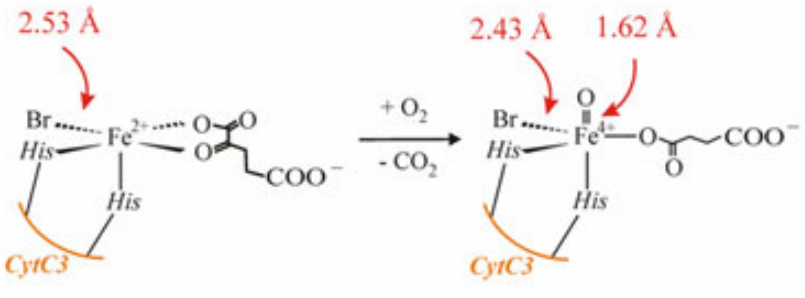
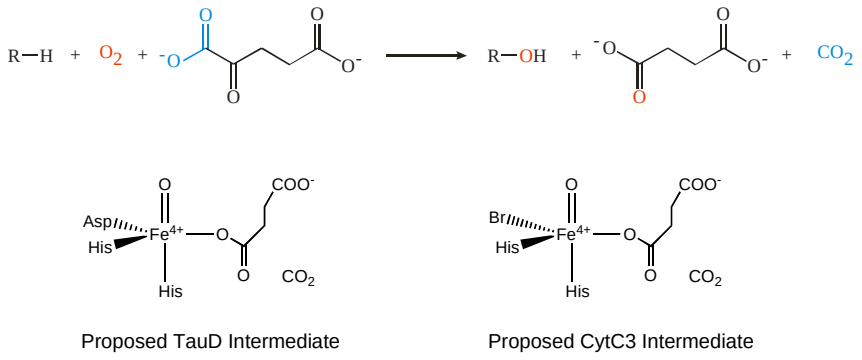
The Structure of a Reaction Intermediate in Enzymatic Halogenation



The reaction catalyzed by the halogenase CytC3

There are over 4,500 known halogenated natural products. The presence of a halogen in the molecular framework tunes a compound's chemical reactivity or biological activity in these natural fungicides and antibiotics. Four classes of enzymes are now known to catalyze halogenation reactions: 1) vanadium haloperoxidases, 2) heme haloperoxidases, 3) flavin-dependent halogenases, and 4) non-heme iron, alpha-ketoglutarate (αKG) dependent halogenases.[1,2] Walsh and coworkers first identified the aKG dependent oxygenases in 2005[3] and noted that they catalyze the insertion of halides into unreactive substrates (for example, the chlorination of a terminal methyl group in barbamide[4]). The chemical logic of the non-heme iron halogenases follows that of the αKG dependent, non-heme iron dioxygenases such as TauD, the *E. coli* enzyme catalyzing the hydroxylation of taurine (2-amino-1-ethanesulfonic acid).[5,6] For TauD, a high valent Fe(IV)-oxo species is generated that abstracts a hydrogen atom from the substrate and a subsequent radical rebound results in the hydroxylation of the substrate.[7-9] A conserved His-His-Asp/Glu "facial triad" provides the iron ligands.

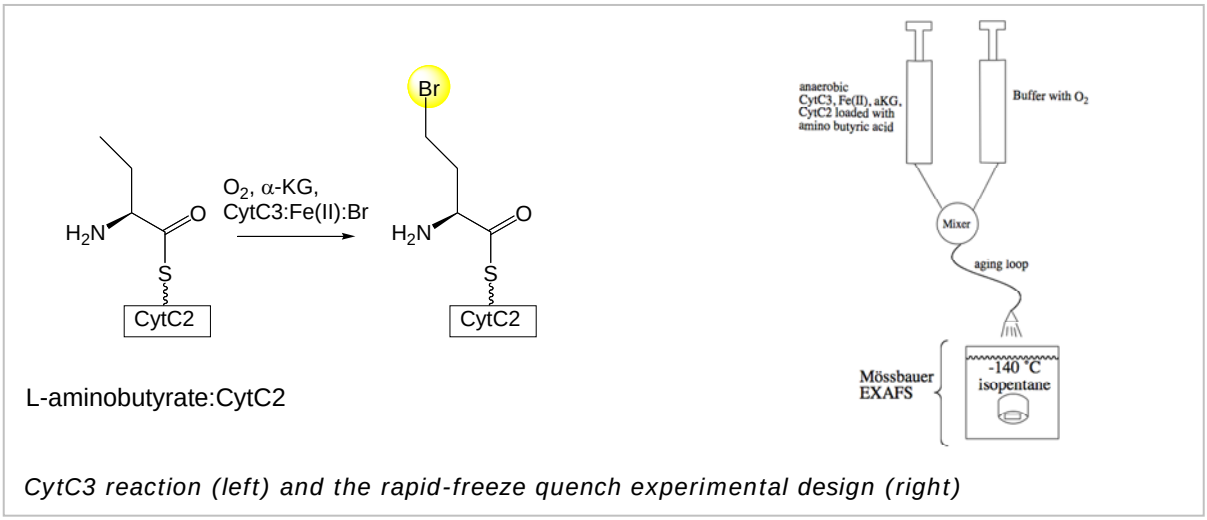


Reaction catalyzed by α-ketoglutarate and Fe(II)-dependent dioxygenases

Key insight into the mechanistic differences and similarities of the αKG-dependent halogenases and oxygenases came from the crystal structure of the halogenase SyrB2, responsible for the chlorination reaction in the synthesis of syringomycin, a compound secreted by *Pseudomonas syringae*.[10] The structure indicated that a halogen (Br or Cl) directly binds to the iron in place of the conserved Asp/Glu. Thus, when a ferryl-oxo intermediate is generated upon decarboxylation of the bound αKG, the intermediate abstracts a hydrogen atom from the substrate and a halogen radical is transferred to the substrate radical. Using the

halogenase CytC3, an enzyme capable of halogenating L-aminobutyrate, the Bollinger, Krebs, and Walsh groups verified a key component of these proposed mechanistic steps. They demonstrated that a high valent iron intermediate accumulates when the halogenase CytC3 is exposed to oxygen in the presence of Cl^-, αKG, and the scaffold enzyme CytC2-substrate complex.[11] They furthermore demonstrated that this Fe(IV) species was indeed responsible for hydrogen atom abstraction from the substrate.

In the present study, X-ray absorption spectroscopy and Mössbauer spectroscopy were used to identify the structural features consistent with the mechanistic model, i.e. the presence of a short Fe(IV)-oxo interaction and a metal bound halide. The Mössbauer spectra collected prior to XAS data collection demonstrated that the quenched samples contained ~80% of the Fe(IV) intermediate. Our XAS spectrum of the intermediate showed a large enhancement in the 1s→3d transition pre-edge intensity relative to the anaerobic, reduced control, which is consistent with the presence of an asymmetrical $Fe(IV)=O^{2-}$ unit. The XANES edge energy is consistent with the 80% Fe(IV): 20% Fe(II) composition determined using Mössbauer spectroscopy. The most compelling evidence for the presence of a formal $Fe(IV)=O^{2-}$ species comes from the fitting analysis of the EXAFS oscillations. Fits to the Fourier-filtered data require a short, 1.62 (± 0.02) Å Fe-O interaction to best model the data. Furthermore, a large scatterer is apparent in the Fourier transformation of the data. This peak can be modeled with an Fe-Br interaction at 2.43 Å. If the coordination number of the short Fe-O interaction and the Fe-Br interactions are systematically varied, the optimal coordination number is 0.7-0.8 for both features, matching the sample composition determined by Mössbauer spectroscopy. In contrast, fits to the reduced control sample were not improved by adding a short Fe-O interaction. The Fe-Br interaction in the control is 2.53 Å, consistent with the distance found in the crystal structure of SyrB2. The structural features we identified using XAS are only consistent with a $Br-Fe(IV)=O^{2-}$ unit and confirms a key component of the proposed mechanism.



CytC3 reaction (left) and the rapid-freeze quench experimental design (right)

Primary Citation

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