Biomimetic Intradiol-Cleavage of Catechols with Incorporation of Both Atoms of O_2: The Role of the Vacant Coordination Site on the Iron Center

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This is the first example of model system for the active site of protocatechuate 3,4-dioxygenase to display intradiol-cleavage of catechols with incorporation of two oxygen atoms of O_2 promoted by iron complexes.

The metabolic conversion of aromatic compounds to aliphatic compounds is of fundamental importance in biology. Catechol dioxygenases are mononuclear non-heme iron enzymes that catalyze the oxygenation of catechols to aliphatic acids via cleavage of aromatic rings.[1] These enzymes can be divided into two types: intradiol-cleaving enzymes which break the catechol C1–C2 bond, and extradiol-cleaving enzymes which break the C2–C3 or C1–C6 bond. Since Hayaishi et al. have revealed that an intradiol-cleaving catechol dioxygenase, pyrocatechase, catalyzes the oxygenation of catechol to muconic acid with incorporation of two oxygen atoms of O_2 (but not of H_2O),[2] the oxygenation mechanisms of catechol dioxygenases have been studied through investigations of model systems[3] as well as the enzymes themselves.[4] However, details of the O_2 insertion and aromatic ring-cleavage reactions are not yet understood. Interestingly, recent crystallographic studies of a protocatechuic acid (PCA)-bound form of an intradiol-cleaving catechol dioxygenase, protocatechuate 3,4-dioxygenase (3,4-PCD)[5] have revealed that the iron atom in the active site has octahedral geometry with PCA, His460, His462, Tyr408, and a vacant coordination site capable of accommodating an exogenous ligand such as an O_2 (Figure 1a). Herein, we report the first example of model system to display intradiol-cleavage of catechols with incorporation of two oxygen atoms of O_2 promoted by iron complexes (Figure 1b): $[Fe^{III}(^3L)(DBC)Cl](PPh_4)$ {**1**, ^3L = *N*-(2-hydroxyphenyl)-*N*-(2-pyridylmethyl)benzylamine, DBC = 3,5-di-*tert*-butylcatecholato} and $[Fe^{III}(^3L)(DBC)(DMF)]$ (**2**, DMF = *N*,*N*-dimethylformamide). The Cl^- and DMF ligands of **1** and **2** are expected to be exchanged for incoming O_2 during the oxygenation.

The new tridentate ligand ^3L was designed and synthesized to mimic specific attributes of the iron coordination site in the PCA-bound form of 3,4-PCD. Complex **1** was synthesized from the reaction of $FeCl_3$ with ^3L, DBC, triethylamine, and PPh_4Cl in DMF. The structure of **1** was unequivocally determined by X-ray crystallographic analysis.[6] An ORTEP drawing of the anion of **1** is shown in Figure 1c. Complex **1** has a distorted octahedral coordination geometry with bonding parameters similar to those of the PCA-bound form of 3,4-PCD.[5] Complex **1** has a trans arrangement of O1(phenolato group) and O2(DBC) atoms and a cis arrangement of O1(phenolato group) and O3(DBC) atoms, i.e., a meridional coordination mode for the three O atoms analogous to the PCA-bound form of 3,4-PCD. The negative-ion ESI (electrospray ionization) mass spectrum of **1** in DMF shows a prominent signal at *m*/*z* 600.2 {relative intensity (*I*) = 100% in the range of *m*/*z* 400–800} which corresponds to $[Fe(^3L)(DBC)Cl]^-$ ($[\mathbf{1}]^-$).

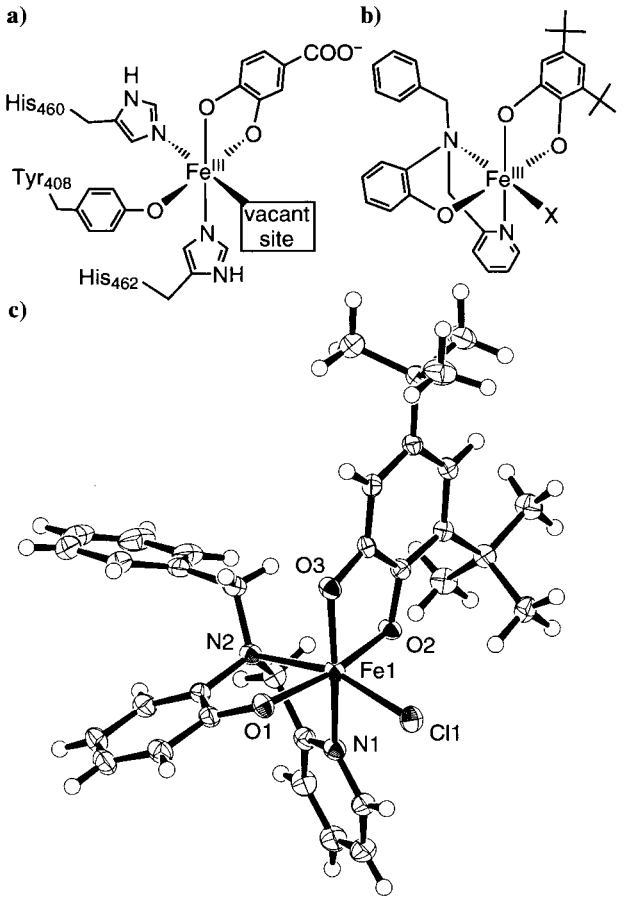


Figure 1. a) Active site structure of PCA-bound form of 3,4-PCD. b) Complexes **1** (X = Cl) and **2** (X = DMF). c) ORTEP drawing of $[Fe^{III}(^3L)(DBC)Cl]^-$ (the anion of **1**). Selected bond lengths (Å): Fe1–O1 1.957(2), Fe1–O2 1.947(2), Fe1–O3 1.949(1), Fe1–N1 2.239(2), Fe1–N2 2.358(2), Fe1–Cl1 2.3572(6).

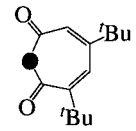
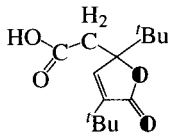
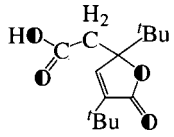
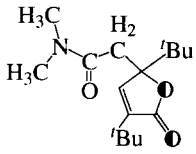
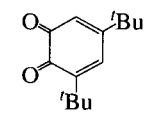
Complex **2** was synthesized from the reaction of **1** with AgOTf (OTf = $CF_3SO_3^-$) in DMF. The positive-ion ESI mass spectrum of **2** shows a prominent signal at *m*/*z* 639.4 (*I* = 100% in the range of *m*/*z* 400–800) which corresponds to $[Fe(^3L)(DBC)(DMF)+H]^+$ ($[\mathbf{2}+H]^+$). To establish the existence

of the DMF ligand in **2**, the same synthesis of **2** has also been carried out in DMF-d_7. ESI-MS results show that the signal at *m*/*z* 639.4 shifts to *m*/*z* 646.4 {[Fe(^{3}L)(DBC)(DMF-d_7) +H]$^+$}, i.e., the labeled DMF is incorporated into **2**.

The UV–vis spectra of **1** and **2** in DMF show two features {**1**: λ_{max} = 708 nm (ε = 3460 $M^{-1}cm^{-1}$, M = mol L^{-1}) and 464 (3660) and **2**: λ_{max} = 710 (3990) and 466 (4260)} which are assigned to ligand-to-metal charge transfer (LMCT) transitions by analogy to those observed in the spectra of other catechol bound complexes.[3] EPR experiments of **1** and **2** observed in DMF at 77 K indicate that they are high-spin ferric complexes.

We recently reported on the oxygenation ability of a catechol-bound iron(III) complex with a tetradentate ligand, [Fe^{III}(^{4}L)(DBC)] {**3**, ^{4}L = 2-hydroxyphenyl-bis(2-pyridylmethyl)amine}.[3a] In DMF at 25 °C, complex **3** reacts with O_2 to yield intradiol-cleavage products, 3,5-di-*tert*-butyl-1-oxacyclohepta-3,5-diene-2,7-dione (**4**, 73% yield based on DBC, Table 1) and 3,5-di-*tert*-butyl-5-(carboxymethyl)-2-furanone (**5**, 26%).[3a] GC–MS and ESI-MS measurements show that only one oxygen atom of $^{18}O_2$ is incorporated into **4** and **5** upon the reaction of **3** with $^{18}O_2$. The hydrolysis of **4** eventually affords **5** containing one ^{18}O atom.

Table 1. Yields/% (based on DBC) of products for the reactions of complexes **1**, **2**, and **3** with O_2 in DMF at 25 °C.[a]

Product		Complex		
		1	**2**	**3**
	(**4**)	0	0	73
	(**5**)[b]	0	0	26
	(**5**)[c]	18	27	0
	(**6**)	34	70	0
	(**7**)	45	0	0

[a] Conditions: **1** or **2** (15 μmol), a large excess of O_2 (1 atm), DMF (1.0 mL), 25 °C. After 24 hours, the reaction was quenched by an addition of 2 M HCl (10 mL). Products were extracted from the aqueous DMF solution with diethyl ether (20 mL × 3). Products **4**, **6**, and **7** were determined by ^{1}H NMR and GC–MS and **5** was determined by ^{1}H NMR and ESI-MS. The isotopic composition (^{18}O: ●) of **4**, **6**, and **7** was determined by GC–MS and that of **5** was determined by ESI-MS. [b] Compound **5** containing one ^{18}O atom. [c] Compound **5** containing two ^{18}O atoms.

The oxygenation ability of **1** and **2** in DMF at 25 °C is shown in Table 1. Complex **1** reacts with O_2 to yield intradiol-products **5** (18% yield based on DBC) and 3,5-di-*tert*-butyl-5-(*N*,*N*-dimethylamidomethyl)-2-furanone (**6**, 34% yield) whose structure was determined by X-ray analysis[7] and a non-intradiol-cleavage product 3,5-di-*tert*-butyl-1,2-benzoquinone (**7**, 45% yield). Complex **2** reacts with O_2 to yield **5** (27% yield) and **6** (70% yield). The negative-ion ESI mass spectra of **5** show that two oxygen atoms of $^{18}O_2$ are incorporated into **5** upon the reaction of **1** or **2** with $^{18}O_2$.

Furthermore, the kinetic study was followed by monitoring the disappearance of the lower energy LMCT bands {λ_{max} = 708 nm (for **1**) or 710 (for **2**) in DMF}. The reaction rates (k_{O_2} = k_{obs}/[O_2] in DMF at 25 °C)[8] of **1** and **2** are 1.80(8) × 10^{-2} and 2.15(9) × 10^{-2} $M^{-1}s^{-1}$, respectively. The oxygenation reactions (decay of **1** and **2**) exhibit pseudo-first-order kinetics.

In summary, what makes **1** and **2** different from **3** is that the intradiol-cleavage product **5** derived from **1** and **2** is shown to incorporate both atoms of O_2. However, the ^{18}O-labeling experiments of **3** show that only one label is found in **5**. Thus, depending on the ligands used, either one or two oxygen atom(s) of O_2 are incorporated into the cleavage product. We attribute these results to the presence and absence of the O_2 binding site in these complexes.

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References and Notes

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5 A. M. Orville, J. D. Lipscomb, and D. H. Ohlendorf, *Biochemistry*, **36**, 10052 (1997).
6 Crystallographic data for **1**: $C_{57}H_{57}ClFeN_2O_3P$, M_r = 940.36, triclinic, space group $P\bar{1}$ (No. 2), a = 9.7300(9), b = 14.0200(5), c = 18.260(2) Å, α = 78.040(2), β = 78.300(1), γ = 86.950(1)°, V = 2386.1(4) Å^3, Z = 2, ρ_{calcd} = 1.309 g cm^{-3}, μ(Mo Kα) = 4.53 cm^{-1}, R = 0.042 and R_w = 0.107, 10573 reflections used, 814 parameters. Crystallographic data for **1** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-153553.
7 The details of the crystal structure of **6** will be reported elsewhere in a full paper.
8 The solubility of O_2 (1 atm) in DMF at 25 °C is 4.86 mM. Japan Chemical Society, *Kagaku-Binran Basic Part II*, 2nd edition, Maruzen, Tokyo, 775 (1975).