A Reaction Mimic of Tyrosine Hydroxylase: Hydroxylation of a Phenoxo Ferric Complex to a Catecholato Complex with mCPBA

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Tyrosine hydroxylase (TH, tyrosine 3-monooxygenase) is a tetrahydropterin-dependent non-heme iron monooxygenase that catalyzes the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (dopa).[1] This reaction is an indispensable reaction step in the biosynthesis of catecholamines, which are important neurotransmitters and hormones. While the reaction mechanism of TH remains unclear, in light of the mechanism accepted for cytochrome P-450,[2] the catalytic cycle is suggested to involve a reductive activation of dioxygen to form a peroxy intermediate. As the most plausible intermediate, Benkovic et al. proposed the formation of peroxytetrahydropterin.[3,4] Support for this mechanism lies on the fact that TH catalyzes the reaction with a peroxide such as H_2O_2,[4] which corresponds to the shunt pathway known for cytochrome P-450. The role of the non-heme iron in the subsequent O–O bond cleavage of the peroxy intermediate is the focus of interests, because few of the mechanistic aspects of non-heme iron monooxygenases have been elucidated to date, though it is well established for cytochrome P-450 that the heterolytic O–O bond cleavage generates a ferryl oxo porphyrin π-cation radical responsible for oxo-transfer reactions.[5] As a plausible mimic for this peroxy oxidation by TH, we now report the hydroxylation of a phenoxo ferric complex to a catecholato complex with a peroxide (mCPBA).[6]

A series of bis(phenoxo) ferric complexes $LFe(OAr)_2$ can be prepared by aerobic oxidation of ferrous complexes LFe(OAr).[7] Because mCPBA is more (or at least comparably) acidic than phenols, it was anticipated that the reaction of the bis(phenoxo) complex with 1 equiv of mCPBA may result in formation of an (acylperoxo)(phenoxo) ferric species by an acid/base ligand displacement. Thus, $LFe(OC_6H_4\text{-}4\text{-}NO_2)_2$ (**1**)[8] was treated with 1 equiv of mCPBA at −78 °C in Et_2O. Upon the addition of mCPBA, the deep blue purple color of **1** immediately turned into reddish brown with formation of 1 equiv of 4-nitrophenol, which was quantitatively identified by *in situ* GC analysis. The solvent was replaced with MeOH, and the solution was warmed to room temperature, resulting in the formation of 4-nitrocatechol essentially in quantitative yield. The same reactions with $LFe(OC_6H_4\text{-}4\text{-}Cl)_2$ and $LFe(OC_6H_4\text{-}4\text{-}Me)_2$ gave 4-chlorocatechol and 4-methylcatechol, respectively, as a solely identified product. Hydroxylation of tyrosine ester was attempted as well. Identification of the product was accomplished by TLC isolation followed by GC–MS analysis in comparison with an authentic sample, establishing the formation of dopa ester in 68% isolated yield. Thus the present reactions mimic the catalysis of TH. Also, the chemistry may be relevant to that known for the mutant protein R2 of ribonucleotide reductase. The site-directed mutagenesis of Phe-208 in R2 to tyrosine causes the hydroxyl-

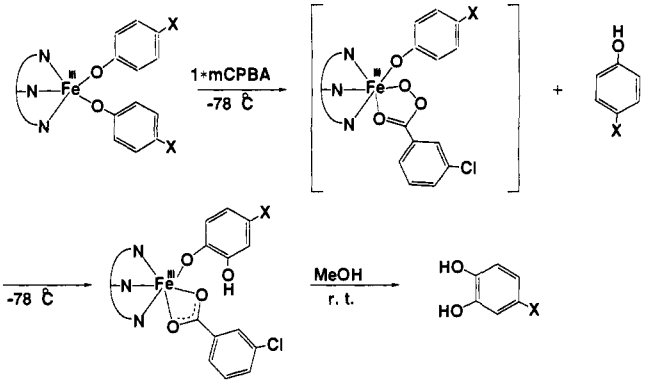

Figure 1. Plausible reaction mechanism of hydroxylation of bis(phenoxo) ferric complex with mCPBA.

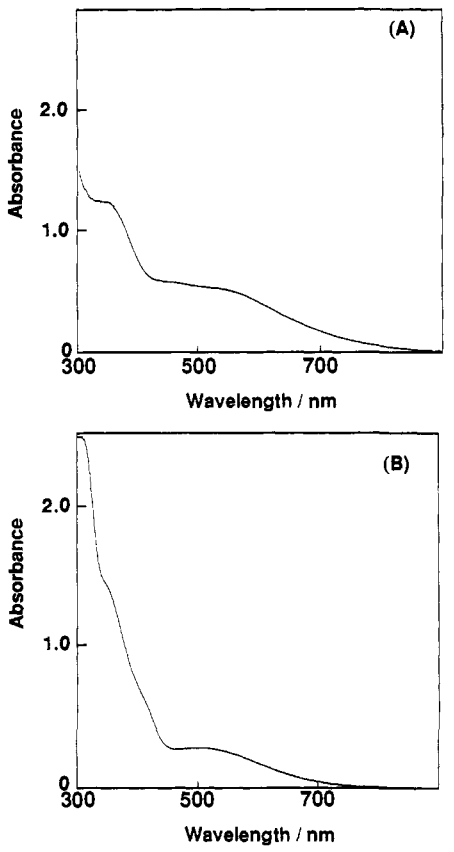

Figure 2. Electronic spectra of **3** (A) and the reaction mixture of **1** and mCPBA (B) recorded at room temperature. Spectrum A: **3**, 0.121 mM in Et_2O. Spectrum B: **1** (0.067 mM) was reacted with 1.2 equiv of mCPBA in Et_2O at −78 °C for 2 h and warmed to room temperature under argon. The intense band at ca. 300 nm is ascribed to overlapping of the bands due to **3**, 4-nitrophenol (λ_{max} 302 nm (ϵ 11 000)), and 4-nitrocatechol (λ_{max} 280 nm (ϵ 34 000)).

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(6) The abbreviations used: mCPBA, *m*-chloroperbenzoic acid; m-CBA, *m*-chlorobenzoate; L=HB(3,5-$iPr_2pz)_3$, hydrotris(3,5-diisopropyl-1-pyrazolyl)borate; 3,5-iPr_2pzH, 3,5-diisopropylpyrazole; Cat-4-NO_2, 4-nitrocatecholate.

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(8) Anal. Calcd for $C_{39}H_{54}N_8BO_6Fe$: C, 58.73; H, 6.82; N, 14.05. Found: 58.87; H, 7.20; N, 14.21. IR (KBr, cm^{-1}): ν(BH), 2555; ν(Ph), 1584; $\nu(NO_2)$, 1285. UV–vis (nm, ϵ/cm^{-1} M^{-1}, in Et_2O): 320 (7150), 528 (2360). The crystal structure of the MeCN adduct of $LFe(OC_6H_4\text{-}4\text{-}Me)_2$ was determined by X-ray crystallography.[7]

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ation of the tyrosine upon dioxygen treatment, generating a ferric catecholate species.[9]

The selective formation of catechols by the oxidation of the bis(phenoxo) complexes with mCPBA contrasts with the results of control experiments: the reactions of the free phenols with 1 equiv of mCPBA under comparable reaction conditions did not proceed, and the reactions at room temperature merely yielded oxidatively coupling products, diphenoquinones, with no catechol formation. Such a dramatic change in reactivity and selectivity is most rationally explained in terms of the mechanism involving an (acylperoxo)(phenoxo) ferric intermediate followed by an intramolecular oxo-transfer reaction that gives a catecholato ferric complex which is easily hydrolyzed to yield the catechol (see the mechanism illustrated in Figure 1).

Since the isolation of the catecholato complex from the reaction mixture was unsuccessful, presumably due to contamination of impurities such as mCBA, we synthesized the authentic catecholato complex to lend support to the working hypothesis. Thus a (phenoxo)(carboxylato) complex LFe(OC_6H_4-4-F)(mCBA) (**2**)[10] was reacted with 1.2 equiv of 4-nitrocatechol at −78 °C. The solution was warmed to room temperature, whereupon the color change from bluish green to reddish brown was noted. After filtration, the filtrate was concentrated and cooled at −20 °C, yielding LFe(OC_6H_3-2-OH-4-NO_2)(mCBA) (**3**) as a brown solid whose structure was identified on the basis of elemental analysis, IR, and FD–MS spectroscopy.[11] Complex **3** exhibits two characteristic absorption bands at 350 and 550 nm, as shown in Figure 2A. This spectral feature is in excellent accord with that of the solution yielded by the reaction of **1** and mCPBA at −78 °C (see Figure 2B). The FD–MS spectrum of the solution also supports the formation of **3**. Moreover, the possibility of formation of a chelated catecholato complex in the reaction of **1** and mCPBA is definitely excluded, since the authentic sample of the chelated catecholato complex LFe(Cat-4-NO_2)(3,5-iPr_2pzH) (**4**) gives distinct spectral features.[12]

All attempts to spectroscopically detect the (acylperoxo)(phenoxo) intermediate in the present oxidation reaction have been unsuccessful. This implies that the formed (acylperoxo)(phenoxo) complex undergoes the subsequent oxo-transfer reaction instantaneously. Therefore, it is not possible as yet to conclude whether the oxo-transfer reaction proceeds via the heterolytic,[2,13] homolytic,[14] or some other concerted mechanism of the peroxy intermediate.[15] In order to gain more mechanistic insight, efforts are being made to replace the phenoxide with a ligand robust against oxidation in hopes of stabilizing and identifying the acylperoxo complex.

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(10) Anal. Calcd for $C_{40}H_{54}N_6BClFO_3Fe$: C, 60.97; H, 6.91; N, 10.66. Found: 61.22; H, 6.86; N, 10.68. IR (KBr, cm^{-1}): ν(BH), 2554; ν(Ph-MCBA), 1591; ν(Ph), 1555; ν(CO), 1537. UV–vis (nm, ϵ/cm^{-1} M^{-1}, in Et_2O): 616 (2300). The X-ray analysis of LFe(OC_6H_4-4-F)(OAc) confirmed the bidentate coordination mode of the carboxylate. The details will be reported elsewhere.

(11) Anal. Calcd for $C_{40}H_{53}N_7BClO_6Fe$: C, 57.88; H, 6.44; N, 11.81. Found: C, 57.70; H, 6.71; N, 12.05. IR (KBr, cm^{-1}): ν(BH), 2544; ν(Ph-MCBA), 1595; ν(Ph), 1555; ν(CO), 1535; $\nu(NO_2)$, 1271. UV–vis (nm, ϵ/cm^{-1} M^{-1}, in Et_2O): 350 (10 400), 548 (4000). FD–MS (m/e): 831.

(12) The complex was prepared by the reaction of LFeCl and 4-nitrocatechol in the presence of triethylamine followed by oxidation with $KMnO_4$. The structure was determined by X-ray crystallography, establishing the chelate coordination of the catecholate: Fe–O1, 1.951(8) and Fe–O2, 1.938(9) Å. Anal. Calcd for $C_{42}H_{64}N_9BO_4Fe$: C, 61.14; H, 7.75; N, 15.27. Found: C, 61.23; H, 7.81; N, 15.03. IR (KBr, cm^{-1}): ν(NH), 3362; ν(BH), 2563; ν(Ph), 1564; $\nu(NO_2)$, 1285. UV–vis (nm, ϵ/cm^{-1} M^{-1}, in Et_2O): 400(5020), 735-(1520).

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