CHEMISTRY LETTERS, pp. 2143-2146, 1991. 

# Strong Radical Character of the Catecholate Ligand of Catecholato(pyridine)iron Complexes Involved in the Catecholdioxygenase-model Reaction

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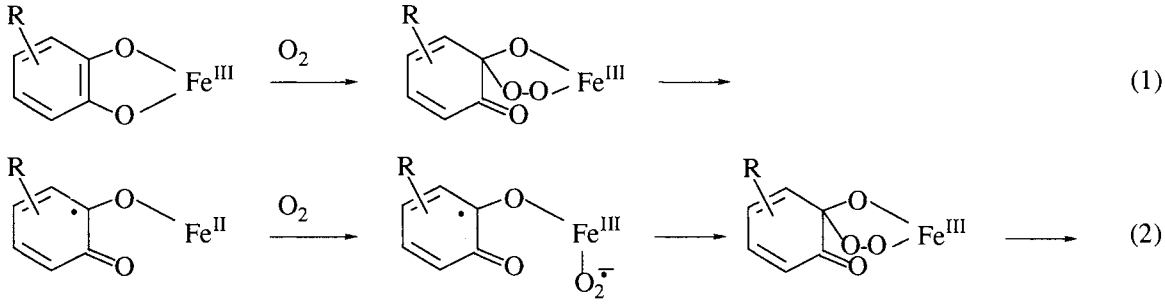
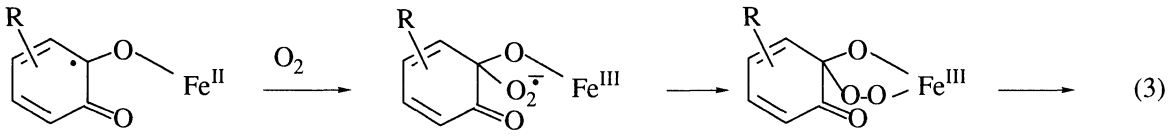
Five-coordinated catecholato(pyridine)iron complexes were isolated from the THF solution of the iron complex used for the catecholdioxygenase model reaction. Formation and structure of species with a catecholate ligand having a strong radical character in the pyridine solution were shown spectroscopically, especially by the large down-field shift of the methyl resonance of 4-methylcatecholate complex in $^2$H NMR and by the large band at >1000 nm in electronic spectra. Effect of $Et_4NCl$ on the spectra indicated that the complexes are in equilibrium with the the chloride-pyridine ligand exchange.

Catecholdioxygenase is one of the oxygenases which have extensively been studied,[1-4] but the mechanism of the oxygenation is still beyond clarification. The model chemistry by using nonheme iron complexes is important not only for development of oxygenation catalysts for aromatic ring cleavage, but also for clarification of the mechanism of the key step in the enzymatic reactions. Previously, we have first clearly shown that the oxygen addition proceeds stepwise *via* a monooxygenated intermediate,[5] but one of the important questions to be solved is how catechol and oxygen are activated by the iron center in the oxygen insertion process. Direct oxygen attack to the catecholate ligand (Eq.1) was proposed based on the non-observation of $Fe^{2+}$ species in the enzymatic system,[6] but we have proposed the activation of the catecholate ligand to the semiquinonate ligand and probability of the activation of oxygen by coordination to $Fe^{2+}$ (Eq. 2).[5,7,8] Another probability is the direct attack of oxygen to the semiquinonate ligand (Eq. 3).[2,9] It is very difficult to get evidence for the oxygen activation by $Fe^{2+}$ in Eq. 2, but very important to make clear the formation of the semiquinonate species as a direct reactant with oxygen. We here first isolated (catecholato)(pyridine)iron complexes from the THF solution of our model system and obtained spectroscopic information showing the strong radical character of the catecholate ligand.






(3)

Two types of complexes were isolated depending on the kind of catechols and solvents. One type is formulated as $[FeCl_2(Cat)(Py)]^-[H^+]$ and the other as $FeCl(Cat)(Py)_2$, in which Cat and Py denote catecholate and pyridine ligands, respectively. The former type was isolated from 4-methyl- and pyrocatechols (**1** and **2**, respectively), and the latter from 3-methyl- and 3,5-di-t-butylcatechols (**3** and **4**, respectively) which have alkyl substituents at the ortho position. As an example, the dark blue solution of 4-methylcatechol (4-Me-$CatH_2$, 0.267 g, 2.30 mmol), $FeCl_3$ (0.376 g, 2.30 mmol), pyridine (Py, 0.36 $cm^3$, 4.45 mmol) in 15 $cm^3$ THF under argon gave the dark blue-violet powder by slow cooling. Analysis (calculated) for $FeCl_2(4\text{-}CH_3\text{-}Cat)(Py)(H)$: C, 43.80 (43.81); H, 3.60 (3.68); N, 4.25 (4.26); Cl, 21.27 (21.55). The complexes of $^2H$ isomers were isolated in the same way by using the deuteriated catechols, *e.g.*, $C(^2H)_3\text{-}C_6H_3(OH)_2$ or $CH_3\text{-}C_6(^2H)_3(OH)_2$.

These complexes exhibited characteristic visible spectra, reproducing the same spectra as those observed with the complexes prepared *in situ*; [5] in THF (**1**, 646; **2**, 630; **3**, 658; **4**, 722 nm, ε: $\approx 10^3$) and in pyridine (**1**, 504 and 924; **2**, 482 and 868; **3**, 532 and 906; **4**, 544 and 975 nm, ε:$\approx 10^3$). As shown by the forth-order differential curves in Fig. 1, we found that the large, broad bands near at 900 nm are separated to two bands; in the case of **1**, to two bands near at 750 and 1020 nm. This large peak at >1000 nm is characteristic in our model system, and ascribed to the inter-valence charge transfer band, indicating the presence of the species of the strong $Fe^{2+}$ character in addition to $Fe^{3+}$. Figure 1 also shows that addition of the increasing concentration of $Et_4NCl$

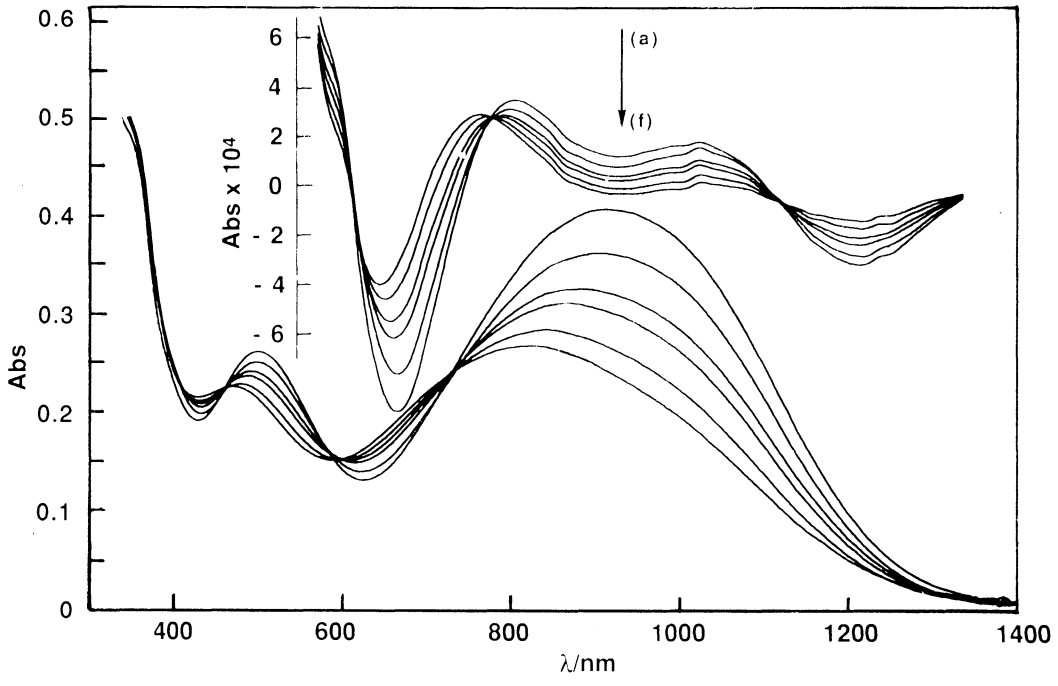



Fig.1. Electronic spectra and their 4th-order differential curves to show the effect of addition of $Et_4NCl$ to 4-methyl catecholatoiron complex in pyridine/acetonitrile $[FeCl_2(4\text{-}CH_3Cat)(Py)]^-[H^+]$=2 mM in pyridine/acetonitrile (1/1), under argon, at room temperature, $[Et_4NCl]/[Fe]$=(a)0, (b)5, (c)10, (d)20, (e)40, (f)60 .

to the pyridine solution brings about the blue shift of the bands with showing the isosbestic points and that the shift is caused by the decrease of the 1020 nm band and the blue shift of the 750 nm band. The result indicates that complexes are in equilibrium with the exchange between the chloride and pyridine ligands.

NMR spectra of the solutions, which were prepared under helium by dissolving the complexes in THF, pyridine or THF/pyridine (1/1, v/v), were recorded by NICOLET NT-300 by varying temperature at -60–40 °C. We measured $^{2}H$ NMR which made possible to observe the sharper peaks in the wider magnetic field range than $^{1}H$ NMR.[7] The complexes of **1** and **3** exhibited a single methyl resonance at 54 and 45 ppm, respectively, in THF, but in the pyridine solution the peak number changed depending on temperature. As shown in Fig. 2, the complex of 4-C($^{2}H$)$_3$-C$_6$H$_3$(OH)$_2$ in pyridine exhibited three main peaks at low temperature (68, 133, 217 ppm at -60 °C) and two in higher temperature (50, 130 ppm at 40 °C). The Curie plots indicated that the species showing 133 and 207 ppm at -60 °C are in equilibrium and exhibit a peak at 130 ppm at 40 °C. The downfield shift to 217 ppm is the largest one observed in the catechol-iron system,[2] and much greater than that of 3-methylcatechol complex (109 ppm at -60 °C). Observation of the same spectra repetitively by varying temperature of the solution in the way of -60 → 40 → -60 °C, dependence of the peak intensities on temperature and the Curie plots of the methyl resonances indicate that three 4-methylcatecholate complexes are in equilibrium. The addition of $Et_4NCl$ decreased the intensities of the resonances at the lower field (e.g. 130 ppm at 40 °C) and increased that of the higher field (50 ppm at 40 °C), indicating that the complexes corresponding to the former have the smaller number

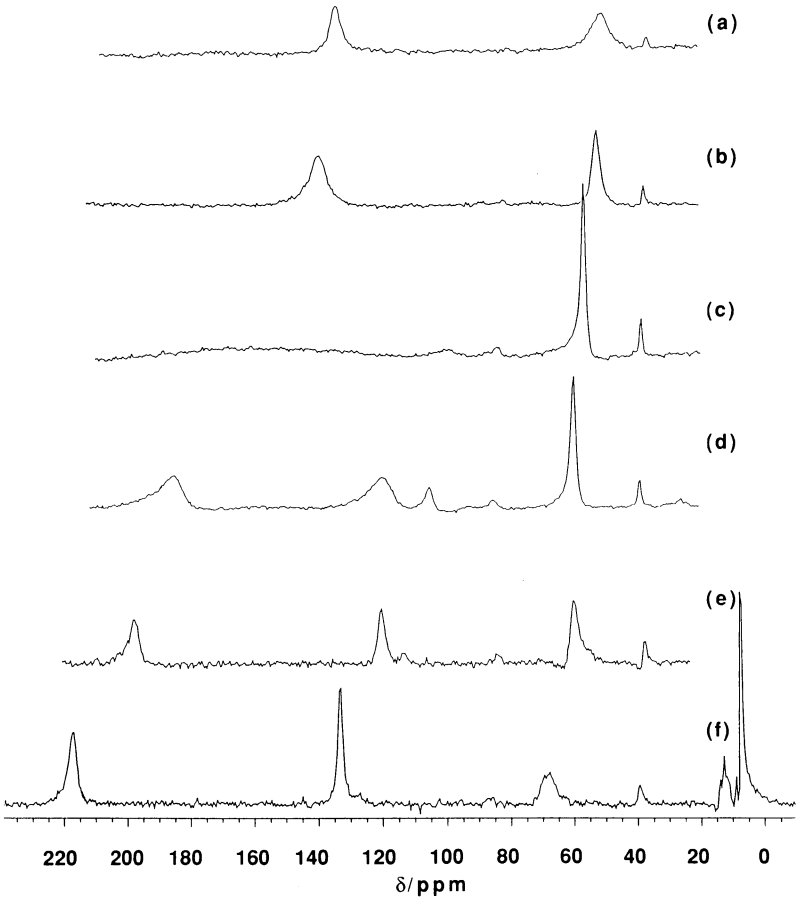



Fig.2. Temperature effect of $^{2}H$ NMR of 4-methylcatecholatoiron complex in pyridine/THF
[FeCl$_2$(4-C($^{2}H$)$_3$Cat)(Py)(H)]=125 mM in pyridine/THF(1/1), under He.
(a)40 °C, (b)23 °C, (c)0 °C, (d)-15 °C, (e)-30 °C, (f)-60 °C

of the chloride ligand than the latter. Temperature dependent spectral changes of the aromatic ring proton resonances were observed in the upfield, *e.g.*, the complex of **1** exhibits peaks at -22, -46, -52, -82, -109 at -30 °C and -19, -26, -43, -66 at 40 °C, though the peak assignment is not easy. These results, *i.e.* appearance of the methyl and aromatic ring proton resonances only in the lower field and higher field, respectively, the large down field shift of the 4-methyl resonances, and the greater shift of 4-methyl than 3-methyl resonances, indicate the strong radical (semiquinonate) character of the catecholate ligand.

As for the structure of the species in the pyridine solution, we have compared the pre-edge peak of the X-ray absorption spectra (XANES). The pre-edge peak area, $(9.7–17.9)\times10^{-2}$ eV, for the complexes in THF supports the five-coordination, and $(4.1–7.2)\times10^{-2}$ eV in pyridine supports the six-coordination.[10] Considering the chloride-pyridine ligand exchange shown by the effect of addition of $Et_4NCl$ on the electronic and NMR spectra and supposing the formation of the six-coordinated species by the additional pyridine coordination to the five-coordinated complexes, the equilibria in pyridine are explained by Eq. 4. In the case of 3-methylcatecholate complex which is isolated as a monochloride complex, two complexes are in equilibrium as supported by the two methyl resonances in NMR.[7]

$$[FeCl_2(Cat)(Py)_2]^- \overset{\text{Py, -Cl}^-}{\rightleftharpoons} FeCl(Cat)(Py)_3 \overset{\text{Py, -Cl}^-}{\rightleftharpoons} [Fe(Cat)(Py)_4]^+ \quad (4)$$

$$[Fe^{III}(Cat)L_4 \longleftrightarrow Fe^{II}(SQ)L_4] \quad (5)$$

$$[Fe^{III}(Cat)L_4] \rightleftharpoons [Fe^{II}(SQ)L_4] \quad (6)$$

The magnitude of the shift reflects the difference of the spin density of the unpaired electron and increases with the number of the pyridine ligand. The radical character may be represented by Eq. 5 or 6, in which SQ and L represents the semiquinonate and other ligands. Further results are necessary, but we prefer, at moment, Eq. 5 which supports the increasing radical character of the $Fe^{III}$ species with the pyridine ligand and the importance of the pyridine ligand in the model system.

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(Received September 21, 1991)