# Activation of Chelated Catecholatoiron Species for Catalytic Oxygenation of Catechols by Catecholdioxygenase-model Iron Complexes

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Reactivity of the isolated catecholatoiron complex with $O_2$ and the spectroscopic observation of the reversible catecholate ligand exchange reveal that a stable chelated catecholate species may be activated to be reactive with $O_2$ in the catalytic system.

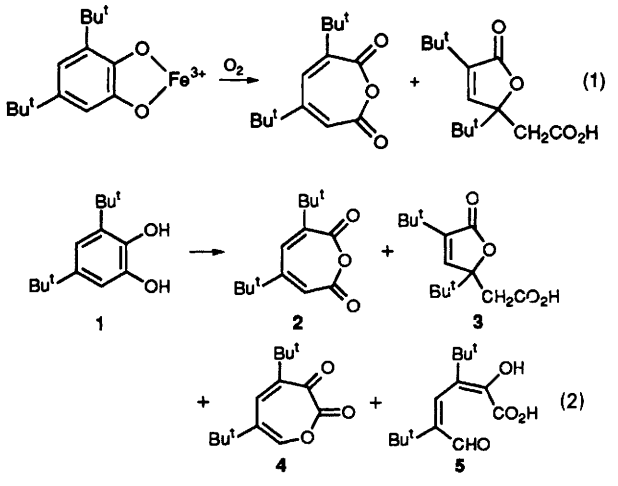
Catecholdioxygenases play key roles in the metabolism of aromatic compounds and are the central objects in studies of dioxygenases. Since the discovery of the oxygenation of 3,5-di-*tert*-butylcatechol (DTBC, **1**) by a bipyridine(pyridine)iron complex,[1] some important results have been obtained with model systems used for oxygen insertion,[2,3] but little work has been devoted to the development of the catalytic process. One of the reasons is that the stability of the intermediate species of the enzymes and models can be different. In the case of iron(III) catecholdioxygenases, it is proposed that oxygenation proceeds *via* a catecholatoiron(III) complex which has a semiquinonatoiron(II) character.[2–4] Formation of oxygenated products from isolated catecholatoiron(III) complexes[3] seems to be accepted as evidence for the chelated catecholate iron species acting as an intermediate in the reaction with oxygen. However, it is not convincing that the catecholatoiron complex, which is stable enough to be isolated, is a direct intermediate in the catalytic systems. We have found that the stable catecholatoiron complex becomes reactive with oxygen in the presence of an excess of catechols to accomplish the catalytic reaction. Spectroscopic studies indicated that catecholate ligand exchange plays an important role in the activation of the catecholate ligand.

Catecholatoiron complexes were isolated from the solutions of $FeCl_3$ with **1**, 4-chlorocatechol (4Clcat) and tetrachlorocatechol ($Cl_4$cat) in THF in the presence of pyridine under argon. X-Ray absorption spectra (EXAFS, XANES) indicated that the DTBC and 4Clcat complexes are dimeric in the solid state but monomeric in THF. The DTBC and 4Clcat complexes in THF are five-coordinated complexes, formulated as $[FeCl(DTBC)py_2]$ and $[FeCl_2(4Clcat)py]^-H^+$, respectively. The $Cl_4$cat complex is a monomeric six-coordinated complex, formulated as $[FeCl_2(Cl_4cat)py_2]^-H^+$ in both the solid state and THF. These complexes (0.125 mmol) were dissolved in THF (9.1 $cm^3$) and pyridine (0.9 $cm^3$) with or without addition of catechols (1 mmol) or *tert*-butylhydroquinone (TBHQ), under 1 atm. $O_2$ and 25 °C. After extraction with $CH_2Cl_2$, the products were quantitatively analysed by $^1H$ NMR. The known characteristic peaks of $^1H$ signals of products[2] were used for estimation of yields using toluene as an internal reference.[5] Electronic spectra of the complexes were recorded using a quartz cell of a 1 mm light path length under argon.

Fig. 1 shows the effect of the concentration of **1** on the formation of the intra- and extra-diol oxygenation products catalysed by $FeCl(DTBC)py_2$. Oxygenation products were not obtained without addition of **1**. Both intra- and extra-diol oxygenation products, **2** + **3** and **4** + **5**, respectively, were obtained as shown in eqn. (2) in addition to some other products. The total yields of products **2**–**5** increased with the concentration of **1**, indicating that oxygenation proceeds catalytically in the presence of an excess of **1**.

The importance of the presence of an excess of **1** for oxygenation was examined either by the reactivity of the 4Clcat and $Cl_4$cat complexes with **1**, or by that of $FeCl(DTBC)py_2$ with chlorocatechols. Table 1 shows these results together with the reactivity of the complex with TBHQ, for comparison. Added **1** was oxygenated by $FeCl(DTBC)py_2$ and $FeCl_2(4Clcat)py$ similarly to the complex prepared *in situ*, but not by $FeCl_2(Cl_4cat)py_2$. The oxygenated products were obtained from the DTBC ligand in $FeCl(DTBC)py_2$ by the addition of 4Clcat, but $Cl_4$cat was not effective. These results indicate that 4Clcat, which is not oxygenated, functions similarly to DTBC in activating the DTBC ligand, but $Cl_4$cat makes the complex inactive.

Fig. 2 shows spectral changes after addition of different catechols to the complexes in THF. Fig. 2(*a*) shows clearly



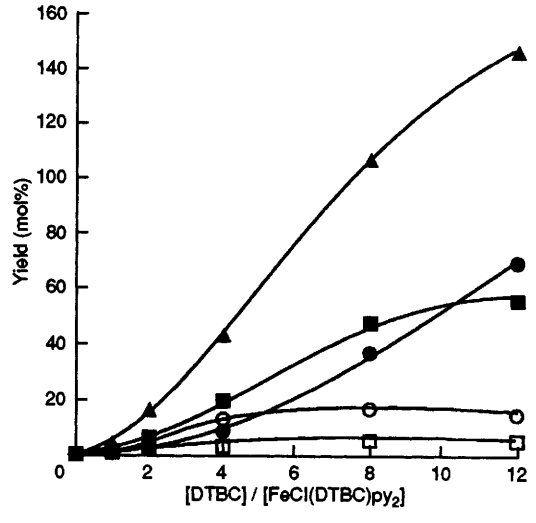




**Fig. 1** Effect of concentration of **1** added to $FeCl(DTBC)py_2$ on the yields of intra- and extra-diol oxygenation products: ○ : 2, ● : 3, □ : 4, ■ : 5, ▲ : total yield of 2–5. Yields are based on [Fe], after 24 h.

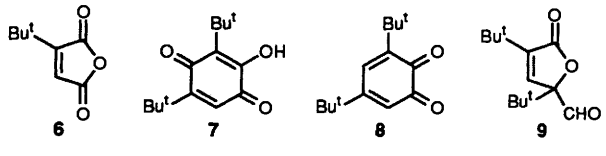


**Table 1** Oxygenation of DTBC catalysed by catecholatoiron complexes in THF–pyridine

| Iron Species[a] | Catechols[b] | Conv.[c] | Yields of products (mol%)[d] Intra- and extra-diol cleavage 2–5 (2:3:4:5)[e,f] | Others 6–7 (6:7)[e] | 8 |
|---|---|---|---|---|---|
| $FeCl_3$ | DTBC | 98 | 132 (0:63:3:33) | 91 (90:10) | 431 |
| $FeCl(DTBC)py_2$ | DTBC | 89 | 106 (16:35:5:44) | 93 (74:26) | 570 |
| $FeCl_2(4Clcat)py$ | DTBC | 96 | 130 (0:57:2:40) | 86 (89:11) | 386 |
| $FeCl_2(Cl_4cat)py_2$ | DTBC | 10 | 0 | 0 | 99 |
| $FeCl(DTBC)py_2$ | 4Clcat | 85 | 6 (0:53:0:47) | 15 (26:74) | 30 |
| $FeCl(DTBC)py_2$ | $Cl_4cat$ | 0 | 0 | 0 | 0 |
| $FeCl(DTBC)py_2$ | TBHQ | 83 | 1 (0:0:0:100) | 44 (100:0) | 23 |

[a] Fe (0.125 mmol). [b] Catechols or hydroquinone (1 mmol). [c] Conversion of **1**. [d] Yields formed after 24 h, based on Fe. [e] Products **2–5**: see text. [f] **5** was mostly converted to **9**.

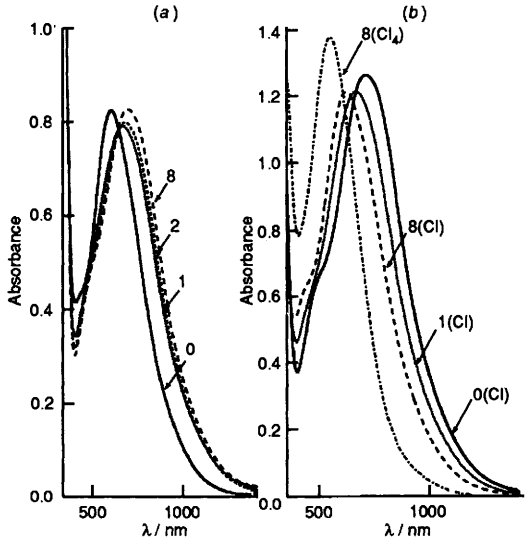
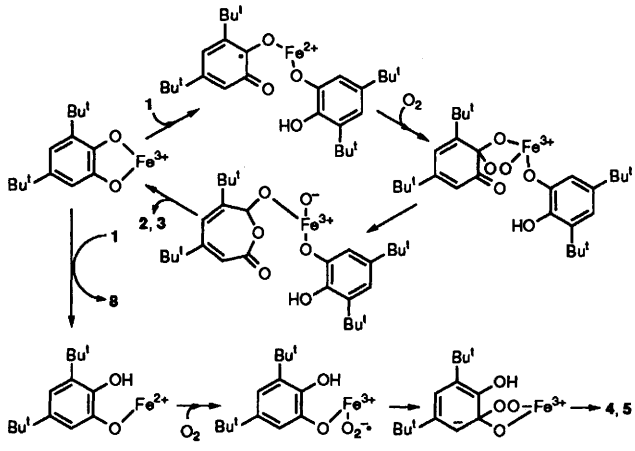



**Fig. 2** Electronic spectra of the catecholatoiron complexes after addition of different catechols. (*a*) Addition of **1** to the solution of $[FeCl_2(4ClCat)py]^-H^+$ in THF [numbers denote the added concentration of **1**: $[FeCl_2(4Clcat)py]^-H^+$ (mol ratio)]. (*b*) Addition of 4Clcat (Cl) and $Cl_4cat$ ($Cl_4$) to $[FeCl(DTBC)py_2]$.



**Scheme 1**

that the 4Clcat ligand is replaced very easily by **1** to form the DTBC complex; this exchange reaction occurs very rapidly. Fig. 2(*b*) shows that the DTBC ligand is replaced by 4Clcat and $Cl_4cat$ ligands. We observed also that the $Cl_4cat$ ligand is not replaced by DTBC, indicating that the $Cl_4cat$ ligand coordinates more tightly than DTBC and irreversibly to $Fe^{III}$.

These results indicate that $FeCl(DTBC)py_2$ which is stable enough to be isolated can not be a direct intermediate for oxygenation. The catecholate ligand, however, is activated during the reversible catecholate ligand exchange. The activation may be caused by the transformation of the bidentate catecholate ligand to the monodentate form during the ligand exchange process,[6] with increasing radical character. The presence of the vacant site is required for the reaction with oxygen, and as suggested by the results the reaction is inhibited by the formation of six-coordinated complexes, *e.g.* $[FeCl_2(Cl_4cat)py_2]^-$. The six-coordinated complex, $[Fe(DTBC)py_4]^+$, which is formed in the presence of an excess of pyridine, is also inactive for oxygenation even though the DTBC ligand has a high radical character.[4] The activated catecholate ligand may react with oxygen to give oxygenation products as shown in Scheme 1. As for the extradiol oxygenation, we noticed that the extradiol oxygenation product is only formed in a small amount when TBHQ is added to $FeCl(DTBC)py_2$ (Table 1). Considering that TBHQ may be effective to reduce the $Fe^{III}$ species, this is a reasonable observation as we have recently found that selective extradiol oxygenation is catalysed by the $Fe^{II}$ species rather than $Fe^{III}$ similarly to the enzymatic system.[5] Although no spectroscopic evidence has been obtained for the coordination of catechols to $Fe^{II}$, the extradiol oxygenation may proceed *via* a catecholatoiron(II) species which is formed prior to the coordination of oxygen as proposed in the enzymatic system.[7] Since the catecholate iron(II) species is much less stable than the corresponding iron(III) species, oxygenation may proceed without activation of the intermediate by extra catechols.

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## References

1 T. Funabiki, H. Sakamoto, S. Yoshida and K. Tarama, *J. Chem. Soc., Chem. Commun.*, 1979, 754.
2 T. Funabiki, A. Mizoguchi, T. Sugimoto, S. Tada, M. Tsuji, H. Sakamoto and S. Yoshida, *J. Am. Chem. Soc.*, 1986, **108**, 2921.
3 D. D. Cox, S. J. Benkavic, L. M. Bloom, F. C. Bradley, M. J. Nelson and L. Que, Jr., *J. Am. Chem. Soc.*, 1988, **110**, 2026; D. D. Cox and L. Que, Jr., *J. Am. Chem. Soc.*, 1988, **110**, 8085.
4 T. Funabiki, H. Kojima, M. Kaneko, T. Inoue, T. Yoshioka, T. Tanaka and S. Yoshida, *Chem. Lett.*, 1991, 2143.
5 T. Funabiki, I. Yoneda, M. Ishikawa, M. Ujiie, Y. Nagai and S. Yoshida, *J. Chem. Soc., Chem. Commun.*, 1994, 1453.
6 P. Barbaro, C. Bianchini, O. Frediani, A. Meli and F. Vizza, *Inorg. Chem.*, 1992, **31**, 1523.
7 P. A. Mabrouk, A. M. Orville, J. D. Lipscomb and E. I. Solomon, *J. Am. Chem. Soc.*, 1991, **113**, 4053.