DALTON COMMUNICATION

Structural model for the Cu_B site of dopamine β-hydroxylase and peptidylglycine α-hydroxylating monooxygenase: crystal structure of a copper(II) complex showing N_3OS coordination and axial sulfur ligation

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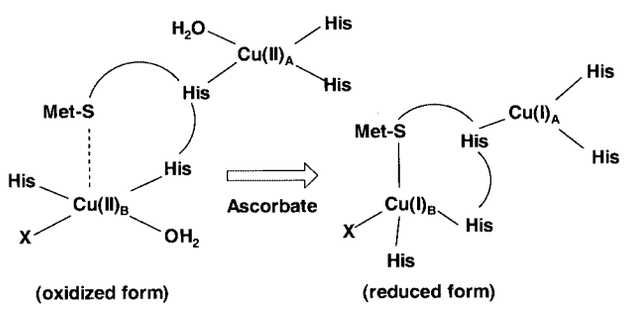
A copper(II) complex [Cu(L)(phen)](ClO_4) (HL, NSO-donor Schiff base ligand) with a $Cu^{II}N_3OS$ geometry showing axial sulfur ligation is a structural model for the Cu_B site of DβH and PHM, and the complex is catalytically active in the oxidation of ascorbic acid by dioxygen mediated by a copper(I) species.

Dopamine β-hydroxylase (DβH; E.C.1.14.17.1) and peptidylglycine α-hydroxylating monooxygenase (PHM; E.C.1.14.17.3) are copper proteins responsible for the benzylic hydroxylation of dopamine to norepinephrine in the biosynthesis of the neurohormone adrenaline and α-amidating bioactive peptides, respectively.[1–3] The active sites of DβH and PHM have structural and functional similarities. The enzymes have two essentially uncoupled copper centres per subunit. The crystal structure of PHM shows a separation of 11 Å between two mononuclear copper sites.[4] While the Cu_A site mediates electron transfer, the Cu_B site is responsible for the functionalization of the organic substrate. The Blackburn model for the oxidized and reduced active sites of DβH and PHM shows a CuN_3OS coordination for the $Cu(II)_B$ site with the MetS ligand occupying the axial site (Scheme 1).[5] The axially bound Met residue



Scheme 1 Blackburn model showing the active site coordination geometries in the oxidized and reduced forms of DβH and PHM [$Cu(II)_BN_3OS$ geometry with X as either His or an azide ion].

is known to be crucial for the catalytic activity.[6] The crystal structure of PHM shows the presence of a weakly bound Met residue at a distance of 2.68 Å.[4] Herein, we present the synthesis, crystal structure and properties of a copper(II) complex, [Cu^{II}(L)(phen)](ClO_4) (**1**) which shows axial sulfur ligation in a CuN_3OS geometry giving a similar Cu–S distance as in PHM. Complex **1** is of significance as a structural model for the $Cu(II)_B$ site of DβH and PHM as the presently known[7] copper(II) complexes with N_3OS coordination have sulfur ligation at the basal plane.

The copper(II) complex [Cu(O-2-C_6H_4CH=NC_6H_4-2′-SMe)(phen)](ClO_4) (**1**) was prepared from the reaction of copper(II) acetate hydrate with 1,10-phenanthroline (phen) and 2-(methylthiophenyl)salicylaldimine (HL) in methanol followed by addition of sodium perchlorate. The copper(I) complex [Cu(HL)(phen)](ClO_4) (**2**) was obtained by reducing **1** with ascorbic acid (H_2A) in an aqueous acetonitrile medium under anaerobic conditions.† Both the complexes show an infrared band near 1090 cm^{-1} assignable to the perchlorate anion. Complex **1** displays a d–d band at 646 nm and a charge transfer transition at 402 nm in MeCN. Complex **2** exhibits a charge transfer transition at 348 nm with a shoulder at 536 nm in MeCN. Complex **1** is one-electron paramagnetic and shows an axial EPR spectrum with $g_{\|}$ = 2.16 ($A_{\|}$ = 114 × 10^{-4} cm^{-1}) and $g_{\perp}$ = 2.02 in MeCN–toluene glass (1 : 1 v/v). Complex **2** is diamagnetic and exhibits a singlet at 13.19 ppm in the ^{1}H NMR spectrum in CD_3CN assignable to the phenolic OH. The non-involvement of the phenolic group in **2** in metal binding is evidenced from the negligible shift of the proton resonance compared to that of the free ligand [OH(ligand) = 13.16 ppm]. The formation of a copper–sulfur bond in **2** is indicated from the significant downfield shift of the S-methyl resonance from 2.28 ppm in HL to 2.52 ppm in **2**.[8,9] The ^{1}H NMR data suggest a four-coordinate geometry for **2** with a CuN_3S coordination geometry which models the Cu_B site structure of DβH and PHM in the reduced state.

The crystal structure of **1** shows a tridentate NSO coordination of the Schiff base and a bidentate NN mode of bonding for the phen ligand (Fig. 1).‡ The coordination geometry is

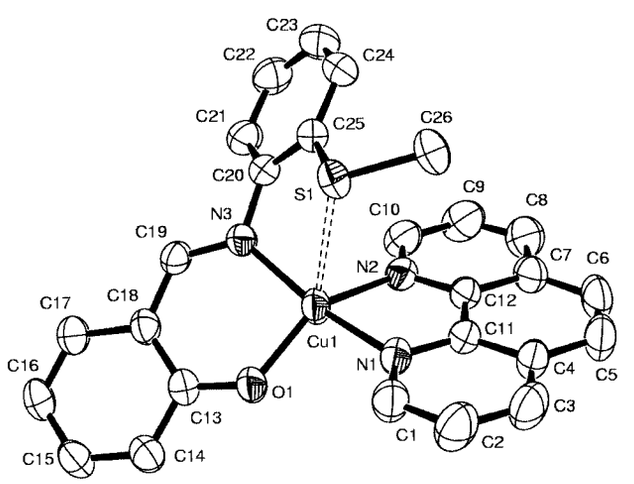


Fig. 1 An ORTEP view of the cation of complex **1** with thermal ellipsoids at the 50% probability level and the atom numbering scheme. Selected bond distances (Å) and angles (°): Cu(1)–N(1) 1.995(3), Cu(1)–N(2) 2.069(3), Cu(1)–N(3) 1.949(3), Cu(1)–O(1) 1.877(3), Cu(1)–S(1) 2.765(1); O(1)–Cu(1)–N(1) 91.66(12), O(1)–Cu(1)–N(2) 142.77(12), O(1)–Cu(1)–N(3) 95.25(11), O(1)–Cu(1)–S(1) 128.65(9), N(1)–Cu(1)–N(2) 81.52(12), N(1)–Cu(1)–N(3) 162.28(13), N(1)–Cu(1)–S(1) 89.01(10), N(2)–Cu(1)–N(3) 102.23(12), N(2)–Cu(1)–S(1) 88.01(9), N(3)–Cu(1)–S(1) 73.94(9).

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distorted square-pyramidal with three nitrogen and one oxygen atoms occupying the basal plane (τ = 0.3). The Cu(1)–O(1) bond length of 1.877(3) Å is the shortest in the basal plane. The sulfur atom is involved in a weak axial ligation giving a Cu–S distance of 2.765(1) Å. The bond length compares well with the Cu_B–M314 distance of 2.68 Å found in the crystal structure of PHM.[4] Complex **1** with axial sulfur coordination in a CuN_3OS geometry exemplifies the first structural model for the Cu_B site of DβH and PHM in the oxidized form. A recent report on the modeling of the Cu_B site shows axial sulfur ligation in a copper(II) complex having a CuN_3SCl coordination geometry.[10]

The redox activity of the complexes has been studied by cyclic voltammetry in both protic and aprotic solvents. In dmf–Tris–HCl/0.1 M KCl buffer medium [1 : 4 v/v, pH 7.2, Tris = tris(hydroxymethyl)aminomethane], the complexes show a quasi-reversible voltammetric response for the Cu(II)/Cu(I) couple at −0.10 and −0.08 V for **1** and **2** respectively, with a ΔE_p value of 120 mV at a 50 mV s^{-1} scan rate (Fig. 2). The ratio

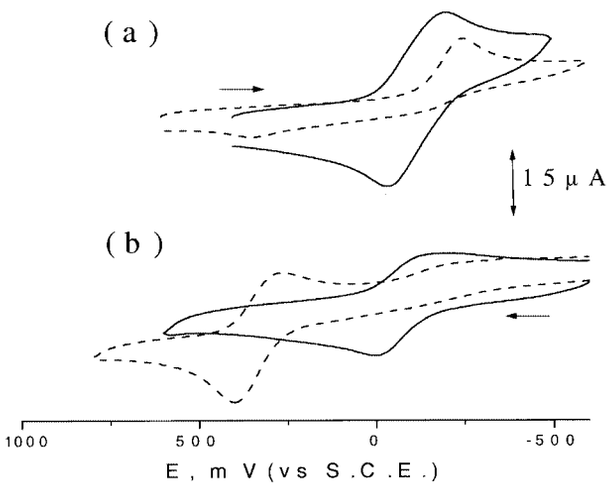
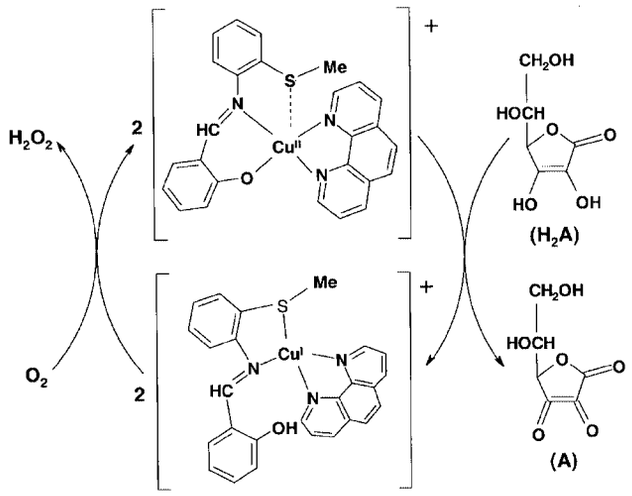


Fig. 2 Cyclic voltammograms of $[Cu^{II}(L)(phen)](ClO_4)$ (**1**, a) and $[Cu^{I}(HL)(phen)](ClO_4)$ (**2**, b) in dmf–Tris–HCl/0.1 M KCl buffer (1 : 4 v/v, pH 7.2) (——) and CH_2Cl_2–0.1 M TBAP (---) at a scan rate of 50 mV s^{-1}.

of anodic (i_{pa}) and cathodic (i_{pc}) peak currents is unity at scan rates of 50–200 mV s^{-1}. In an aprotic solvent such as CH_2Cl_2–0.1 M TBAP (tetrabutylammonium perchlorate), complex **1** displays an irreversible reduction peak at −0.25 V due to the formation of $[Cu^{I}(L)(phen)]$ ($\mathbf{1}^-$) (Fig. 2a). An anodic response with much reduced current ($i_{pa}/i_{pc} \approx 0.1$) is observed at 0.35 V. This anodic peak is assignable to the oxidation of complex **2** formed in trace quantity from the unstable $\mathbf{1}^-$ species during the voltammetric scan. A better reversibility for the Cu(II)/Cu(I) couple in the buffer medium could be due to the presence of an electroprotic reaction: $[Cu^{II}(L)(phen)]^+ + H^+ + e^- \rightleftharpoons [Cu^{I}(HL)(phen)]^+$. In CH_2Cl_2–0.1 M TBAP complex **2** exhibits a quasireversible voltammogram at 0.34 V (ΔE_p = 120 mV) with a i_{pa}/i_{pc} ratio of unity due to the Cu(II)/Cu(I) couple involving $[Cu^{II}(HL)(phen)]^{2+}$ and $[Cu^{I}(HL)(phen)]^+$ (Fig. 2b). An additional cathodic response near −0.25 V with a reduced peak current is observed, possibly due to the formation of a trace quantity of **1** during oxidation of complex **2**. The high positive potential for the Cu(II)/Cu(I) couple in **2** in CH_2Cl_2 is due to stabilization of the copper(I) state in a CuN_3S coordination geometry.

Complex **1** readily reacts with ascorbic acid (H_2A) to form the reduced complex **2**. The reduced species converts to **1** on exposure to dioxygen. This cyclic process is effective with a 1 : 100 mol ratio of **1** and H_2A in a dmf–Tris–HCl buffer (1 : 4 v/v, pH 7.2). In the presence of excess H_2A, **1** degrades to a non-catalytic copper(II) species. The catalytic process is of significance as it involves two copper species having $Cu^{II}N_3OS$ and $Cu^{I}N_3S$ coordination geometries that model the structural changes associated with the Cu_B site of DβH and PHM in enzymatic reactions (Scheme 2).



Scheme 2 Catalytic oxidation of ascorbic acid by dioxygen medicated by five-coordinate $[Cu^{II}(L)(phen)](ClO_4)$ and four-coordinate $[Cu^{I}(HL)(phen)](ClO_4)$ species in a dmf–Tris–HCl buffer (1 : 4 v/v, pH 7.2).

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Notes and references

† The complex $[Cu(L)(phen)](ClO_4)$ (**1**) was prepared in 75% yield from the reaction of $Cu_2(O_2CMe)_4(H_2O)_2$ (0.5 mmol) with phen (1.0 mmol) in 15 cm^3 MeOH. The mixture was left stirring for 0.5 h at 25 °C at which point Schiff base (HL, 1.0 mmol), obtained from the condensation of salicylaldehyde and 2-(methylthio)aniline, was added. The complex was isolated as a green solid by addition of a methanolic solution of $NaClO_4$ (1.0 mmol). Found: C, 53.6; H, 3.6; N, 7.5. Calc. for $C_{26}H_{20}N_3O_5SClCu$ (**1**): C, 53.3; H, 3.4; N, 7.2%. λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) in MeCN: 646 (190), 402 (6350), 270 (42900), 225 (51200). Complex **2** was isolated in 70% yield as a brown solid by reacting complex **1** (0.34 mmol) in MeCN (15 cm^3) with ascorbic acid (0.34 mmol) in H_2O (1 cm^3) under a dinitrogen atmosphere. Solvents were removed under vacuum and the solid was thoroughly washed with deoxygenated cold water and finally dried *in vacuo* over P_4O_{10}. Found: C, 53.6; H, 4.0; N, 7.5. Calc. for $C_{26}H_{21}N_3O_5SClCu$ (**2**): C, 53.2; H, 3.6; N, 7.2%. λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) in MeCN: 536 sh, 348 (11400), 267 (52200), 225 (61600). ^1H NMR in CD_3CN, δ: 2.52 (s, 3H, S–Me), 7.01–7.57 (16H, aromatic protons), 8.84 (s, N=CH), 13.19 (s, OH) (s, singlet). **CAUTION!** Perchlorate salts are potentially explosive. Single crystals of **1**, suitable for X-ray studies, were obtained by slow evaporation of an aqueous methanolic solution of the complex.

‡ Crystal data for **1**: $C_{26}H_{20}N_3O_5SClCu$, M = 585.50, triclinic, space group $P\bar{1}$ (no. 2), a = 9.366(2), b = 10.706(6), c = 13.686(3) Å, α = 100.20(3), β = 105.57(2), γ = 101.83(3)°, U = 1254.1(8) $Å^3$, Z = 2, D_c = 1.551 g cm^{-3}, T = 293(2) K, 1.59 ≤ θ ≤ 24.97°, μ = 11.04 cm^{-1}, $F(000)$ = 598, $R1$ = 0.0461, $wR2$ = 0.1514 for 3929 reflections with $I > 2\sigma(I)$ and 415 parameters [$R1$ (F^2) = 0.0506 (all data)]. Weighting scheme: $w = 1/[\sigma^2(F_o^2) + (0.0969P)^2 + 1.0991P]$, where $P = (F_o^2 + 2F_c^2)/3$. Intensity data from a crystal of dimensions 0.41 × 0.36 × 0.16 mm were obtained on an Enraf-Nonius CAD4 diffractometer using graphite-monochromated Mo-Kα radiation (λ = 0.7107 Å). Data were corrected for Lorentz, polarization and absorption effects. Structure solution and refinement were performed using the SHELX system of programs.[11] The perspective view of the molecule was obtained using ORTEP.[12] CCDC reference number 172492. See http://www.rsc.org/suppdata/dt/b1/b109332k/ for crystallographic data in CIF or other electronic format.

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