DALTON COMMUNICATION

Ascorbate oxidation leading to the formation of a catalytically active oxalato bridged dicopper(II) complex as a model for dopamine β-hydroxylase

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A dicopper(II) complex $[Cu_2(bpy)_2(\mu\text{-ox})]X_2$ ($X = ClO_4^-$, PF_6^-; $ox = C_2O_4^{2-}$) has been found to be catalytically active in the oxidation of ascorbic acid by dioxygen involving a copper(I) intermediate species and the process is also effective in the presence of benzylamine to form benzaldehyde.

Dopamine β-hydroxylase is a copper containing glycoprotein that hydroxylates dopamine to norepinephrine.[1,2] Based on spectroscopic studies, the active site of the metalloenzyme is proposed to have two copper atoms. While one copper centre (Cu_A) is bonded to three histidine ligands and one water, the other copper atom (Cu_B) is ligated to two histidines and one water. The enzyme in the oxidised dicopper(II) form gets reduced to the dicopper(I) unit by ascorbate and in the second step, dopamine and dioxygen bind to the enzyme at the Cu_B site to form a ternary complex resulting in the oxidation of dopamine and the formation of the oxidised form of the enzyme. Herein we report a catalytic reaction involving the oxidation of ascorbic acid (vitamin C) by dioxygen mediated by copper complexes. The reaction models the functional property of dopamine β-hydroxylase (DβH).

The catalytically active dicopper(II) complex $[Cu_2(bpy)_2(ox)]X_2$ ($ox = C_2O_4^{2-}$; $X = ClO_4^-$, **1a**; PF_6^-, **1b**) was obtained from the reaction of $[Cu_2(OH)(OH_2)(O_2CMe)(bpy)_2]X_2$[3] with ascorbic acid in aqueous methanol.† Complex **1b** has been structurally characterized.[4]‡ In the dimeric core, the $Cu(bpy)^{2+}$ units are bridged by an oxalate anion to give a Cu···Cu separation of 5.123(1) Å (Fig. 1). The PF_6 anion shows an axial mode of binding giving a Cu–F distance of 2.441(2) Å. The infrared spectra of **1** show bands characteristic of the oxalate ligand at 1653 [ν_{as}(CO)]; 1354, 1314 [ν_s(CO)] and 775 cm^{-1} [ν(OCO)].[5] The intense bands at 1091 and 841 cm^{-1} are assignable to the ClO_4 and PF_6 anions, respectively. The complex exhibits a visible spectral d–d band at 650 nm ($\varepsilon = 140$ dm^3 mol^{-1} cm^{-1}) in MeCN and the dimeric core shows antiferromagnetic spin-coupling between two metal centres. The fitting parameters for **1a** from the $\chi_m T$ *vs.* T plot are $J = -179$ cm^{-1}, $g = 2.18$, $\theta = 8$ with a paramagnetic impurity $\rho = 0.01$, where $-2J$ is the singlet–triplet energy separation.[6,7] The J value for **1b** is -185 cm^{-1}. A 400 MHz ^{1}H NMR spectrum of **1** in CD_3CN shows a broad peak at δ 10.64 due to 2,2′-bipyridine ligands.

Complex **1** on reaction with ascorbic acid forms an unstable brown diamagnetic copper(I) species ($\chi_g = -0.03 \times 10^{-6}$ cm^3 g^{-1} at 298.5 K) which rapidly converts to **1** on exposure to air.[8,9] The catalytic cycle is found to be effective with a turnover number of *ca.* 20 (Scheme 1). The reduced species which shows a visible band at 435 nm ($\varepsilon \approx 5200$ dm^3 mol^{-1} cm^{-1} in MeOH–H_2O) is formulated as $[Cu(bpy)(H_2O)X]$ ($X = ClO_4^-$, **2a**; PF_6^-, **2b**) from the analytical and spectral data. It shows a strong infrared band at 1089 cm^{-1} for ClO_4^- and at 840 cm^{-1} for the PF_6 anion. The ^{1}H NMR spectra of **2** in CD_3CN show a broad

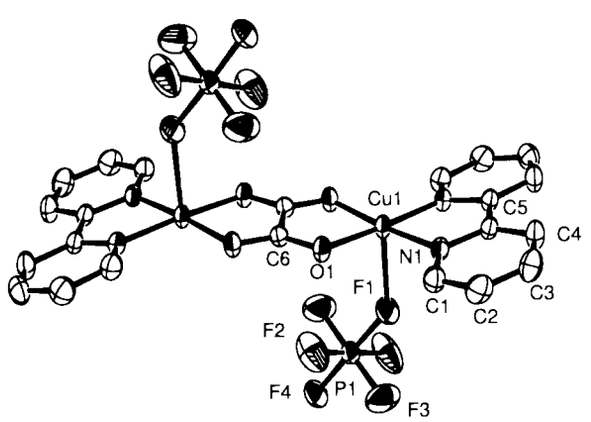
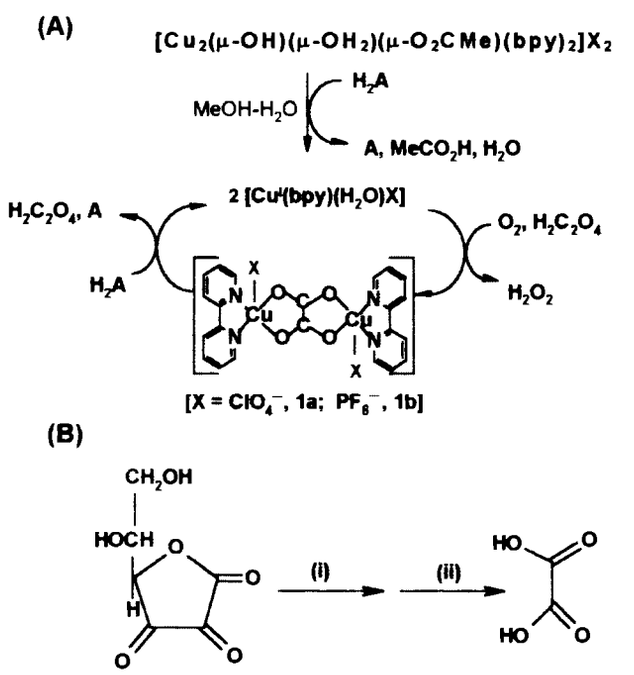


Fig. 1 An ORTEP[12] view of complex **1b**. Selected bond distances (Å) and angles (°): Cu(1)–N(1) 1.965(2), Cu(1)–O(1) 1.960(2), Cu(1)···Cu(1′) 5.123(1), Cu(1)–F(1) 2.441(2), O(1)–C(6) 1.245(2), C(6)–C(6′) 1.550(6); Cu(1)–F(1)–P(1) 140.01(1), O(1)–Cu(1)–O(1′) 85.08(9), O(1)–Cu(1)–N(1) 95.95(7), O(1)–Cu(1)–N(1′) 175.21(7), O(1)–Cu(1)–F(1) 93.57(3), N(1)–Cu(1)–F(1) 91.04(3), C(6)–O(1)–Cu(1) 111.3(2), O(1)–C(6)–O(1′) 127.6(3), O(1)–C(6)–C(6′) 116.2(2).



Scheme 1 H_2A = ascorbic acid. (A) Catalytic reaction, (B) oxalate formation from dehydroascorbic species: (i) hydrolytic ring rupture; (ii) oxidation.

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peak at δ 8.34 which corresponds well to the 2,2′-bipyridine spectrum appearing in the range of δ 7.3 to 8.7. The observed broadening could be due to the formation of a trace quantity of a copper(II) species from the oxidation of **2**. The precursor dicopper(II) complex has a $[Cu_2(\mu\text{-}OH)(\mu\text{-}OH_2)(\mu\text{-}O_2CMe)]^{2+}$ core.[3] The cleavage of this core as well as the oxalato bridged dimeric unit in complex **1** takes place presumably due to protonation of the bridging ligand(s) by ascorbic acid, thus forming complex **2** by a concomitant two-electron, two-proton transfer process: $H_2A \longrightarrow A + 2e^- + 2H^+$. The oxalate anion in complex **1** is formed from the oxidation of L-threonic acid, an oxidation product of 2:3-diketo-l-gulonic acid, which is a product from the hydrolytic ring rupture of the dehydroascorbic acid in solution (Scheme 1).[10]

In an attempt to model the functional properties of dopamine β-hydroxylase, the catalytic reaction has been carried out in the presence of benzylamine. The reaction of benzylamine with the reduced copper(I) complex in the presence of dioxygen forms an initial green solution which then converts to the oxidized blue dicopper(II) species, **1**. The organic product has been identified as benzaldehyde from the mass spectral studies, showing peaks with *m*/*z* values of 105.3 and 106.3 corresponding to $PhCO^+$ and PhCHO.† It is presumed that the reaction proceeds through the attacks of the peroxo moiety on the benzylic carbon to form an α-hydroxylamine which being unstable undergoes deamination to form benzaldehyde.[11] A detailed study using other amines towards understanding the kinetic and mechanistic aspects of this catalytic reaction of bioinorganic relevance is in progress.

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Notes and references

† The complex $[Cu_2(bpy)_2(ox)](ClO_4)_2$ (**1a**) was prepared in 40% isolated yield from a reaction of $[Cu_2(OH)(OH_2)(O_2CMe)(bpy)_2](ClO_4)_2$ (0.14 mmol) with ascorbic acid (H_2A, 0.28 mmol) in aqueous methanol (35 cm^3, 7:3 v/v) under stirring for 1 h at 25 °C. The complex was obtained as a sky blue crystalline solid. Found: C, 36.5; H, 2.5; N, 7.6. Calc. for $C_{22}H_{16}N_4O_{12}Cl_2Cu_2$ (**1a**): C, 36.6; H, 2.2; N, 7.7%. **CAUTION!** Perchlorate salts are potentially explosive. The PF_6 salt (**1b**) was prepared by following a similar procedure to that above. The MALDI mass spectrum of **1b** shows molecular ion peaks at 817 (^{63}Cu) and 820 (^{65}Cu) *m*/*z* values. Single crystals of **1b**, suitable for X-ray studies, were obtained by slow evaporation of the complex from an aqueous methanolic medium. Attempts to isolate the reduced species in crystalline form were unsuccessful as the reduced solution was found to be unstable and the copper(I) species is susceptible to disproportionation. The organic product from the benzylamine reaction was isolated by solvent extraction using diethyl ether after removal of methanol from the reaction mixture by rotary evaporation. The diethyl ether solution was concentrated before mass spectral measurements.

‡ Crystal data for **1b**: $C_{22}H_{16}N_4O_4F_{12}P_2Cu_2$, $M = 817.41$, monoclinic, space group $C2/m$ (no. 12), $a = 14.463(2)$, $b = 13.941(2)$, $c = 7.918(1)$ Å, $\beta = 121.61(1)°$, $U = 1359.7(4)$ Å^3, $Z = 2$, $D_c = 1.997$ g cm^{-3}, $T = 293$ K, $4.4 \leq 2\theta \leq 60°$, $\mu = 18.05$ cm^{-1}, $F(000) = 808$, $R1 = 0.0357$, $wR2 = 0.0965$ for 2060 reflections with $I > 2\sigma(I)$. Intensity data from a crystal of dimensions 0.3 × 0.4 × 0.5 mm were obtained in the 2θ range of 4 to 60° on an Ernaf-Nonius CAD4 diffractometer using graphite-monochromated Mo-Kα radiation ($\lambda = 0.7107$ Å). Data were corrected for Lorentz, polarization and absorption effects. CCDC reference number 186/1905.

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