Oxygen Activation and Arene Hydroxylation by Functional Mimics of α-Keto Acid-Dependent Iron(II) Dioxygenases

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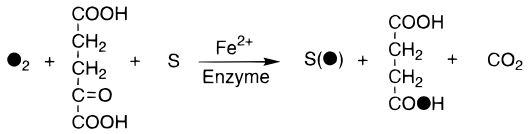
α-Keto acid-dependent enzymes, which represent the largest and most diverse class of non-heme iron enzymes, utilize an α-keto acid cofactor to effect dioxygen activation in numerous biochemical pathways (Scheme 1).[1,2] Crystallographic studies of one member of this class, deacetoxycephalosporin C synthase (DAOCS),[3] reveal a 2-His-1-carboxylate facial triad at the iron center, an emerging motif among mononuclear nonheme iron enzymes.[4] In the enzyme-cofactor complex, the α-ketoglutarate (α-KG) cofactor is chelated to the iron through the carboxylate and the α-keto oxygen. These crystal structures confirm the model previously proposed based on various spectroscopic and biochemical studies.[2,5] While the few model iron(II)−α-keto acid complexes available[6,7] have provided important mechanistic insight, they have not demonstrated the dioxygenase reactivity exhibited by the enzymes. Here we report the structure and properties of [Fe(Tp^{Ph2})(BF)] (**1**),[8] which reacts with O_2 to effect intramolecular arene hydroxylation, incorporating both atoms of O_2 into the products and thus demonstrating for the first time the dioxygenase activity exhibited by α-keto acid-dependent enzymes. In addition, the reactivity of **1** is contrasted with that of [Fe(Tp^{Ph2})(OBz)], emphasizing the unique function of the α-keto acid cofactor in oxygen activation.

Scheme 1



Complex **1** was synthesized by the anaerobic, sequential addition of equimolar amounts of $FeCl_2$, K(Tp^{Ph2}),[9] and the α-keto acid sodium benzoylformate (BF) in an acetonitrile slurry (Supporting Information). The crystal structure of **1**[10] (Figure 1) reveals a 5-coordinate iron(II) center with a monoanionic, face-capping Tp^{Ph2} and a chelated BF, similar to that of the related complex [Fe(Tp$^{t-Bu,i-Pr}$)(BF)];[11] two pyrazole nitrogens and a carboxylate oxygen define the equatorial plane of the distorted trigonal bipyramid ($\tau = 0.65$).[12] The optical spectrum of **1** (Figure 2A) exhibits an absorption band at 531 nm (340 $M^{-1}cm^{-1}$) with shoulders at 476 (210 M^{-1} cm^{-1}) and 584 nm (300 M^{-1} cm^{-1}). This feature, which is diagnostic of an α-keto acid chelated to an iron(II) center through the α-keto and one carboxylate oxygen, is blue-shifted (Figure 2B) when BF is replaced with an aliphatic α-keto acid, pyruvate (Ph → Me), consistent with its assignment to MLCT transitions.[5,6,11]

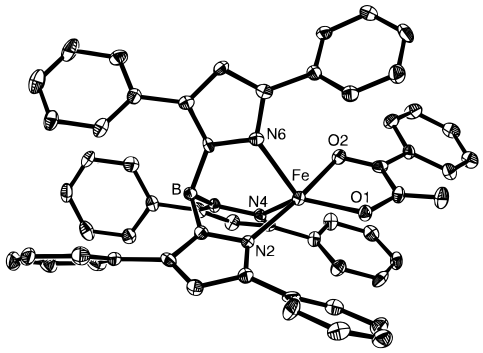


Figure 1. X-ray structure of [Fe(Tp^{Ph2})(BF)] (**1**) showing 30% probability thermal ellipsoids. Hydrogen atoms and two acetone solvent molecules are omitted for clarity. Selected bond lengths (Å) and distances (deg) are: Fe−O1, 1.968(4); Fe−O2, 2.206(5); Fe−N2, 2.188(5); Fe−N4, 2.068(5); Fe−N6, 2.068(5); O1−Fe−O2, 77.3(2); N2−Fe−N4, 86.4(2); N2−Fe−N6, 89.0(2); N4−Fe−N6, 91.1(2); O1−Fe−N2, 110.1(2); O2−Fe−N2, 171.7(2); O1−Fe−N4, 132.6(2); O2−Fe−N4, 85.7(2); O1−Fe−N6, 131.5(2); O2−Fe−N6, 88.5(2).

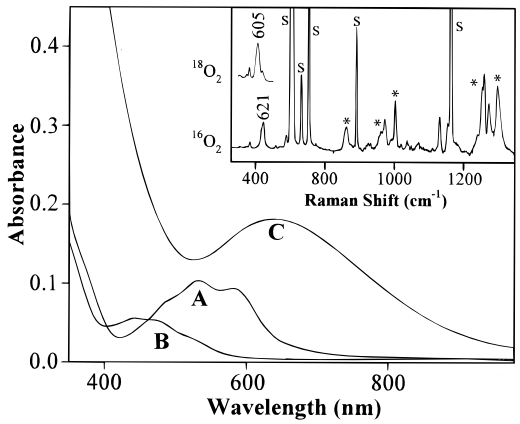


Figure 2. Optical spectra of (A) a 0.25 mM solution of **1** (R = Ph), (B) a 0.25 mM solution of the corresponding pyruvate complex (R = Me), and (C) the product **3** formed from the reaction of 0.25 mM **1** with excess O_2. Inset: resonance Raman spectra of **3** obtained from frozen CH_2Cl_2 solutions with 632.8 nm excitation.

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(8) Abbreviations: BF, benzoylformate; DAOCS, deacetoxycephalosporin C synthase; α-KG, α-ketoglutarate; MLCT, metal-to-ligand charge transfer; Tp$^{t-Bu,i-Pr}$, hydrotris(3-*tert*-butyl-5-isopropylpyrazol-1-yl)borate; Tp^{Me2}, hydrotris(3,5-dimethylpyrazol-1-yl)borate; Tp^{Ph2}, hydrotris(3,5-diphenylpyrazol-1-yl)borate.
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(10) X-ray quality violet needles were obtained over a period of several weeks from an acetone solution at −20 °C. **1** crystallizes in the monoclinic space group $P2_1$, with $a = 10.0654(3)$ Å, $b = 21.2126(5)$ Å, $c = 12.0796(3)$ Å, $\beta = 97.307(1)°$, $V = 2558.21(12)$ Å^3, and $Z = 2$. The structure was solved and refined with SHELXTL 5.0 (Siemens Industrial Automation: Madison, WI). Two molecules of acetone cocrystallized with each molecule of **1**. 7996 unique reflections were collected to 25.07° 2θ at 173 K with Mo Kα (λ = 0.71073 Å radiation) on a Siemens SMART Platform CCD. The structure was solved by direct methods and refined to $R = 0.0713$ and $R_w = 0.1083$ ($I > 2\sigma(I) = 4890$).
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(12) According to this formalism, a perfect square pyramid would have a τ-value of 0.00, while a perfect trigonal bipyramid would have a τ-value of 1.00 (Addison, A. W.; Rao, T. N.; Reedijk, J.; van Rijn, J.; Verschoor, G. C. *J. Chem. Soc., Dalton Trans.* **1984**, 1349−1356).
(13) In a typical experiment, 1 mg of **1** was dissolved in 1 mL of anaerobic, distilled benzene and then diluted with an additional 2 mL of O_2-saturated benzene.

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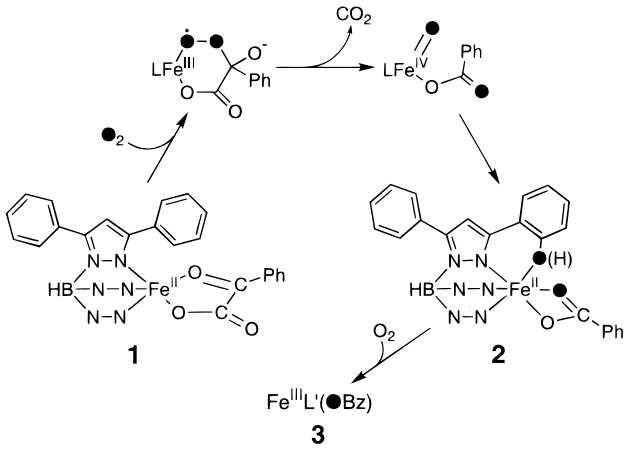
Scheme 2



Complex **1** reacts with O_2 over the course of 30 min[13] affording the quantitative decarboxylation of BF to benzoic acid.[14] Concomitant with this transformation is the generation of a green species (**3**) (λ_{max} = 650 nm, Figure 2C) whose resonance Raman spectrum (Figure 2 inset) exhibits features characteristic of an iron(III) coordinated to an ortho-substituted phenolate.[15] The identical product is also obtained when [Fe(Tp^{Ph2})(OBz)] is exposed to O_2, but the reaction takes approximately 14 h. The analogous green species generated from the reaction of O_2 with [Fe(Tp^{Ph,Me})(OOCR)] has been identified by Fujisawa et al. to be an iron(III) complex in which one of the 3-phenyl groups of the Tp ligand is hydroxylated.[16] Combined, these results demonstrate that **1** reacts with O_2 to form **2**, which autoxidizes to green **3** (Scheme 2).

The fate of dioxygen was established by $^{18}O_2$-labeling experiments (95%, Cambridge Isotope). Analysis of the OBz product via GC-mass spectrometry demonstrated that approximately 80% of the newly formed carboxylate contained one atom of oxygen from O_2. The substoichiometric incorporation is consistent with previously published results and is attributed to a competing autoxidation reaction.[6] The other ^{18}O atom is incorporated into the phenolate product as evidenced by the diagnostic shifts in key peak frequencies in the resonance Raman spectrum.[17,18] Incorporation of the label into the phenolate is nearly quantitative (90%) as deduced from the intensity of the residual 621 cm^{-1} peak ($\nu_{Fe-16OAr}$) relative to that of the 605 cm^{-1} peak ($\nu_{Fe-18OAr}$) (Figure 2 inset). Thus, **1** reproduces the dioxygenase nature of α-keto acid-dependent enzymes (Scheme 1) and, in particular, mimics *p*-hydroxyphenylpyruvate dioxygenase in its ability to hydroxylate a phenyl ring.[19]

Steric effects play a key role in modulating the reactivity of α-keto acid complexes. Whereas **1** reacts with O_2 within 30 min, the sterically encumbered [Fe(Tp$^{t-Bu,i-Pr}$)(BF)] is completely unreactive toward O_2.[11] Consistent with this trend, [Fe(Tp^{Me2})(BF)] reacts with O_2 within minutes.[7] Furthermore, when the phenyl group of the BF moiety on **1** is replaced with Me or *i*-Pr,[20] the resulting complexes effect arene hydroxylation significantly faster. The order of reactivity for the different R groups is Ph < *i*-Pr < Me, with R = Me reacting almost an order of magnitude faster than BF. This is the opposite of what is expected based on previous results where electron-withdrawing groups on ring-substituted BFs enhanced reactivity, which was consistent with a partially rate-limiting nucleophilic attack of an Fe-bound superoxide on the α-carbon.[6] Taken together, these results indicate that steric constraints are an important determinant of the reactivity and suggest that the transformation of the O_2 adducts into products entails molecular motions that require ample space around the iron center.

The fact that both **1** and [Fe(Tp^{Ph2})(OBz)] react with O_2 to produce **3** suggests that these complexes generate related, if not identical, oxidizing agents during the course of the reaction. An attractive intermediate is a [(Tp^{Ph2})Fe^{IV}=O] species. The 30-fold difference in reactivity between these two complexes (0.5 h vs 14 h) thus can be rationalized by the differing mechanisms each utilizes to form the putative oxene species. [Fe(Tp^{Ph2})(OBz)] very likely generates the iron−oxo species via O−O bond lysis of a (μ-1,2-peroxo)diiron(III) intermediate, which has previously been observed and crystallized for a Tp^{i-Pr2} derivative.[21,22] Complex **1**, on the other hand, is proposed to form the iron−oxo species via lysis of a mononuclear iron−peroxo species, derived from nucleophilic attack of the Fe^{III} -bound superoxide onto the α-carbon of the keto acid (Scheme 2).[6] Significantly, the activation energy for O−O bond cleavage may be lowered considerably by coupling this step to the loss of CO_2. Thus, **1** effects arene hydroxylation significantly faster than [Fe(Tp^{Ph2})(OBz)], highlighting the importance of the α-keto acid in activating O_2.

In conclusion, [Fe(Tp^{Ph2})(BF)] is both a structural and functional mimic of α-keto acid-dependent enzymes. The monoanionic, tridentate, facially capping Tp^{Ph2} ligand approximates the endogenous 2-His-1-carboxylate facial triad found in DAOCS, BF chelates to the iron center, and an available coordination site is maintained for binding O_2. Upon exposure of **1** to O_2, BF undergoes oxidative decarboxylation, and the Tp^{Ph2} ligand is hydroxylated, with 1 atom of oxygen being incorporated into each product, mimicking for the first time the dioxygenase nature of the enzymatic reaction. The use of the α-keto acid cofactor thus allows these enzymes to activate dioxygen and generate a versatile oxidant via a mechanism which is distinct from those of enzymes that require a porphyrin ring[23] or a second metal ion.[24]

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Supporting Information Available: Further experimental details concerning the synthesis and characterization of **1**, [Fe(Tp^{Ph2})(pyruvate)], [Fe(Tp^{Ph2})(O$_2$CC(O)CH(CH$_3$)$_2$)], and [Fe(Tp^{Ph2})(OBz)], full resonance Raman spectra of **3**, and tables of crystal data, data collection, structure solution and refinement, atomic coordinates, and bond lengths and angles for **1** are available (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) Benzoate was formed stoichiometrically as determined via GC. Quantification entailed decomposing the inorganic complex with 0.1 M H_2SO_4, extracting the organic products with Et_2O and $CHCl_3$, and esterifying with diazomethane according to standard procedures. Methyl 3-chlorobenzoate was added after degradation of the complex as an internal standard.

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