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Direct Probe of Iron Vibrations Elucidates NO Activation of Heme Proteins

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Nuclear resonance vibrational spectroscopy (NRVS) is an emerging site-specific probe of active site vibrational dynamics in metalloproteins.[1,2] NRVS is a synchrotron-based technique that uniquely targets the vibrations of a Mössbauer nucleus, such as ^{57}Fe, without interference from vibrations of other atoms, and reveals not only the frequency, but also the (mean squared) amplitude,[1b,2a] of all vibrations of the probe nucleus along the direction of the incident X-ray beam. Quantitative characterization of vibrational modes involving a reactive probe atom can illuminate mechanisms of complex biomolecules.

Reactions with heme proteins mediate the physiological effects of nitric oxide (NO). The proposed trigger for activation of soluble guanylate cyclase (sGC) is rupture of the covalent Fe-His bond[3a,b] in the heme-containing domain[3c,d] upon NO binding to the Fe. A thermodynamic consequence of NO-induced weakening of a trans Fe-imidazole bond, as observed in several heme systems, is that imidazole binding should weaken a trans Fe-NO bond. Model compound structures support such a reciprocal negative trans interaction,[4a] although protein structural data[4b-d] may not have sufficient precision to resolve the 3 pm increase in Fe-NO bond length due to imidazole binding.

On the other hand, vibrational frequencies respond sensitively to bond length changes of this magnitude, and it is therefore puzzling that the frequency attributed to stretching of the Fe-NO bond in six-coordinate imidazole-ligated heme proteins[5] is higher, rather than lower, than the frequencies observed for five-coordinate iron nitrosyl hemes. For example, the assigned Fe-NO stretching frequencies of the 5- and 6- coordinate NO complexes with myoglobin (MbNO) are 521 cm^{-1} and 552 cm^{-1}, respectively.[5c]

NRVS measurements on 6-coordinate MbNO suggest reexamination of this issue (Fig. 1A). The Fe-weighted vibrational density of states (VDOS) $D(\nu)$ samples the vibrational kinetic energy distribution (KED), with each mode contributing an area e_{Fe}^2 equal to the fraction of mode energy associated with Fe motion.[2a,d] The $e_{Fe}^2 = 0.11$ area of the feature at 547 cm^{-1} is well below the $e_{Fe}^2 = 0.23$-0.33 range that we observed for the FeNO stretching mode in a series of 5-coordinate nitrosyl porphyrins,[2d] but is clearly visible because of the distinctly improved signal quality compared to previously published NRVS measurements on myoglobin.[1a,b,d] In contrast, a mode with an area $e_{Fe}^2 = 0.25$ appears at 443 cm^{-1}, near a

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Supporting Information Available: Experimental information and fits to NRVS and Raman data. This material is free of charge via the Internet at http://pubs.acs.org.

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mode in the MbNO Raman spectrum previously shown to be sensitive to NO isotope substitution.[5b,c]

The frequency shift upon isotopic substitution of atom j provides an indirect estimate[2a] of e_j^2, assuming an unaltered mode composition. Raman measurements on isotopically enriched MbNO at ambient temperature (Fig. 1B, C) reveal a 3 cm^{-1} ^{54}Fe/^{57}Fe isotope shift for a mode at 451 cm^{-1}, and no significant Fe isotope sensitivity for the 556 cm^{-1} feature, in qualitative concord with the NRVS results. Furthermore, the value $e_{Fe}^2 = 0.29$ calculated[2a] from the isotope shift of the 451 cm^{-1} mode is in reasonable quantitative agreement with that determined for the 443 cm^{-1} mode observed in the NRVS data. The value $e_{Fe}^2 = 0.18$ determined from the isotope shift of an additional Fe-sensitive Raman feature at 360 cm^{-1} can be reconciled with a significantly larger value for the corresponding NRVS feature (Table 1) if both consist of two unresolved modes. These may be the (nominally degenerate) in-plane Fe-N$_{pyr}$ vibrations, which we previously observed to contribute prominently to the Fe VDOS of deoxyMb[1b] and of iron porphyrins.[2] NRVS and Raman isotope shift measurements reveal the KED (e_{Fe}^2, e_N^2, and e_O^2) over the FeNO fragment (Table 1).

The 556 cm^{-1} feature is characteristic for six-coordinate hemes with NO and imidazole as axial Fe ligands, and has successfully monitored interconversion between five- and six-coordinate states.[7a-c] One component of this complex feature undergoes a large frequency shift upon $^{14}\rightarrow^{15}$NO substitution (Fig. S2), and has previously been identified as Fe-NO stretching.[5] The KED (Table 1) indicates that 80-90% of the mode energy is localized on the FeNO fragment, but with the majority associated with motion of the NO nitrogen atom. This contrasts with the relatively uniform distribution of kinetic energy among all three atoms predicted[2d] for Fe-NO stretching in five-coordinate iron nitrosyls. On the other hand, Fe motion accounts for 25-30% of the 451 cm^{-1} mode energy, similar to the value $e_{Fe}^2 = 0.30$ determined experimentally for the Fe-NO mode in Fe(TPP)(NO).[2d]

NRVS measurements on oriented samples provide additional insight into vibrational mode character.[2b,1d] Fig. 2 compares the NRVS excitation probability measured on a powder and on a single crystal of Fe(TPP)(1-MeIm)(NO), oriented to enhance the contribution of modes involving Fe motion perpendicular to the mean plane of the porphyrin, and suppress in-plane Fe vibrations.

The data in Fig. 2 identify a mode at 440 cm^{-1} with dominant Fe motion perpendicular to the mean porphyrin plane. Normal mode analyses[2b,d,5a] indicate significant vibrational mixing between Fe-NO stretching and FeNO bending, and the reduced amplitude (e_{Fe}^2=0.16) of the 440 cm^{-1} mode determined from the VDOS of Fe(TPP)(1-MeIm)(NO) (Table S2) relative to the 443 cm^{-1} mode in MbNO and the 540 cm^{-1} mode in Fe(TPP)(NO) might reflect an enhanced contribution from FeNO bending. Nevertheless, the absence of any other mode above 200 cm^{-1} with dominant out-of-plane Fe motion supports a dominant Fe-NO stretching contribution. The 100 cm^{-1} frequency decrease from the 540 cm^{-1} frequency identified[2a,b,d] with Fe-NO stretching in the analogous 5-coordinate complex Fe(TPP)(NO) indicates that this mode is highly sensitive to the strength of the Fe-NO bond. DFT calculations on Fe(TPP)(NO) also revealed a strong sensitivity of the Fe-NO frequency to the Fe-N bond distance.[2b]

It would be desirable to confirm the negative trans interaction between the NO and Im ligands by monitoring the vibration of the Fe-Im bond in the presence and absence of NO. In fact, the Fe(TPP)(1-MeIm)(NO) single crystal data identify several modes below 200 cm^{-1} whose Fe motion is primarily orthogonal to the porphyrin plane. However, none approach the area $e_{Fe}^2 = 0.59$ predicted for a two-body Fe-1-MeIm oscillator. Further work will be needed to determine whether any of these modes correlates with the strength of the Fe-His bond.

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The vibrational data presented above identify modes with FeNO stretching character in the 440-450 cm^{-1} region for MbNO and Fe(TPP)(1-MeIm)(NO). The 70-100 cm^{-1} decrease relative to the Fe-NO frequencies in the corresponding 5-coordinate NO complexes[2a,5] confirms the weakening of the Fe-NO bond in the presence of a trans imidazole ligand. These results support a proposed mechanism[3a,b] for NO activation of heme proteins, and underline the value of NRVS as a direct probe of metal reactivity in complex biomolecules. The revised assignment for the Fe-NO stretch mode may also facilitate attempts[7b,d] to identify correlations between Fe-NO and N-O stretching vibrations, analogous to the well-established vibrational Fe-CO/C-O anticorrelation[7e] in heme-CO complexes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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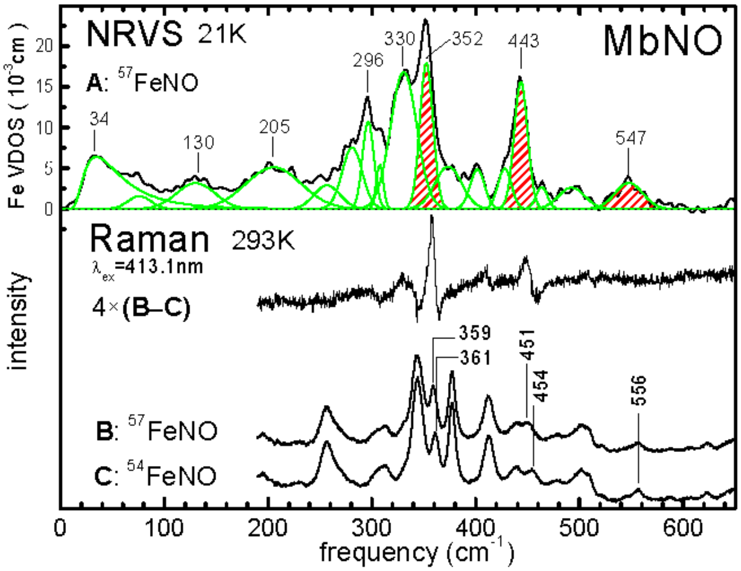

Figure 1.
Fe-weighted VDOS determined from NRVS data on ^{57}Fe-enriched horse MbNO at 21 K (A) using the program PHOENIX and Raman data on ^{57}Fe- and ^{54}Fe-enriched MbNO at ambient temperature (B, C) reveal modes with significant Fe contribution. An expanded trace shows the calculated Raman difference spectrum between B and C.

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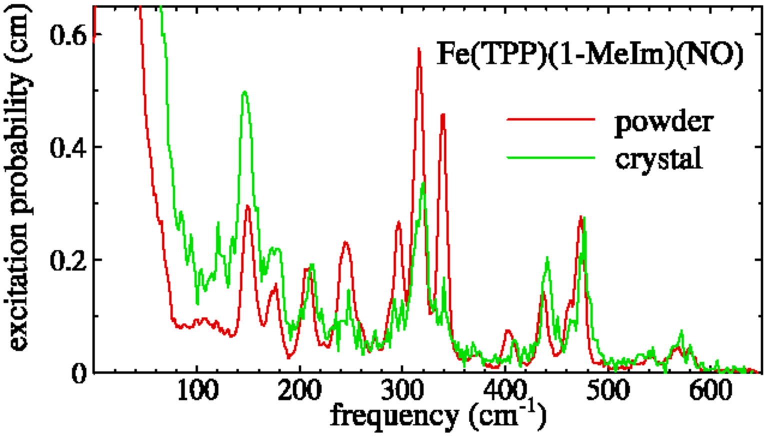

Figure 2.

Excitation probabilities determined from NRVS measurements on a powder (13 K, red) and on a crystal (82 K, green) of Fe(TPP)(1-MeIm)(NO), oriented with the X-ray beam incident along the {001} direction, which lies 13.8° from the normal of all porphyrins. The single crystal spectrum only includes motion along the X-ray beam, so that modes with Fe motion perpendicular to the porphyrin plane are therefore enhanced, and in-plane modes suppressed, in the crystal spectrum relative to the powder spectrum.

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Table 1

Vibrational kinetic energy distributions on the FeNO fragment in MbNO

NRVS (21 K)		**Raman (293 K)**				
frequency[a]	**Σe_{Fe}^2**	**frequency**[a]	**e_{Fe}^2**	**e_N^2**	**e_O^{2}**[b]	**Σe_j^2**
352 cm^{-1}	0.28	360 cm^{-1}	0.18	0.03	0.02[c]	0.2
443 cm^{-1}	0.25	451 cm^{-1}	0.29	0.10	0.04	0.43
547 cm^{-1}	0.11	556 cm^{-1}	0.14[c]	0.8	0.06	0.9

[a] Temperature-dependent frequency shifts may indicate structural changes,[6] and are under further investigation.

[b] Calculated from reported[5c] $N^{18}O/N^{16}O$ frequency shift.

[c] Estimated upper limit based on noise level; no shift observed.