A Haemocyanin Model: a Synthetic Copper(I) Complex Having Imidazole Ligands and Reversible Dioxygen Activity

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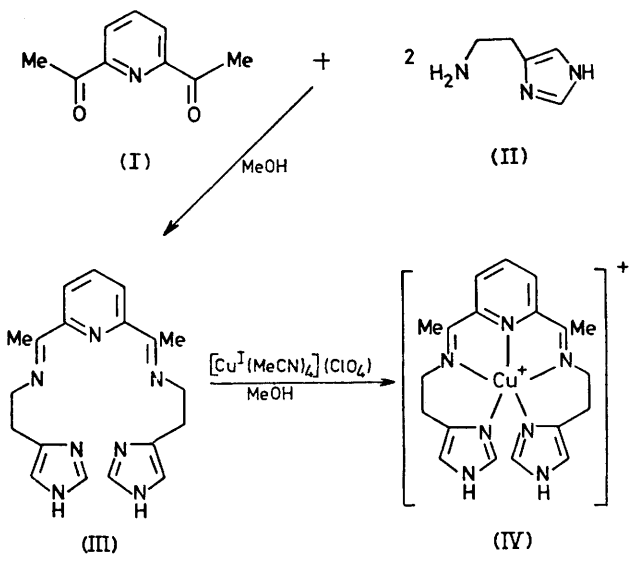
Summary A synthetic Cu^{I} compound (IV) having two imidazole ligands has been found to bind dioxygen reversibly in both the solid and solution states at room temperature.

HAEMOCYANINS[1] are large copper-based metalloproteins which function to transport dioxygen during the respiratory cycle of some molluscs and arthropods. Thus, in function, they are related to the iron-based haemoglobins of higher life forms. However, in comparison to Fe(haem)–O_2 in the haemoglobins, relatively little is known about the molecular structure of the active site in the haemocyanins, except that it is thought to contain at least two imidazole ligands per copper.[2,3] In addition, the reaction stoicheiometry ($Cu : O_2$, 2 : 1),[4] the diamagnetism,[5] and the resonance Raman spectrum[2] of oxyhaemocyanin suggest a Cu^{II}–O_2^{2-}–Cu^{II} formalism for the oxygenated active site. One approach toward a more detailed understanding of dioxygen activity in the haemocyanins is to develop and study small synthetic Cu^{I} model compounds which accurately mimic the reversible dioxygen carrying capacity of the protein. To date, efforts in this direction have met with little success[6] and no reversible oxygenation reaction of a synthetic Cu^{I} species in solution has been established. In this work we describe initial results which demonstrate that the bis-2,6-[1-(2-imidazol-4-ylethylimino)ethyl]pyridinecopper(I) cation (IV) $\{[Cu^{I}(bimp)]^{+}\}$ is the first such synthetic Cu^{I} complex to bind dioxygen reversibly in both the solid and solutions states, with the degree of reversibility being *ca.* 80% per oxy–deoxy cycle in solution at room temperature.

The bimp ligand (III) was generated by the Schiff base condensation of 2,6-diacetylpyridine (I) and 2 mol of histamine (II) in refluxing methanol (Scheme). Addition of $[Cu^{I}(MeCN)_4](ClO_4)$, under N_2, produces a dark red solution from which analytically pure $[Cu^{I}(bimp)]ClO_4$ can be isolated as a red solid in 60% yield.† As indicated in the Scheme the ligand is assumed to be pentaco-ordinate



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with the complex being monomeric in solution as indicated by its 1 : 1 electrolyte behaviour in deoxygenated MeCN, dimethylformamide (DMF), or Me_2SO. As a solid, the compound is diamagnetic. Use of $Cu(ClO_4)_2 \cdot 6H_2O$ and $Zn(ClO_4)_2 \cdot 6H_2O$ in the synthesis produces analytically pure $[Cu^{II}(bimp)](ClO_4)_2$† [green solid with μ_{eff} (25 °C) = 1·8 B.M.] and $[Zn^{II}(bimp)](ClO_4)_2$‡ (yellow solid and diamagnetic), respectively, which have molar conductivities in solution corresponding to 1 : 2 electrolytes. Finally, the Zn^{II} complex exhibits a ^1H n.m.r. spectrum in solution consistent with the proposed pentaco-ordinate structure.

If a deoxygenated red Me_2SO solution of (IV) is exposed to dioxygen (1 atm; room temp.), the solution quickly turns green absorbing 1 mol of O_2 per 2 mol of available Cu, as measured manometrically.[7] The reaction is complete in *ca.* 2 min and can be readily reversed by gently

† Satisfactory elemental analyses (C, H, N, Cu) were obtained for this compound.

‡ This compound analysed satisfactorily for C, H, and N; for Zn: found 10·08, calc. 10·65%.

heating (*ca.* 40 °C) and degassing (N_2) the solution or under reduced pressure with vigorous stirring. Under these conditions the original red colour returns and the solution will again absorb dioxygen (*ca.* 0·8 mol of O_2 per 2 mol of Cu). Thus, the reaction stoicheiometry (Cu : O_2, 2 : 1) is that of the haemocyanins and suggests a Cu–O_2–Cu bridging structure for the reversibly oxygenated product. The oxy–deoxy cycling process can be repeated up to 6 times, with *ca.* 20% decrease in the volume of O_2 absorption accompanying each successive recycling and, ultimately, after continuous cycling the solution turns brown and no further O_2 uptake is observed. The same degree of O_2 reversibility is also observed in MeCN, DMF, pyridine, and 2,6-lutidine at room temperature although, qualitatively, the rate of O_2 uptake is solvent dependent: MeCN ≃ DMF ≃ Me_2SO (1—2 min) > pyridine ≃ 2,6-lutidine (5 min). In the solid state the O_2 absorption rate is much slower, requiring at least 1 h at 1 atm. of O_2 for a powdered sample of red (IV) to become totally green. (The red colour can be regenerated under reduced pressure at 60 °C, but the sample is no longer fully diamagnetic with μ_{eff} = *ca.* 1·2 B.M. per Cu). The fact that oxygen is released from the solid during this vacuum–heat treatment has been verified by g.l.c.–mass spectrometry. The same reaction stoicheiometry (Cu : O_2, 2 : 1) is obtained in all the solvents

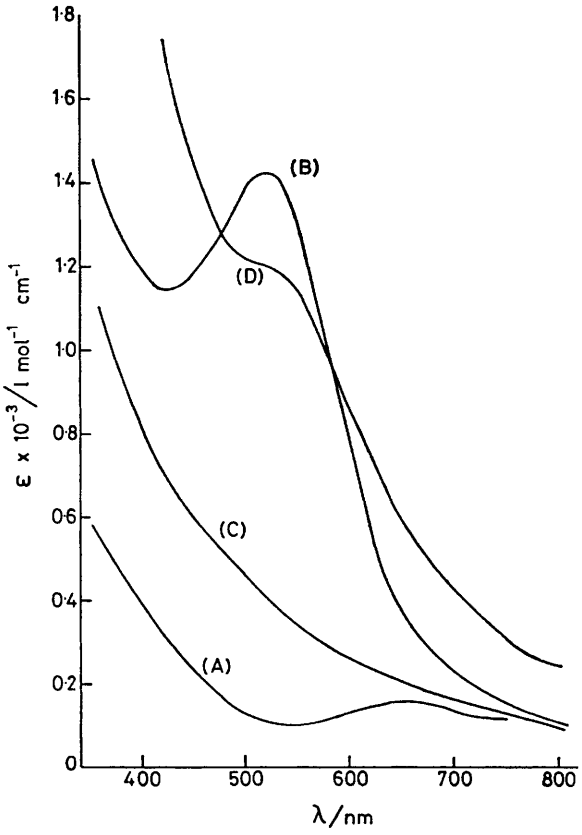

FIGURE 1. Electronic absorption spectrum of (A) $[Cu^{II}(bimp)](ClO_4)_2$ in Me_2SO with [Cu] = 5 × 10^{-3} mol l^{-1}; (B) $[Cu^{I}(bimp)](ClO_4)$ in deoxygenated Me_2SO with [Cu] = 1 × 10^{-3} mol l^{-1}; (C) $[Cu^{I}(bimp)](ClO_4)$ in oxygenated Me_2SO with [Cu] = 1 × 10^{-3} mol l^{-1} and Cu : O_2 ratio of 2 : 1; (D) solution (C) degassed with N_2/gentle heating (*ca.* 45 °C) for 10 min, *i.e.*, after one oxy–deoxy cycle.

and the same brown solutions ultimately appear with the demise of O_2 uptake activity. The nature of the brown product has not yet been established, but clearly it is not simply the oxidized form of the green complex, $[Cu^{II}(bimp)]^{2+}$. The Cu^{II} and Zn^{II} complexes show no reactivity toward dioxygen in any of the solvents or as solids and none of the three metal complexes reacts with carbon monoxide under ambient conditions, although several synthetic Cu^{I}–CO species are well documented,[8] as is carboxy-haemocyanin.[9]

Figure 1 shows the electronic absorption spectrum in the visible region for $[Cu^{II}(bimp)]^{2+}$ (A) and $[Cu^{I}(bimp)]^{+}$ (B—D) under various conditions in Me_2SO solution. The spectrum of the Cu^{II} compound (A) consists of a weakly intense absorption band (ϵ 160 l mol^{-1} cm^{-1}) centred at 660 nm which is assigned as a *d–d* transition. For Cu^{II} haemocyanin, a similar band is also present, but at a higher energy (570 nm) and greater intensity (ϵ 500 l mol^{-1} cm^{-1}), suggesting a more highly distorted ligand field environment for the Cu centre in the protein.[10] Identical spectra are obtained for $[Cu^{II}(bimp)]^{2+}$ in Me_2SO, MeCN, DMF, and pyridine, so that the complex appears inert towards potential ligating solvent molecules, and thus, probably remains pentaco-ordinate in solution. The spectrum of the red Cu^{I} compound (B) has a moderately intense charge transfer band (ϵ 1415 l mol^{-1} cm^{-1}) at 520 nm. Again, the band position and intensity are relatively insensitive to solvent, but the band does appear somewhat broadened in 2,6-lutidine. If solution (B) is exposed to dioxygen, it absorbs 1 mol of O_2 per 2 mol of Cu, turns green, and produces spectrum (C). Deoxygenation of solution (C) with N_2 at 40 °C for 10 min generates spectrum (D) and the reappearance of the red colour which, judging from the ratio of the B(ϵ 1415) : D(ϵ 1200) band intensities, reflects *ca.* 80% recovery of the original Cu^{I} complex. Further deoxygenating of solution (D) has little additional effect on the spectrum, although once deoxygenated the solution will again absorb O_2 (but only *ca.* 80% of the original volume), turn green, and reproduce a spectrum similar to (C). Thus, the manometric and electronic spectral data are in good agreement in establishing *ca.* 80% reversibility factor per oxygenation cycle.

It is now generally accepted, largely on the basis of Co^{II}–dioxygen work,[11] that dioxygen adds to metal ions *via* an 'internal oxidative addition' reaction [equation (1); L = ligand, M = metal] in which the metal is formally

$$LM^{n+} + O_2 \overset{K_1}{\rightleftharpoons} LM^{(n+1)+}\text{–}O_2^{-} \overset{K_2}{\rightleftharpoons} LM^{(n+1)+}\text{–}O_2^{2-}\text{–}M^{(n+1)+}L \quad (1)$$

oxidized and O_2 formally reduced to O_2^- or O_2^{2-}. For the present $[Cu^{I}(bimp)]^{+}$ case, the dioxygen adduct would then be viewed as a LCu^{II}–O_2^- or LCu^{II}–O_2^{2-}–$Cu^{II}L$ species with the binuclear form apparently being the final product. In either case it is likely (but not certain) that the adduct would be diamagnetic *via* antiferromagnetic exchange between the formally Cu^{II} (S = 1/2) centres in the binuclear complex or between Cu^{II} (S = 1/2) and O_2^- (S = 1/2) in the mononuclear form. The former situation is usually invoked to explain the diamagnetism and absence of e.s.r. signal of the oxyhaemocyanins.[12] The e.s.r. spectrum of $[Cu^{II}(bimp)]^{2+}$ (i) and that of $[Cu^{I}(bimp)]^{+}$ plus 1/2 O_2

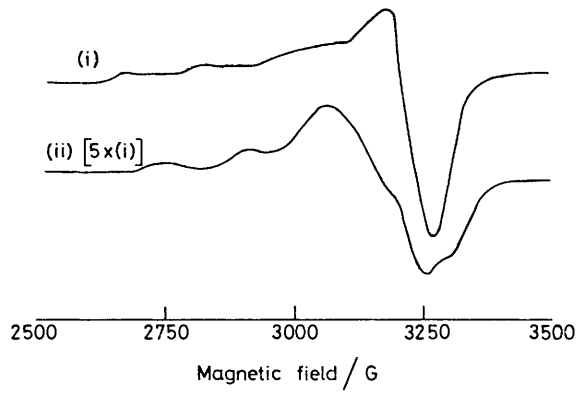

FIGURE 2. *X*-Band e.s.r. spectrum of (i) $[Cu^{II}(bimp)](ClO_4)_2$ in Me_2SO at −196 °C with [Cu] = 10^{-3} mol l^{-1}; (ii) $[Cu^{I}(bimp)](ClO_4)$ plus 1/2 O_2 in Me_2SO at −196 °C with [Cu] = 10^{-3} mol l^{-1}; spectrum amplitude is 5 times that of spectrum (i).

(ii) in Me_2SO is shown in Figure 2, where [Cu] = 10^{-3} mol l^{-1}. Both spectra have the characteristic features of a magnetically dilute Cu^{II} centre with normal (but different) $g_{\|}$ ($A_{\|}$) and $g_{\perp}$ values. However, if the spectra are compared and quantified by integration, the spectrum (ii) accounts for only *ca.* 20% of available Cu. Thus, it would appear that the reversibly oxygenated Cu^{II}–O_2 and/or Cu^{II}–O_2–Cu^{II} species are indeed e.s.r. inactive, with the observed signal probably occurring owing to some decomposition product(s) of the Cu^{II} complex.

It has not yet been possible to isolate an analytically pure dioxygen adduct of $[Cu^{I}(bimp)]^{+}$, and crystal growth attempts for an *X*-ray structure[13] have also been unsuccessful since the oxygenated solutions turn brown with time, reminiscent of their behaviour after multiple oxy–deoxy cycles. We are at present trying low-temperature crystallization techniques and also the preparation of other related derivatives in an attempt to overcome this problem.

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