Volume 34
Number 14
July 5, 1995

Inorganic Chemistry

Communications

Structure of a Mononuclear Iron(II)−Catecholate Complex and Its Relevance to the Extradiol-Cleaving Catechol Dioxygenases

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Received December 9, 1994

The catechol dioxygenases are iron enzymes that catalyze the oxidative cleavage of catechols as part of Nature's strategy to degrade aromatic molecules in the environment.[1] There has been significant progress toward understanding the mechanism of Fe(III)-containing intradiol-cleaving enzymes, such as protocatechuate 3,4-dioxygenase (3,4-PCD), but less is known of the Fe(II)-requiring extradiol-cleaving catechol dioxygenases.[2,3] Spectroscopic studies of catechol 2,3-dioxygenase (2,3-CTD) indicate the presence of a square pyramidal high-spin iron(II) center,[4−6] while EPR studies of the 2,3-CTD·NO and 2,3-CTD·substrate·NO complexes show the availability of three sites on the enzyme-active site for exogenous ligand binding, two for substrate and one for NO (and presumably O_2).[7−9] To date, there is no synthetic mononuclear iron(II)−catecholate complex with which to compare spectroscopic data on the enzyme−substrate complexes of extradiol dioxygenases. In this paper, we report the structure and properties of the first mononuclear iron(II)−catecholate complex, $[Fe^{II}(6TLA)(DBCH)](ClO_4)$ (**1**).[10]

Complex **1** was synthesized by reacting equimolar amounts of $Fe(ClO_4)_2{\cdot}6H_2O$, 6TLA, $DBCH_2$, and Et_3N in methanol under argon to afford a light green solid which can be recrystallized from EtOH/Et_2O.[11] The crystal structure of **1**[12] shows that the iron center is in a distorted octahedral environment featuring a tripodal tetradentate 6TLA ligand and a bidentate catecholate ligand (Figure 1). The 6TLA ligand coordinates to the Fe(II) center in a manner typical for high-spin Fe(II)−6TLA complexes with the Fe1−N1 and Fe1−N21 bonds shorter than the other two Fe−N bonds.[13−16] To minimize steric interactions among the α-methyl groups of 6TLA, the two trans pyridine nitrogen atoms are pushed apart, giving rise to the longer Fe−N bonds.

The catecholate ligand binds to the iron *asymmetrically* with two distinct Fe−O bond lengths of 2.263(8) and 1.953(8) Å. The shorter Fe1−O2 bond is trans to the amine (N1) ligand, while the longer Fe1−O1 bond is trans to a pyridine nitrogen (N21). This 0.31-Å difference in Fe−O bonds and the presence of one ClO_4^- anion per molecule unit suggest that the catechol is a monoanion. The shortness of the Fe−O2 bond is consistent with its being the anionic oxygen of the DBCH ligand; its length of 1.953(8) Å is typical of Fe(II)−OAr bond distances,[17−20] which are among the shortest for high-spin Fe(II)−O bonds. Dissociation of the O2−H bond appears to be preferred despite steric interactions between the 3-*tert*-butyl group of the DBCH ligand and the α-methyl groups of the 6TLA ligand which give

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(10) Abbreviations used: 2,3-CTD, catechol 2,3-dioxygenase; $DBCH_2$, 3,5-di-*tert*-butylcatechol; 3,4-PCD, protocatechuate 3,4-dioxygenase; py, pyridine; TPA, tris(2-pyridylmethyl)amine; 6TLA, tris((6-methyl-2-pyridyl)methyl)amine.
(11) Anal. Calcd for **1**·EtOH, $C_{37}H_{51}ClFeN_4O_7$: C, 58.85; H, 6.81; N, 7.42; Cl, 4.69. Found: C, 58.58; H, 6.81; N, 7.37; Cl, 4.93. *Caution*! Metal complexes with organic ligands and perchlorate anions are potentially explosive.
(12) Crystal data for **1** at 173 K: monoclinic, space group $P2_1/c$ (No. 14), a = 20.566(9) Å, b = 8.92(2) Å, c = 20.830(6) Å, β = 92.37(3)°, V = 3820(10) Å^3, Z = 4. For 4951 unique, observed reflections with $I > 2\sigma(I)$ and 451 parameters, the current discrepancy indices are R = 0.085, R_w = 0.096.
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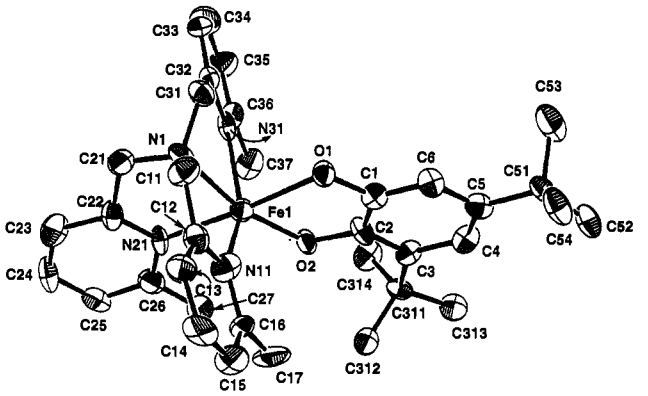

Figure 1. ORTEP drawing of the cation of **1** with atom-labeling scheme. Selected bond lengths (Å) are as follows: Fe1–O1, 2.263(8); Fe1–O2, 1.953(8); Fe1–N1, 2.21(1); Fe1–N11, 2.30(1); Fe1–N21, 2.172(9); Fe1–N31, 2.31(1).

rise to an O2–Fe1–N21 angle of 110.9(3)°, instead of the expected 90° value for an ideal octahedron.

The structure of **1** is unprecedented in having a chelated but monoanionic catecholate. The only other known Fe(II)–catecholate complexes are $[Fe_2(DBC)_2(py)_4]$ (**2**), $[Fe_2(DBC)_2(py)_6]$ (**3**), and $[Fe_4(DBC)_4(py)_6]$ (**4**), which have coordination environments similar to that of **1** but are polynuclear.[21] In **2**–**4**, each dianionic DBC ligand is chelated to an iron(II) center and bridged to at least one other Fe(II) center via one of the DBC oxygens; this coordination mode gives rise to a short Fe–O(DBC) bond (1.94–1.97 Å) for the terminal DBC oxygen and a longer Fe–O(DBC) bond (2.08–2.24 Å) for the bridging oxygen. The significant asymmetry in catecholate binding found in **1** is also observed for **3** and **4**, the effect of a proton being approximately equivalent to that of having at least one other Fe(II) center coordinated to the bridging catecholate oxygen.

The crystal structure of **1** provides insight into the enzyme–substrate complex of catechol 2,3-dioxygenase (2,3-CTD) from *Pseudomonas putida*. Fe K-edge EXAFS analysis of the enzyme–substrate complex reveals an iron environment that consists of one ligand at 1.93 Å and four others at 2.10 Å.[6] The 1.93-Å iron–ligand distance is amazingly similar to the 1.95 Å Fe–O(catecholate) distance found in **1**, suggesting that the substrate is bound to the iron active site in a manner similar to that found in **1**. Complex **1** thus represents a plausible structural model for the enzyme–substrate complexes of extradiol catechol dioxygenases and suggests that the substrate may bind as a monoanion in 2,3-CTD.

We have also investigated whether **1** serves as a functional model for an extradiol-cleaving dioxygenase. The UV–vis spectrum of **1** (Figure 2) consists of only one dominant absorption band at 395 nm (ϵ 2200 M^{-1} cm^{-1}), giving rise to the typical light yellow color of other high-spin Fe^{II}6TLA and Fe^{II}TPA complexes.[14,16,22] Upon exposure to O_2 at 20 °C, **1** is converted within minutes ($t_{1/2}$ = 1.1 min under pseudo-first-order conditions) to an intense purple-blue species with two intense bands at 600 and 1020 nm (Figure 2) which are similar to those of $[Fe^{III}L(DBC)]$ complexes and assigned as catecholate-to-iron(III) charge-transfer transitions.[23–25] The purple-blue species also exhibits EPR signals at g = 7.5, 4.3, and 1.9 which are typical of a high-spin iron(III) species ($S = {}^5/_2$, E/D = 0.06). The purple-blue species further reacts with O_2 ($t_{1/2}$ = 2.5 h under pseudo-first-order conditions) to afford two major organic products, 3,5-di-*tert*-butyl-1-oxacyclohepta-3,5-diene-2,7-dione (53%) and 3,5-di-*tert*-butyl-5-(carboxymethyl)-2-furanone (36%), both of which derive from the oxidative cleavage of the C1–C2 bond of the catecholate (intradiol cleavage). O_2 uptake studies show that 1.3(1) mol of O_2 is consumed/mol of **1**. This result is consistent with the oxidation of 4 molecules of **1** by 1 molecule of O_2 to $[Fe^{III}(6TLA)DBC]^+$ in the intital fast phase (i.e. 0.25 O_2 per **1** with O_2 being reduced to H_2O) and, in the slower phase, the intradiol cleavage of the bound DBC on each $[Fe^{III}(6TLA)DBC]^+$ by another molecule of O_2, as observed for $[Fe^{III}(L)DBC]$ complexes.[23,25] We ascribe the observed reactivity of **1** to its coordinative saturation. We note that the enzyme–substrate complex of 2,3-CTD is five-coordinate according to MCD and XAS studies,[5,6] so the availability of a vacant coordination site on the Fe(II) complex may allow O_2 to bind to the iron(II) center and facilitate its attack on the bound catecholate to afford extradiol cleavage.[26] Current efforts are directed toward the synthesis of five-coordinate Fe(II)–catecholate complexes to test this hypothesis.

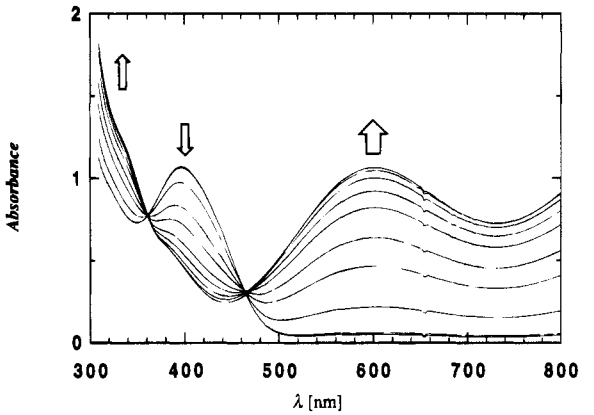

Figure 2. UV–vis absorption data showing the formation of $[Fe^{III}(6TLA)DBC]^+$ upon exposure of **1** to an O_2 atmosphere in CH_3CN at 20 °C. The pseudo-first-order rate constant of formation is 0.64(1) min^{-1}.

Acknowledgment. This work was supported by the National Institutes of Health (Grant GM-33162). We thank Professor Doyle Britton for sharing his expertise in the crystallographic study.

Supporting Information Available: Tables of the crystallographic data, atomic coordinates, thermal parameters, and intramolecular bond lengths and angles for **1** (15 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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