EXAFS Spectroscopic Evidence for an Fe=O Unit in the Fe(IV) Intermediate Observed during Oxygen Activation by Taurine:α-Ketoglutarate Dioxygenase

Pamela J. Riggs-Gelasco,*,† John C. Price,‡ Robert B. Guyer,‡ Jessica H. Brehm,† Eric W. Barr,‡ J. Martin Bollinger, Jr.,*,‡,§ and Carsten Krebs*,‡,§

Department of Chemistry and Biochemistry, College of Charleston, Charleston, South Carolina 29424, and Department of Biochemistry and Molecular Biology and Department of Chemistry, Penn State University, University Park, Pennsylvania 16802

Received March 26, 2004; E-mail: GelascoP@cofc.edu; ckrebs@psu.edu; jmb21@psu.edu

The α-ketoglutarate-dependent dioxygenases represent a large and functionally diverse family of enzymes that catalyze many reactions with environmental, pharmacological, and medical importance. For example, these enzymes catalyze steps in the biosynthesis of antibiotics and collagen, degradation of xenobiotics, repair of alkylated DNA, and cellular sensing of oxygen and response to hypoxia.[1,2] These enzymes couple the reductive activation of dioxygen to the hydroxylation of their substrates and the decarboxylation of the cosubstrate, α-ketoglutarate (αKG^3), to succinate.[2,4] In each member of the family, the reaction is carried out at a mononuclear non-heme Fe center, which is facially coordinated by a conserved His_2Asp/Glu motif from the protein. The mechanism is also thought to be conserved and is shown below in Scheme 1, adapted for the subject of this study, *Escherichia coli* taurine:αKG dioxygenase (TauD). Key features of this mechanism are (1) addition of oxygen to the square pyramidal Fe(II) center of the quaternary TauD:Fe(II):αKG:taurine complex to yield **I**, (2) attack of the uncoordinated O-atom of the O_2-moiety on C2 of αKG to form the bicyclic species **II**, (3) cleavage of the O–O bond and decarboxylation resulting in the $Fe(IV)=O^{2-}$ species **III**, (4) abstraction of an H-atom from the substrate to yield **IV**, (5) hydroxylation via oxygen rebound, and (6) dissociation of the products and re-binding of substrate and cosubstrate. We recently detected an intermediate in the TauD reaction following mixing of the quaternary complex with O_2 and showed that it contains formally an Fe(IV) center in the high-spin configuration.[5] We then demonstrated that decay of the intermediate exhibits a large kinetic isotope effect

upon substitution of the H-atoms bonded to C1 with deuteria ($k_H/k_D \approx 35$). This observation suggests an $Fe(IV)=O^{2-}$ assignment for the intermediate, because this species is proposed to activate the C–H/D bond of taurine.[6] The detection by Hausinger and coworkers of an O-isotope sensitive band (821 cm^{-1} for ^{16}O and 787 cm^{-1} for ^{18}O) using continuous-flow resonance Raman spectroscopy is consistent with this assignment.[7] In this work, we have characterized this intermediate by rapid freeze-quench X-ray absorption spectroscopy. We detect a short, 1.62 Å Fe–O interaction, which is present in the spectra of samples containing large amounts of the intermediate but not in the spectra of samples that were prepared with rigorous exclusion of oxygen. This result corroborates the hypothesis that the intermediate contains an Fe=O structural motif.

Samples containing large amounts of the intermediate, **1**, were prepared as previously described, but with minor modifications.[8] Mössbauer spectra reveal that 79 ± 3% of the total Fe in these samples is in form of the Fe(IV)-intermediate (see Figure S1). The Mössbauer spectrum of the anaerobic control sample, **2**, shows that it does not contain the intermediate. Figure 1 shows the XANES spectrum of **2** (thin line) and of **1** (bold line). The edge of **1** is substantially higher in energy than that of **2**, indicating that the Fe of the intermediate is more oxidized than the Fe(II) present in the starting complex. Although the edge energy can be indicative of the oxidation state of the absorber, an assignment of oxidation number from XAS alone is complicated in this case by several factors.[9] First, the intermediate gives rise to the largest isomer shift ($\delta = 0.31$ mm/s) reported for relevant Fe(IV) complexes, suggesting that its Fe center may have substantial Fe(III) character. Also, the sample contains 21% Fe(II) initially, and partial photoreduction of the intermediate was demonstrated by Mössbauer characterization of the sample after X-ray exposure. Any or all of these complications may skew the edge toward lower energy. Nevertheless, the edge energy (7123 eV, defined as the inflection point of the rising edge) and, more importantly, its general shape are consistent with other

Scheme 1. Proposed Mechanism for Oxygen Activation by TauD*a*
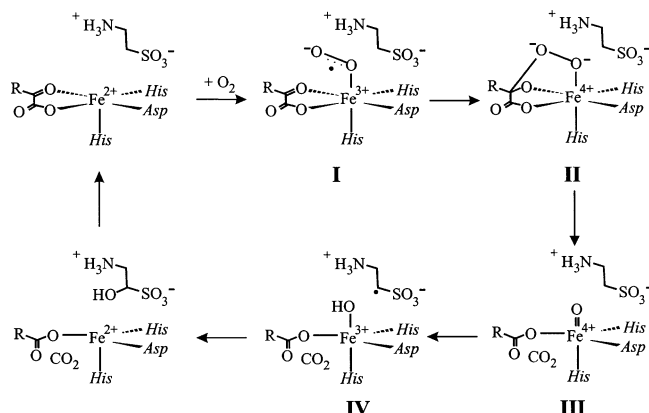
a Species **I**–**IV** are proposed reaction intermediates, and the structures shown for **I**, **II**, and **III** are only one of several possible resonance structures.
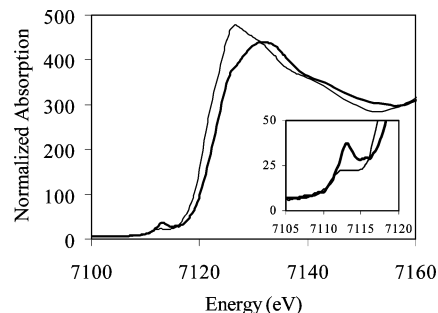
† Department of Chemistry and Biochemistry, College of Charleston.
‡ Department of Biochemistry and Molecular Biology, Penn State University.
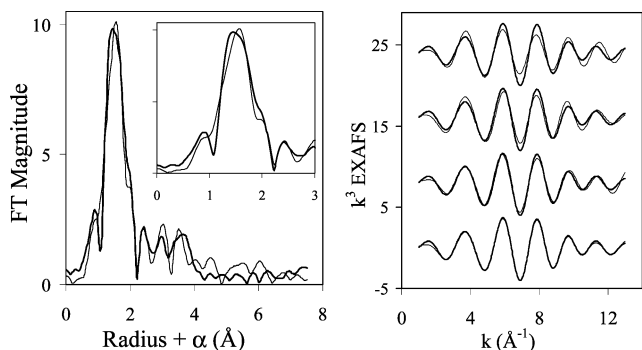§ Department of Chemistry, Penn State University.

Figure 1. X-ray absorption spectra (Fe K-edge) of **1** (bold line) and **2** (thin line). The inset shows a magnification of the preedge feature.

Figure 2. Left panel: FT of the Fe K-edge EXAFS data [$k^3\chi(k)$] for **1** (bold line) and **2** (thin line). FT range: 1−13 Å. Right panel: Fourier-filtered EXAFS spectra [$k^3\chi(k)$] of **1** (bold line) and fits **A**, **B**, **C**, and **D** (top to bottom, thin lines, see Table S1 for fit parameters). **A**: one shell at 2.05 Å; **B**: two shells at 2.06 and 2.42 Å, **C**: two shells at 1.62 and 2.05 Å, and **D**: three shells at 1.62, 2.05, and 2.42 Å.

examples in the literature of Fe(IV) complexes and protein intermediates.[10,11]

The inset of Figure 1 shows a closeup of the feature arising from the 1s−3d transition.[12] The increased intensity in sample **1** is indicative of a less centrosymmetric environment and may be caused by the presence of a short Fe−O bond. Although the edges of Fe-(IV)=O complexes reported in the literature lack the very intense preedge features characteristic of other M=O complexes (M = Mo, V, Cr),[13,14] in all cases, the preedge feature is still sharper and larger in area than the corresponding control samples of lower oxidation state.[10,11,15]

By far the most compelling evidence for the presence of a short Fe−O bond in the formally Fe(IV) intermediate is the fitting analysis of the EXAFS oscillations. Fits to the Fourier-filtered first-shell data and unfiltered data require a short, 1.62 ± 0.01 Å Fe−O interaction to best model the data (Figure 2, right panel and Tables S1 and S3). A two-shell fit (1.62 and 2.05 Å, fit **C**) is significantly better than either a one-shell fit (2.05 Å, fit **A**) or a two-shell fit with two long Fe−O interactions (2.06 and 2.42 Å, fit **B**). The quality of the fit is further enhanced by using a three-shell model (1.62, 2.05, and 2.42 Å, fit **D**). Furthermore, if the coordination number of the short Fe−O interaction is systematically varied in 0.1 increments, the optimal coordination number ranges from 0.5 to 0.9 in the different samples examined. These optimal coordination numbers, albeit rather uncertain, are consistent with the sample composition determined by Mössbauer spectroscopy. In contrast, fits to the data for the control sample are not improved by adding a short Fe−O interaction. If a short interaction is included, the Debye−Waller factor refines to a large and unreasonable value (σ^2 = 0.01 Å^2 for the control vs 0.002 Å^2 for the intermediate). Figure 2 (left panel) shows the FT of data for **1** and **2**.[16] The short Fe−O interaction is not apparent as a resolved peak in the FT, but a shift of the overall first-shell peak to lower distance relative to the control is apparent. In the several Fe(IV)=O^{2-} protein and model complexes that have been examined by XAS, the short Fe−O interaction that is apparent in the fitting analysis has never been obvious from a visual inspection of the FT.[11,15,17]

In conclusion, the EXAFS data presented in this study clearly demonstrate the presence of a 1.62 Å interaction between the Fe center of the high-valent TauD intermediate and one of its ligands. This structural feature is consistent only with an Fe=O unit, thus providing the most compelling evidence for the assignment of the intermediate as an Fe(IV)=O^{2-} species (**III**). The XANES spectra and the high Mössbauer isomer shift indicate, however, that the oxidation state of the Fe in this intermediate may be between +III

and +IV. Ongoing experimental and computational studies will address this intriguing possibility.

Supporting Information Available: Description of EXAFS data analysis, Mössbauer spectra of samples used for XAS spectroscopy, design of the new Mössbauer/XAS sample holder, a figure demonstrating the effect of photoreduction on XAS spectra, a figure of the unfiltered data, and tables with the parameters for the fits of the EXAFS data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ryle, M. J.; Hausinger, R. P. *Curr. Opin. Chem. Biol.* **2002**, *6*, 193−201.
(2) Costas, M.; Mehn, M. P.; Jensen, M. P.; Que, L., Jr. *Chem. Rev.* **2004**, *104*, 939−986.
(3) Abbreviations used: αKG: α-ketoglutarate; TauD: taurine:α-ketoglutarate dioxygenase; XAS: X-ray absorption spectroscopy; XANES: X-ray absorption near edge spectrum; EXAFS: extended X-ray absorption fine structure; FT: Fourier transform.
(4) Solomon, E. I.; Brunold, T. C.; Davis, M. I.; Kemsley, J. N.; Lee, S.-K.; Lehnert, N.; Neese, F.; Skulan, A. J.; Yang, Y.-S.; Zhou, J. *Chem. Rev.* **2000**, *100*, 235−349.
(5) Price, J. C.; Barr, E. W.; Tirupati, B.; Bollinger, J. M., Jr.; Krebs, C. *Biochemistry* **2003**, *42*, 7497−7508.
(6) Price, J. C.; Barr, E. W.; Glass, T. E.; Krebs, C.; Bollinger, J. M., Jr. *J. Am. Chem. Soc.* **2003**, *125*, 13008−13009.
(7) Proshlyakov, D. A.; Henshaw, T. F.; Monterosso, G. R.; Ryle, M. J.; Hausinger, R. P. *J. Am. Chem. Soc.* **2004**, *126*, 1022−1023.
(8) **1** was prepared by the rapid freeze-quench method (reaction time: 115 ms) according to the procedures described in references (5) and (6) and with the following final concentrations of reactants: 1.78 mM TauD, 1.33 mM Fe, 4.87 mM αKG, 4.87 mM 1,1-[^{2}H]$_2$-taurine, and 1.26 mM O$_2$. **2** was prepared under identical conditions, except that the reaction time was 20 ms and oxygen was excluded. Samples were packed into a sample holder that can be used for Mössbauer and XAS spectroscopies. Mössbauer experiments were carried out as described in reference (5). XAS data were collected at the National Synchrotron Light Source on Beamline X9B. Samples were maintained at T = 14 K with a displex cryostat and the Fe Kα fluorescence was detected with a Canberra 13 element Ge detector. The general protocols for data reduction and curve fitting were described in Riggs-Gelasco et al., *J. Am. Chem. Soc.* **1996**, *118*, 2387−2399 and minor modifications are described in the Supporting Information.
(9) Riggs-Gelasco, P. J.; Shu, L.; Chen, S.; Burdi, D.; Huynh, B. H.; Que, L., Jr.; Stubbe, J. *J. Am. Chem. Soc.* **1998**, *120*, 849−860.
(10) Penner-Hahn, J. E.; McMurry, T. J.; Renner, M.; Latos-Grazynsky, L.; Eble, K. S.; Davis, I. M.; Balch, A. L.; Groves, J. T.; Dawson, J. H.; Hodgson, K. O. *J. Biol. Chem.* **1983**, *258*, 12761−12764.
(11) Wolter, T.; Meyer-Klaucke, W.; Müther, M.; Mandon, D.; Winkler, H.; Trautwein, A. X.; Weiss, R. *J. Inorg. Biochem.* **2000**, *78*, 117−122.
(12) Single 45-minute scans were collected at six different sample positions and were averaged for the intermediate edge spectrum shown. Progressive photoreduction both in the edge position and in the intensity of the preedge feature is apparent after several scans on a single spot as shown in Figure S3.
(13) Tullius, T. D.; Gillum, W. O.; Carlson, R. M. K.; Hodgson, K. O. *J. Am. Chem. Soc.* **1980**, *102*, 5670−5676.
(14) Penner-Hahn, J. E.; Benfatto, M.; Hedman, B.; Takahashi, T.; Doniach, S.; Groves, J. T.; Hodgson, K. O. *Inorg. Chem.* **1986**, *25*, 2255−2259.
(15) Lim, M. H.; Rohde, J.-U.; Stubna, A.; Bukowski, M. R.; Ho, R. Y. N.; Münck, E.; Nam, W.; Que, L., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3665−3670.
(16) The spectrum of **1** shown is the average of 20 first and second scans of several replicate samples. Similar results are obtained if all scans, including scans after 10 h of collection, are averaged for a single sample. Also, if first and second scans are excluded from the average, the short Fe−O distance can still be detected, despite the apparent photoreduction in these scans.
(17) Penner-Hahn, J. E.; Smith Eble, K.; McMurry, T. J.; Renner, M.; Balch, A. L.; Groves, J. T.; Dawson, J. H.; Hodgson, K. O. *J. Am. Chem. Soc.* **1986**, *108*, 7819−7825.

JA048255Q