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NO-Bound Myoglobin: Structural Diversity and Dynamics of the NO Ligand

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The structural properties of the heme−Fe−N−O moiety have been widely investigated since the discovery that the simple diatomic molecule nitric oxide (NO) coordinated to the heme prosthetic group is involved in a number of critical physiological processes such as message transduction (soluble guanyl cyclase) or NO transport and oxidation (hemoglobin, nitrophorin, myoglobin).[1] The issue how NO precisely coordinates to heme and how it interacts with the surrounding protein environment has been treated by numerous structural (see Table 1, Supporting information) and spectroscopic studies.[2−5] It was found that the structure of the Fe−N−O unit in model porphyrin complexes[6] is noticeably different from those observed in heme proteins (see Table 1, Supporting Information). A large variation of Fe−N−O angles ranging from 109 to 160° has been reported for different iron(II) six-coordinated heme proteins. The heme prosthetic group is chemically very similar in these proteins; thus, the conformational diversity of coordinated NO must arise from different steric and electronic interactions of the bound NO with the protein residues. It is therefore of fundamental importance to understand the extent to which the experimentally found variation in geometry originates from the specificity of the heme pocket environment and if that translates into the different ligand recognition strategies of the regulatory heme proteins.

To elucidate the structure of the Fe−N−O moiety in the myoglobin adduct with NO (Mb−NO) we applied femtosecond infrared polarization spectroscopy (previously used for the myoglobin−CO adduct[7,8]), from which the angle Φ between the IR transition dipole moment of bound NO and the normal to the heme plane was determined. A pump pulse excitation into the Soret band (centered at 400 nm) was employed, and the spectrally resolved IR probe pulse was used to measure the absorbance change at parallel ($\Delta A_{\parallel}$) and perpendicular ($\Delta A_{\perp}$) polarizations as a function of pulse delay, from which the angle Φ was obtained as[8] $\Delta A_{\perp}/\Delta A_{\parallel} = (4 - \sin^2\Phi)/(2 + 2\sin^2\Phi)$.

A temporal resolution of 150 fs allowed for an accurate determination of this angle not corrupted by geminate recombination of NO and by diffusional motion of the protein. Figure 1A shows the polarization-sensitive transient spectra recorded at 10 ps delay. The ratio of the signal at perpendicular and parallel polarizations enabled us to derive a value of 22.7 ± 4° for the angle Φ (Figure 1A). The bleach recovery dynamics, simulated with a multiexponential decay with time constants of 1, 4, 42, and 238 ps (Figure 1B, red), are at long delay times in accordance with previously reported work.[2] The polarization ratio, solely determined by the bound NO bleach as the photoinduced IR intensities of underlying

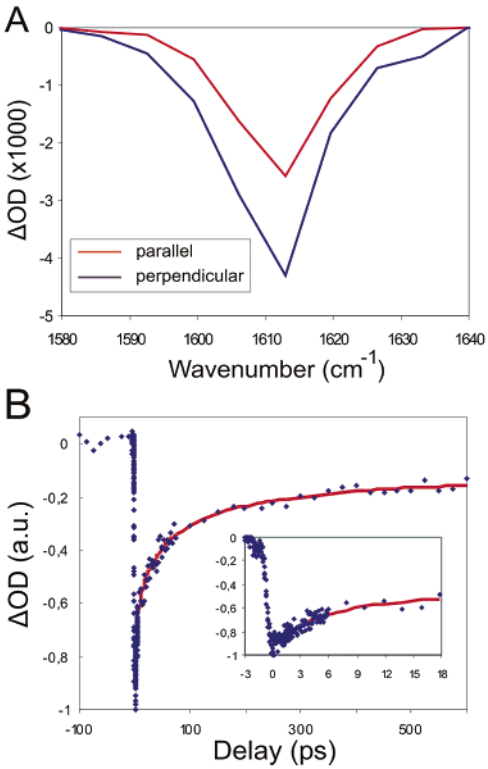

Figure 1. Polarized absorption spectra around the bound NO stretching band (A) measured at 10 ps after Mb−NO photolysis and the transients ascribing NO rebinding dynamics over 0.1−600 ps (B).

heme or protein vibrations have been shown to be small,[9] is identical at all delays. Interestingly, our data constitute the first evidence of a significant fraction (35%) of NO recombining with the heme−Fe(II) within the first 5 ps after the photolysis, making myoglobin a very efficient NO scavenger.[1] This observation allows us to conclude that the quantum yield for NO dissociation induced by a pump at 400 nm is between 0.8 and 1.0, which is much higher than the previously reported value of 0.5 obtained from an optical pump−probe measurement.[3] The difference lies primarily in the fact that in the latter study, the geminate recombination yield is derived by estimating the early time ligand dynamics from an extrapolation of the data from 10 ps delay down to 0 ps.

The femtosecond infrared polarization technique provides unique spectroscopic characteristics of the Fe−X−O moiety (X = C or N), in terms of relative directions of transition dipole moments. However, the IR transition dipole moment direction deviates significantly from the X−O bond direction if the X−O ligand is bound to Fe^{II} in a bend-on conformation. This has previously been shown[10] in a DFT analysis of distorted models of Mb−CO. To

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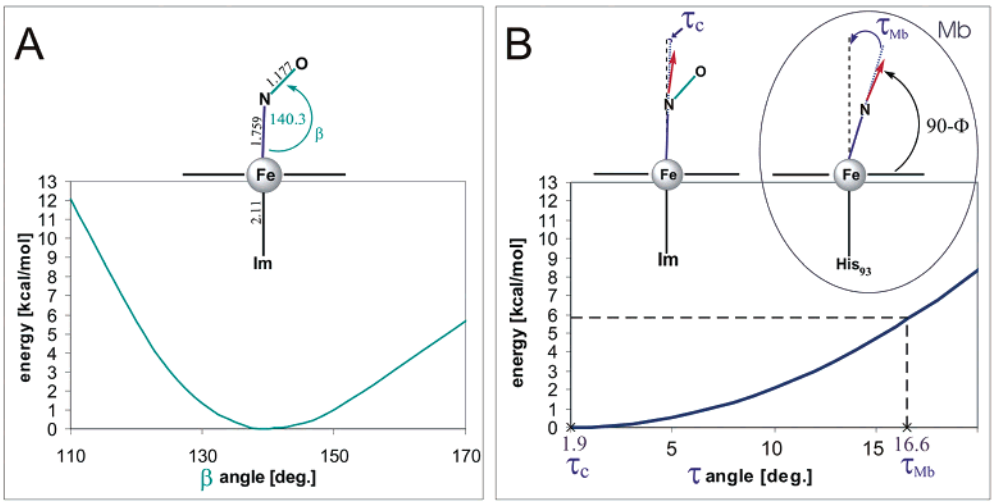

Figure 2. Distortion energies for bending alone (A), tilting alone (B), and transition dipole moment direction calculated via DFT (B). (A) Energy cost for bending of the Fe−N−O unit. Calculated Im−(Fe^{II}P)−NO minimum energy structure. (B) Energy cost for tilting of the Fe−N unit. Transition dipole moment direction (red arrow) essentially lies in the direction of the Fe−N(NO) (with around 6.1° deviation). Left structure: τ_c − value of tilt angle for structure from A. Right structure: figure visualizing data from the polarization experiment. Measured angle $\Phi = 22.7°$ (see text). The value of the tilt angle in Mb is $\tau_{Mb} = 16.6°$.

establish how the spectroscopic properties of the Fe−N−O unit correlates with its actual structure, the active site of Mb−NO was modeled by an Im−(Fe^{II}P)−NO complex, containing porphyrine (P) and imidazole (Im) as the axial ligand. The structure of the Im−(Fe^{II}P)−NO complex was first optimized and the Fe−N−O bending potential computed with respect to minimum energy (Figure 2A). The distortion energy for variation of the bend angle (β) from 140.3° (corresponding to minimum energy) is very small. Approximately 1 kcal/mol energy is required to change this angle by about 10°. Full vibrational analysis established the direction of the calculated IR transition dipole moment to be much closer to the Fe−N bond (6.1° away) than to the N−O eigenvector associated with the NO stretching motion (Figure 2B). This finding is consistent with the previous analysis for Fe−C−O and has a rationale in terms of Fe/d_π−NO/π^* back-bonding.[10] Thus, the direction of the IR transition dipole moment of the bound NO vibration with respect to the normal to the heme plane obtained from the experiment corresponds essentially to off-axis tilting of the Fe−N bond.

The estimated value of the Fe^{II}−N(NO) tilt angle (τ) for myoglobin acquired from the polarization experiment is unexpectedly high and equal to 16.6° (Figure 2B), constituting the first example of off-axis tilting of a nitrosyl ligand bound to a heme protein. This finding is consistent with a recently reported high-quality crystal structure study of 6-coordinated ferrous heme derivatives[6] resulting in values for the angle τ between 1.8 and 6.5°. The larger extent of the Fe−N tilting observed by us in Mb−NO in solution at room temperature most likely reflects the influence of strengthened proximal bonding[11] (Fe−His 93) and the polarity of the binding pocket,[4,5] including hydrogen bonding[11] to the distal histidine (His 63). On the other hand, the tilting of Fe−N unit from 1.9° (corresponding to minimum energy) to 16.6° (recorded for Mb) requires a modest amount of energy, estimated with a harmonic potential analysis to be no more than 6 kcal/mol (Figure 2B). The actual value of energy decreases even further due to the cooperativity that exists between bending and tilting coordinates, as revealed by normal coordinate analysis. The off-diagonal interaction force constant couples these two distortion coordinates, as in the case of Fe−CO.[10] However, due to the uncertainties associated with the Fe−NO bend angle, a more rigorous energy estimation is not possible.

The direct comparison of the level of energy stored in form of electrostatic forces of the heme pocket environment (+30 to −12 kcal/mol for Mb[12]) with the DFT-predicted distortion energy of the Fe−N−O unit (Figure 2) allows one to conclude that this amount is sufficient to control the structure of the Fe−NO moiety. This is reflected in a wide range of values of the Fe−N−O angle in different heme proteins (see Table 1, Supporting Information). Therefore, bound NO can be seen as a local probe of electrostatic properties of the heme pocket. In some regulatory heme proteins, electrostatic interactions with a substrate located in a vicinity of the active site alter not only the geometry of the diatomic ligand but also the binding kinetics,[13] resulting in a specific NO recognition strategy.

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Supporting Information Available: Experimental Section and Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Brunori, M. *Trends Biochem. Sci*. **2001**, *26*, 21−3.
(2) Walda, K. N.; Liu, X. Y.; Sharma, V. S.; Magde, D. *Biochemistry* **1994**, *33*, 2198−209.
(3) Ye, X.; Demidov, A.; Champion, P. M. *J. Am. Chem. Soc*. **2002**, *124*, 5914−24.
(4) Tomita, T.; Hirota S., Ogura; T., Olson, J. S.; Kitagawa, T. *J. Phys. Chem. B* **1999**, *103*, 7044−7054.
(5) Coyle, C. M. et al. *Biochemistry* **2003**, *42*, 4896−903.
(6) Wyllie, G. R.; Schulz, C. E.; Scheidt, W. R. *Inorg. Chem.* **2003**, *42*, 5722−5734.
(7) Hansen, P. A.; Moore, J. N.; Hochstrasser, R. M. *Chem. Phys.* **1989**, *131*, 49−62.
(8) Lim, M.; Jackson, T. A.; Anfinrud, P. A. *Science* **1995**, *269*, 962−6.
(9) Miller, L. M.; Pedraza A. J.; Chance M. R. *Biochemistry* **1997**, *36*, 12199−207.
(10) Spiro, T. G.; Kozlowski, P. M. *Acc. Chem. Res.* **2001**, *34*, 137−44.
(11) Brucker, E. A.; Olson, J. S.; Ikeda-Saito, M.; Phillips, G. N., Jr. *Proteins* **1998**, *30*, 352−6.
(12) Phillips, G. N., Jr.; Teodoro, M. L.; Li, T.; Smith, B.; Olson J. S. *J. Phys. Chem. B* **1999**, *103*, 8817−8829.
(13) Negrerie, M. et al. *J. Biol. Chem.* **1999**, *274*, 24694−702.

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