-1} vibration of Fe along x with no corresponding vibration along y . This mode was also observed to have substantial iron motion along z ; the 394 cm^{-1} mode is clearly the Fe–N–O bending mode.

Measurements as a function of direction within the porphyrin plane allow for a more detailed examination of the mode assignments than previous (general) in-plane studies had permitted. The strongly developed directionality of the observed NRVs spectra provide strong constraints on the spectral predictions by DFT calculations in the issue of the appropriate choice of functional. One feature of major importance is the description of the magnetic properties of the $S = 1/2$ system; the treatment of the unpaired spin systems is a well-known difficulty of DFT calculations, which seems especially true for nitrosyl systems.[33] Although these issues will be investigated in more detail in future calculations, the results from the present calculation using the BP86 functional appears to have captured the essentials of the vibrational directionality.

A comparison of the observed and predicted x and y spectra is given in Figure 3; the analogous diagram for the z component is given in Figure S9. As can be seen in these figures, the peak at 394 cm^{-1} clearly has only x and z observed components, a directional dependency identical to that of the 417 cm^{-1} predicted mode. The components of motion[34] are illustrated in Figure 4 and as noted above are those expected for an FeNO bend, although only 60% of the energy is localized on the FeNO unit. This vibration was not cleanly identified in a previous study on [Fe(TPP)(NO)]. Interestingly, experimentally the x and z components are equal in intensity (Figure 1) whereas the predicted intensities are not quite equal.

The predicted modes in the $200\text{--}380\text{ cm}^{-1}$ region are consistent with the observed peaks in both their directional character and intensities (Figure 3). The minimally resolved peaks at 307 and 314 cm^{-1} Fe vibrations along x correspond to the predicted modes at 297 and 318 cm^{-1} as they are x -only in predicted character and have appropriate separation. The predicted mode at 348 cm^{-1} agrees quite well with the observed 350 cm^{-1} Fe vibration along x and even has predicted modes consistent with the observed asymmetry at 331 cm^{-1} and 336 cm^{-1} . The observed 308 cm^{-1} Fe vibration along y correlates well with predicted modes at 295 and 300 cm^{-1} , all with y -only character. The largest amplitude Fe vibration along y at 342 cm^{-1} is bracketed by predicted modes at 328 cm^{-1} and 355 cm^{-1} , which evidently have a smaller than predicted separation.

The experimental vibrational data for [Fe(OEP)(NO)] over the entire range of iron motion are thus seen to be strongly polarized. The polarized in-plane modes are best referred to a coordinate system where the Fe–N–O group defines the xz plane. The contributions of Fe motion along the x , y , and z directions for all modes for which Fe motion constitutes more than 1% of the total kinetic energy are tabulated in Table S1 of the Supporting Information. We have examined the character of the predicted vibrations and find that there are a number of approximately degenerate pairs. These are found at predicted frequencies 295 and 297 cm^{-1} , 300 and 318 cm^{-1} , 328 and 348 cm^{-1} , 336 and 355 cm^{-1} , 503 and 522 cm^{-1} , and 158 and 160 cm^{-1} . These are illustrated in the Supporting Information (Figures S10 to S15). Only the last pair has iron motion that has both substantial x and y character; the remaining five pairs have motion that is either along x (underlined values) or along y . Only two modes have predicted directional character that is not seen in the experimental spectra; the peaks at 297 and 348 cm^{-1} are predicted to have some z character that however is not apparent in the experimental directional data. Still, the z -component is a relatively small predicted component of these two modes.

Figure 5 depicts the predicted x , y , and z components of all modes with significant iron contributions and which are in strong agreement with the experimental data. This stacked bar graph clearly shows the predominant x OR y character of all major in-plane modes. All but one of the in-plane iron motions, and that with relatively low intensity, are in the directions between adjacent pairs of equatorial Fe–N_p bonds, that is either in the direction parallel or perpendicular to the Fe–N–O plane. What features of the [Fe(OEP)(NO)] molecule lead to such strongly polarized vibrational spectra? We believe that the vibrational spectra reflect the strong asymmetry in the interaction between the Fe $d\pi$ orbitals and the NO π^* orbitals parallel and perpendicular to the FeNO plane, owing to the nonlinear Fe–N–O group. The in-plane iron to porphyrin nitrogen bonds reveal the same asymmetry.[26,35] The strong asymmetry in the in-plane as well as the out-of-plane spectra reveal the wealth of information available in oriented crystal NRVS data. These data can now be used for the construction of more definitive tests of theoretical calculations and ultimately to better define ligand association and dissociation pathways.[36] Further experiments will determine how widespread the axial-ligand-based anisotropy is and the bonding character required for its observation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References and Notes

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methanol as the nonsolvent. The complex crystallizes in the triclinic space group $P1$, $a = 10.4204(1) \text{ \AA}$, $b = 10.5562(7) \text{ \AA}$, $c = 14.0425(7) \text{ \AA}$, $\alpha = 79.830(5)^\circ$, $\beta = 89.585(4)^\circ$, $\gamma = 80.264(4)^\circ$, $V = 1498.10(13) \text{ \AA}^3$, $Z = 2$.

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Figure 1.

Directional contributions to the VDOS of [Fe(OEP)(NO)] for x , y and z directions. For “ x ”, the beam is parallel to the porphyrin plane and to the Fe–N–O plane, for “ y ” the beam is parallel to the porphyrin plane and perpendicular to the Fe–N–O plane and for “ z ”, the beam is perpendicular to the porphyrin plane.

Figure 2. Observed contributions to the Fe VDOS in the 125–480 cm^{-1} region. The inset porphyrin diagrams illustrate direction of the incident X-ray beam. The two portions of the mirror diagram are on the same scale.

Figure 3. Measured (lines) and predicted (shaded) contributions to the VDOS from Fe motion along x (green) and y (red) directions, together with calculated $e^2_{Fe_x}$, $e^2_{Fe_y}$ values (bars) over the 125–480 cm^{-1} region. Porphyrin diagrams to the left present the in-plane orientations, parallel to Fe-NO (top panel) and perpendicular to Fe-NO (bottom panel).

Figure 4.

Depiction of the predicted 417 cm^{-1} mode. Color code: cyan, iron; blue, nitrogen; green, carbon. Hydrogen atoms have been omitted for clarity. In this and subsequent figures, each arrow is $100(m_j/m_{Fe})^{1/2}$ times longer than the zero-point vibrational amplitude of atom j , and the bonds to the Fe are omitted to enhance the visibility of the Fe motion.

Figure 5.

Bar graph showing DFT-predicted directional characteristics of all 32 modes with $e_{Fe}^2 > 0.01$. Color code is NRVS_x (green), NRVS_y (red), and NRVS_z (blue). The values of the predicted frequencies are given at each tick mark, but the horizontal scale is only approximately linear in frequency in order to avoid overlaps.