pH Dependence of the Formation of Simple Imidazolate-bridged Binuclear Copper(II) Complexes

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It is revealed by e.s.r. spectroscopy that the formation of many imidazolate-bridged binuclear copper(II) complexes of aminocarboxylates is pH-dependent with an apparent pK_a of 8.3; this closely resembles an unusual pH-dependent property of zinc-free bovine erythrocyte superoxide dismutase.

The preparation and characterisation of discrete imidazolate-bridged (im-bridged) complexes, especially of copper(II), are of current interest,[1-4] primarily because the enzyme bovine erythrocyte superoxide dismutase (BESOD) has a histidine-bridged copper(II)–zinc(II) active site.[5] Here we show e.s.r. results for the formation of many simple im-bridged binuclear copper(II) complexes in aqueous solutions, and offer an explanation for an unusual pH-dependent property of zinc-free BESOD.[6]

On addition of imidazole (0.5 mol) to aqueous solutions of 1 : 1 complexes of copper(II) with N-glycylglycine, N-(2-amino-ethyl)-1,2-ethanediamine, and related tridentate ligands (1 mol; 1.0×10^{-2} M) at pH $\geqslant 9$, the e.s.r. signals of monomer species at *ca.* 0.3 T change into single broad absorptions which are superimposed by weak monomer spectra, while half-field spectra at *ca.* 0.15 T are too weak to be observed. The intensities of the remaining monomer spectra are always minimized at the concentration ratio of imidazole to copper(II) complexes of *ca.* 0.5 : 1.0. We have found that the 1 : 1 complexes of copper(II) with aminocarboxylates, such as N,N'-1,2-ethanediylbisglycinate (edda), N,N-bis(carboxymethyl)-glycinate, N-(carboxymethyl)-N-(2-hydroxyethyl)glycinate, N-{2-[(carboxymethyl)amino]ethyl}-N-(2-hydroxyethyl)glycin-ate, and related ligands, also behave like the above copper(II) complexes with addition of imidazole, except that the half-field spectra are clearly observable. Figure 1 shows representative e.s.r. spectra with the most well-resolved half-field spectrum. This e.s.r. result demonstrates the formation of some kind of copper(II) dimer in solution; broad spectra at *ca.* 0.3 T and half-field ones at *ca.* 0.15 T correspond to $\Delta M = 1$ and 2 transitions, respectively. Spectrum (A) in Figure 1, which is of a monomer type, is almost invariable in line shape in the pH 7—10 region. A similar spectrum with slightly smaller g values is obtained for the solution of spectrum (B) by a change of pH from 10.0 to 7.0. Addition of 1-methylimidazole instead of imidazole does not lead to the appearance of dimer spec-
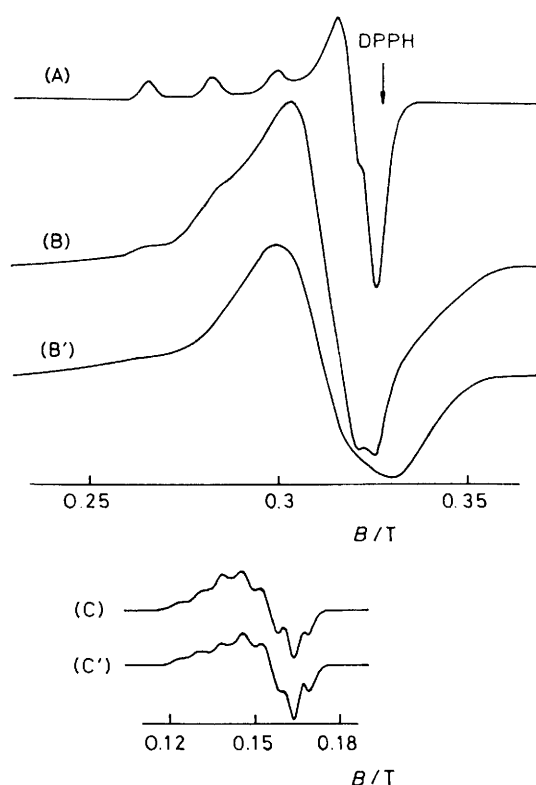

Figure 1. E.s.r. spectra of frozen aqueous solutions of (A) Cu–edda and (B), (C) Cu–edda + 0.5 imidazole at 77 K (pH 10.0, borate buffer; [Cu] = 10 mM; [NaNO₃] = 0.30 M). The curves of (B′) and (C′) show simulation spectra calculated with the parameters $r = 6.0$ Å, $\xi = 90°$, $D = 0.02$ cm^{-1}, $E = 0.015$ cm^{-1}, $g_{\parallel} = 2.285$, $g_{\perp} = 2.07$, $A_{\parallel} = 0.008$ cm^{-1}, $A_{\perp} = 0.001$ cm^{-1}, $\Delta H_1 = 75 \times 10^{-4}$ T, and $\Delta H_2 = 20 \times 10^{-4}$ T (ΔH_1 and ΔH_2 express the line widths of $\Delta M = 1$ and 2 spectra, respectively).

tral lines. All these results are consistent with the formation of im-bridged complexes.†

An analysis of the above e.s.r. results was attempted by the computer simulation method developed for the system of parallel planar copper(II) dimers,[7,8] with the intention of obtaining further evidence for im-bridged complexes. Two structural parameters, r and ξ, are introduced in this simulation, where r is the Cu–Cu distance, and ξ the angle between the Cu–Cu direction and the normal to the molecular plane. The r values of some im-bridged binuclear copper(II) complexes in crystals are known to be ca. 6.0 Å, while ξ is always ca. 90°.[1,4] Simulated spectra for both $\Delta M = 1$ and 2 transitions [Figure 1(B') and (C'), respectively] were obtained with the same r and ξ values as above and reasonable D and E values.‡ In addition, there seems to be some similarity in e.s.r. spectral pattern between the present im-bridged complexes and zinc-free BESOD.

The pH dependence of the formation of the im-bridged complexes has been investigated from the intensity variation of $\Delta M = 2$ spectra with pH. The plot of the relative $\Delta M = 2$ spectral intensity versus pH for the case of Figure 1 is shown in Figure 2; almost the same results are obtained for the other cases. These plots are similar in shape to a titration curve with an apparent pK_a of ca. 8.3. This property strongly resembles the pH-dependent migration of Cu^{2+} to the empty zinc-binding site in zinc-free BESOD, with an apparent pK_a of 8.2, to form an im-bridged binuclear copper(II) centre.[6] The present complexes, accordingly, may be considered as models for the active site of BESOD with respect to stability and e.s.r. behaviour. Lippard et al. synthesized a similar model complex using a structurally complicated macrocyclic ligand,[2] but such ligands are not always needed for this purpose. In conclusion,

Figure 2. The pH dependence of the $\Delta M = 2$ spectral intensity of Cu–edda + 0.5 imidazole [Figure 1(C)]{pH, (borate + HEPES) buffer; [Cu] = 10 mM; [NaNO$_3$] = 0.30 M }.

the pH-dependent migration of Cu^{2+} in zinc-free BESOD may not be a phenomenon in which the protein part of BESOD plays an essential role, but which depends mainly on the stability of the im-bridged active centre itself.

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† The formation constants (K) of the im-bridged complexes at ca. 0 °C can be roughly estimated by comparison between the real integrated spectral intensities of monomer and dimer species at ca. 0.3 T. Most K values, determined as in the caption to Figure 1, were of the order of 10^7.

‡ The zero-field splitting term in the spin Hamiltonian for this simulation consists of two terms. One is the electron spin dipole–dipole interaction term, and the other is the pseudo-dipolar term, which is J-dependent and was included here as the generalized expression $DS_z^2 + E(S_x^2 - S_y^2)$ (ref. 9). A value of $|J| < 20$ cm^{-1} results in negligibly small D and E values, giving no remarkable effects on dimer e.s.r. line shapes. The half-field spectral intensity is theoretically expected to be proportional to the square of the zero-field splitting parameter, so that, under a constant r value of 6.0 Å, the intensity depends only on the D and E values. The exchange interaction of $J = -(38 \pm 3)$ cm^{-1} for the im-bridged complex in Figure 1 was determined as previously from the temperature variation of the half-field spectral intensity (refs. 8 and 10). This large $|J|$ value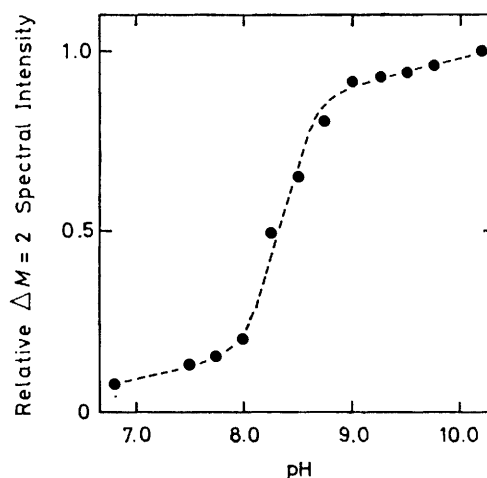 allows an easy observation of its half-field signal.