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Oxygenative cleavage of catechols including protocatechuic acid with molecular oxygen in water catalysed by water-soluble non-heme iron(III) complexes in relevance to catechol dioxygenases

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Catechol dioxygenase model oxygenations have been performed for the first time in water by using water-soluble nonheme iron(III) complexes, enabling the oxygenation of protocatechuic acid and other catechols.

Catechol dioxygenases play key roles in the metabolism of various aromatic compounds, converting aromatics to aliphatics with insertion of molecular oxygen between a C–C bond of a benzene ring. Since the first example of cleavage of 3,5-di-*tert*-butylcatechol by a simple nonheme iron complex,[1–3] various types of functional models have been developed.[4–7] These model reactions have been performed exclusively in organic solvents such as acetonitrile. Because of growing interest in the development of catalytic reactions in aqueous solutions, we here synthesized water-soluble tripodal ligands, with which we first successfully performed the intradiol cleavage of catechols *via* water-soluble catecholatoiron(III) complexes. Catechols examined here are 4-*tert*-butylcatechol (**1a**), 4-chlorocatechol (**1b**) and protocatechuic acid (**1c**), which are fairly soluble in water. **Ic** is a typical substrate in the enzymatic oxygenations, but has been hard to oxygenate by model complexes. Here we first show that not only **1c** but also other catechols are catalytically oxygenated by water-soluble iron complexes in water (Scheme 1) and that the reactivity and selectivity are greatly dependent on both catechol and ligand.

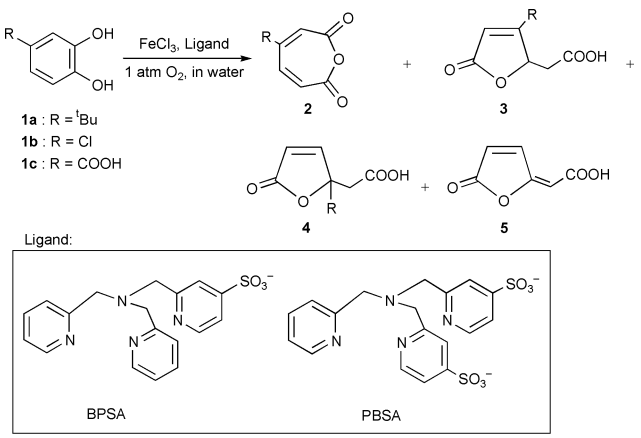
The water-soluble ligands used are bis(2-pyridylmethyl)((4-sulfo-2-pyridyl)methyl)amine (BPSA) and (2-pyridylmethyl-)bis((4-sulfo-2-pyridyl)methyl)amine (PBSA).† Oxygenation was started by mixing aqueous buffer solutions of catechols and of the complexes prepared *in situ* ([$FeCl_3$]:[ligand] = 1:2 ~ 3) under 1 atm O_2 at pH 3.4 (glycine buffer) or 6.0 (MES buffer: 2-morpholinoethanesulfonic acid–NaOH) (the solutions keep homogeneous at pH 3.4 ~ 7.0).‡

Fig. 1 shows the effects of pH and the ligand in the oxygenation of **1a** ([Fe]:[ligand]:[**1a**] = 1:2:4) at 25 °C. We found that 4-*tert*-butylcatechol is a good substrate which is readily oxygenated in aqueous media without being accompanied by miscellaneous reactions such as polymerization. **1a** was quantitatively and selectively converted to the intradiol cleavage product **3a** (398% yield after 14 h, based on [Fe]) at pH 3.4.§ **2a** was not detected in aqueous conditions. At higher pH, the reaction stopped at an earlier stage to give a lesser yield of **3a** accompanied with **4a** (214% yield, **3a** :**4a** = 84:16, at pH 8.0). We expected that PBSA having two SO_3^- groups forms a more active complex than BPSA by forming an iron center with higher Lewis acidity, but as shown in Fig. 1A the promoting effect of the increasing number of SO_3^- was not significant and the higher yield of product was obtained rather by the BPSA system.

4-Chlorocatechol (**1b**) was selectively converted to **5**, indicating that only **4b** is initially formed and dehydrochlorinated.[8] This result is very different from the case in acetonitrile, in which both **3b** and **5** are products in the catalytic oxygenation by Fe(TPA)Cl.[8] The yields of **5** at 50 °C and pH 6.0 were 33% (2 h) and 110% (6 h) in the BPSA system and 59% (2 h) and 75% (6 h) in the PBSA system, indicating that the former gives higher yields than the latter. **2b** was not detected as an intermediate.

No model complex in organic solvents has oxygenated protocatechuic acid (**1c**), but we here found that **1c** is selectively and catalytically oxygenated to give only **3c** in aqueous solution. As shown in Fig. 1B, the reaction is very slow and the promoting effect of the SO_3^- group is significant: the yields of **3c** are 59% (BPSA) and 123% (PBSA) after 36 h, at 50 °C and pH 6.0. A small amount of **2c** was detected only in the early stage (7% at 2 h), suggesting the oxygenation proceeds by the stepwise oxygen insertion as proposed.[4,7] However, different from the reactions in acetonitrile, the yields of **2a** ~ **2c** are too low to support the formation of free **2** as a precursor of lactonic acid.[3,9]

It is noteworthy that the type of cyclization to form lactonic acid is greatly dependent on the catechol substituent. It is known



Scheme 1

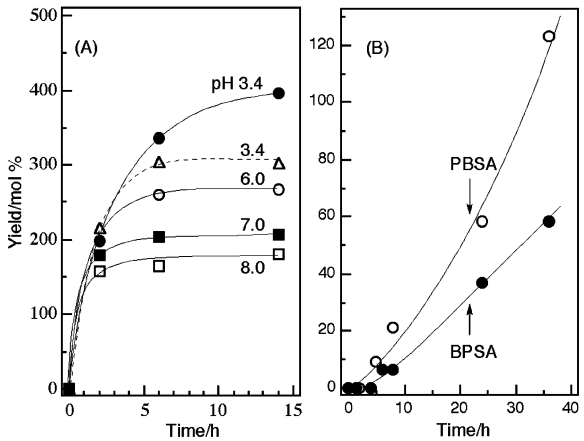


Fig. 1 Time course of the oxygenations of **1a** at 25 °C and **1c** at 50 °C with O_2, catalysed by the water-soluble Fe(III) complexes. Yields of **3a** or **3c** [mol%] are based on the amount of Fe used. (A) Effect of pH in the oxygenation of **1a** (8 mM) in the BPSA system ([$FeCl_3$]:[BPSA]:[**1a**] = 1:2:4) (solid line) and comparison with the PBSA system (broken line): (B) Effect of the ligand at pH 6.0 in the oxygenation of **1c** (12 mM) ([$FeCl_3$]:[ligand]:[**1c**]) = 1:3:3).

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that the cyclization of muconic acid is catalysed by muconate cycloisomerase in the case of the enzyme,[10] but in the model systems free muconic acids as precursors of lactonic acid have not been detected. To determine if free muconic acid is involved in controlling the type of cyclization, we have studied whether 3-chloro-*cis*,*cis*-muconic acid (**6**) is converted to **5** under the reaction conditions in a similar way to muconate cycloisomerase II.[10,11] Interestingly, we found that **6** gave mainly **3b** (86%) rather than **5** (14%) at pH < 6.0 and no cyclization was observed at pH > 8.0. This indicates that **6** is not a precursor of **5** in the model system and suggests that the cyclization of muconate ligands takes place before elimination of free muconic acids. The coordination mode of muconate ligand may affect the type of cyclization, controlling the selectivity.

Formation of catecholatoiron complexes as reactive intermediates was shown by the characteristic LMCT bands[3,12,13] and by the diminishment of their peak intensities under oxygen, though the complexes have not been isolated. The complexes of **1a** exhibited bands at 694 nm (BPSA) and 701 nm (PBSA) while the higher energy bands were not clear. Complexes of **1b** exhibited peaks at 416 and 734 nm (BPSA) and 417 and 738 nm (PBSA) (Fig. 2A), and those of **1c** at 504, 753 nm (BPSA) and 543, 784 nm (PBSA) (Fig. 2B). The spectra of the complexes prepared *in situ* in aqueous media are less clear-cut than those in organic solvents. This is because the water-soluble catechols form catecholatoiron complexes in water even without addition of ligands,[14] exhibiting bands at 404 and 757 nm (**1a**), 423 and 725 nm (**1b**), 566 nm (**1c**), and can be in equilibrium with catecholate complexes with BPSA or PBSA ligands even in the presence of excess of these ligands. Oxygenations of catechols proceed no doubt preferentially *via* catecholatoiron complexes with BPSA or PBSA ligands because the complexes without these ligands are stable under oxygen and give no oxygenated products.

The present results reveal that water-soluble iron complexes oxygenate catechols in a different mode from those in organic media and achieve oxygenation of protocatechuic acid. As seen from the selective oxygenation of 4-*tert*-butylcatechol, water was found effective in preventing miscellaneous radical reactions of catechols, enabling the use of catechols other than 3,5-di-*tert*-butylcatechol which are not substrates in the enzymatic system. Since enzymatic reactions proceed in aqueous media, this new model system will give the more direct information about oxygenation mechanisms than those in organic solvents. In addition, this system holds out the possibility of oxygenatively decomposing water-soluble aromatic pollutants in the environment.

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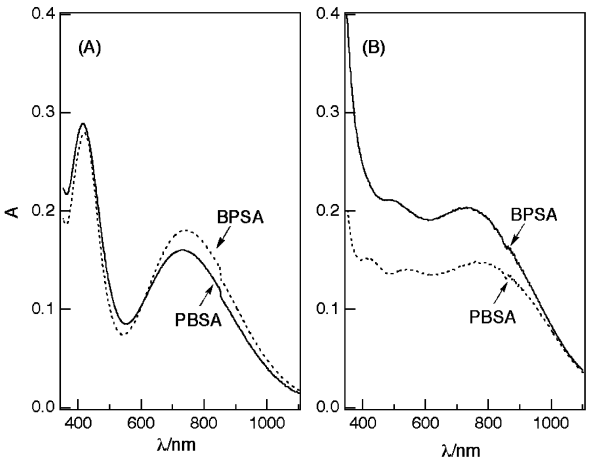

Fig. 2 Absorption spectra of catecholate complexes with water-soluble ligands. (A) Complexes of **1b** at pH 3.4 ([$FeCl_3$]:[ligand]:[**1b**] = 1:2:4). (B) Complexes of **1c** at pH 6.0.

Notes and references

† The sodium salt of BPSA was synthesized by the reaction of ((4-chloro-2-pyriryl)methyl)bis(2-pyridylmethyl)amine with sodium bisulfate in water–methanol in the 79% yield: ^{1}H NMR (400 MHz, 25 °C, D_2O) δ 3.94 (s, 4H), 3.95 (s, 2H), 7.25(m, 2H), 7.48 d, 1H, *J* 5.37 Hz), 7.51 (d, 2H, *J* 7.81 Hz), 7.68 (s, 1H), 7.73 (m 2H), 8.35 (d, 2H, *J* 4.89 Hz), 8.45 (d, 1H, *J* 5.37 Hz); ^{13}C NMR (100 MHz, 25 °C, D_2O) δ 60.5, 60.7, 118.4, 119.8, 123.1, 124.4, 138.3, 147.4, 149.0, 151.2, 157.1, 159.7; ESI-MS: *m/z* 393.2 ($[C_{18}H_{17}N_4(SO_3Na)H]^+$), 415.2 ($[C_{18}H_{17}N_4(SO_3Na)Na]^+$). The sodium salt of PBSA was synthesized by the reaction of bis((4-chloro-2-pyridyl)methyl)(2-pyridiylmethyl)amine with sodium bisulfate in water–methanol in 64% yield: ^{1}H NMR (400 MHz, 25 °C, D_2O) δ 3.95 (s, 2H), 4.03 (s, 4H), 7.21 (m, 1H), 7.47 (d, 1H, *J* 7.38 Hz), 7.49 (d, 2H, *J* 5.38 Hz), 7.61 (s, 2H), 7.70 (m, 1H), 8.32 (d, 1H, *J* 5.38 Hz), 8.51 (d, 2H, *J* 5.38 Hz); ^{13}C NMR (100 MHz, 25 °C, D_2O) δ 60.0, 60.1, 117.3, 118.8, 121.7, 123.3, 136.5, 146.6, 148.1, 150.0, 156.2, 158.4; ESI-MS: *m/z* 448.8 ($[C_{18}H_{17}N_4(SO_3)(SO_3H)]^-$), 470.8 ($[C_{18}H_{17}N_4(SO_3)(SO_3Na)]^-$).

‡ Oxygenations were performed in a 20 cm^3 cylindrical flask at 25 or 50 °C and under 1 atm O_2. In a typical case, reactions were started by addition of 1 cm^3 buffer solution of **1** (0.008 mmol) to 1 cm^3 buffer solution containing $FeCl_3$ (0.004 mmol) and ligand (0.008 mmol). Product extraction and analysis were performed as reported previously.[15]

§ Oxygenated products from **1b** were identified and quantitatively analyzed as reported before.[8] The product from **1a** was identified as follows. 4-*tert*-butyl-5-carboxymethyl-2-furanone (**3a**): ^{1}H NMR (400 MHz, 25 °C, $CDCl_3$) δ 1.26 (s, 9H), 2.56 (dd, 1H, *J* 9.27, 16.60 Hz), 3.11 (dd, 1H, *J* 2.93, 16.60 Hz), 5.42 (ddd, 1H, *J* 1.46, 2.93, 9.27 Hz), 5.90 (d, 1H, *J* 1.46 Hz); ^{13}C NMR (100 MHz, 25 °C, $CDCl_3$) δ 29.4, 33.6, 38.3, 79.2, 116.3, 172.4, 173.6, 179.3; DI-MS; *m/z* 198. 5-*tert*-butyl-5-carboxylmethyl-2-furanone (**4a**): ^{1}H NMR (400 MHz, 25 °C, $CDCl_3$) δ 1.02, 2.91, 3.00, 6.17, 7.53; ^{13}C NMR (100 MHz, 25 °C, $CDCl_3$) δ 25.3, 30.9, 37.8, 92.3, 122.6, 156.5, 172.4, 174.1; DI-MS: *m/z* 198. The product from **1c** was identified as 4-carboxyl-5-carboxymethyl-2-furanone (**3c**): ^{1}H NMR (400 MHz, 25 °C, CD_3CN) δ 2.65(dd, 1H, *J* 16.6, 8.1 Hz), 3.14 (1H, *J* 16.6, 2.9 Hz), 5.47 (ddd, 1H, *J* 8.1, 2.9, 2.0 Hz), 6.32 (d, 2H, *J* 2.0 Hz); ^{13} C NMR (100 MHz, 25 °C, CD_3CN) 37.1, 79.4, 128.0, 157.3, 162.2, 170.0, 171.5; DI-MS; *m/z* 186. 4-carboxyloxacyclohepta-3,5-diene-2,7-dione (**2c**) was not isolated, but detected by ^{1}H NMR (400 MHz, 25 °C, $CDCl_3$) δ 6.51 (d, 1H, *J* 15.6 Hz), 6.65 (s, 1H), 8.05 (dd, 1H, J 13.5, 1.3 Hz).

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