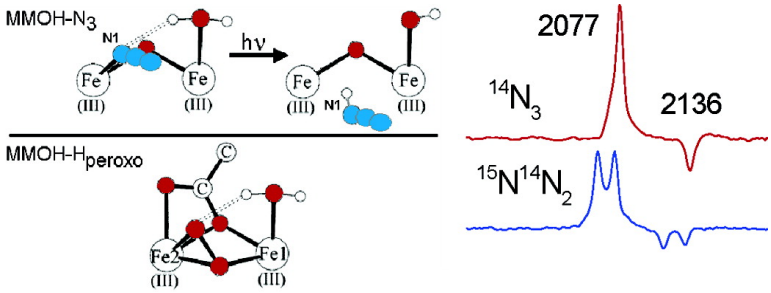
Communication

Fourier Transform Infrared Characterization of the Azido Complex of Methane Monooxygenase Hydroxylase from *Methylococcus capsulatus* (Bath)

Shen Lu, Matthew H. Sazinsky, James W. Whittaker, Stephen J. Lippard, and Pierre Monne-Loccoz

J. Am. Chem. Soc., **2005**, 127 (12), 4148-4149• DOI: 10.1021/ja044414u • Publication Date (Web): 03 March 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 03/03/2005

Fourier Transform Infrared Characterization of the Azido Complex of Methane Monooxygenase Hydroxylase from *Methylococcus capsulatus* (Bath)

Shen Lu,[†] Matthew H. Sazinsky,[‡] James W. Whittaker,[†] Stephen J. Lippard,*[,‡] and Pierre Moënne-Loccoz*[,†]

Department of Environmental & Biomolecular Systems, OGI School of Science & Engineering, Oregon Health & Science University, Beaverton, Oregon 97006-8921, and Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received September 14, 2004; E-mail: Lippard@mit.edu; ploccoz@ebs.ogi.edu

The hydroxylase enzyme component of soluble methane monooxygenase (MMOH) in methanotrophic bacteria utilizes O_2 and a carboxylate-bridged diiron center to oxidize methane to methanol. In the course of this reaction with O_2, the diiron(II) unit (H_{red}) forms a diiron(III) peroxide intermediate (H_{peroxo}), which evolves into a diiron(IV) di-μ-oxo species (Q), which in turn reacts with methane. The enzyme is an $\alpha_2\beta_2\gamma_2$ heterodimer and requires the presence of a 16-kDa regulatory protein, MMOB, to optimize the hydroxylase activity.[1] Here, we investigate the azido complex of oxidized MMOH (H_{ox}) by using FTIR spectroscopy. Because the highest occupied π^{nb} orbitals of azide are homologous to the filled π^* orbitals of peroxide, the diiron(III) azido complex can provide information about the coordination geometry of transient peroxo species that form in carboxylate-bridged diiron proteins.[2]

Incubation of MMOH with a 50-fold excess of sodium azide results in the appearance of an ~440-nm absorbance band in the UV−vis spectrum, which may be readily assigned to an azide-to-iron(III) charge-transfer band (Figure S1, Supporting Information).[3] Attempts to define the geometry of azide binding by using X-ray crystallography were unsuccessful (Experimental Details, Supporting Information). Although a low-frequency band in the resonance Raman spectra of MMOH−N_3 was tentatively assigned to an Fe−N_3 stretching mode, the internal symmetric and asymmetric azido stretches could not be detected (Figure S2, Supporting Information). However, the ν_{as}(NNN) mode was extracted from FTIR spectra by taking advantage of the photolabile character of iron-azido complexes. This approach has often been applied to iron carbonyl complexes where the dissociated state can be trapped at low temperature.[4,5] For example, a myoglobin-CO "dark" minus "illuminated" FTIR difference spectrum shows a positive band at ~1940 cm^{-1} corresponding to the heme iron carbonyl ν(C−O) and a negative signal at ~2130 cm^{-1} from the photolyzed CO that docks within internal cavities of the protein.[4] We recently applied this technique to the characterization of iron nitrosyl complexes in the R2 protein of ribonucleotide reductase.[6] To validate this approach with non-heme iron-azido complexes, we first present FTIR data obtained on the structurally well-characterized terminal azido complex of iron superoxide dismutase from *Echerichia coli*.[7] Using RR spectroscopy, the ν_{as}(NNN) mode is observed at 2057 cm^{-1}.[8] When the complex is formed with $^{15}NN_2$, ν_{as}(NNN) splits into two components at 2039 and 2051 cm^{-1}.[8] Accordingly, the FTIR difference spectrum "dark" minus "illuminated" shows these same frequencies (±1 cm^{-1}) as positive bands, associated with negative bands at 2025 and 2014 cm^{-1} assigned to the dissociated $^{14}N_3^-$ and $^{15}NN_2^-$, respectively (Figure 1A,B). These frequencies are 20 cm^{-1} lower than ν_{as}(NNN) for azide ion in solution. The 10-cm^{-1} bandwidth of these negative signals is only a third of that of ν_{as}(NNN) from bulk azide. These results indicate that the photolyzed azide remains within the protein matrix and experiences a well-defined environment that favors the [$^-$N=N$^+$=N$^-$] resonance form.

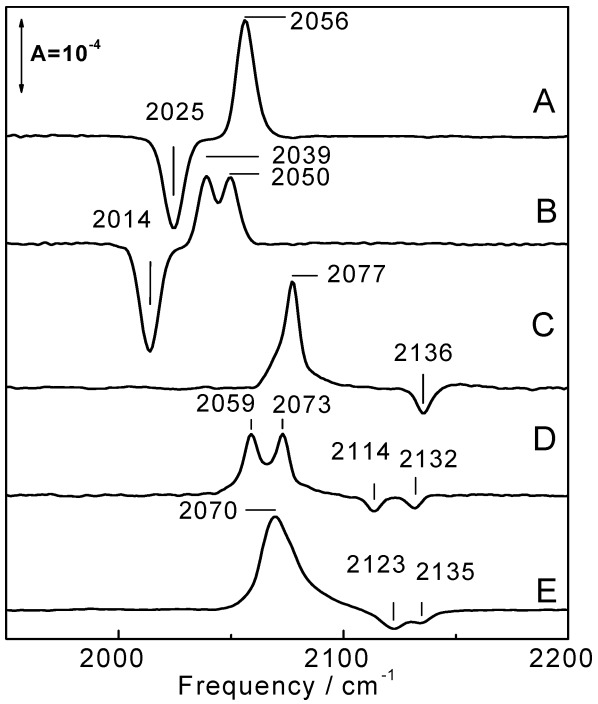


Figure 1. FTIR difference spectra ("dark" minus "illuminated") of the azido complexes in FeSOD and MMOH at 15 K. FeSOD−N_3 (A), FeSOD−$^{15}NN_2$ (B), MMOH−N_3 (C), MMOH−$^{15}NN_2$ (D), and MMOH−N_3 in D_2O (E). The FeSOD−N_3 spectra were divided by 3 to facilitate comparison with the MMOH−N_3 data (Experimental Details, Supporting Information).

Comparison of the intensity of the 2056 cm^{-1} band in the FTIR spectrum of FeSOD−N_3 before and after illumination indicates that the photolyzed state is trapped in 17 ± 5% of the protein (Figure S3, Supporting Information). This photolyzed population is obtained after a few minutes of white light illumination at 15 K, and subsequent illumination does not produce any further changes. Because the frequency and spectral envelope of the ν_{as}(NNN) mode before illumination match that of the difference spectrum, the low apparent yield of the photodissociation process does not appear to be due to a subpopulation of photolabile azido complex distinct from nonlabile complexes. Rather, it must reflect differences in ligand migration and geminate rebinding of the photolyzed ligand. Different side chain configurations sampled by protein dynamics can be trapped at cryogenic temperatures and produce an inhomogeneous distribution where only a fraction of protein

[†] Oregon Health & Science University.
[‡] Massachusetts Institute of Technology.

10.1021/ja044414u CCC: $30.25

conformers present a migration route for the dissociated ligand. In the fraction of protein conformers that do not offer an escape route, the larger size of azide compared to CO and the electrostatic attraction between the photolyzed azide anion and the iron(III) are expected to promote geminate rebinding at 15 K.

In the azido complex of MMOH, ν_{as}(NNN) occurs at 2077 cm^{-1} and splits into two bands at 2059 and 2073 cm^{-1} with $^{15}NN_2$ (Figure 1C,D). The ν_{as}(NNN) band is 21 cm^{-1} higher than that in FeSOD−N_3 and 5 cm^{-1} higher than the η^1-terminal azido complex formed in steroyl-acyl carrier protein Δ^9 desaturase (Δ^9D).[2] The 14 cm^{-1} splitting of ν_{as}(NNN) with $^{15}NN_2$ is comparable to that observed in the FeSOD−N_3 and Δ^9D−N_3 complexes and is consistent with an η^1-terminal binding of azide to the diiron(III) cluster in MMOH. In contrast, alternative μ-1,1 or μ-1,3 bridging geometries are expected to give rise to greater and smaller splittings, respectively.[2] The vibrational data define an azide binding that favors the [Fe^{3+}−N^{2-}−N^{+}≡N] resonance form relative to the symmetric [Fe^{3+}−N^{-}=N^{+}=N^{-}] one. Incubation of the MMOH-azide complex in D_2O results in a 6-cm^{-1} downshift of the ν_{as}(NNN) and suggests that the azido group is engaged in hydrogen bond interaction(s) at the diiron site (Figure 1E). Such a hydrogen bonding interaction at the 1N atom of the azido group is expected to increase the asymmetry of the two N−N bonds and to upshift ν_{as}(NNN). Deuterium exchange at the hydrogen bond donor will diminish the strength of the hydrogen bond and result in a downshift of the ν_{as}(NNN). The terminal water molecule bound to Fe1 in all MMOH crystal structures[9] is a likely source of the H-bond donor.

The FTIR difference spectra of the $^{14}N_3$ and the $^{15}N^{14}N_2$ complexes of MMOH also display negative signals at 2136 cm^{-1} (Figure 1C) and at 2114 and 2132 cm^{-1} (Figure 1D), respectively. These frequencies are 6 cm^{-1} higher than ν_{as}(NNN) from hydrazoic acid in aqueous solution.[10] As in deuterated hydrazoic acid, Fermi resonance splitting of the ν_{as}(NNN) is observed with deuterated samples (Figure 1E). Thus, the photodissociation of the iron-azido complex in MMOH is accompanied by a proton transfer that results in the formation of an N_3H molecule that remains trapped within the substrate pocket.

Analyses of the signal intensities in the FTIR difference spectra obtained at 15 K suggest that 15 ± 10% of photolyzed state can be trapped in MMOH, a yield comparable to that of FeSOD−N_3. In contrast, the temperature dependence for rebinding is different in the two metalloproteins. Rebinding starts to occur at 50 K in FeSOD, but the temperature must be raised above 200 K for rebinding in MMOH. This observation supports the formation of distinct products of photolysis in FeSOD−N_3 and MMOH−N_3. Because the photolysis is associated with transfer of a proton in MMOH, which does not occur in FeSOD, the rebinding process in MMOH faces a larger energy barrier that includes reorganization energy from the proton donor to the azido group. Comparisons of UV−vis absorption spectra obtained before and after the FTIR photolysis experiments and of successive FTIR difference spectra obtained after annealing above 200 K confirm the reversibility of the photolytic process (Figures S4 and S5). The FTIR experiments on MMOH−N_3 were reproduced with 2 and 4 equiv of MMOB, but the presence of this regulatory protein had no effect on the results described above (data not shown). Perturbation upon addition of MMOB might have been anticipated, since MMOB is believed to dock at the cleft of the $\alpha_2\beta_2\gamma_2$ dimer interface of MMOH and strongly influences the O_2 reactions of the diiron cluster.[11]

The vibrational signature of MMOH−N_3 indicates that the azide is bound terminally to one iron(III) and suggests that the azide-1N atom is engaged in a hydrogen bonding interaction. An aqua ligand would constitute a good candidate for a hydrogen bond donor to the azido group and could also act as proton donor during photolysis. A proton transfer from the aqua ligand to the leaving azide group would allow the photolysis to occur without a change in the overall charge of the diiron cluster. This proposal has mechanistic significance to the O_2 activation in MMOH. Recently, a theoretical study of the MMOH reaction with O_2 has predicted the presence of a hydrogen bond between an aqua ligand to Fe1 and the oxygen atom bound to Fe2 in the superoxo and peroxo complexes.[12] Our interpretation supports these predictions, but alternative models involving other ionizable groups at the diiron site are equally reasonable. For example, rather than N_3^-, HN_3 may react with the oxidized cluster to from the azido complex, converting a bridging μ-hydroxo group to a μ-aqua ligand. The data suggest an azide binding that differs significantly from what was recently observed in a crystal structure of the azido complex of toluene/*o*-xylene monooxygenase.[13] In this related diiron enzyme, the azido group is bound asymmetrically in an μ-1,1 semibridging geometry and engages a hydrogen bond interaction at its N3 atom with a water molecule. A comparative FTIR study of this complex and further characterization of MMOH−N_3 and its diiron center after photolysis are underway.

Acknowledgment. This work was supported by the NIH (P.M.-L., GM18865; S.J.L., GM32134; and J.W.W., GM42680).

Supporting Information Available: Experimental details, UV−vis spectra of FeSOD-N_3 and MMOH-N_3, low-frequency RR spectra of Hox and MMOH-N_3, and successive FTIR difference spectra of MMOH−N_3 obtained after an annealing cycle. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Feig, A. L.; Lippard, S. J. *Chem. Rev.* **1994**, *94*, 759−805. (b) Wallar, B. J.; Lipscomb, J. D. *Chem. Rev.* **1996**, *96*, 2625−2658. (c) Merkx, M.; Kopp, D. A.; Sazinsky, M. H.; Blazyk, J. L.; Müller, J.; Lippard, S. J. *Angew. Chem., Int. Ed.* **2001**, *40*, 2782−2807.
(2) (a) Pate, J. E.; Ross, P. K.; Thamann, T. J.; Reed, C. A.; Karlin, K. D.; Sorrell, T. N.; Solomon, E. I. *J. Am. Chem. Soc.* **1989,** *111*, 5198−5209. (b) Kurtz, D. M., Jr.; Shriver, D. F.; Klotz, I. M. *Coord. Chem. Rev.* **1977**, *24*, 145−178. (c) Freier, S. M.; Duff, L. L.; Shriver, D. F.; Klotz, I. M. *Arch. Biochem. Biophys.* **1980**, *205*, 449−63. (d) Ai, J.; Broadwater, J. A.; Loehr, T. M.; Sanders-Loehr, J.; Fox, B. G. *J. Biol. Inorg. Chem.* **1997**, *2*, 37−45. (e) Dori, Z.; Ziolo, R. F. *Chem. Rev.* **1973**, *73*, 247−254.
(3) Atta, M.; Fontecave, M.; Wilkins, P. C.; Dalton, H. *Eur. J. Biochem.* **1993**, *217*, 217−223.
(4) (a) Antonini, E., Brunori, M. *Hemoglobin and myoglobin in their reactions with ligands*; North-Holland: Amsterdam, 1971. (b) Bagley, K. A.; Van Garderen, C. J.; Chen, M.; Duin, E. C.; Albracht, S. P. J.; Woodruff, W. H. *Biochemistry* **1994**, *33*, 9229−9236.
(5) (a) Alben, J. O.; Moh, P. P.; Fiamingo, F. G.; Altschuld, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 234−237. (b) Ansari, A.; Berendzen, J.; Braunstein, D.; Cowen, B. R.; Frauenfelder, H.; Hong, M. K.; Iben, I. E.; Johnson, J. B.; Ormos, P.; Sauke, T. B.; Scholl, R.; Steinbach, P. J.; Vittitow, J.; Young, R. D. *Biophys. Chem.* **1987**, *26*, 337−355.
(6) Lu, S.; Libby, E.; Saleh, L.; Xing, G.; Bollinger, J. M., Jr.; Moënne-Loccoz, P. *J. Biol. Inorg. Chem.* **2004**, *9*, 818−827.
(7) Lah, M. S.; Dixon, M. M.; Pattridge, K. A.; Stallings, W. C.; Fee, J. A.; Ludwig, M. L. *Biochemistry* **1995**, *34*, 1646−1660.
(8) Xie, J.; Yikilmaz, E.; Miller, A. F.; Brunold, T. C. *J. Am. Chem. Soc.* **2002**, *124*, 3769−3774.
(9) (a) Rosenzweig, A. C.; Frederick, C. A.; Lippard, S. J.; Nordlund, P. *Nature* **1993**, *366*, 537−543. (b) Whittington, D. A.; Lippard, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 827−838. (c) Elango, N.; Radhakrishnan, R.; Froland, W. A.; Wallar, B. J.; Earhart, C. A.; Lipscomb, J. D.; Ohlendorf, D. H. *Protein Sci.* **1997**, *6*, 556−568.
(10) Moore, C. B.; Rosengren, K. *J. Chem. Phys.* **1966**, *44*, 4108−4115.
(11) (a) Walters, K. J.; Gassner, G. T.; Lippard, S. J.; Wagner, G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7877−7882. (b) Wallar, B. J.; Lipscomb, J. D. *Biochemistry* **2001**, *40*, 2220−2233.
(12) Gherman, B. F.; Baik, M. H.; Lippard, S. J.; Friesner, R. A. *J. Am. Chem. Soc.* **2004**, *126*, 2978−2990.
(13) Sazinsky, M. H.; Bard, J.; Di Donato, A.; Lippard, S. J. *J. Biol. Chem.* **2004**, *279*, 30600−30610.

JA044414U