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# Towards a model for galactose oxidase: the crystal structure of {2-[bis(2-pyridylethyl)aminomethyl]phenolato} copper(II) perchlorate

Harry Adams, Neil A. Bailey, David E. Fenton* and Qingyu He

*Department of Chemistry, University of Sheffield, Sheffield S3 7HF (UK)*

Masaaki Ohba and Hisashi Okawa

*Department of Chemistry, Faculty of Science, Kyushu University, HakozaKi, Higashi-ku, Fukuoka 812 (Japan)*

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**Abstract**

The synthesis and X-ray crystal structure of a copper(II) complex derived from a tripodal ligand bearing pyridyl and phenolic arms is described.

*Key words:* Crystal structures; Copper complexes; Galactose oxidase complexes

Galactose oxidase is a monomeric enzyme that catalyses the stereospecific oxidation of a broad range of primary alcohol substrates and possesses a unique mononuclear copper site essential for catalysing a two-electron transfer reaction during the oxidation of primary alcohols to corresponding aldehydes [1]. The active site in galactose oxidase, crystallised from acetate buffer (pH ~ 4.5), has been shown, by X-ray structural analysis, to be quite distinct from those in other copper-containing enzymes, ascorbate oxidase and superoxide dismutase [2]. The site has five ligands – Tyr-272, which is uniquely modified by a covalent bond to nearby Cys-228, His-496, His-581 and a monodentate acetate anion form an almost perfect square and the fifth axial ligand is Tyr-295 (Fig. 1). A crystal grown from PIPES buffer (pH ~ 7.0) has essentially the same structure but the acetate has been replaced by a water molecule; at a distance of 2.8 Å from the metal, this is probably not coordinated. The unusual bond between Tyr-272 and Cys-228 is suggested to play an essential role in the catalytic mechanism [2].

We have initiated a search for a small molecule model for the site in galactose oxidase, and have begun by using a series of tripodal ligands bearing pyridyl and phenolic arms (LH). These readily form copper(II) complexes of the type $[CuL]X$ ($X = ClO_4^-$, $BF_4^-$, $Br^-$, $NO_3^-$, $CF_3SO_3^-$) indicating the ease of phenol deprotonation. The synthesis and X-ray crystal structure for one of these complexes are reported here.

To date only a limited number of investigations have considered complexes that might be regarded as models for galactose oxidase [3]. The first example, the crystal structure of the mononuclear copper complex [Cu(L)Cl] (L has $R = NO_2$, $x = y = 1$) was recently reported [4] and shows that the complex has an axial phenolate–copper(II) bond that may mimic the axial copper(II) phenolate bond in the enzyme. More recently, Alilou *et al.* [5] reported a series of monomeric copper(II) amide complexes, $[Cu\{OC[(CH_2)_2N(CH_2C_6H_4X)(CH_2CH_2C_5H_4N)]NH(CH_2)_nPh\}(Solv)][CF_3SO_3]_2$ ($X = H$, $Solv = H_2O$, $n = 2$ (**1**) or 1(**2**); $X = OH$, $Solv = H_2O$, $n = 2$ (**3**); $X = OH$, $Solv = MeCN$, $n = 1$ (**4**)), which were investigated as models for the $Cu_B(II)$ site of the monooxygenase dopamine $\beta$-hydroxylase. These have square-based pyramidal geometries derived from an equatorial $N_2O_2$ or $N_3O$ ligand donor set with an axial phenolate–copper(II) bond and so may also be considered as models for galactose oxidase.

In this study, the ligand $L^1$ ($L^1$ has $R = H$, $x = y = 2$) was synthesised by the reaction of bis[2-(2-pyridyl)ethyl]amino with *o*-acetoxybenzyl bromide in the presence of triethylamine followed by hydrolysis with NaOH to give the phenol. Purification of the ligand was effected by using flash chromatography [6] with silica gel (60–240 mesh). The copper complex $[CuL^1]ClO_4$ was prepared by refluxing a methanolic solution of the ligand with $Cu(ClO_4)_2 \cdot 6H_2O$ in methanol and triethylamine for 4 h. The resulting solution was

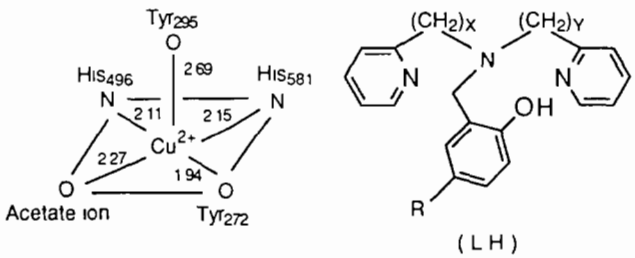


Fig 1. The structure of the copper site in galactose oxidase [2] Bond lengths in Å Ligands $R = H$ or $NO_2$; $x$, $y = 1$ or 2.

*Author to whom correspondence should be addressed.


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concentrated, and dark green crystals were obtained either by leaving the solution standing at room temperature for three days or by using diethyl ether vapour diffusion techniques. The fast atom bombardment (FAB) mass spectrum of the complex was recorded using glycerol and 3-nitrobenzyl alcohol (NOBA) matrices. The highest molecular peak found in NOBA, ($m/z = 395$), is attributable to the monomeric species $[Cu(L^1)]^+$ whereas the highest peak detected in glycerol is at $m/z = 475$ that may be assigned to $[Cu(L^1)OCu]^+$. The presence of this peak (albeit at 5% of the intensity of the signal found at 395 in the same spectrum) suggests a possible dimeric structure that is readily broken down during the FAB experiment.

An X-ray structure of $[CuL^1]ClO_4$ has shown that the complex is indeed a dimer in the solid state (Fig. 2)**. The copper atom is coordinated by two pyridine nitrogen atoms, the tertiary amine nitrogen atom and two phenolate oxygen atoms that coordinate to the second copper in the dimer. The geometry around copper(II) is best described as a distorted square pyramid with a small trigonal bipyramidal component, $\tau = 0.17$ ($\tau = (\beta - \alpha)/60$, where $\beta = O(1)$–$Cu(1)$–$N(3)$ 167.6° and $\alpha = N(1)$–$Cu(1)$–$O(1a)$ 157.7°). The basal plane is comprised of one pyridine and one tertiary amine nitrogen atom and two phenolate oxygen atoms Copper is 0.252 Å above the plane and is axially coordinated to the second pyridine nitrogen (Cu–N(2) 2.168 Å). The two parts of the dimer in the present complex are *trans* to each other.

The structure is different to that of the related copper(II) chloride complex [Cu(L)Cl], in which two pyridyl nitrogen atoms coordinate to copper equatorially, as one of the pyridine nitrogen atoms now occupies

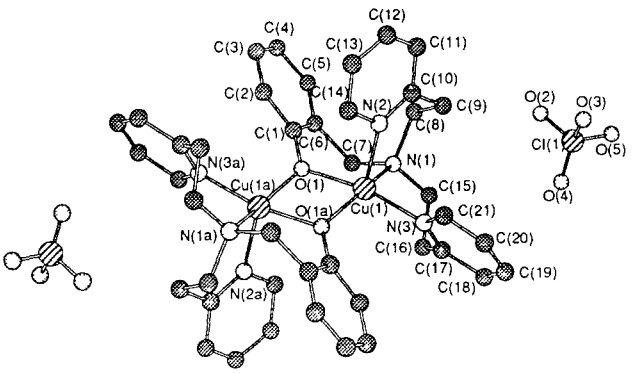


Fig 2 The molecular geometry of the complex The relevant bond lengths and bond angles are Cu(1)–O(1) 1 922(12), Cu(1)–O(1a) 2.019(12), Cu(1)–N(1) 2 085(13), Cu(1)–N(2) 2.168(13), Cu(1)–N(3) 1.983(15), Cu(1a)–O(1) 2 019(12) Å, N(1)–Cu(1)–N(2) 96.6(5), N(1)–Cu(1)–N(3) 90 8(6), N(2)–Cu(1)–N(3) 98 1(6), N(1)–Cu(1)–O(1) 94 2(5), N(2)–Cu(1)–O(1) 92 8(5), N(3)–Cu(1)–O(1) 167.5(6), N(1)–Cu(1)–O(1a) 157 6(5), N(2)–Cu(1)–O(1a) 103 3(5), N(3)–Cu(1)–O(1a) 96 3(5), O(1)–Cu(1)–O(1a) 74 9(6), Cu(1)–O(1)–Cu(1a) 105 1(6)°.

the axial position occupied by the phenolate in [Cu(L)Cl] [4]. It is however in accord with the structure predicted by Karlin and Cohen of the complex $[Cu(L^1)BF_4]$ [8]. It is possible that in the related complex [Cu(L)Cl] introduction of the $-NO_2$ group *para* to the phenolic group causes a change from equatorial to axial at the site of the coordinated phenolate ion. Alternatively extension of the tripodal arms may be involved in the stereochemical modification as has been noted in the two monomeric amide copper(II) complexes **3** and **4** where the large flexibility of one of the tripodal arms enables the phenolate oxygen to coordinate to copper at the axial position [5]. The $N_{py}$–Cu–$N_{py}$ angle in the present complex is 98.1°, larger than that for the monomeric azide complex (95.5°) with the same coordination geometry [8, 9]. The short Cu(1)–O(1) bond (1.922 Å) demonstrates the strong coordination of phenolic oxygen to copper in this molecule relative to that in analogous mononuclear [4, 5, 8, 9] or dinuclear [10–13] complexes. The bond between the two parts of the dimer is not very strong; this is shown in the bond length of Cu(1)–O(1a) (2.019 Å) which is longer than that of Cu(1)–O(1) and those of other phenolic–copper equatorial bonds [5, 9, 11].

The electronic spectrum of the complex (in acetonitrile) shows two clear absorption bands at 262 and 440 nm, respectively. The former, intense absorption peak, is assigned as a $\pi$–$\pi^*$ aromatic transition, the latter may be a charge transfer transition from phenolate to the copper(II) ions. A variable temperature magnetic study of the dimer indicates that the two coppers are strongly antiferromagnetically coupled, $J = -330$ cm$^{-1}$ based on $\mathscr{H} = -2JS_1 \cdot S_2$, and that there is a small monomeric impurity present (~2.5%).

**Crystal data for $\{[CuL^1]ClO_4\}_2$: $C_{42}H_{44}O_{10}N_6Cl_2Cu_2$, $M = 990$ 83, crystallises from methanol as blue blocks, crystal dimensions $0.35 \times 0.25 \times 0.15$ mm, orthorhombic, $a = 12$ 875(9), $b = 15$ 066(11), $c = 21.432(12)$ Å, $U = 4157(5)$ Å$^3$, $Z = 4$, $D_c = 1.583$ g cm$^{-3}$, space group *Pbca* ($D_{2h}^{15}$, No 61), Mo K$\alpha$ radiation ($\lambda = 0.71069$ Å), $\mu$(Mo K$\alpha$) = 12 19 cm$^{-1}$, $F(000) = 2039$ 60 Three-dimensional, room temperature X-ray data were collected in the range $3.5 < 2\theta < 40°$ on a Nicolet R3 diffractometer by the $\omega$-scan method The 1209 independent reflections (of 3121 measured) for which $|F|/\sigma(|F|) > 4.0$ were corrected for Lorentz and polarisation effects, and for absorption by analysis of 6 azimuthal scans (minimum and maximum transmission coefficients 0 374 and 0.553) The structure was solved by direct methods and refined by blocked cascade least-squares methods Hydrogen atoms were detected and refined in riding mode. Refinement converged at a final $R = 0$ 0975 ($R_w = 0.0963$, 281 parameters, mean and maximum $\Delta/\sigma$ 009, 0.045), with allowance for the thermal anisotropy of all non-hydrogen atoms. Minimum and maximum final electron density $-0$ 59 and 1 05 e Å$^{-3}$ A weighting scheme $w^{-1} = \sigma^2(F) + 0$ 00261$(F)^2$ was used in the latter stages of refinement Complex scattering factors were taken from the program package SHELXTL [7] as implemented on the Data General DG30 computer

We are now attempting to break down the dimer as this would lead to mononuclear copper(II) complexes having unfilled and available coordination sites. Through the subsequent complexation of bulky and strongly coordinating solvates or, alternatively, more strongly coordinating anions than perchlorate and tetrafluoroborate, our objective is the building of a close synthetic analogue for the metal site in galactose oxidase.

## Supplementary material

Tables of bond lengths and angles, anisotropic temperature factors, atom coordinates and temperature factors, hydrogen atom coordinates and temperature factors, and observed and calculated structure factors are available from the authors on request.

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## References

1 D J Kosman, in R. Lontie (ed ), *Copper Proteins and Copper Enzymes*, Vol 1, CRC, Boca Raton, FL, USA, 1984, p. 1.
2 N Ito, S E.V. Phillips, C. Stevens, Z B. Ogel, M.J. McPherson, J N Keen, K.D.S. Yadav and P.F. Knowles, *Nature (London), 350* (1991) 87.
3 N Kitajima, *Adv Inorg Chem , 39* (1992) 1
4 U Rajendran, R. Viswanathan, M. Palaniandavar and M. Lakshminarayanan, *J Chem Soc , Dalton Trans ,* (1992) 3563
5 E.I Alilou, E Amadei, M. Giorgi, M. Pierrot and M. Reglier, *J. Chem Soc , Dalton Trans* (1993) 549
6 W C. Still, M. Kahn and A Mitra, *J Org Chem , 43* (1978) 2923
7 G.M. Sheldrick, *SHELXTL,* an integrated system for solving, refining and displaying crystal structures from diffraction data (Revision 5.1), University of Gottingen, Germany, 1985.
8 K.D. Karlin and B I Cohen, *Inorg Chim Acta, 107* (1985) L17; B I. Cohen, *Ph D Dissertation*, SUNY at Albany, 1987.
9 K D Karlin, B.I. Cohen, J C. Hayes, A. Farooq and J. Zubieta, *Inorg Chem , 26* (1987) 147.
10 J. Reedijk, W.L. Driessen and J. Wan Rijn, in K.D. Karlin and J Zubieta (eds.), *Biological and Inorganic Copper Chemistry*, Vol. 2, Adenine, Guilderland, NY, 1986, p 143.
11 K.D Karlin, J C. Hayes, Y. Gultneh, R W Cruse, J.W McKown, J.P. Hutchinson and J. Zubieta, *J Am Chem. Soc , 106* (1984) 2121.
12 K D Karlin, R W. Cruse, Y. Gultneh, A. Farooq, J.C. Hayes and J Zubieta, *J. Am. Chem Soc , 109* (1987) 2668.
13 Y. Nishida, H. Shimo, H Maehara and S Kida, *J Chem Soc , Dalton Trans* (1985) 1945.