ortho-Hydroxylation of 4-*tert*-butylphenol by nonheme iron(iii) complexes as a functional model reaction for tyrosine hydroxylase

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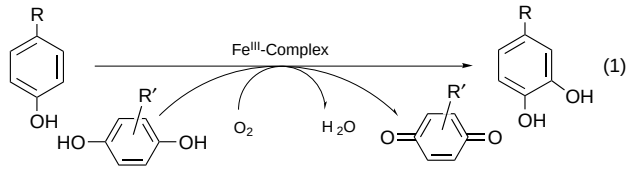
Hydroxylation of 4-*tert*-butylphenol to 4-*tert*-butylcatechol is performed by a catecholatoiron(III) complex–hydroquinone–O_2 system which mimicks the roles of Fe^{2+} and Fe^{3+} in tyrosine hydroxylase; the *ortho*-hydroxylation of phenols by iron–oxygen active species is suggested.

Tyrosine hydroxylase (TH) is one of the pteridine-dependent nonheme iron monooxygenases and converts tyrosine to DOPA by hydroxylation with molecular oxygen. Very little is known about not only the active site environment of the enzyme, but also the role of iron in oxygen activation. TH is isolated mostly in the form of Fe^{3+} [TH(Fe^{3+}), coordinated by DOPA],[1–4] but Fe^{2+} [TH(Fe^{2+})] is suggested to be an active species.[1,5–7] Scheme 1[1,3] shows that Fe^{3+} is reduced to an active TH(Fe^{2+}) by tetrahydropterine (BPH_4)[1] and coordinated by catecholamine to give an inert species.[3,4,8,9] The active species has been proposed to be a peroxytetrahydropterin [(BPH_4)–OOH] rather than an iron–oxygen species.[5,10,11]

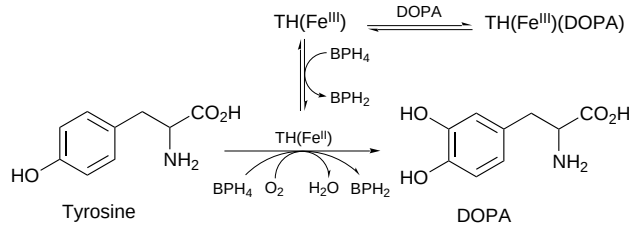
In model studies, on the other hand, results suggest that the hydroxylation proceeds *via* an iron—oxygen species in the enzymatic system. Inner-sphere transfer of a peroxo oxygen in HOC_6H_4O–Fe^{III}–OOR[12] or of the OH ligand in HOC_6H_4O–Fe^{IV}–OH[13] to the phenolate ligands has been proposed. In the latter case, Fe^{II} complexes such as bis(2,6-dicarboxypyridine)iron(ii) and reductants such as PhNHNHPh have been used.[13] Considering that cytochrome P450-type monooxygenations are catalysed by Fe^{III} species, with donation of two electrons and two protons in a catalytic cycle, it is probable that the tyrosine hydroxylase-type *ortho*-hydroxylations are catalysed by Fe^{III} species in the presence of electron and proton donors. In mimicking the processes of reduction of Fe^{3+} to Fe^{2+} by BPH_4 and inhibition by coordination of catechol, and in excluding the formation of the (BPH_4)–OOH type active species, we have performed hydroxylation of phenol with O_2 by iron(iii) complexes in the presence of hydroquinones as proton and electron donors [eqn. (1)].



Catecholatoiron complexes formed *in situ*†[14,15] were used as the ferric complex, and 4-*tert*-butylphenol ($Bu^tC_6H_4OH$ **1**) was used as substrate.‡ The effect of four different hydroquinones was studied here: 2,6-di-*tert*-butylhydroquinone (DTBHQ), *tert*-butylhydroquinone (TBHQ), hydroquinone (HQ) and 1,4-dihydroxynaphthalene (DNH). Hydroxylation was performed at 25 °C with O_2 (1 atm). In a typical case, the reaction of **1** was started by addition of a MeCN solution (7.5 cm^3) of $FeCl_3$ (0.125 mmol) and pyridine (0.25 mmol) to **1** (25 mmol), catechol (0.125–1.25 mmol) and hydroquinone (5.0 mmol).

4-*tert*-Butylcatechol **2** was formed as the sole detectable product from **1**.§ The yield of **2** was greatly dependent on the substituents on catechol: hydroxylation was promoted by electron-withdrawing substituents. Thus, the yield of **2** per mol% Fe decreased in the order: 3,4,5,6-Cl_4 (560) > 4-NO_2 (410) > 4-Cl (230) > H (120) > 3,5-Bu^t_2 (80) > none (*i.e.* without catechol, 52), with [Fe] = [catechol] = 0.125 mmol and [DTBHQ] = 5 mmol. The reverse order was found for the relative rate of consumption of hydroquinones: 3,4,5,6-Cl_4 (0.08) < 4-NO_2 (0.24) < 4-Cl (0.65) < 3,5-Bu^t_2 (0.91) < none (2.18), indicating that electron-withdrawing substituents on catechols, which stabilize the catecholatoiron(iii) complexes, are efficient for the *ortho*-hydroxylation of phenols.

It was noticed that after consumption of hydroquinone, **2** was converted to an unidentified polymeric product. As shown in Fig. 1(*a*), this consecutive reaction of **2** was prevented by addition of excess catechol which also, brought about an increase in the yield of **2**. This is probably because the excess catechol effectively controls the competitive coordination of **2**



Scheme 1

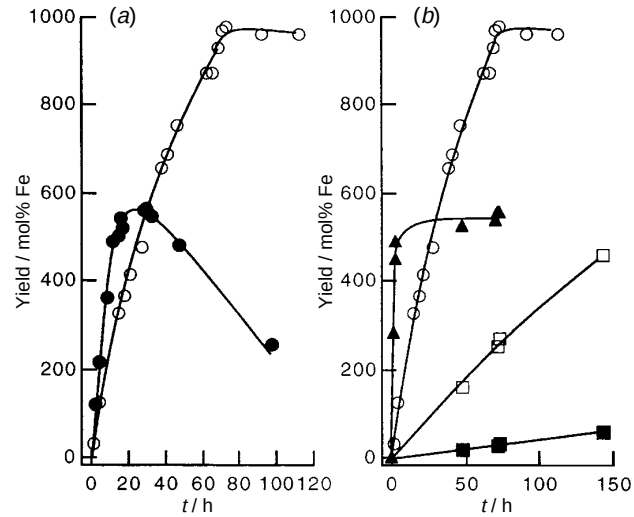


Fig. 1 Formation of 4-*tert*-butylcatechol from 4-*tert*-butylphenol catalysed by a tetrachlorocatecholatoiron(iii) complex under O_2 (1 atm). (*a*) Effect of tetrachlorocatechol concentration: [tetrachlorocatechol]:[Fe] = (●) 1 : 1 and (○) 10 : 1, $FeCl_3$ = 0.125 mmol, pyridine = 0.25 mmol, DTBHQ = 5.0 mmol, $Bu^tC_6H_4OH$ = 25 mmol in MeCN (7.5 cm^3). (*b*) Effect of different hydroquinones: (○) DTBHQ, (▲) DHN, (□) TBHQ and (■) HQ = 5.0 mmol, tetrachlorocatechol = 1.25 mmol, $FeCl_3$ = 0.125 mmol, pyridine = 0.25 mmol, $Bu^tC_6H_4OH$ = 25 mmol in MeCN (7.5 cm^3).

and hydroquinone to Fe^{III}. The end of hydroxylation does not mean the deactivation of the active species. The reaction is repeatedly performed by addition of hydroquinone, indicating that the active species is maintained in the form of a catecholatoiron(iii) complex.

Hydroxylation is greatly influenced by the hydroquinones. As shown in Fig. 1(*b*), the initial rate of hydroxylation is correlated with the electron-transfer affinity of the hydroquinone (DHN > DTBHQ > TBHQ > HQ), as shown by the decrease in the intensity of the characteristic band of the catecholatoiron(iii) complexes [Fig. 2(*a*)]. The rapid quenching of the reaction in spite of the initial rapid formation of **2** is caused by the oxidation of DHN to the corresponding ineffective quinone. Reduction of Fe^{III} to Fe^{II} by hydroquinones was also shown by the formation of a characteristic phenanthrolineiron(ii) complex. In the presence of phenanthroline, the hydroxylation does not proceed, indicating that the active iron(ii) species is trapped by phenanthroline. The effect of the catecholate ligand on the electron transfer shown in Fig. 2(*a*) indicates that tetrachlorocatecholatoiron(iii) is less susceptible to the electron transfer from DTBHQ than the 4-chlorocatecholatoiron(iii) complex.

Regardless of the importance of the iron(ii) species for hydroxylation, the rapid formation and high yield of **2** was not observed when $FeCl_2$ was used in place of $FeCl_3$ in the tetrachlorocatechol–DTBHQ system. Since Fe^{2+} can be oxidized to an Fe^{3+} species under oxygen, it is difficult to perform the reaction only in the presence of Fe^{II} species, but the result indicates that the $FeCl_3$ system is much better at hydroxylation than the $FeCl_2$ system.

The above results indicate not only that electron transfer to Fe^{III} is essential for hydroxylation, but also that the presence of Fe^{III} species in equilibrium with Fe^{II} is important for the catalytic hydroxylation. This is similar to the participation of both Fe^{3+} and Fe^{2+} in the activity of tyrosine hydroxylase. The most favourable combination of conditions studied here is the use of the tetrachlorocatecholatoiron(iii) complex and DTBHQ, which combine to form the most efficient active iron(ii) species.

The importance of the presence of Fe^{III} in equilibrium with Fe^{II} suggests the mechanism shown in Scheme 2 for the hydroxylation of phenols. Unlike reactions catalysed by Fe^{II} complexes,[13] the present results suggest that the Fe^{III} species [$(L)Fe^{3+}$] is involved in the catalytic cycle. Support for formation of a free radical intermediate was not found, but addition of triphenylmethane did not affect the reaction. Observation of characteristic LMCT bands at around 600 nm indicated that phenols, including hydroquinones, coordinate to Fe^{3+}. Fig. 2(*b*) shows the coordination of phenol to Fe^{III}, either together with or replacing a catecholate ligand. The electron-withdrawing substituents on catechol might be effective in making the ferrous as well as ferric centre a better Lewis acid towards the phenolic substrate. It is very likely that the *ortho*-hydroxylation proceeds inside the coordination sphere, but whether the oxygen transfer to phenol proceeds directly *via* a hydroperoxoiron(iii) or oxoiron(v) species is a problem to be solved in future.

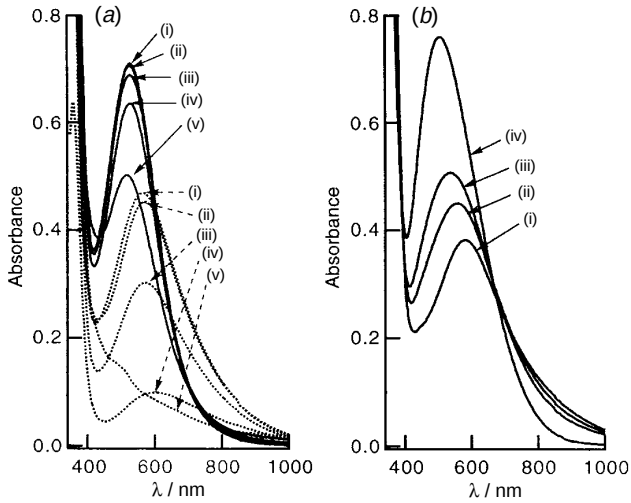
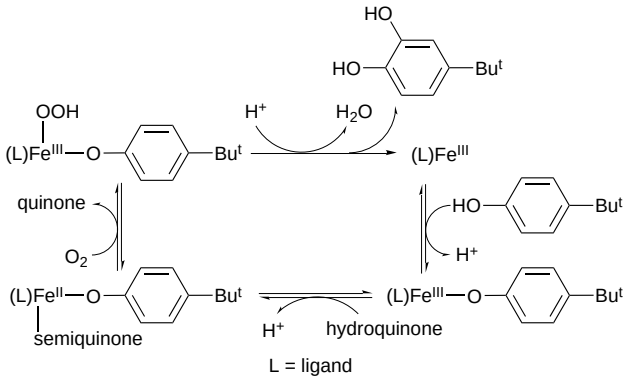


Fig. 2(*a*) Visible spectral changes caused by addition of hydroquinones to (- - - - -) tetrachloro- and (——) 4-chloro-catecholatoiron (iii) complexes under argon: (i) none, (ii) HQ, (iii) TBHQ, (iv) DTBHQ and (v) DHN. $FeCl_3$ = catechol = 0.025 mmol, hydroquinone = 1.0 mmol, pyridine = 0.05 mmol in MeCN (7.5 cm^3). (*b*) Visible changes caused by addition of *tert*-butylphenol to a catecholatoiron (iii) complex under argon: (i) $[Bu^tC_6H_4OH]:[catechol]$ = 0 : 1, (ii) 100 : 1, (iii) 250 : 1 (iv) 250 : 0. $FeCl_3$ = catechol = 0.025 mmol, pyridine = 0.05 mmol in MeCN (75 cm^3).



Scheme 2

Footnotes

† Formation of three types of catecholatoiron complexes in solution, depending on the catechols used, has been reported:[13,14] $[FeCl(Cat)py_2]$ ($CatH_2$ = 3,5-di-*tert*-butylcatechol), $[FeCl_2(Cat)py]$ ($CatH_2$ = pyrocatechol, 4-methylcatechol, 4-chlorocatechol), $[FeCl_2(Cat)py_2]$ ($CatH_2$ = tetrachlorocatechol).

‡Although chlorophenol is reported to be more reactive than **1**, (ref. 12) we could not detect chlorocatechol in our conditions.

§ No product other than **1** has been detected, indicating that **2** and *tert*-butyl-1,2-benzoquinone **3** are converted to polymeric products.

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