CHEMISTRY LETTERS, pp. 461-464, 1993.

A Possible Model Reaction of Dopamine β-Monooxygenase

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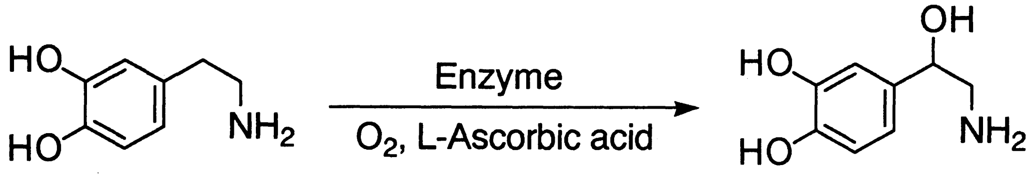
Dopamine (3,4-dihydroxyphenethylamine) was oxidized with O_2 in the presence of copper (II) sulfate and L-ascorbic acid to yield norepinephrine under ambient conditions. The active species generated from this system was assigned to OH radical by means of OH radical scavenging method.

Dopamine β-monooxygenase (E.C.1.14.17.1) catalyzes the oxygenation of dopamine (DPA) to norepinephrine (NEP) in the presence of L-ascorbic acid (AsA).[1, 2] Dopamine β-monooxygenase is one of the copper-containing proteins and the ESR studies of this enzyme showed that the valence state of the copper species changes between Cu (II) and Cu (I) during the enzymatic oxidation, depending on the relative concentrations of O_2 and AsA.[3,4] Though the active species in the catalytic cycle of the oxygenation of DPA is believed to contain Cu (I) species,[1,5] no details of the active species and mechanism of the oxygenation have been elucidated. In the present communication, we would like to demonstrate that DPA is chemically oxidized to give NEP in the presence of both O_2 and Cu (I) ion, which was formed by the reduction of Cu (II) ion with AsA.



DPA NEP

Into a 100 ml Erlenmeyer flask, 25 ml of 0.1 mol dm^{-3} acetate buffer (pH 4.5) containing 5 mmol dm^{-3} (0.125 mmol) of DPA 60 mmol dm^{-3} (1.5 mmol) of copper (II) sulfate was placed. When the solution was shaken for 2 h at a rate of 170 oscillation per min under aerial atmosphere at 25 °C, NEP was not formed at all.[6] As seen in Fig. 1, the addition of 0.25–0.75 mmol of AsA to the mixture of DPA and copper (II) sulfate promoted the formation of NEP. The yield of NEP based on DPA consumed was ca. 1%.[7] Spectroscopic determination of copper (I) species by use of bathocuproine sulfate method[8] revealed that AsA effectively reduced copper (II) ion to copper(I) species under the present reaction conditions, as shown in Fig. 2. The result of Fig. 2 indicates that the following stoichiometry (2) holds for the reaction of AsA with copper (II) sulfate and that copper (II) sulfate and AsA can be replaced by copper (I) salt. In fact, the aerial oxidation of DPA in the presence of copper

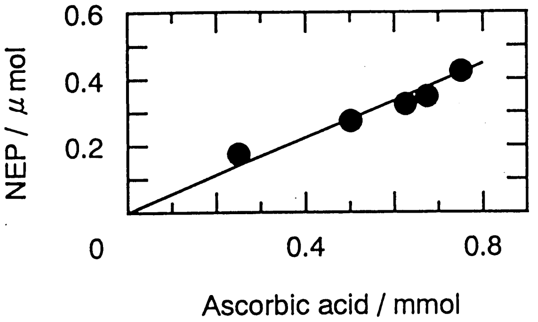


Fig. 1. Formation of NEP in the aerial oxidation of DPA in the presence of copper (II) sulfate and ascorbic acid.

Solvent : 25 ml of acetate buffer (0.1 mol dm^{-3}, pH 4.5), DPA : 5 mmol dm^{-3} (0.125 mmol), copper (II) sulfate : 40 mmol dm^{-3} (1.0 mmol), atmosphere : air, reaction time: 2 h, temp : 25 °C.

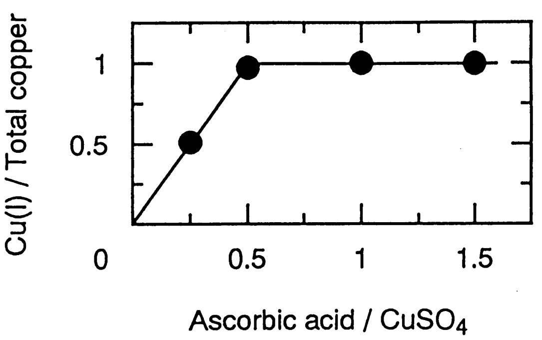
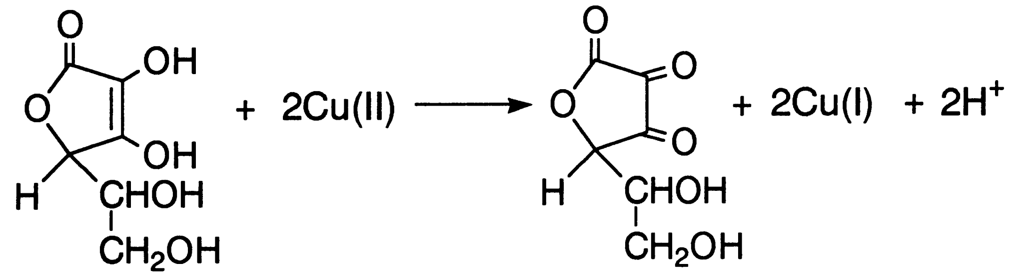


Fig. 2. Formation of copper (I) species by the reaction of copper (II) sulfate with ascorbic acid.

Solvent : 4 ml of acetate buffer (0.1 mol dm^{-3}, pH 4.5), bathocuproine sulfonate : 0.3 mmol dm^{-3}, copper (II) sulfate : 30–60 μmol dm^{-3}, atmosphere : N_2, reaction time : 1 min, temp : 25 °C.



Ascorbic acid (AsA) Dehydroascorbic acid (1)

(I) chloride, which is known to promote the oxidation of benzene with O_2,[9] produced NEP as shown in Table 1. In the anaerobic conditions, however, NEP was not appreciably formed, while the combination of copper (I) chloride and H_2O_2 gave NEP even under N_2 atmosphere. These results suggest that the Cu (II)/AsA/O_2 system will generate hydroxyl radical according to equations (2) and (3), as was already proposed for the oxidation of benzene with the copper (I) chloride/O_2 system.[9]

$$2\,Cu(I) + O_2 + 2\,H^+ \longrightarrow 2\,Cu(II) + H_2O_2 \qquad (2)$$

$$Cu(I) + H_2O_2 + H^+ \longrightarrow Cu(II) + {\cdot}OH + H_2O \qquad (3)$$

The active species was determined by the OH radical scavenging method[9] as follows. Provided the oxidation of DPA with the Cu(II)/AsA/O_2 system involves OH radical as an active species, the addition of a OH radical scavenger such as ethyl and t-butyl alcohols would decrease the yield of NEP according to Eq. 4:

$$\frac{[NEP]_0}{[NEP]} = 1 + \frac{k_D}{k[DPA]} + \frac{k_A}{k[DPA]}[Alcohol] \qquad (4)$$

where $[NEP]_0$ and [NEP] stand for the theoretical and observed concentration of NEP in the oxidation mixture, respectively, k_A, k and k_D are the rate constants for the reaction of hydroxyl radical with

Table 1. Oxidation of DPA to NEP with the Cu(II)/AsA/O_2 and related systems[a)]

Copper salt	AsA/mmol	Atmosphere	pH	NEP/μmol
$CuSO_4$	———	Air	4.5	0
$CuSO_4$	0.5	Air	4.5	0.4
$CuCl_2$	0.5	Air	4.5	0.8
$Cu(CH_3COO)_2$	0.5	Air	4.5	2.6
CuCl	———	Air	4.5	2.4
CuCl	———	Air	1.5	2.4
CuCl	———	N_2	4.5	0
CuCl[b)]	———	N_2	4.5	1.2

a) Solvent : 25 ml of acetate buffer (0.1 mol dm^{-1}, pH 4.5) or 25 mmol dm^{-3} H_2SO_4 (pH 1.5), DPA : 5 mmol dm^{-3} (0.125 mmol), copper salt : 40 mmol dm^{-3} (1.0 mmol), reaction time : 2 h, temp : 25 °C.

b) H_2O_2 (0.25 mmol) was added.

alcohols, β-hydrogen of DPA and the third compound(s) (which consumes hydroxyl radical without formation of NEP), respectively. When DPA was oxidized in the presence of various concentration of alcohols [Alcohol], the yield of NEP decreased with increasing [Alcohol]. The plots of $[NEP]_0$ /[NEP] against [Alcohol] for methyl, ethyl, propyl, isopropyl, isobutyl and t-butyl alcohols gave linear relationship (not shown in Figure) and the slopes of each straight line corresponds to $k_A/k[DPA]$ in Eq. 4.

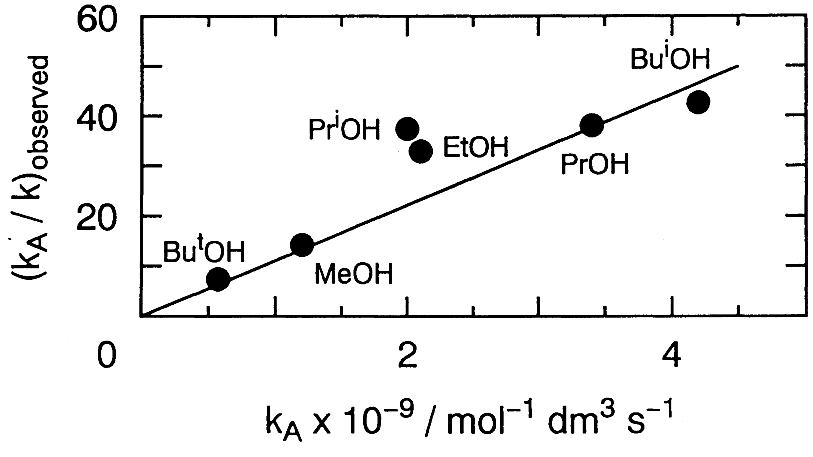


Fig. 3. Plot of k_A/ k obtained from the $[NEP]_0$/[NEP] vs. [Alcohol] against the k_A reported by Walling.[10)]

Solvent : 25 ml of 25 mmol dm^{-3} H_2SO_4 (pH 1.5), DPA : 5 mmol dm^{-3}, copper (I) chloride : 1.0 mmol, alcohols : 5–200 mmol dm^{-3}, atmosphere : air, reaction time : 2 h, temp : 25 °C.

The plot of the slope divided by [DPA] against the authentic k_A reported by Walling showed a linear relationship (Fig. 3). The k value obtained from the slope of Fig. 3 was 1×10^8 mol^{-1} dm^3 s^{-1}, which is comparable with the rate constant (3×10^8 mol^{-1} dm^3 s^{-1}) of hydroxyl radical with α-hydrogen of toluene.[6,11]

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6) The oxidation of toluene with the Cu(I)/O_2 system gave benzylalcohol in 1.2% yield (based on copper(I) chloride added) accompanied with the formation of benzaldehyde and three isomeric cresols. The molar ratio of benzylalcohol among the products was ca. 0.1. In the oxidation of DPA, therefore, NEP should be a minor product.
7) NEP was determined with a reversed-phase HPLC (column : Cica-Merck Lichrosorb RP-18 (5 μm), eluent : 50 mmol dm^{-3} phosphate buffer (pH 3.5), detector : UV at 283 nm).
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(Received December 11, 1992)