An Accurate Synthetic Model of Oxyhaemocyanin

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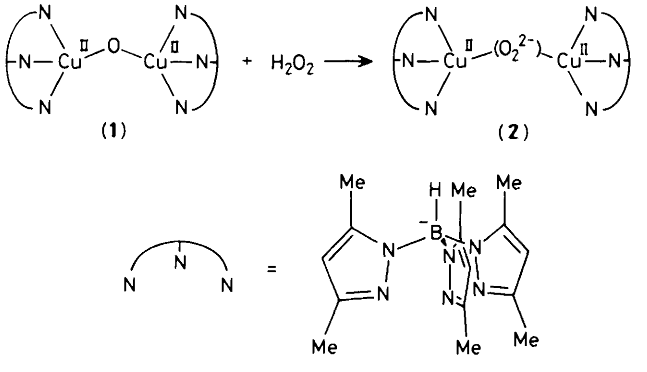
A novel μ-peroxo binuclear copper(II) complex which can serve as an accurate synthetic model of oxyhaemocyanin has been synthesised and characterised.

Haemocyanin is an oxygen transport protein found in arthropods and molluscs. The recent structural determination[1] of the reduced haemocyanin showed that the dioxygen binding site consists of two copper ions each of which co-ordinates to three histidyl nitrogen atoms. Although dioxygen is known to bind as peroxide at this site,[2] the detailed geometric and electronic structure of oxyhaemocyanin remained to be solved. Hence, the synthesis of a μ-peroxo binuclear copper(II) complex as a chemical model has been a current topic in bioinorganic chemistry.[3] So far, however, there has been no plausible model which can mimic closely the characteristic physicochemical properties of oxyhaemocyanin: diamagnetic properties; the characteristic absorption spectrum, and the low oxygen–oxygen stretching frequency. Here, we report the synthesis of a novel μ-peroxo binuclear copper(II) complex which shows many similarities to oxyhaemocyanin in its physicochemical properties.

Several oxo complexes of transition metals are known to react with hydrogen peroxide to give the corresponding peroxo complexes. When a solution of the μ-oxo binuclear copper(II) complex (**1**)[4] was treated with an equimolar amount of hydrogen peroxide at −35 °C, an immediate colour change from greenish blue to purple was noted. From this solution, the novel μ-peroxo binuclear copper(II) complex (**2**) was isolated as a dark purple crystalline solid (*ca.* 80% yield). This is the first example of μ-peroxo binuclear complex in which the copper atoms are bridged solely by a peroxide ion.† The structure of (**2**) was confirmed by satisfactory elemental analysis, and i.r. and field desorption mass spectroscopy (FD-MS). The Resonance Raman spectrum of (**2**) shows a characteristic band at 725 cm^{-1} whose frequency shifted to 686 cm^{-1} for (**2**) prepared from $H_2{}^{18}O_2$. This establishes the peroxide co-ordination in (**2**).‡ The O–O stretching frequency of (**2**) is distinctly lower than the values for the two previously reported μ-peroxo complexes,§ and close to that of oxyhaemocyanin, 744—752 cm^{-1}.[9] As shown in Figure 1, the electronic spectrum of (**2**) exhibits two characteristic absorption bands at 530 nm (ε 840 mol^{-1} dm^{-1} cm^{-1}) and 338 nm (20800). This mimics the absorption spectrum of oxyhaemocyanin; two bands appear at 570 nm (*ca.* 1000) and 345 nm (*ca.* 20000).[10] Compound (**2**) does not show any e.s.r. signal but gives a ^{1}H n.m.r. spectrum consisting of sharp peaks at 1.98 (s, 3H, Me), 2.05 (s, 3H, Me), 5.72 [s, 1H, Pz (Pz = pyrazolyl)] p.p.m. (δ, in $CDCl_3$, at −30 °C), indicating the diamagnetism of (**2**). The magnetic susceptibility measurement of (**2**) by the Evans[11] method also supports this diamagnetism.



(**1**) (**2**)

The similarities between (**2**) and oxyhaemocyanin imply that the structure of (**2**) resembles that of the oxygen binding site in oxyhaemocyanin and also suggests that the existence of an endogeneous ligand generally believed[12] to cause the diamagnetism in oxyhaemocyanin is not necessary.

The complex (**2**) is moderately stable in the crystalline state at room temperature, but undergoes rapid decomposition to

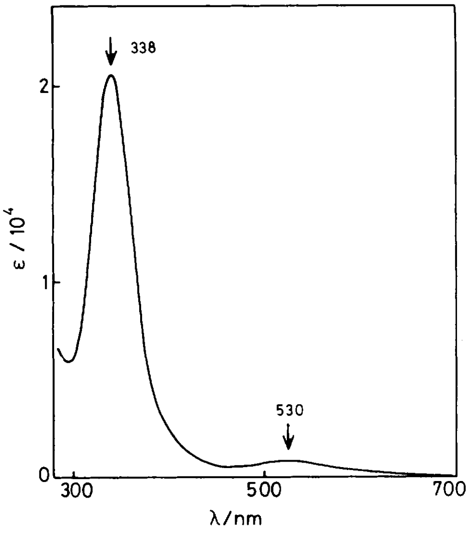


Figure 1. Electronic spectrum of (**2**) in CH_2Cl_2.

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† Karlin has reported the reversible[5] and irreversible[6] reaction of dioxygen with binuclear copper(II) complexes, and has suggested the generation of such μ-peroxo dicopper(II) complexes, although spectroscopic data are not available.

‡ There are two possible modes of peroxide co-ordination on the two copper atoms in (**2**): one is μ-1,1; the second is μ-1,2. To date we have not established the actual co-ordination mode.

§ Only two μ-peroxo binuclear copper(II) complexes whose O–O stretching frequency has been determined have been reported. Karlin[7] has observed the formation of a thermally unstable μ-peroxo complex in solution, in which two copper atoms are bridged by phenoxide (absorption band at 803 cm^{-1}). A water bridged μ-peroxo complex (825 cm^{-1}) was prepared by Thompson.[8] These frequencies are, however, considerably higher than that of oxyhaemocyanin (744—752 cm^{-1}). Furthermore, neither complex has an electronic spectrum similar to that of oxyhaemocyanin.

(**1**) in solution above −30 °C.¶ The interconversion between μ-oxo and μ-peroxo binuclear copper(II) complexes provides evidence for the involvement of μ-oxo species in type III copper protein systems. Haemocyanin inactivated towards dioxygen binding can be reactivated by hydrogen peroxide treatment to form oxyhaemocyanin.[13] Similarly, the resting state of tyrosinase, a mono-oxygenase having a similar binuclear copper site to that of haemocyanin, becomes active upon treatment with hydrogen peroxide.[14] It is noteworthy that μ-oxo complex (**1**) possesses similar spectroscopic properties to these proteins, (**1**) being e.s.r. silent and showing two electronic absorption bands at 660 and 335 nm.[4]

This research was supported in part by grants from the Ministry of Education, Science and Culture, and the Asahi Glass Foundation for Industrial Technology, for which we are grateful.

Received, 7th July 1987; Com. 964

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¶ The conversion of (**2**) into (**1**) was established by n.m.r. and electronic spectroscopy. The half-life of (**2**) in CH_2Cl_2 at room temperature is *ca.* 0.5 h.

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