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Structural studies on dicopper(II) compounds with catechol oxidase activity†

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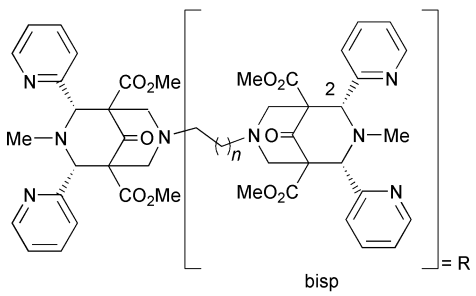
The X-ray crystal structures of three low molecular weight models of catechol oxidase with three different coordination modes are reported; and the compound with a bridging catecholate is shown to be the catalytically most active form.

A range of biological dicopper sites have similar structures, with three histidine donors for each of the two Cu sites and Cu–Cu distances of *ca.* 3.5 Å. These include the oxygen transport protein hemocyanin, the oxygenation enzyme tyrosinase and the oxidation enzyme catechol oxidase. The active sites of these dicopper proteins are structurally well characterized;[1–6] thorough spectroscopic studies, combined with computational investigations, have defined the electronic structures of the active sites,[7,8] and extensive kinetic studies[9,10] have led to the proposal of mechanisms for oxygen transport and activation, as well as oxygen and electron transfer. The assumption of a bridging catecholate as the active species in catechol oxidase[7] was recently challenged on the basis of crystallographic data, which suggested an active state with a monodentate catecholate.[6]

Low molecular weight model compounds have helped to understand structural, electronic and mechanistic features and are expected to be useful for the development of new catalysts. A number of Cu^{II}-based models with catechol oxidase activity have been reported,[11–16] but only few relevant experimental structures with coordinated catecholate have appeared so far.[11] In particular, there is no example where the various coordination modes of catecholate, including the putative intermediates with bridging or monodentate catecholate, have been analyzed with identical coligands, and for some relevant coordination models there have not been any structural data available so far.

We have successfully used mono- and di-nuclear Cu^{I} and Cu^{II} compounds with bispidine-type ligands to stabilize μ-peroxodicopper(II) compounds.[17,18] We now present preliminary results on the catechol oxidase activity of the corresponding Cu^{II} compounds with 3,5-dtbc in MeOH (3,5-dtbc = 3,5-di-*tert*-butylcatechol; spectrophotometric analysis of the *o*-quinone product).[14,15] The mononuclear Cu^{II} complex of L^1 is inactive, in contrast to the dinuclear compounds with L^2 and L^3.



L^1: R = Me, **L^2**: R= bisp, *n* = 1; **L^3**: R = bisp, *n* =2;
L^4: R = bisp, *n* = 2, carbon atoms 2 inverted

One equivalent of $[Cu_2(L^3)(solv)_2]^{4+}$ (solv = solvent) produces in a stoichiometric process 2 equivalents of quinone, while 13 equivalents of quinone are produced per h in a catalytic reaction with $[Cu_2(L^2)(solv)_2]^{4+}$. It emerges that the catalytic activity is a function of the catechol binding mode and stability, and this may differ for all three Cu^{II} compounds.

To examine this, the electronically deactivated substrate $tccH_2$ ($tccH_2$ = tetrachlorocatechol) was added in various concentrations to methanolic solutions of the three Cu^{II} compounds, and substrate binding was monitored spectrophotometrically. For $[Cu(L^1)(solv)]^{2+}$ a strong absorption band appeared at *ca.* 450 nm; for $[Cu_2(L^2)(solv)_2]^{4+}$ and $[Cu_2(L^3)(solv)_2]^{4+}$ equilibria between species with absorptions at *ca.* 450 nm and *ca.* 530 nm were established; with L^2 the species with the lower energy transition was more stable than with L^3, where it disappeared with an excess of catechol (see ESI†). These results are in accord with the assumption that $[Cu_2(L^2)(solv)_2]^{4+}$ and $[Cu_2(L^3)(solv)_2]^{4+}$ lead to catecholate-bridged active compounds, while $[Cu(L^1)(solv)]^{2+}$ leads to a mononuclear catecholate compound. A molecular model[19] (see Fig. 1) indicates that the ethylene-bridged dicopper(II) compound is suitable and highly preorganized for a bridging catecholate.

Single crystals of $[Cu(L^1)(tccH)](ClO_4)$ **1**, $[Cu(L^1)(tcc)]$ **2** and $[Cu_2(L^3)(tcc)](ClO_4)_2$ **3** were obtained by reaction of the Cu^{I} precursors with the fully chlorinated quinone (tcbq) or with $tccH_2$ and O_2, followed by slow evaporation of the solvent (full experimental details, including synthetic procedures, crystal growth, spectroscopic (IR, UV–VIS) and elemental analytical data, are given as ESI†).[20,21] ORTEP plots are shown in Fig. 2. The structure of **1** is of poor quality, but catecholate binds unambiguously as a monodentate, monoprotonated ligand (see analytical data in the ESI†). Also shown in Fig. 2 is an ORTEP plot of $[Cu_2(L^4)(tcc)_2]$ **4**. L^4 has a non-coordinating pyridyl substituent at each copper center and, therefore, leads to copper(II) chromophores with the usual in-plane chelating catecholate coordination mode.

The C–C and C–O bond lengths of tcc^{2-} and $tccH^{-}$ confirm the assignment as coordinated catecholate in all four structures.

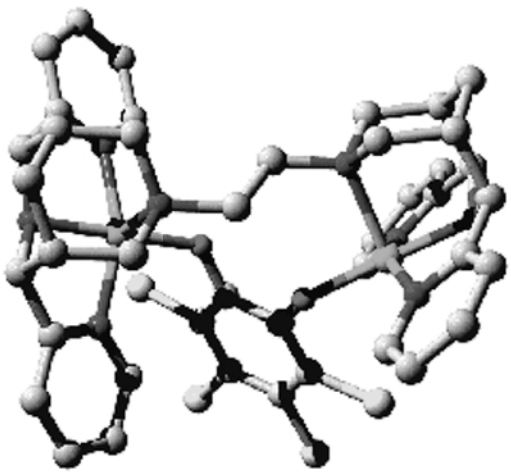


Fig. 1 Molecular model of $[Cu_2(L^2)(tcc)]^{2+}$.

† Electronic supplementary information (ESI) available: S1–S3: titration data; S4: experimental; S5: colour version of Fig. 1. See http://www.rsc.org/suppdata/cc/b0/b008714i/

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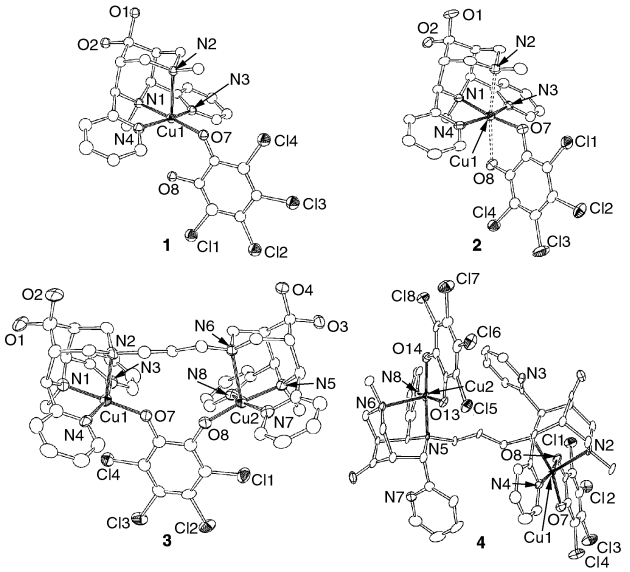

Fig. 2 ORTEP plots of $[Cu(L^1)(tccH)]^+$ **1**, $[Cu(L^1)(tcc)]$ **2**, $[Cu_2(L^3)(tcc)]^{2+}$ **3** and $[Cu_2(L^4)(tcc)_2]$ **4** (50%) probability level). H-atoms, ester groups and counter ions have been omitted for clarity. Selected bond lengths (Å) and angles (°) for **1**; **2**; **3**; **4**; Cu(1)–N(1): 2.020(11); 2.042(2); 2.027(5); 2.094(5). Cu(1)–N(2): 2.320(12); 2.433(2); 2.359(5); 2.293(5). Cu(1)–N(3) 1.987(11); 2.009(2); 2.004(6); —. Cu(1)–N(4) 1.989(11); 2.031(2); 1.985(6); 1.995(5). Cu(1)–O(7) 1.915(9); 1.909(2); 1.898(4); 1.947(4). Cu(1)···O(8) 2.76; 2.46; —; 1.898(4). N(1)–Cu(1)–N(2) 84.06(44); 80.97(8); 83.93(19); 82.24(17). N(1)–Cu(1)–N(3) 81.71(44); 80.56(9); 81.5(2); —. N(1)–Cu(1)–N(4) 81.94(44); 82.15(9); 82.4(2); 80.37(18). N(1)–Cu(1)–O(7) 176.85(47); 172.97(8); 178.1(2); 164.10(18). N(2)–Cu(1)–N(3) 96.66(44); 91.42(8) 95.6(2); —. N(3)–Cu(1)–N(4) 161.28(48); 161.58(9); 161.7(2); —. **4**: O(8)–Cu(1)–O(7) 86.70(17).

The geometry around the Cu^{II} centers can be described as square pyramidal, with N1, the pyridine N-atoms and one of the catecholate O atoms in the square plane and N2 at the axial position; in **2** O8 completes the coordination sphere to an elongated octahedron, and in **4** the second catecholate-O substitutes one of the pyridine-N atoms. **2** is only sparingly soluble in most solvents; the UV–VIS spectrum of a very dilute solution (MeCN) indicates an equilibrium between the mono- and bidentate coordination modes of catecholate, *i.e.* structures **1** and **2** (Fig. 2).

The most prominent structural difference between **3** and the other known structure of a catecholate-bridged dicopper(II) complex is the orientation of the catecholate bridge (angle between the line through the two metal ions and the line through the two catecholate O-atoms: 13.6° in **3**, 32.0° in $[Cu_2(L^2)(TCC)]^{2+}$ (computed), 63.1° in[11]). It is interesting that the increasing puckering of the catecholate bridge correlates with the catalytic activity in the 3,5-dtbc to 3,5-dtbq reaction.

For the model reactions involving bispidine-based ligands it appears that catecholate oxidation occurs at a catecholate-bridged dicopper(II) site by electron transfer from the catechole to the Cu^{II} ions; reoxidation of the Cu centers by molecular oxygen produces water and the active catalyst (the absence of H_2O_2 has been checked by reaction with KI). An interesting question is how thermally stable copper(II) peroxo compounds, generated during the catalytic process, affect the reaction. This and a thorough analysis of the electronic structures of the various structural modes are the subject of further studies in this area.

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20 *Crystal structure determination*: data were collected at −100 °C with a Bruker-AXS CCD diffractometer (Mo-Kα radiation, λ = 0.71073 Å, ω-scans). Structures were solved by direct methods and refined against F^2 (SHELXTL V5.10). **2**: $C_{31}H_{31}N_5O_8Cl_4Cu$, M = 806.9, monoclinic $P2_1/c$, a = 16.5741(9), b = 13.5311(7), c = 16.0711(8) Å, β = 110.439(1)°, V = 3377.3(3) Å^3, Z = 4, 8154 independent reflections, θ_{max} = 28.3°, 597 parameters, $R1$ = 0.042, $wR2$ = 0.112. **3**: $C_{55}H_{61}N_9O_{23}Cl_6Cu_2$, M = 1555.9, monoclinic, $P2_1/n$, a = 12.1045(2), b = 33.7116(5), c = 15.8529(2) Å, β = 103.798(1)°, V = 6282.3(2) Å^3, Z = 4, 7686 independent reflections, θ_{max} = 22°, 901 parameters, $R1$ = 0.054, $wR2$ = 0.154. **4**: $C_{75}H_{75}N_{15.50}O_{14}Cl_8Cu_2$, M = 1828.2, triclinic, $P\bar{1}$, a = 19.3832(5), b = 19.5174(5), c = 24.2065(7) Å, α = 71.184(2), β = 80.390(2), γ = 76.041(2)°, V = 8372.5(4)Å^3, Z = 4, 28535 independent reflections, θ_{max} = 24.7°, 2017 parameters, $R1$ = 0.069, $wR2$ = 0.205. CCDC 182/1863. See http://www.rsc.org/suppdata/cc/b0/b008714i/ for crystallographic files in .cif format.
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