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A Hemocyanin Model Compound, Copper Complex with 1,3-bis[N,N-bis(2-benzoimidazolylmethyl)aminomethyl] cyclohexane

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Hemocyanins are the copper proteins of high molecular weight which function as the oxygen carriers in the hemolymph of molluscs and arthropods. These nonheme proteins reversibly bind dioxygen with a stoichiometry of one oxygen molecule for every two copper atoms [1, 2]. The protein, colored blue when oxygenated, becomes colorless when deoxygenated. Although no X-ray structure determination has been reported, the following facts have been revealed with respect to the copper coordination sites [3–8]. The coordination geometries of copper(II) were assumed to be rhombic from the simulation of ESR spectra of the mononuclear species in which one of the two copper ions in the active site is oxidized [3]. Simulations including superhyperfine coupling indicated that the donor atoms of copper(II) ions include at least two nitrogen atoms [4], and there are many chemical evidences for imidazole coordination [5–7]. The distance between the two copper ions was estimated to be in the range 3.4 ~ 3.7 Å on the basis of EXAFS [8].

Some hemocyanin model complexes have recently been prepared by Wilson *et al.* [9] and Osborn *et al.* [10], however no blue copper-O_2 complex has yet been reported.

Considering the above facts we have designed binucleating ligands (pxb, mxb, and cyb, see Fig. 1), for the synthesis of hemocyanin model compounds. They all contain benzoimidazole groups, which can

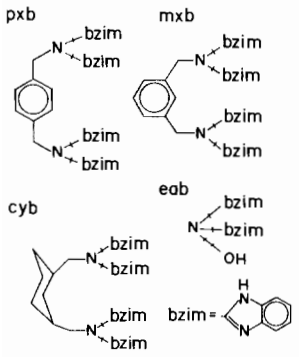

Fig. 1. Structures of the ligands cited in this paper.

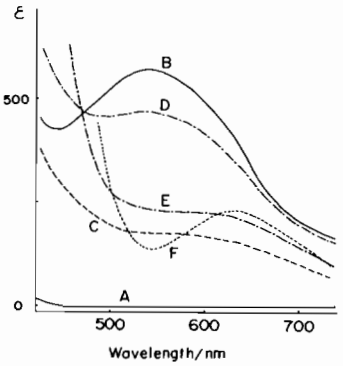

Fig. 2. Absorption spectra of copper–cyb complexes (CH_3OH:CH_3CN (1:14) solution): A ——— $Cu^{I}(CH_3CN)_4BF_4$ + cyb (in N_2); $[Cu^{I}] = 2.67 \times 10^{-3}$ mol dm^{-3} and [cyb] = 1.33×10^{-3} mol dm^{-3}; B ——— Bubbled O_2 in solution A at 5 °C; C – – – – Bubbled N_2 in solution B at 20 °C for 10 minutes; D – · – · – Bubbled O_2 in solution C at 5 °C; E, – · – · – Bubbled N_2 in solution D at 20 °C; F, ------- $Cu^{II}(H_2O)_6(BF_4)_2$ + cyb; $[Cu^{II}] = 2.67 \times 10^{-3}$ mol dm^{-3} and [cyb] = 1.33×10^{-3} mol dm^{-3}.

be yielded by the general reaction [11, 12]. The ligand, cyb, was prepared by the following procedure. 1,2-Diaminobenzene and 1,3-bis(aminomethyl)cyclohexane-N,N,N′,N′-tetraacetic acid were finely ground and throughly mixed together in a (4:1) ratio, followed by heating to 200 °C until no more water vapour was evolved. After cooling, the mixture was dissolved in hot methanol (containing charcoal) and filtered, followed by crystallization with water. The crude ligand was recrystallized from a CH_3OH–H_2O solution. *Anal.* Found: C; 70.99%, H; 6.52%, N; 20.51%. Calcd. for cyb·H_2O, C; 70.56%, H; 6.51%, N; 20.57%.

The reaction of Cu(I) complex of pxb, mxb, or eab, with O_2 results in irreversible oxidation of Cu(I), yielding a green solution. However, the Cu(I) complex with cyb undergoes a reversible (though not completely) color change with bubbling O_2 and N_2 alternately. Under an atmosphere of N_2, a CH_3OH–CH_3Cn (1:14) solution (75 ml) containing cyb (67 mg, 0.001 mol) and $Cu^{I}(CH_3CN)_4BF_4$ (63 mg, 0.0002 mol) was pale yellow. Deep violet color gradually developed when the above solution was exposed to air at 5 °C. When O_2 was bubbled in the pale yellow solution, the color immediately changed to violet. The absorption spectrum of the violet solution is shown in Fig. 2, a broad band being observed around 550–600 nm with $\epsilon \sim 540$, where ϵ was calculated for the two-copper unit. Here, it should be remarked that this spectrum resembles that of oxyhemocyanin of *Sepioteuthis lessomana* where a broad band was observed around 560–610 nm with

$\epsilon \sim 500$ at 20 °C [13]. The violet solution showed no noticeable change at 0 °C at least 30 minutes. When N_2 was bubbled in this solution the violet color gradually faded and the solution became a yellowish green in about 10 minutes. Bubbling O_2 again in the yellowish green solution at 5 °C resulted in a brownish blue solution (see Fig. 2). This solution turned green when N_2 was bubbled at 20 °C. The green solution showed any remarkable color change no longer with O_2 bubbling.

In Fig. 2, the absorption spectrum of a solution of copper(II) complex with cyb, which was prepared by dissolving copper(II) tetrafluoroborate and cyb in CH_3OH–CH_3CN in the (2:1) ratio, is also shown, a broad absorption band being observed around 630 nm. This spectrum is entirely different from that of violet species mentioned above.

Thus, it is most likely that the violet species formed from the reaction mixture of Cu_2^I–cyb and O_2, is due to the formation of a Cu_2–cyb–O_2 complex, and the fading of the violet color corresponds to the deoxygenation of the Cu_2–cyb–O_2 complex.

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