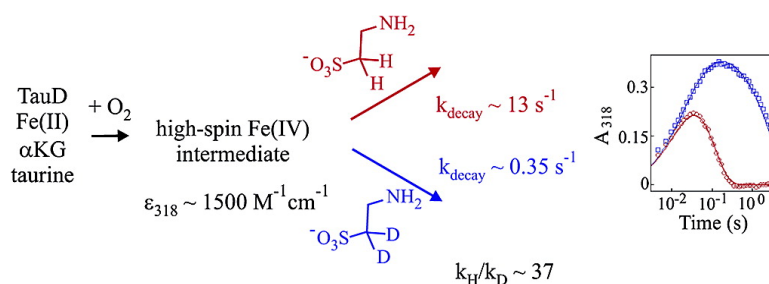Communication

Evidence for Hydrogen Abstraction from C1 of Taurine by the High-Spin Fe(IV) Intermediate Detected during Oxygen Activation by Taurine:￼-Ketoglutarate Dioxygenase (TauD)

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Evidence for Hydrogen Abstraction from C1 of Taurine by the High-Spin Fe(IV) Intermediate Detected during Oxygen Activation by Taurine:α-Ketoglutarate Dioxygenase (TauD)

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The Fe(II)- and α-ketoglutarate-dependent dioxygenases catalyze hydroxylation of unactivated carbon atoms on a variety of substrates by coupling reductive activation of dioxygen with oxidative decarboxylation of α-ketoglutarate (2-oxopentane-1,5-dioic acid; hereafter denoted αKG[1]). This elegant strategy for alkyl group functionalization is orchestrated by a conserved, His$_2$Asp-facially coordinated, mononuclear Fe(II) center to which αKG and (presumably) oxygen bind. A consensus mechanism for these enzymes, in which an oxo-ferryl (Fe(IV)=O^{2-}) intermediate is formed upon decarboxylation of αKG and initiates hydroxylation by abstraction of a hydrogen atom from the target carbon of the substrate, has repeatedly been proposed, but neither the Fe(IV)=O^{2-} complex nor any of the several other postulated intermediates between addition of O$_2$ to the Fe(II) center and substrate hydroxylation has ever been directly demonstrated for any member of this family.[2,3] Recently, we used kinetic and spectroscopic methods to characterize a high-spin Fe(IV) complex in the catalytic cycle of one of these enzymes, taurine:αKG dioxygenase (TauD) from *Escherichia coli*, which catalyzes hydroxylation of taurine (2-aminoethane-1-sulfonic acid) at C1.[4] A minimal kinetic mechanism involving three steps (formation of the Fe(IV) intermediate in a bimolecular reaction between the quaternary TauD·Fe(II)·αKG·taurine complex and O$_2$, decay of the complex to an Fe(II)-containing second intermediate, and conversion of this second intermediate back to the reactant complex; Scheme S1[5]) was shown to account well for both the transient-state kinetic/spectroscopic data and the steady-state catalytic rate. Although the oxidation and spin state of the novel Fe-(IV) complex were unambiguously defined, its position in the catalytic cycle and relationship to hypothetical intermediates in the consensus mechanism were not. In this work, we demonstrate that substitution of the C1 hydrogen atoms of taurine with deuterium slows decay of the Fe(IV) intermediate by ~37-fold without affecting the rates of the two other resolved steps of the minimal mechanism. A kinetic isotope effect (KIE) of this magnitude requires that decay of the intermediate occur concomitantly with, or be tightly kinetically coupled to, cleavage of the C1−H bond. Thus, the results prove that the novel Fe(IV) complex either is the hydroxylating intermediate or rapidly and reversibly converts to the hydroxylating species. Chemical logic dictates that the former possibility is more likely.

Absorption spectra from a control reaction between O$_2$ and the quaternary TauD·Fe(II)·αKG·taurine complex, which was formed with synthetic 1-[^{1}H]$_2$-taurine (^{1}H-taurine) obtained by a procedure mimicking that used to prepare the 1-[^{2}H]$_2$-taurine (^{2}H-taurine),[6] are essentially indistinguishable from those previously obtained with commercial taurine.[4] Changes are dominated by a transient, positive
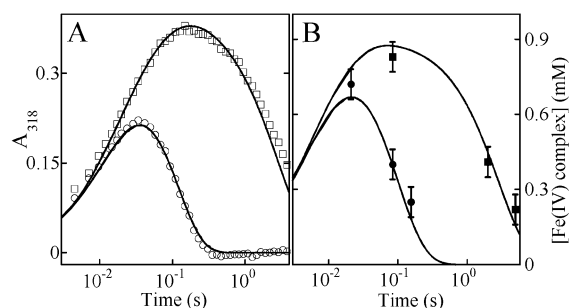

Figure 1. Kinetics of, and C1-deuterium KIE on the reaction at 5 °C of, the quaternary TauD·Fe(II)·αKG·taurine complex with limiting O$_2$ monitored (A) by stopped-flow absorption and (B) by freeze-quench Mössbauer. In (A), a solution of the quaternary complex (in 50 mM Tris buffer, pH 7.6) containing 2.0 mM TauD, 1.8 mM Fe(II), 17 mM αKG, and 17 mM synthetic ^{1}H-taurine (○) or ^{2}H-taurine (□) was mixed with air-saturated buffer in a volume ratio of 2:5. The path length was 1 cm. In (B), a solution of the quaternary complex (same buffer) containing 4 mM TauD, 3.6 mM ^{57}Fe(II), 10 mM αKG, and 10 mM synthetic ^{1}H-taurine (●) or ^{2}H-taurine (■) was mixed with an equal volume of O$_2$-saturated buffer. The solid lines are simulations according to Scheme S1 and assumptions (0.87 equiv of Fe(II) bound, 72% of complex active) indicated by previous work.[4] The initial concentrations of O$_2$ were calculated according to ref 7.

feature at 318 nm (Figure S1A). Previous work showed that this feature is attributable to the Fe(IV) intermediate.[4] Difference spectra from short times (less than 0.030 s) in the otherwise-identical reaction employing the synthetic ^{2}H-taurine are very similar and are also dominated by the positive 318-nm feature of the Fe(IV) complex (Figure S1B). However, this feature persists for much longer in the ^{2}H-taurine reaction. Comparison of the 318-nm kinetic traces (○ and □ in Figure 1A) illustrates that the amplitude is nearly twice as great (under these conditions) and the decay phase is much slower with ^{2}H-taurine (□). These stopped-flow data, as well as all other stopped-flow and freeze-quench kinetic data from experiments carried out under different reaction conditions (see below), can be accounted for by the same kinetic scheme simply by adjustment of the rate constant for decay of the Fe(IV) complex from (13 ± 2) s^{-1} for ^{1}H-taurine to (0.35 ± 0.05) s^{-1} for ^{2}H-taurine, setting limits of $28 < k_H/k_D < 50$ on the KIE.

The freeze-quench Mössbauer method was used to confirm that the observed effect on decay of the 318-nm absorption is indeed a reflection of a large KIE on decay of the Fe(IV) intermediate. Samples were prepared by rapidly freezing the reaction solution after 0.021, 0.084, and 0.16 s for the ^{1}H-taurine reaction and after 0.084, 2.0, and 5.0 s for the otherwise-identical ^{2}H-taurine reaction. Weak-field (40 mT applied parallel to the γ-beam), 4.2-K spectra of these samples are superpositions of the quadrupole doublet of the Fe(IV) species and the (at least two) incompletely resolved doublets characteristic of Fe(II)-containing complexes (reactant and intermediate). The concentrations of the Fe(IV) complex determined from its contribution to each spectrum (● and ■ in Figure 1B)
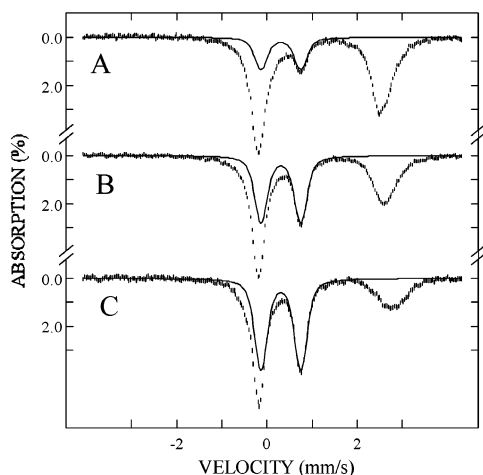
Figure 2. Selected 4.2 K, weak-field Mössbauer spectra of samples prepared by rapid freezing of the reaction at 5 °C of the quaternary TauD·Fe(II)·αKG·taurine complex with O_2. Spectra A and B are of the 0.084 s time-points from the ^1H-taurine and ^2H-taurine reactions, respectively, of Figure 1B. Spectrum C is of a sample frozen 0.11 s after mixing a solution of 3.6 mM TauD, 3.25 mM $^{57}Fe(II)$, 12.3 mM αKG, and 12.3 mM ^2H-taurine with O_2-saturated buffer in a volume ratio of 2:5. The solid lines are the theoretical spectrum of the Fe(IV) intermediate[4] ($\delta = 0.31$ mm/s, $\Delta E_Q = 0.88$ mm/s) scaled to 22%, 46%, and 63% of the total iron absorption in (A), (B), and (C), respectively. The Mössbauer spectrometer has been described.[4]

confirm the existence of a large KIE on decay of the intermediate. For example, at 0.084 s, the contribution of the intermediate has already decayed to approximately one-half of its maximum value in the ^1H-taurine reaction (Figure 2, spectrum A) but remains at its (greater) maximum value in the ^2H-taurine reaction (spectrum B), due to its much slower decay. For both reactions, the kinetics of the Fe(IV) complex agree well with those predicted by Scheme S1 (solid traces in Figure 1B). Under optimized reaction conditions (i.e., excess O_2, rather than the limiting-O_2 conditions employed for the above kinetic experiments), the increased lifetime of the complex with ^2H-taurine permits its accumulation to >60% of the iron in the sample (Figure 2, spectrum C).[8] This augmentation of lifetime and purity should prove extremely useful for ongoing spectroscopic characterization of the complex.

The steady-state rates of decarboxylation of αKG and release of sulfite from taurine (the ultimate result of C1 hydroxylation) were determined for both ^1H-taurine and ^2H-taurine.[9] With the former, a k_{cat} of 1.3 ± 0.2 s^{-1} was obtained, irrespective of which product was quantified. As previously noted, this value agrees well with that predicted by Scheme S1, taking into account (1) that our preparations of TauD bind 0.87 equiv of Fe(II) and (2) that apparently only 72% of the incorporated Fe(II) is competent to activate O_2. The agreement of the two assays establishes that the reaction is, within the uncertainty of our measurements, completely coupled under these conditions with ^1H-taurine. With ^2H-taurine, a value of 0.11 ± 0.02 s^{-1} was obtained for CO_2 production, whereas the rate constant for sulfite release was 0.07 ± 0.01 s^{-1}. Slower sulfite production suggests that the ^2H-taurine reaction is partially uncoupled as a result of the very large KIE. This uncoupling could explain why the observed steady-state rate constant for decarboxylation is less than the value of (0.19 ± 0.04) s^{-1} predicted by Scheme S1: uncoupled decay may produce an off-pathway form of the enzyme (e.g., an Fe(III)-containing form by one-electron reduction of the Fe(IV) intermediate) that may be slow to re-enter the catalytic cycle. The occurrence of uncoupling would also imply that the measured KIE underestimates the true KIE on C1—H bond cleavage, because k_{obs} for decay of the Fe(IV)

complex in the ^2H-taurine reaction would represent the sum of the rate constants for coupled and uncoupled decay, whereas it is likely that only the former involves C1—H cleavage. Thus, the true KIE could be as large as 58 ($37 \times 0.11/0.07$), or larger if error limits are considered.

Because the ^2H-taurine has deuterium atoms at both prochiral positions on C1, the observed KIE is expected to represent the product of primary and secondary effects. If an upper limit of 2 is taken for the (normal) secondary KIE, the primary effect is calculated to be at least 16 (considering all error limits). The magnitude of this effect suggests that quantum-mechanical tunneling may contribute to C—H bond cleavage.

The combined spectroscopic and kinetic data establish that productive decay of the high-spin Fe(IV) intermediate requires cleavage of the C1—H bond of taurine. The simplest interpretation of this result is that the Fe(IV) complex is the H-abstracting intermediate. Alternatively, the Fe(IV) complex may undergo rapid and reversible conversion to the H-abstracting species. However, in the consensus mechanism, C—H bond cleavage is preceded immediately by decarboxylation, and the latter is expected to be irreversible. Therefore, we consider it extremely likely that the Fe(IV) complex is the C1—H abstracting intermediate. Ongoing spectroscopic characterization of this novel species will reveal whether it is the generally postulated Fe(IV)=O^{2-} complex or some other structure.

Supporting Information Available: Characterization of the synthetic ^1H-taurine and ^2H-taurine, procedure for measuring CO_2 production from αKG, the scheme used for simulation of the kinetic data, kinetic-difference absorption spectra from reactions of the quaternary TauD·Fe(II)·αKG·taurine complex with limiting O_2 for both ^1H-taurine and ^2H-taurine, and Mössbauer spectra of all six samples from the experiment of Figure 1B (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Abbreviations used: αKG, α-ketoglutarate; TauD, taurine:α-ketoglutarate dioxygenase; KIE, kinetic isotope effect; k_{obs}, observed first-order rate constant.
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(5) In the cited manuscript, the second, Fe(II)-containing intermediate was ascribed slightly greater absorptivity at 318 nm than the reactant quaternary complex ($\Delta\epsilon_{318} = 100$ M^{-1} cm^{-1}). The greater kinetic simplicity resulting from the use of limiting-O_2 (single-turnover) conditions in this study has made it clear that the second intermediate has $\Delta\epsilon_{318} = 0$ M^{-1} cm^{-1}. In addition, the mechanism presented previously contained no error estimates, whereas the additional experiments conducted in this work have allowed error limits to be estimated.
(6) The synthetic procedures will be described elsewhere; characterization of the synthetic substrates is provided in the Supporting Information.
(7) Hitchman, M. L. *Chemical Analysis, Vol. 49: Measurement of Dissolved Oxygen*; Wiley: New York, 1978.
(8) The kinetic constants imply that the Fe(IV) complex should accumulate to nearly 100% of the Fe, but an upper limit of 70% is imposed by the previously noted heterogeneity in the reactant complex (only 72% of the bound Fe(II) is competent to react rapidly with O_2).
(9) The assay for sulfite production has been described.[4] $^{14}CO_2$ release was measured by a procedure derived from that reported by Jones et al. (ref 10). The modified procedure is described in the Supporting Information.
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