A Reaction Intermediate Involved in Oxygenation of Catecholatoiron(III) Complexes with Molecular Oxygen — Relevance to Catechol Dioxygenases

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Formation of an intermediate in the step of O_2 binding to a catecholatoiron(III) complex was first clearly shown by construction of a labile coordination site on the ferric center. In addition, spectral changes indicated reversible interconversion between the catecholatoiron(III) complex and the intermediate.

Intradiol catechol dioxygenases are nonheme Fe^{III}-containing enzymes that catalyze the oxidative cleavage of the intradiol C–C bond of catechols utilizing molecular oxygen.[1,2] X-ray crystallographic studies reveal that binding of a catechol to the enzyme forms a catecholatoiron(III) complex with a vacant coordination site.[3] In order to explore the reactivity of the catecholatoiron(III) species with molecular oxygen, various types of catecholatoiron(III) complexes have been synthesized and found to produce oxygenated products in some model systems.[2,4] Some O_2-bound intermediates have so far been postulated in the step of O_2 binding to the catecholatoiron(III) species,[5] but no such intermediate has been detected.[4] The spectral changes suggesting the formation of an intermediate species was once reported,[6] but the structure of the species has not been well characterized. In contrast to the model system, stopped-flow kinetic studies on the enzymatic system suggested the formation of an O_2-bound intermediate, although the species has not been characterized beyond its absorption spectrum.[7] Here, we newly synthesized a highly reactive catecholatoiron(III) complex aiming to observe a reaction intermediate by accelerating the O_2 binding step. In this study we first observed spectral changes indicating interconversion between the catecholatoiron(III) complex and the reaction intermediate.

We prepared a five coordinate catecholatoiron(III) complex, $[Fe^{III}(Me_3TACN)(3,6\text{-DTBC})]BF_4$ (**1**), where Me_3TACN and 3,6-DTBC denote the 1,4,7-trimethyl-1,4,7-triazacyclononane and 3,6-di-*tert*-butylcatecholate ligands, respectively, by removing a chloride ion from $[Fe^{III}(Me_3TACN)(3,6\text{-DTBC})Cl]$ (**2**) by adding $AgBF_4$. EPR spectra of **1** and **2** showed signals at $g = 6.3$ and 2.0 at 2 K typical of a high-spin Fe^{III} complex with axial symmetry. This result indicates that complex **1** retains the octahedral coordination structure of **2** and has a labile coordination site.

Complex **2** showed low reactivity with O_2. A solution of **2** in CH_3CN solvent changed its color very slowly from purple to yellow even at room temperature upon exposure to 1 atm O_2, and its catecholate-to-iron(III) LMCT bands ($\lambda_{max} = 516$ and 773 nm) showed an exponential decay as reported for most of the catecholatoiron(III) complexes.[4] The rate constant was estimated to be $k_{obs} = 6.9 \times 10^{-4}\ s^{-1}$. In contrast to **2**, complex **1** reacted in very different way. Under anaerobic conditions, a solution of **1** in CH_3CN solvent exhibited an intense purple color, and its electronic spectrum showed two LMCT bands at 605 and 968 nm, as shown in Figure 1 (spectrum A). Upon exposure to 1 atm O_2 at room temperature, the solution of **1** immediately changed color from purple to green, and then gradually turned to yellow.[8] We followed the electronic spectral changes at $-40\,^{\circ}C$, since the rate of the initial color change was too rapid to be measured at room temperature. The spectral change shown in Figure 1 is distinct from the exponential spectral decay.

Computer analysis of this spectral change revealed the formation of a reaction intermediate **3** that exhibits an intense absorption at around 705 nm (spectrum B in Figure 2). It is to be noted that the spectrum of the intermediate **3** resembles that assigned as an O_2-bound intermediate for the enzymes.[7] Kinetic

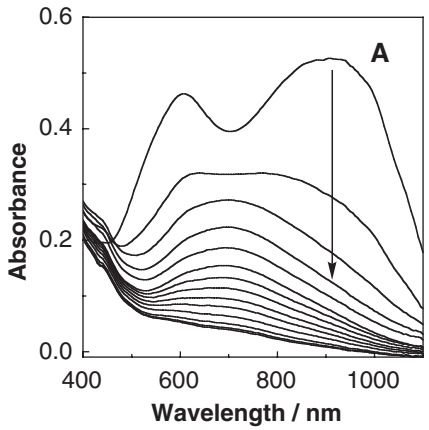


Figure 1. Progress of the reaction of complex **1** with molecular oxygen in CH_3CN at $-40\,^{\circ}C$. Interval: 4 min.

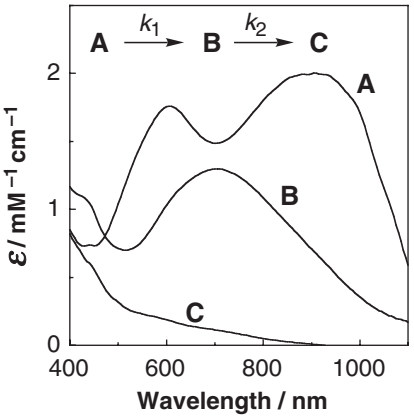


Figure 2. Absorption spectra of three components resolved by singular value decomposition of the spectral change during the oxygenation reaction of complex **1** at $-40\,^{\circ}C$. Complex **1** (A), the green intermediate **3** (B), and the final solution (C).

analysis also revealed that the reaction proceeds via the green intermediate **3** with rate constants of $k_1 = 4.3 \times 10^{-3}\ s^{-1}$ and $k_2 = 9.4 \times 10^{-4}\ s^{-1}$.

Remarkably, we have found that the spectral change between **1** and the green intermediate **3** is reversible. When the solution containing **3**, which was prepared by oxygenation of **1** at $-40\,^{\circ}C$, was bubbled with argon and warmed to room temperature, the two LMCT bands characteristic of **1** reappeared as shown in Figure 3. This spectral change indicates that **3** is converted to **1** with the release of O_2. The reversible spectral changes can be repeated several times ($A \rightarrow B \rightarrow A' \rightarrow B' \rightarrow A'' \rightarrow B''$). The decrease in the peak intensity after each cycle is thought to reflect that the oxygenative decomposition of **3** proceeds under these experimental temperatures.

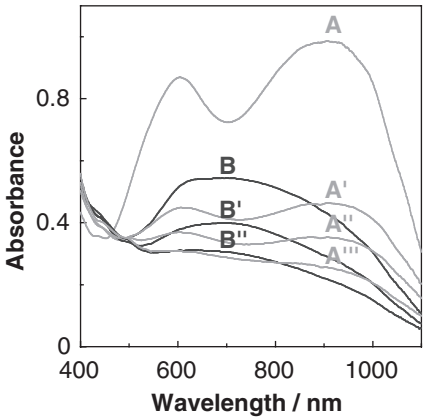
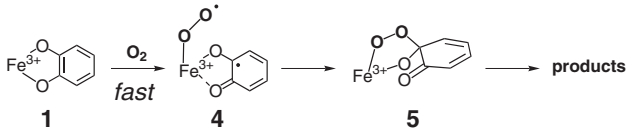


Figure 3. Reversible spectral changes between **1** and **2**. Spectrum B was obtained after the anaerobic solution of complex **1** (spectrum A) was exposed to 1 atm of O_2 at $-40\,^{\circ}C$ for 300 s. Spectrum A′ was obtained after bubbling with Ar gas and warming the solution to room temperature. Spectra B′, A″, and B″ were obtained after the second and third cycles of the repetition of the above described procedure.



Scheme 1. Oxygen activation scheme. The observed intermediate **3** could be **4** or **5**.

The above results suggest an oxygen activation mechanism involving the initial binding of O_2 to the ferric center rather than a substrate activation mechanism involving the initial binding of O_2 to the catecholate ligand (Scheme 1).[5,9] In the former mechanism, the attack of O_2 on the ferric center generates a Fe^{III}-superoxide-semiquinonate complex (**4**). The labile site of **1** would be favorable to the binding of O_2 to the ferric center and allow the rapid formation of the O_2-bound intermediate **3**. On the other hand, the coordinatively saturated structure of **2** would be much less favorable to the O_2 binding to the ferric center, that makes the O_2 binding step rate-limiting and the detection of **3** impossible. The species **4** will be converted to a Fe^{III}–peroxide complex (**5**) by recombination of the superoxide with the semiquinonate ligand, and afford oxygenated products. Thus, both **4** and **5** are considered to be observable intermediates; however, the only one intermediate was observed in this study. At present, it is hard to assign the absorption band of **3** to either **4** or **5** since both **4** and **5** are expected to exhibit a similar intense absorption band at around 600 nm.[10]

In summary, we successfully observed a reaction intermediate in the oxygenation of the catecholatoiron(III) complex **1** by constructing a labile coordination site. The unoccupied or labile coordination site of the catecholatoiron(III) complex formed in enzyme active site would be required for efficient O_2 binding on the ferric center. Furthermore, our experimental results show that the O_2-bound species is fairly stable under controlled conditions, and that the bound O_2 can be released. We are now conducting a further characterization of the intermediate **3**, and exploring the mechanism of O_2 binding to the ferric center of the catecholatoiron(III) complexes.

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