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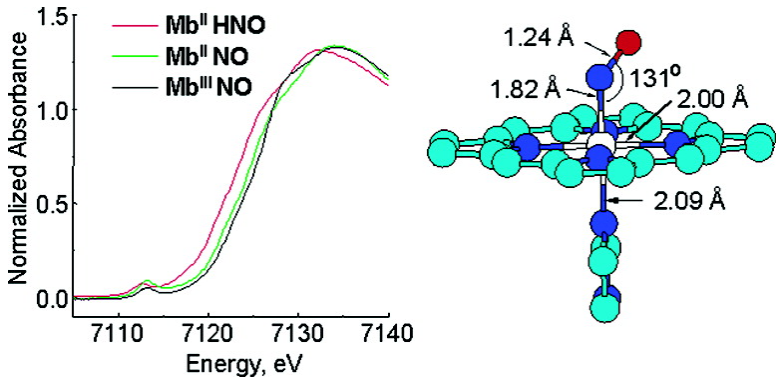

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Bonding in HNO-Myoglobin as Characterized by X-ray Absorption and Resonance Raman Spectroscopies

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Like nitric oxide (NO), many biological roles have been suggested for nitroxyl (NO^- or HNO) that involve its interaction with heme proteins.[1−7] Nitroxyl intermediates have been proposed in the catalytic cycles of the heme enzymes nitric oxide synthase[2] and the nitrite and nitric oxide reductases.[3,4] The pharmacological activity of nitroxyl-releasing drugs has likewise been suggested to be due to their reactivity with the various heme targets.[5−7] Isolable metal complexes of nitroxyl are rare, with only a handful of well-characterized examples.[7] Recently, an unusually stable HNO adduct of myoglobin, Mb-HNO (**1**), has been isolated,[8] and this provides a unique opportunity to study such a species in a biologically relevant system. Herein, we report resonance Raman (RR) and X-ray absorption characterizations[9] of **1**, which yields new information on bonding within this species.

The XANES spectra and the energies of the XANES features of **1**, $Mb^{II}NO$,[10] and $Mb^{III}NO$[10] are compared in Figure 1 and Table 1. The edge energy increased in the order **1** < $Mb^{II}NO$ < $Mb^{III}NO$, and the significantly lower edge energy for **1** suggests a metal-centered reduction.

Experimental and calculated XAFS spectra of **1** (Figure 2) led to the optimized heme structure in Figure 1, and a summary of XAFS parameters, in comparison with those for $Mb^{II}NO$ and Mb^{III}-NO,[10] is given in Table 1 (Supporting Table S1).[11]

The Fe−N(NO) bond lengths decrease and the Fe−N−O bond angles increase in the order **1**, $Mb^{II}NO$, $Mb^{III}NO$. The optimized Fe−N(His) bond is also longer for **1** as compared to that of Mb^{II}-NO or $Mb^{III}NO$, while there are no significant differences in the Fe−N_p bond lengths (Table 1). The N−O bond (1.24 Å) is longer than that for HNO in the gas phase (1.21 Å),[12] closer to a typical single N−O bond (1.25 Å).[13]

Resonance Raman spectra in the high- (1300−1700 cm^{-1}) and low-frequency (500−800 cm^{-1}) regions were obtained by Soret excitation (λ_{ex} = 413 nm) of natural abundance **1** ($H^{14}NOMb$) and $H^{15}NO$-enriched samples (Supporting Figure S3). The isotope difference spectrum revealed strong features in the 1350−1410 cm^{-1} region, with weaker features in the 630−660 cm^{-1} region.[14] The low-frequency feature is due to a single band that downshifts from 649 to 636 cm^{-1} upon ^{15}N substitution. On the basis of its frequency and isotope shift, this band is assigned to ν(Fe−N(H)O). The frequency of this vibration is significantly higher than those of Mb^{II}-NO and $Mb^{III}NO$ (554 and 595 cm^{-1}, respectively).[14b] This does not necessarily mean that the Fe−N bond is strongest in **1**, as the low Fe−N−O bond angle in **1** lowers the effective mass for ν(Fe−N(H)O), thereby increasing the frequency independent of any change in bond order.[15] Likewise, in highly bent geometries, the ν(Fe−N) and Fe−N−O bending modes may be highly mixed. Regardless, the frequency of the ν(Fe−N) mode of $H^{15}NO$-enriched **1** (at 636 cm^{-1}) is significantly higher than that of the ν(Fe−O) in isoelectronic oxyMb (at 575 cm^{-1}).[14]

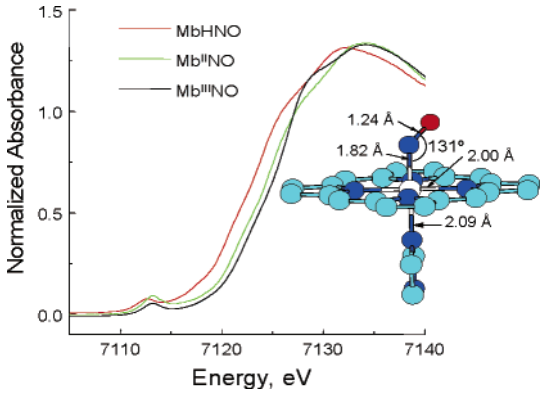

Figure 1. XANES spectra (10 K) of 40% aqueous glycerol solutions of **1** (inset: XAFS derived heme structure), and published spectra[10] of $Mb^{II}NO$ and $Mb^{III}NO$.

Table 1. XANES and XAFS Data for the HNO/NO Mb Adducts

	Mb-HNO	Mb^{II}-NO	Mb^{III}-NO
Fe−N (por), Å	2.00(2)[a]	1.99(2)[b]	2.00(2)[b]
Fe−N (His), Å	2.09(3)[a]	2.05(2)[b]	2.04(2)[b]
Fe−N (xNO), Å	1.82(2)[a]	1.76(2)[b]	1.68(2)[b]
edge energy, eV	7122.5[a]	7124.7[b]	7125.4[b]
N−O, Å	1.24(1)[a]	1.12(1)[b]	1.13(1)[b]
Fe−N−O, deg	131(6)[a]	150(2)[b]	180(4)[b]
ν_{N-O}, cm^{-1}	1385[a]	1613[c]	1927[c]
ν_{Fe-N}, cm^{-1}	651[a]	551[c]	595[c]

[a] From this work. [b] From ref 10. [c] From ref 14b.

In the high-frequency isotope difference data, shifts upon ^{15}N substitution were apparent in several bands. Spectral simulations obtained satisfactory fits (Supporting Figure S4) only by modeling the difference band envelope with a minimum of three features: a dominant one due to the very strong ν_4 heme mode, which *upshifts* slightly (1374 to 1375 cm^{-1}) upon ^{15}N substitution; a second feature due to a weaker band of uncertain origin (possibly the ν_{12} or ν_{29} heme mode)[16] that *downshifts* from 1408 to 1403 cm^{-1} upon ^{15}N substitution; and a third feature of comparable intensity that downshifts from 1385 to 1355 cm^{-1}. This last feature could not be resolved in the spectrum of natural abundance **1** but becomes visible as a shoulder for ^{15}N-labeled **1**. On the basis of its frequency and isotope shift, the 1385 cm^{-1} band is assigned to ν(N−O) of the bound HNO group.[17]

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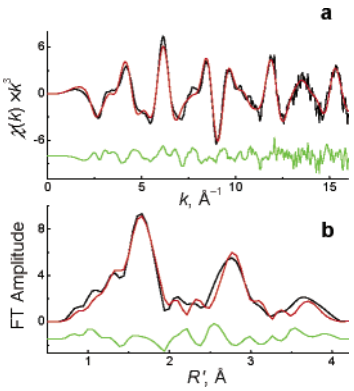

Figure 2. Experimental (black lines) and calculated (red lines) (a) XAFS and (b) Fourier transform (FT) of XAFS spectra of **1** (10 K). Fit residuals are shown by green lines. Applied window functions are shown in Figure S2 in the Supporting Information.

The Fe−N(H)O (1.82 Å), Fe−N_p (2.00 Å), and Fe−N_ϵ (2.09 Å) bond lengths obtained by EXAFS fitting compare well with those of crystallographically characterized Fe−porphyrin RNO complexes.[18] Likewise, the N−O bond length (1.24 Å) and Fe−N−O bond angle (131°) are within 1% of values reported for two recent crystallographically characterized HNO−metal complexes.[7d,e]

The frequency of the ν(N−O) band of **1** at 1385 cm^{-1} is close to that of a recently reported HN(O)−Ru^{II} porphyrin (1380 cm^{-1})[19] and is within the range of those reported for nonheme HNO−metal complexes.[7] The ν_4 heme mode, which is sensitive to both the Fe oxidation state and the extent of back-bonding, occurs at 1375 cm^{-1} for **1**, essentially the same frequency as observed for both Mb-NO and Mb-NO$^+$.[20] However, the XANES data suggest considerable metal-based reduction, as does the longer Fe−N_ϵ and Fe−N(H)O bonds of **1**, as compared to the other NO adducts. The lowering of the observed stretching ν(NO) (1385 cm^{-1}), relative to the value for free HNO (1563 cm^{-1}),[21] indicates the ligand acts as a π-acceptor, but much less so than its two redox siblings, NO and NO$^+$. The π-acidity of the HNO ligand in **1** is also demonstrated by its orthogonal orientation to proximal His93, which minimizes π-back-bonding competition;[8c] similar orthogonal ligand orientations have been observed in structures of RNO adducts of metalloporphyrins.[18] While these bonding aspects require further exploration, the results reported here provide definitive evidence for the structure of **1**, which supports the structure deduced on the basis of NMR experiments,[8c] and it is the first structural characterization of an HNO bound to a heme protein.

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Supporting Information Available: Experimental details, XAFS fitting details, resonance Raman spectra, and fitting details. This material is available free of charge via the Internet at http://pubs.acs.org.

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