Functional Model of Dopamine β-Hydroxylase. Quantitative Ligand Hydroxylation at the Benzylic Position of a Copper Complex by Dioxygen†

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There has been considerable interest in the modeling of metalloprotein functions and their active site structures in order to understand their catalytic mechanisms and unusual spectroscopic characteristics, which is essential to the development of an efficient catalyst and/or functional material.[1] Among them, copper proteins have long attracted many chemists because of their versatile functions in several living systems.[2] In particular, copper monooxygenases have recently merited much attention owing to their beautiful achievements in Cu/O_2 chemistry.[3] Several peroxo dinuclear copper complexes have been prepared, providing valuable information about the binding mode of O_2 to the dinuclear copper center of hemocyanin and tyrosinase. The redox chemistry in such Cu_2O_2 complexes has also been studied extensively in order to elucidate how O_2 is activated in oxygenation reactions. Thus a number of efficient model systems mimicking tyrosinase function (*aromatic hydroxylation*) have been reported.[4,5] As far as *aliphatic hydroxylation* is concerned, however, functional models of dopamine β-hydroxylase (DβH) or peptidylglycine α-amidating monooxygenase (PAM) are still very rare,[6] and no quantitative aliphatic hydroxylation has so far been achieved. Here we report the first example of quantitative ligand hydroxylation at the benzylic position of a Cu complex by O_2, which can be regarded as an efficient functional model of DβH.

DβH catalyzes the ascorbate dependent benzylic hydroxylation of phenylethylamines such as dopamine to the corresponding norepinephrine.[7] The enzyme contains two coppers per catalytic unit, but these Cu ions have recently been suggested to have different roles, one (Cu_A) being an electron acceptor (ascorbate binding site) and the other (Cu_B) acting as a substrate as well as O_2 binding site.[8,9] In order to mimic the enzyme active center, we employed a tridentate ligand Py2Phe (*N,N*-bis[2-(2-pyridyl)ethyl]-2-phenylethylamine) in which a phenethylamine (substrate) moiety is incorporated into the ligand molecule.[10] Cu(II) complex **1** was prepared by treatment of the Py2Phe ligand with $Cu(ClO_4)_2 \cdot 6H_2O$ in methanol and crystallized from CH_2Cl_2-ether, whereupon an X-ray crystal analysis was carried out.[11,12] The Cu(II) ion has a nearly square pyramidal structure and the benzene ring is located far from the copper center (the distance between the benzylic carbon and Cu(II) is about 4.36 Å) as shown in Figure 1.

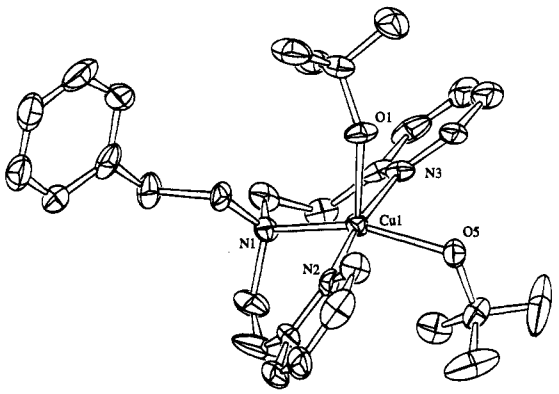

Figure 1. ORTEP drawing of Cu(II) complex **1**.

In the enzymatic system, two electrons are supplied to the Cu_B active center from ascorbate through the Cu_A center during the hydroxylation event. Thus, we employed a 1,2-enediolate, derived from benzoin and a base, as a model of the electron donor, ascorbate. Cu(II) complex **1** (84 μmol) was treated with an equimolar amount of benzoin and triethylamine in CH_2Cl_2 (5 mL) at room temperature for 2 h under Ar, and then the mixture was stirred under an atmospheric pressure of O_2 for several hours. Allowing the reaction mixture to stand for a few days gave a single, green crystal, whose structure was determined by X-ray crystallographic analysis as shown in Figure 2.[11,13] The product is a dinuclear Cu(II) complex **2** in which ligand hydroxylation occurred selectively at the benzylic position of the substrate moiety and this O^- group acts as a bridge for both copper ions. The dimer has an approximate C_2 symmetry, and both copper ions also have nearly square pyramidal structures. The yield of the ligand hydroxylation was 100%; the modified ligand was isolated quantitatively after an ordinary workup treatment of the reaction mixture with aqueous NH_4OH and following extraction by CH_2Cl_2 as shown in Scheme

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(1) (a) Lippard, S. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 344. (b) Sorrell, T. N. *Tetrahedron* **1989**, *45*, 3. (c) Kurtz, D. M. *Chem. Rev.* **1990**, *90*, 585. (d) Solomon, E. I.; Baldwin, M. J.; Lowery, M. D. *Chem. Rev.* **1992**, *92*, 521. (e) Reedijk, J., Ed. *Bioinorganic Catalysis*; Marcel Dekker, Inc.: New York, 1993. (f) Karlin, K. D. *Science* **1993**, *261*, 701.

(2) Karlin, K. D., Tyeklàr, Z., Eds. *Bioinorganic Chemistry of Copper*; Chapman & Hall: New York, 1993.

(3) For recent reviews, see: (a) Karlin, K. D.; Gultneh, Y. *Prog. Inorg. Chem.* **1987**, *35*, 219. (b) Karlin, K. D.; Tyeklár, Z. *Adv. Inorg. Biochem.* **1993**, *9*, 123. (c) Kitajima, N. *Adv. Inorg. Chem.* **1992**, *39*, 1. (d) Kitajima, N.; Moro-oka, Y. *Chem. Rev.* **1994**, *94*, 737.

(4) (a) Gagne, R. R.; Gall, R. S.; Lisensky, G. C.; Marsh, R. E.; Speltz, L. M. *Inorg. Chem.* **1979**, *18*, 771. (b) Karlin, K. D.; Hayes, J. C.; Gultneh, Y.; Cruse, R. W.; McKown, J. W.; Hutchinson, J. P.; Zubieta, J. *J. Am. Chem. Soc.* **1984**, *106*, 2121. (c) Nasir, M. S.; Karlin, K. D.; McGowty, D.; Zubieta, J. *J. Am. Chem. Soc.* **1991**, *113*, 698. (d) Casella, L.; Gullotti, M.; Pallanza, G.; Rigoni, L. *J. Am. Chem. Soc.* **1988**, *110*, 4221. (e) Gelling, O. J.; Meetsma, A.; Feringa, B. L. *Inorg. Chem.* **1990**, *29*, 2816. (f) Menif, R.; Martell, A. E.; Squattrio, P. J.; Clearfield, A. *Inorg. Chem.* **1990**, *29*, 4723. (g) Sorrell, T. N.; Vankai, V. A.; Garrity, M. L. *Inorg. Chem.* **1991**, *30*, 207.

(5) Kitajima, N.; Koda, T.; Iwata, Y.; Moro-oka, Y. *J. Am. Chem. Soc.* **1990**, *112*, 8833.

(6) (a) Thompson, J. S. *J. Am. Chem. Soc.* **1984**, *106*, 8308. (b) Patch, M. G.; McKee, V.; Reed, C. A. *Inorg. Chem.* **1987**, *26*, 776. (c) Capdevielle, P.; Maumy, M. *Tetrahedron Lett.* **1991**, *32*, 3831. (d) Amadéi, E.; Alilou, E. H.; Eydoux, F.; Pierrot, M.; Réglier, M.; Waegell, B. *J. Chem. Soc., Chem. Commun.* **1992**, 1782.

(7) Stewart, L. C.; Klinman, J. P. *Annu. Rev. Biochem.* **1988**, *57*, 551.

(8) Brenner, M. C.; Klinman, J. P. *Biochemistry* **1989**, *28*, 4664.

(9) (a) Pettingill, T. M.; Strange, R. W.; Blackburn, N. J. *J. Biol. Chem.* **1991**, *266*, 16996. (b) Blackburn, N. J.; Hasnain, S. S.; Pettingill, T. M.; Strange, R. W. *J. Biol. Chem.* **1991**, *266*, 23120. (c) Reedy, B. J.; Blackburn, N. J. *J. Am. Chem. Soc.* **1994**, *116*, 1924.

(10) Réglier, M.; Amadeï, E.; Tadayoni, R.; Waegell, B. *J. Chem. Soc., Chem. Commun.* **1989**, 447.

(11) Analytical data for **1**: Anal. for $[Cu^{II}(Py2Phe)(ClO_4)_2]$. Calcd for $C_{22}H_{25}Cl_2CuN_3O_8$: C, 44.49; H, 4.24; N, 7.08. Found: C, 44.33; H, 4.23; N, 7.00. UV-vis [CH_2Cl_2, λ_{max}, nm (ε, M^{-1} cm^{-1})]: 266 (15 400), 664 (234). IR (KBr, cm^{-1}): 1614 (s), 1574 (w) [ν (aromatic ring)], 1116 (vs br), 624 (s) [ν (ClO_4)]. **2**: Anal. for $[Cu^{II}_2(Py2Phe\text{-}O)_2](ClO_4)_2 \cdot 2H_2O$. Calcd for $C_{44}H_{52}Cl_2Cu_2N_6O_{12}$: C, 50.10; H, 4.97; N, 7.97. Found: C, 50.07; H, 4.69; N, 8.00. UV-vis [CH_2Cl_2, λ_{max}, nm (ε, M^{-1} cm^{-1})]: 259 (18 700), 382 (2840), 666 (234). IR (KBr, cm^{-1}): 1606 (s), 1574 (w) [ν (aromatic ring)], 1094 (vs br), 624 (s) [ν (ClO_4)].

(12) Crystal data of **1**: $C_{22}H_{25}N_3O_8Cl_2Cu$, FW = 593.9, orthorhombic, space group $Pna2_1$, a = 18.003(1) Å, b = 15.376(1) Å, c = 9.054(1) Å, V = 2506.3(6) Å^3, Z = 4, D_c = 1.574 g cm^{-3}, μ(Cu Kα) = 36.6 cm^{-1}, and $4° < 2\theta < 120°$. Final R and R_w values were 0.055 and 0.049, respectively.

(13) Crystal data of **2**: $C_{45.5}H_{51}N_6O_{10}Cl_5Cu_2$, FW = 1146.29, monoclinic, space group $P21/c$, a = 16.176(2) Å, b = 15.082(2) Å, c = 41.608(2) Å, β = 96.54(1)°, V = 10084(1) Å^3, Z = 8, D_c = 1.51 g cm^{-3}, μ(Cu Kα) = 39.94 cm^{-1}, and $4° < 2\theta < 120°$. Final R and R_w values were 0.070 and 0.069, respectively.

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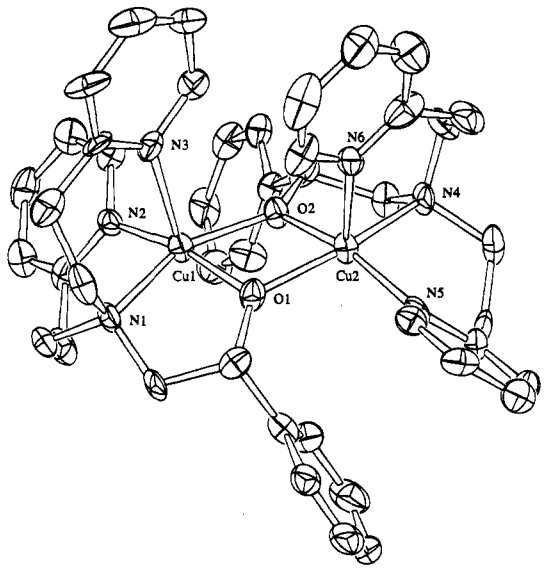
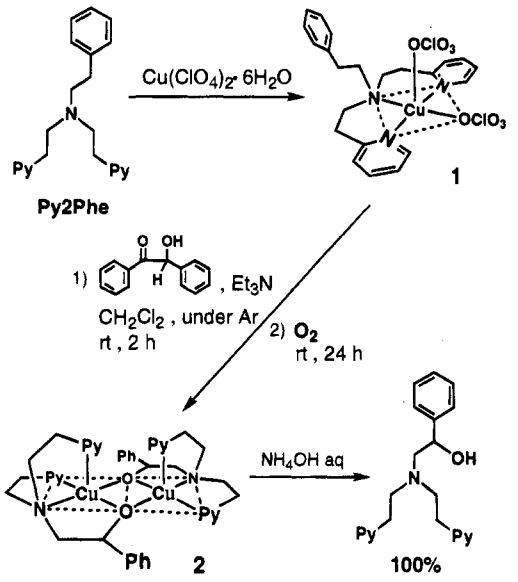


Figure 2. ORTEP drawing of Cu(II) complex **2**.

Scheme 1



1.[14] The mass spectroscopic analysis of the modified ligand obtained in the same reaction with $^{18}O_2$ (96% content) clearly showed that the oxygen atom of the OH group comes from molecular oxygen (more than 95% of ^{18}O was introduced; peak height: $M^+:(M^+ + 2) = 11:100$). The stoichiometry of O_2 to copper was determined to be 1:1 by manometry. The ligand hydroxylation was only observed in the presence of all the reaction components, i.e., benzoin, base, and O_2 in addition to the copper complex.

The same reaction as in Scheme 1 also occurred when the ligand (Py2Phe, 84 μmol) was treated with an equimolar amount of $[Cu^I(CH_3CN)_4]PF_6$ under O_2 in CH_2Cl_2 (5 mL). In this case, however, the yield of hydroxylation was 50% based on the Cu ion and the stoichiometry of $Cu:O_2$ was 2:1, indicating clearly that 2 equiv of electrons and 1 equiv of O_2 are required for the quantitative ligand hydroxylation. Starting from Cu(I), the isolated yield of the hydroxylated ligand reached the maximum value (50%) in a few hours, while starting from Cu(II) in the presence of benzoin and triethylamine, it took more than 24 h to reach completion. When the reductant was hydroquinone (1 equiv), a more powerful reductant, instead of benzoin, the rate of the reaction became as fast as that starting from Cu(I).[15] These results indicate that the reduction of Cu(II) to Cu(I) is the rate-determining step in the benzoin/triethylamine system.

The hydroxylated ligand was also obtained in 33% yield when Cu(II)-complex **1** was treated with H_2O_2 (10 equiv) in CH_3OH for 18 h under Ar. On the other hand, *tert*-butyl hydroperoxide, cumene hydroperoxide, and *m*-CPBA gave no hydroxylation product under the same experimental conditions,[16] indicating that a (μ-peroxo)dicopper(II) complex is formed as an intermediate. Karlin and co-workers studied the reaction of a Cu(I) complex of Py2Bz (**3**, Py2Bz (L) = *N,N*-bis[2-(2-pyridyl)ethyl]-benzylamine) with molecular oxygen in CH_2Cl_2,[17] when four-electron reduction of O_2 took place to give $LCu^{II}-O-Cu^{II}L$ (**4**), which reacted further with water to afford stable dihydroxo-bridged dinuclear Cu(II) complex $LCu^{II}-(OH)_2-Cu^{II}L$ (**5**), resulting in no ligand hydroxylation.[18] They have reported that **3** forms a μ-η^2:η^2-type peroxodicopper(II) complex at −80 °C but it readily disproportionates into **4** and $^1/_2O_2$ at higher temperatures (e.g., 0 °C).[18] Kitajima and his co-workers reported that (μ-η^2:η^2-peroxo)dicopper(II) complex $[Cu[HB(3,5\text{-}Me_2pz)_3]]_2(O)_2$ spontaneously decomposes into the corresponding oxodicopper(II) complex via O−O bond homolysis.[5] From these results, we presume that a $Cu^{II}-O^\bullet$ species produced via homolytic O−O cleavage of the initially formed (μ-η^2:η^2-peroxo)dicopper(II) complex may be responsible for the present ligand hydroxylation. The $Cu^{II}-O^\bullet$ species may abstract a hydrogen atom from the benzylic position to give $Cu^{II}-OH$ and a carbon-centered radical which another $Cu^{II}-O^\bullet$ species attacks to yield the final product. The $Cu^{II}-OH$ complex thus formed is reduced to a cuprous state that may participate in the hydroxylation reaction again. According to this mechanism the stoichiometry of $1:O_2$:benzoin is 1:1:1, agreeing with the experimental results. In the enzymatic system, it has been suggested that $Cu-O^\bullet$ generated by homolytic cleavage of $Cu^{II}-O-OH$ is an active species of the hydroxylation reaction.[19] The present reaction may be regarded as mimicking such an enzymatic process. At present, however, an alternative reaction pathway which involves direct hydrogen atom abstraction from the benzylic position by the peroxodicopper(II) species itself cannot be ruled out.[20] Details of the reaction mechanism are now under investigation.

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Supplementary Material Available: Tables of fractional atomic coordinates and interatomic bond distances and angles for **1** and **2** and text giving the details of X-ray crystal analysis (33 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(14) Spectral data of the modified ligand: ^1H NMR (270 MHz, $CDCl_3$, δ, TMS) 2.57−3.16 (m, 10 H, $CH_2 \times 5$), 4.60 (dd, J = 3.5, 9.7 Hz, 1 H, CHOH), 7.02 (d, J = 7.7 Hz, 2 H, Py-H_3), 7.10 (dd, J = 4.8, 7.7 Hz, 2 H, Py-H_5), 7.22−7.38 (m, 5 H, Ph), 7.54 (dt, J = 1.7, 7.7 Hz, 2 H, Py-H_4), 8.52 (d, J = 4.8 Hz, 2 H, Py-H_6); IR (neat, cm^{-1}) 3396 (OH); MS (CI, *m/z*) 348 ($M^+ + 1$).

(15) The yield of hydroxylation was lower (62%), since hydroquinone itself reacted with O_2 to be oxidized to benzoquinone under the reaction conditions.

(16) In the reaction of the Cu(I) complex of Py2Phe with iodosylbenzene, Réglier *et al.*[10] reported the ligand hydroxylation only at the ortho position of one pyridine nucleus to occur.

(17) Karlin, K. D.; Gultneh, Y.; Hayes, J. C.; Zubieta, J. *Inorg. Chem.* **1984**, *23*, 521.

(18) More recently, they observed benzaldehyde formation (20%) in the same reaction: Sanyal, I.; Mahroof-Tahir, M.; Nasir, M. S.; Ghosh, P.; Cohen, B. I.; Gultneh, Y.; Cruse, R. W.; Farooq, A.; Karlin, K. D.; Liu, S.; Zubieta, J. *Inorg. Chem.* **1992**, *31*, 4322.

(19) Klinman, J. P.; Berry, J. A.; Tian, G. In *Bioinorganic Chemistry of Copper*; Karlin, K. D., Tyeklàr, Z., Eds.; Chapman & Hall: New York, 1993; pp 151− 163.

(20) Direct attack of a (μ-η^2:η^2-peroxo)dicopper(II) species at the ligand isopropyl groups has recently been reported to occur in a new peroxodicopper(II) complex: Mahapatra, S.; Halfen, J. A.; Wilkinson, E. C.; Que, L.; Tolman, W. B. *J. Am. Chem. Soc.* **1994**, *116*, 9785.