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Catecholase Activity in a Model Binuclear Copper Phthalazine Derivative

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We report here a series of binuclear copper(II) complexes of 1,4-(di-2'-pyridyl)aminophthalazine (PAP) (1), which have been previously characterised [1] but whose catalytic catecholase activity had not been previously recognised. We report details of the catalytic oxidation of 3,5-di-tert-butylcatechol to 3,5-di-tert-butylquinone (referred to henceforth as 'catechol' (cat) and 'quinone' (Q) respectively). The importance of this system lies in the ability to identify spectroscopically, putative intermediate stages in the catalytic process and thereby to deduce a mechanism.

This phthalazine system is one of the very few, *in vitro*, binuclear copper systems in which the binuclear framework retains its integrity upon reduction of the complex to copper(I) [2, 3], a necessary condition to mimic binuclear copper proteins [4]. Binuclear PAP-$[Cu(I)]_2(X)$ (2) (where X = OAc, halide, ClO_4, PF_6) [5] may be prepared by reaction of an appropriate copper(I) salt with PAP or by reduction of (1) with reducing agents such as hydrazine hydrate. In oxygen, such solutions of (2) re-oxidise, fairly slowly (see below) to regenerate binuclear copper(II), (1), cleanly, in a solution which, when followed spectroscopically yields isosbestic points (Fig. 1A). Most importantly the binuclear copper(II) complexes (1) (X = Cl, OAc), which exhibit a spin-triplet esr spectrum may be reduced to the esr quiet copper(I) complex, (3), with one equivalent of the 'catechol'. Complex (3) may also be produced directly by reaction of (2) with the 'quinone' (Fig. 1B). This complex (3) is spectroscopically uniquely identified and considered to be a copper(I) quinone derivative, PAP-$[Cu(I)]_2(Q)(X)$.

Addition of excess 'catechol', and oxygen, to this solution results in catalytic oxidation of this excess 'catechol' to the 'quinone' at a relatively fast rate (Fig. 2). When all the 'catechol' has been consumed, complex (1) is converted to esr active PAP$[Cu(II)]_2$-(Q) (4). This complex is also identifiable by electronic spectroscopy in the 350–500 nm range, and may alternatively be prepared by direct reaction of (1) with 'quinone'. These data are summarised in Scheme I.

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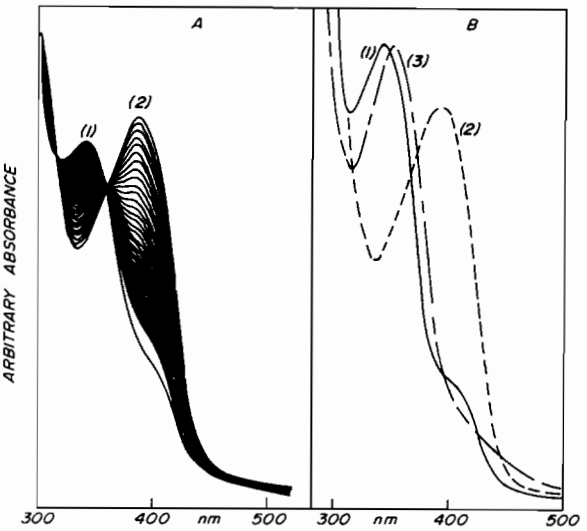


Fig. 1. A: The electronic spectrum of complex (2), in the 300–500 nm range, and its reaction with molecular oxygen to yield complex (1). B: The electronic spectra of complexes (1), (2) and (3) in the range 300–500 nm. Both sets of spectra are recorded in aqueous acetonitrile (50:50).

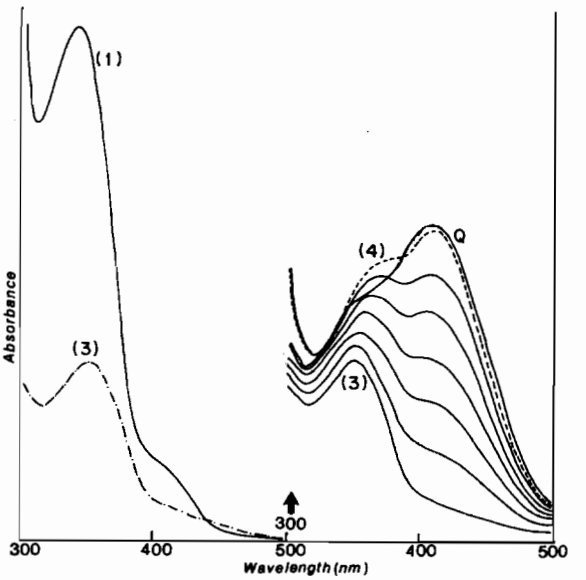
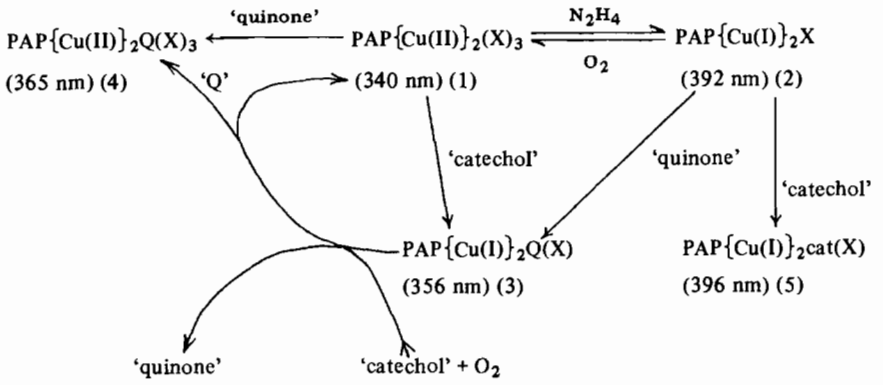


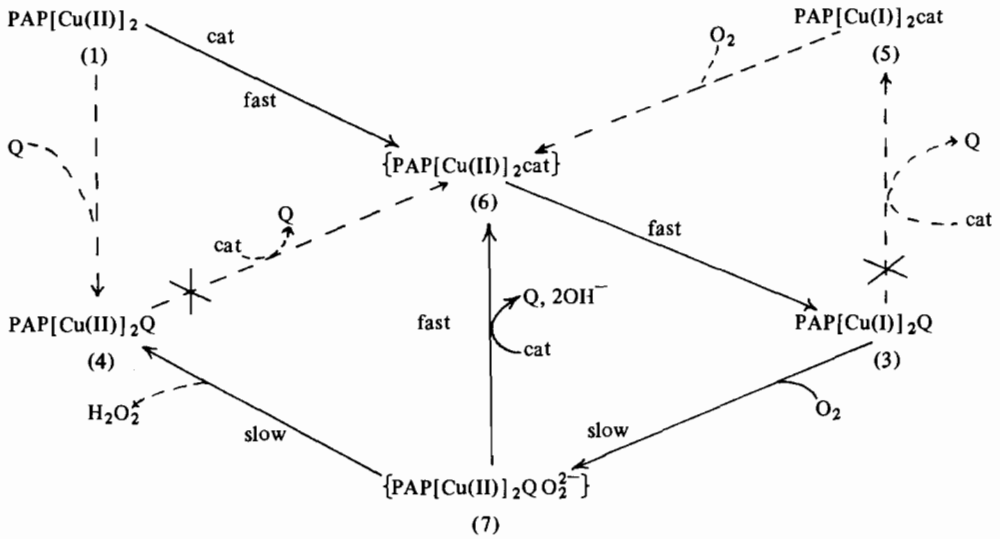
Fig. 2. The electronic spectra obtained during the catalytic oxidation of 'catechol' with complex (1) in aqueous acetonitrile (50:50), in the range 300–500 nm. Notes: *i*) the initial reduction of (1) to (3); *ii*) the build up of 'quinone' as indicated by absorption just above 400 nm; *iii*) the ultimate build up of (4) when all the 'catechol' has been exhausted.

The catalysis reaction may be followed to complete oxidation of 'catechol'. The system is stable for up to 30 hours. Beyond this time, esr spectroscopy reveals some decomposition to mononuclear copper-(II).



SCHEME I

(The number of X groups associated with each complex is known for (1) and (2), but presumed for the remaining species)



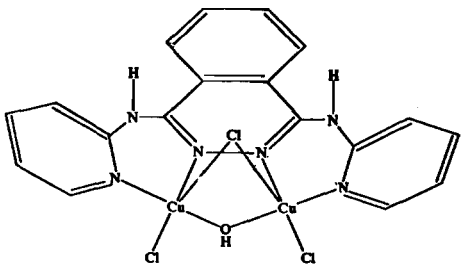
SCHEME II (X groups omitted for clarity)

The gross mechanism of this reaction may be deduced by noting that there are four possible combinations of binuclear copper(I), and copper(II), with 'catechol' and 'quinone'. Three of these may be prepared as stable solution species under the conditions noted in Scheme I. The fourth member, PAP-$[Cu(I)]_2(cat)(X)$ (5) may be prepared by reaction of (2) with 'catechol'. The electronic spectrum is very similar to that of (2), but is sufficiently different to imply coordination of 'catechol' to binuclear copper-(I). While we have not been able yet to isolate any of these complexes in the solid state, the solution spectra provide definitive evidence for the reaction of 'quinone' or 'catechol' with copper(I) or copper(II) in each of the four cases. The exact stoichiometry, however, is uncertain.

Preliminary kinetic data for the catalytic oxidation of 'catechol' reveal that the reaction shows a first order dependence upon the initial copper complex (1), but no dependence upon 'catechol', when the latter is in reasonable excess. In excess oxygen and catechol the rate constant is *ca.* 5×10^{-4} atm^{-1} sec^{-1} for X = Cl at room temperature in 70% (v/v) acetonitrile/water.

The overall chemistry contained with Fig. 2 and summarised in Scheme I, may be broken down into several possible mechanistic pathways as shown in Scheme II.

The solid line pathway is tentatively identified as the catalytic cycle. Complex (1) is rapidly converted to (3) *via* the transient copper(II) catechol complex (6). Oxidation of (3) to (4) is about 5 times slower



than the catalytic rate. Moreover (4) will not react to any significant degree with 'catechol'. The catalytic cycle cannot then proceed through (4). It is necessary to postulate an intermediate (7). The rate determining step is the conversion of (3) to (7) which is rapidly scavenged by 'catechol' to return into the cycle *via* the copper(II) catechol intermediate (6). When all the 'catechol' has been consumed, (7) more slowly forms the terminal product (4). Intermediate (6) is presumed to be some kind of ternary complex. Its formulation as a binuclear copper(II)/'quinone'/oxygen complex is tentative. We are searching for direct evidence of its existence through low temperature esr and electronic spectroscopy. Either its electronic spectrum is very similar to (3), or (4), or it is present in only small equilibrium amounts therewith.

Several pathways do not occur. Thus there is no reaction of 'catechol' with (4) nor any apparent reaction with (3). If (1) is first converted to (4) with 'quinone' and subsequently 'catechol'/oxygen added, there is no immediate reaction. After a lengthy induction period, however, catalysis does occur. We have no direct evidence for the formation of hydrogen peroxide in the termination step. However note that oxidation of 'catechol' with hydrogen peroxide is much slower than reaction with (1) implying that hydrogen peroxide is not involved in this pathway.

Complex (1) represents a unique mimic of catecholase binuclear copper enzymes [6], though its mechanism of reaction may not necessarily parallel enzymic mechanisms. Moreover, as we shall demonstrate elsewhere [7], complex (1) also possesses phenolase activity. It therefore behaves as a mimic of a mixed function oxidase/oxygenase enzyme.

Acknowledgement

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