Spectroscopic characterisation of a copper(II) complex of a thioether-substituted phenoxyl radical: a new model for galactose oxidase

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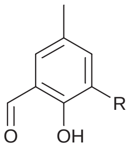
The EPR-silent species [Cu(L^2)(Tp^{Ph})]$^+$ exhibits a UV–VIS–NIR spectrum that is very similar to that of active galactose oxidase.

Galactose oxidase ('GOase') is a fungal enzyme that catalyses the oxidation of primary alcohols by molecular oxygen.[1] The active site of GOase contains a [Cu(His)$_2$(Tyr)$_2$(OH$_2$)] centre, in which a basal tyrosinate ligand has been chemically modified by an *ortho*-thioether crosslink formed from a cysteine residue, and is involved in a π-stacking interaction with a neighbouring tryptophan side chain.[2] In active enzyme this modified phenoxide ligand is oxidised to a very long-lived radical,[3] whose oxidation potential is +0.40 V *vs.* NHE (compared to +0.9 V for a 'normal' tyrosine side-chain). We describe here a Cu(II) phenoxide complex containing a thioether-substituted phenoxide ligand, designed as a model for the GOase copper complex, and the spectroscopic characterisation of its Cu(II) phenoxyl oxidation product.



R = H, HL^1
R = SMe, HL^2

2-Hydroxy-3-methylsulfanyl-5-methylbenzaldehyde (HL^2) was prepared from 2-hydroxy-5-methylbenzaldehyde (HL^1)[4] by the method of Wang and Stack.†[5] Complexation of hydrated Cu(BF$_4$)$_2$ by HL (HL = HL^1, HL^2) and K[Tp^{Ph}] ([Tp^{Ph}]$^-$ = tris-3-phenylpyrazolylborate)[6] in CH$_2$Cl$_2$ at room temperature affords dark green solutions, from which deep green microcrystals of [Cu(L)(Tp^{Ph})] ([L]$^-$ = [L^1]$^-$, **1**, [L]$^-$ = [L^2]$^-$, **2**)† can be obtained in 40–45% yield after filtration and addition of a large excess of hexanes. Weakly diffracting single crystals of **2** were grown from toluene–hexanes.‡ The structure shows a square pyramidal Cu(II) centre with a N$_3$O$_2$ donor set and unexceptional metric parameters (Fig. 1).

The visible spectra of **1** and **2** in CH$_2$Cl$_2$ at 293 K each show a d–d absorption at λ_{max} = 685 nm (ε_{max} = 92–93 dm^3 mol^{-1} cm^{-1}). The X- and Q-band EPR spectra of **1** and **2** in 10 : 1 CH$_2$Cl$_2$–toluene solution at 110 K exhibit the $g_\parallel > g_\perp > g_e$ pattern expected of a {$d_{x^2-y^2}$}^{1} or {d_{xy}}^{1} Cu(II) ion (for **1**; $g_\parallel$ = 2.284, $g_\perp$ = 2.065, $A_\parallel$\{^{63,65}Cu\} = 160 G: for **2**; $g_\parallel$ = 2.286, $g_\perp$ = 2.065, $A_\parallel$\{^{63,65}Cu\} = 163 G), only one species being detected in solution for both compounds. These spectra are consistent with **1** and **2** possessing essentially identical tetragonal coordination spheres in CH$_2$Cl$_2$. Hence, in this solvent the [L^2]$^-$ ligand in **2** is coordinated *via* both O-donors, with no isomerisation to a form containing O,S-coordinated [L^2]$^-$ taking place. While the lack of observable A\{^{14}N\} couplings for **1** and **2** prevents more detailed EPR studies, we have recently proven that related [Cu^{II}(L)(Tp^{Ph})] (L = bidentate ligand) complexes retain their square-pyramidal solid state geometries upon dissolution in CH$_2$Cl$_2$.[7] It is therefore probable that the solution structures of **1** and **2** closely resemble those in the crystal.

The cyclic voltammogram (CV) of **2** in CH$_2$Cl$_2$/0.5 M NBu$^n_4$PF$_6$ at 293 K shows a one-electron couple at $E_{1/2}$ = +0.53 V *vs.* Fc–Fc$^+$, which is chemically reversible for 10 mV s^{-1} ⩽ ν ⩽ 1 V s^{-1} and which we assign to a [L^2]$^-$/L$^{2\cdot}$ oxidation. The observation of a chemically reversible oxidation for coordinated [L^2]$^-$ in **2** is very unusual for a phenoxide without encumbering *tert*-butyl substituents.[8] The CV of **2** also exhibits an irreversible secondary oxidation of variable broadness and intensity centered near E_{pa} = + 0.85 V, which is characteristic of partial adsorption of the initial oxidised species onto the Pt electrode;[9] and an irreversible Cu(II/I) reduction at E_{pc} = −1.29 V with associated daughter peaks at E_{pa} = −0.41 and −0.11 V.

Electrooxidation of **2** in CH$_2$Cl$_2$–0.5 M NBu$^n_4$PF$_6$ at 243 K at a potential corresponding to the **2**/[**2**]$^+$ couple yields a brown solution exhibiting only a very weak residual EPR signal from

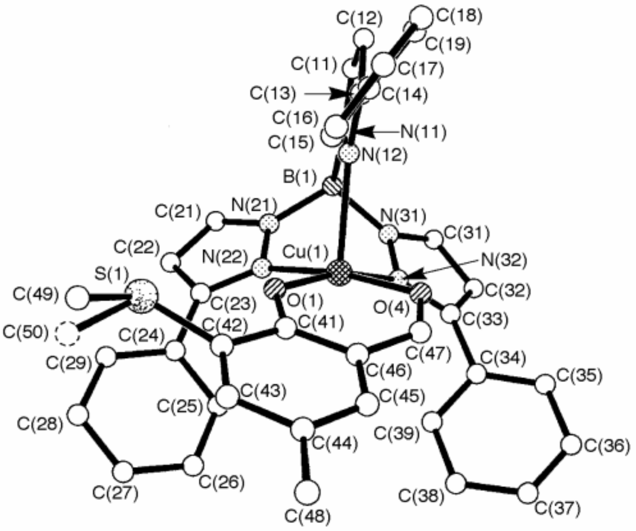


Fig. 1 View of the complex molecule in the crystal of **2**, showing the disordered thioether group. For clarity, all H atoms have been omitted. Selected distances (Å) and angles (°): Cu(1)–N(12) 2.337(6), Cu(1)–N(22) 2.009(6), Cu(1)–N(32) 1.996(7), Cu(1)–O(1) 1.941(7), Cu(1)–O(4) 1.967(5), N(12)–Cu(1)–N(22) 89.7(2), N(12)–Cu(1)–N(32) 90.0(2), N(12)–Cu(1)–O(1) 102.8(2), N(12)–Cu(1)–O(4) 98.7(2), N(22)–Cu(1)–N(32) 87.7(3), N(22)–Cu(1)–O(1) 91.5(3), N(22)–Cu(1)–O(4) 171.3(2), N(32)–Cu(1)–O(1) 167.1(2), N(32)–Cu(1)–O(4) 89.9(2), O(1)–Cu(1)–O(4) 88.9(3).

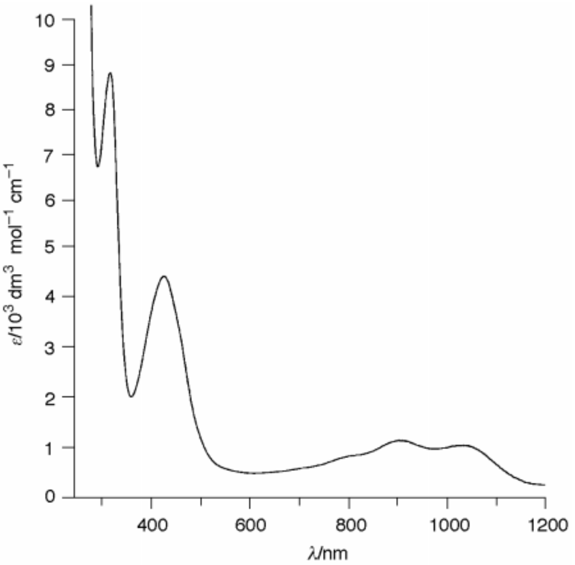


Fig. 2 UV–VIS–NIR spectrum of [**2**]$^+$ in CH_2Cl_2–0.5 M $Bu^n{}_4NPF_6$ at 243 K.

unreacted **2**. A similar experiment using an optically transparent electrode results in a blue-shift of the $[L^2]^-$-derived absorptions in the UV, and the ingrowth of new peaks in the visible and near-IR regions. The oxidised solution shows λ_{max} = 317 nm (ε_{max} ≈ 9 000 dm^3 mol^{-1} cm^{-1}), 419 (4400), 470 (sh), 725 (sh), 818 (sh), 907 (1200) and 1037 (1100) at 243 K (Fig. 2). Rereduction of this solution at 0 V results in the near-quantitative regeneration of **2**. We ascribe these observations to the generation of an EPR-silent $[\mathrm{Cu^{II}}(L^{2\cdot})(\mathrm{Tp^{Ph}})]^+$ species [**2**]$^+$. The **2**/[**2**]$^+$ preparative oxidation is not quite reversible, since [**2**]$^+$ decomposes with a half-life of *ca.* 10 h under these conditions; the absorption coefficients quoted above may therefore be slightly underestimated.

Other known Cu(II) phenoxyl complexes, although usually also EPR-silent, give electronic spectra significantly different from [**2**]$^+$, with peaks at λ_{max} = 400–450 nm (ε_{max} = 3000–16 000 dm^3 mol^{-1} cm^{-1}) and 600–680 nm (300–8000).[10] None of these examples contains a thioether side-chain to the phenoxyl ligand, however. Active GOase exhibits two spectroscopic features attributable to the modified tyrosyl radical: a peak at λ_{max} = 444 nm (ε_{max} = 5200 dm^3 mol^{-1} cm^{-1}) and a broad absorption between 600 and 1200 nm, centred at 800 nm (3200) with several low- and high-wavelength shoulders.[11] The similarity of this spectrum to that shown by [**2**]$^+$ (Fig. 2) is striking. The VIS–NIR feature in the spectrum of GOase has been attributed to an inter-ligand charge transfer process between the tyrosyl and tyrosinate ligands.[12] However, the observation of an equivalent broad, structured band for [**2**]$^+$, which lacks a second phenoxide ligand, suggests that π→π*, MLCT and/or LMCT transitions involving the tyrosyl radical should also contribute to this absorption.

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Notes and references

† Correct analytical and NMR data were obtained for HL^2. Analytical data for the complexes. **1**: Found: C, 59.7; H, 4.3; N, 11.5; Calc. for $C_{35}H_{29}BCuN_6O_2.CH_2Cl_2$: C, 59.7; H, 4.3; N, 11.6 %. **2**: Found: C, 63.0; H, 4.6; N, 13.4; Calc. for $C_{36}H_{31}BCuN_6O_2S$: C, 63.0; H, 4.6; N, 12.3 %.

‡ *Crystal data* for **2**: $C_{36}H_{31}BCuN_6O_2S$, triclinic, space group $P\bar{1}$, dark green block, 0.30 × 0.25 × 0.20 mm, a = 12.536(8), b = 13.90(2), c = 9.760(4) Å, α = 99.00(7), β = 90.75(4), γ = 102.48(9)°, U = 1638(3) Å^3, Z = 2, T = 150(2) K, μ(Mo-Kα) = 0.773 mm^{-1}; Rigaku AFC7-R diffractometer, 5405 measured reflections, 5126 independent, R_{int} = 0.0941; $R(F)$ = 0.079, $wR(F^2)$ = 0.233, S = 1.076. The thioether methyl C atom of the $[L^2]^-$ ligand was disordered over two sites C(49) and C(50) in a 60:40 occupancy ratio, which were restrained to common S(1)–C(X) and C(42)···C(X) (X = 49, 50) distances of 1.85(1) and 2.77(1) Å, respectively. All non-H atoms except C(49) and C(50) were refined anisotropically. CCDC 182/1047.

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