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The phenol *ortho*-oxygenation by mononuclear copper(I) complexes requires a dinuclear μ-η²:η²-peroxodicopper(II) complex rather than mononuclear CuO_2 species

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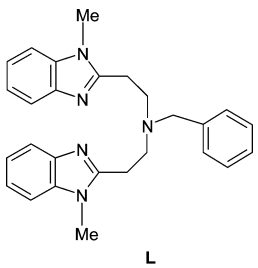
The mononuclear complex [Cu(L)]⁺ performs the *ortho*-oxygenation of an exogeneous phenol through the formation of a dinuclear μ-η²:η²-peroxodicopper(II) intermediate, which is so far the only type of copper–dioxygen complex that mediates the tyrosinase monophenolase reaction.

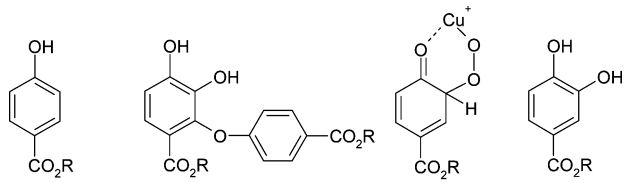
Copper mediated phenol *ortho*-oxygenation is an essential step in the mechanism of tyrosine oxidative polymerization to melanin induced by tyrosinase[1] and cofactor biogenesis by copper amine oxidases.[2] Strong similarities exist between tyrosinases, hemocyanins and catechol oxidases,[3] in that they all contain dinuclear type-3 copper centres and bind reversibly molecular dioxygen as peroxide, in a μ-η²:η² bridging mode,[4] while copper amine oxidases contain mononuclear type-2 copper centres. Synthetic model systems in which dioxygen binds to Cu(I) centres producing adducts with Cu_2O_2 cores contain either μ-η²:η²-peroxo, μ-1,2-peroxo, or bis-μ-oxo arrangements.[5–11] Unfortunately, the activity of most of these systems is limited to ligand hydroxylation[12–17] or affords biologically not relevant C–C coupling products when the reaction occurs with exogenous phenolic substrates.[8,18,19] Despite there have been a number of reports in the literature that demonstrated *o*-catechol and/or *o*-quinone formation from phenol mediated by copper(I) and dioxygen,[20–25] only in two cases is there direct information about the oxygen intermediates involved, which invariably are of μ-η²::η²-peroxo type.[11,16] Recently, Sayre and coworkers reported that reaction between the Cu(I) complex of *N*,*N*-bis(2-*N*-methylbenzimidazol-2-yl)ethyl)benzylamine (**L**) with the sodium salt of phenol **1b** and O_2 at room temperature produces catechol **2b**, which results from coupling of the initial *ortho*-oxygenation product with the starting phenolate.[21]



L

The reaction was proposed to proceed through a 6-peroxy-2,4-cyclohexadienone intermediate **3**.[21] We report herein that conversion of the sodium salt of phenol **1a** to catechol **4a** by [Cu(**L**)]⁺ and O_2 depends on the *dinuclear* μ-η²:η²-peroxo intermediate $[Cu_2(\mathbf{L})_2O_2]^{2+}$.

Treatment of [Cu(**L**)]⁺ with dioxygen in anhydrous acetone at −85 °C slowly produces a green–brown solution exhibiting a moderately intense absorption band at 356 nm and a weaker band near 560 nm, due to a peroxo complex (Fig. 1). Under saturating O_2 conditions, the development of the bands follows a first order behavior, with an observed rate constant $k_1 \times [O_2] = 1.1 \times 10^{-3}$ s⁻¹.† The oxygenation can be slowly but fully reversed upon application of vacuum/argon cycles at low temperature, with no detectable decomposition after several



a: R = Me
b: R = Et

oxygenation/deoxygenation cycles.† The complex is not observed at temperatures higher than about −80 °C. From the position of the dominant absorption band, peroxide must be bound in a μ-η²:η² fashion,[4] the absorption corresponding to the $\pi^*_\sigma(O_2) \rightarrow$ Cu(II) charge-transfer (CT) transition of the Cu_2O_2 moiety. The low intensity of the second CT band at about 560 nm, $\pi^*_v \rightarrow$ Cu(II), further indicates that the $Cu(O_2)Cu$ core of the dioxygen adduct including the four equatorial nitrogen ligands must be highly planar.[22] As a consequence, the ε value of the 356 nm band should be about 20000 M^{-1} cm^{-1}, and from Fig. 1 the relative fraction of peroxo adduct can then be estimated as about 30% of the theoretical amount. This is confirmed by bleaching experiments of the UV band at low temperature, by the addition of a small excess acid, and analysis of the hydrogen peroxide produced by the peroxidase/ABTS assay,[16] which yielded 0.30 equiv. H_2O_2 with respect to the theoretical amount. Therefore, the μ-η²:η²-peroxodicopper(II) intermediate[7–9,16] only partially forms in solution. ¹H-NMR spectroscopy at low temperature suggests the complex is diamagnetic.‡ Due to fluorescence from the large fraction of Cu(I) precursor present in solution, no Raman data of the peroxo adduct could be obtained at excitation wavelengths of either 350 or 413 nm, where in the case of the analogous $[Cu_2(L\text{-}66)(O_2)]^{2+}$ complex (L-66 is α,α′-bis{bis[2-(1′-methyl-2′-ben-

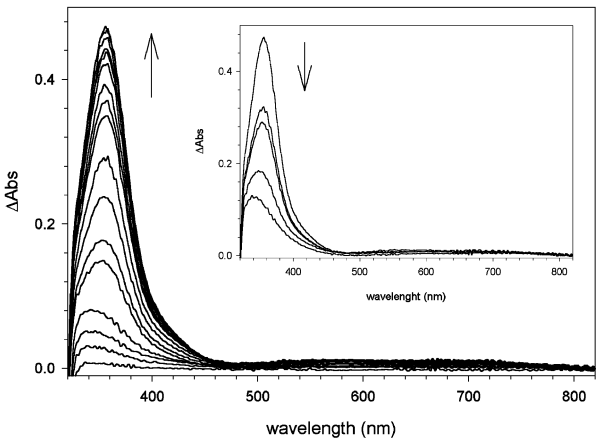


Fig. 1 Difference spectra recorded during the oxygenation of [Cu(**L**)]⁺ in acetone at −85 °C (concentration 3.8×10^{-4} M, 0.5 cm path length cell). The inset shows the reversibility of the process upon application of several vacuum/argon cycles at the same temperature.

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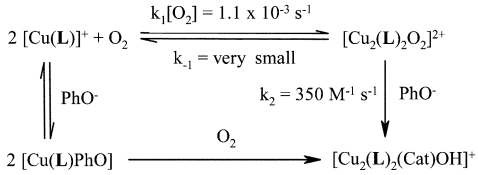

Scheme 1 Possible paths in the phenol monooxygenation.

zimidazolyl)-ethyl]amino}-*m*-xylene) the metal–ligand and O–O stretches could be observed.[16]

Upon adding sodium 4-carbomethoxyphenolate (PhONa) to a solution of $[Cu_2(\mathbf{L})_2O_2]^{2+}$ formed *in situ* in dry acetone at −85 °C, the UV band at 356 nm rapidly decreases in intensity until it completely disappears in about 1 min. The rate constant of this process is k_2 = 350 M^{-1} s^{-1}. The fast reaction of $[Cu_2(\mathbf{L})_2O_2]^{2+}$ with PhO^- is followed by a slower reaction characterized by an increase in absorbance at 334 nm, which is due to the formation of $[Cu_2(\mathbf{L})_2(Cat)OH]^+$, where Cat represents the dianion of **4a**. Under saturating O_2 (1 atm), the latter reaction occurred with a first order rate constant of k_r = 8.9 × 10^{-4} s^{-1}.

These results are consistent with a reaction mechanism involving an electrophilic attack on the phenolate by the μ-η^2:η^2-peroxodicopper(II) intermediate. The small amount of this species initially formed is rapidly consumed and does not accumulate in solution; further hydroxylation of the phenolate requires reformation of the peroxo complex. The high reactivity of the $[Cu_2(\mathbf{L})_2O_2]^{2+}$ complex (k_2) and the similarity between the values of the Cu(I) oxygenation rate (k_1×[O_2]) and k_r suggest that the phenol monooxygenation proceeds through a rate-limiting continuous formation of $[Cu_2(\mathbf{L})_2O_2]^{2+}$ which reacts rapidly with further phenolate according to Scheme 1.

Therefore, formation of the product proceeds in a clockwise, and not in a counter-clockwise manner in Scheme 1. Thus, in spite of its limited stability, $[Cu_2(\mathbf{L})_2O_2]^{2+}$ is highly reactive toward the phenol and its consumption in the monooxygenase reaction shifts the Cu(I)/Cu_2O_2 equilibrium driving the formation of the peroxo intermediate.

When the low-temperature phenol oxygenation was continued for 1 h and then quenched, HPLC analysis of the products showed conversion of **1a** into catechol **4a** in 44% yield based on the starting Cu(I) complex (88% based on the theoretical amount of $[Cu_2(\mathbf{L})_2O_2]^{2+}$).§ Furthermore, neither the corresponding *o*-quinone derivative nor the C–C or C–O coupling dimers were detected in the product mixture. Isotope labelling experiments using $^{18}O_2$ confirmed that the origin of the inserted oxygen atom into the catechol product derives from molecolar oxygen.¶

The behavior of $[Cu(\mathbf{L})]^+$ in the phenol hydroxylation parallels the reactivity of its dinuclear analogue $[Cu_2(L\text{-}66)]^{2+}$, which when reacted with the sodium salt of **1a** afforded **2a** at room temperature,[20,23] but gave **4a** when the oxygenation was performed at −60 °C.[16] In conclusion, the phenol *ortho*-oxygenation reaction of $[Cu(\mathbf{L})]^+$ depends on the intermediate formation of a dinuclear μ-η^2:η^2-peroxodicopper(II) complex, which is currently the only type of copper–dioxygen complex exhibiting tyrosinase-like activity.

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Notes and references

† The oxygenation of the complex was carried out at −85 °C by bubbling chilled O_2 (1 atm) in a 6.5 × 10^{-4} M solution of $[Cu(\mathbf{L})](PF_6)$ (20 ml) in acetone previously kept under Ar. The deoxygenation process was performed by repeated applications of a vacuum to the oxygenated solution and purging with an inert gas. The reactions were monitored through the appearance/disappearance of the band at 356 nm of the peroxo complex, with a custom-designed fiber-optic quartz probe (Hellma).

‡ ^{1}H-NMR measurements were obtained with a Bruker AVANCE spectrometer operating at proton Larmor frequency of 400.13 MHz. A degassed solution of $[Cu(\mathbf{L})]^+$ (~1 mM) in $(CD_3)_2CO$ was exposed to dioxygen at −85 °C, and the spectral evolution was followed for approximately 1 h at the same temperature. Only a few weak new signals in the aromatic region (7–10 ppm) attributable to the peroxo species could be detected under the much more intense envelope of signals of the starting Cu(I) complex.

§ The phenol monooxygenation reaction was carried out at −85 °C by slowly adding, through a syringe, a solution of sodium 4-carbomethoxyphenolate (6.1 mg) in acetone (1 ml) to an oxygenated solution of $[Cu(\mathbf{L})](PF_6)$. After about 80 min, a sample of the reaction mixture (1 ml) was withdrawn and rapidly quenched with 0.04 M H_3PO_4 (1 ml). A solution of 4-hydroxybenzoic acid as internal standard was added and the resulting solution was analyzed by HPLC, using a Supelco LC18 semipreparative column (250 × 10 mm). Elution was carried out at 5 ml min^{-1} starting with water containing 0.1% trifluoroacetic acid for 4 min, followed by a linear gradient from 0% to 100% acetonitrile containing 0.1% trifluoroacetic acid during 20 min. Spectrophotometric detection of the HPLC elution profile in the range 200–650 nm was performed with a Jasco MD-1510 diode array instrument. The identity of the catechol was confirmed by comparison with an authentic sample and UV/ESI-MS analysis. ESI-MS spectra were acquired using a Finnigan MAT system equipped with an ion trap detector.

¶ The oxygen incorporation experiment was carried out by reaction of $^{18}O_2$ (ISOTEC®, > 99% atom ^{18}O) with the Cu(I) complex and the phenolate salt in dry acetone at low temperature as described above. The ^{18}O-atom incorporation into the phenol proved to be essentially complete (> 90%) by ESI-MS analysis (*m*/*z* [(catechol$^{18}O^{16}O$)+H^+]$^+$ = 171.1).

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