ELECTRON SPIN RESONANCE AND OPTICAL SPECTRA OF COPPER (II)-PEPTIDE-AMINO ACID MIXED-LIGAND COMPLEXES

IMPORTANCE of copper ion in biological processes has led in recent years to investigate the naturally occurring systems and model compounds[1]. The activity of biological macromolecules containing copper ion depends upon the selective binding of different functional groups with the metal[2]. For instance enzymatic reactions involving copper occur with the formation of mixed-ligand complexes[3]. Mixed-ligand complexes of copper with amino acids have been isolated from human serum and the complexes appear to play a role in transporting copper through biological membranes[4]. We have undertaken to study the properties of copper (II)-peptide-amino acid mixed-ligand complexes in a systematic way using ESR and visible spectral measurements This communication presents the results obtained using glycylglycine, glycine and β-alanine as ligands. Simple complexes of these ligands with copper (II) are well studied and the spectral properties are known. Yokoi *et al.* have reported the e.s.r., spectra of several mixed-ligand complexes of copper (II) with amino acids and amines[5].

Experimental

Simple complexes were prepared according to the procedure in the literature. E.s.r. spectra were measured in a Varian E-4 X-band instrument using a 10^{-2} M solution of copper – simple complexes in 1 : 1 water-methanol solution. The spectra of the mixed-ligand complexes were obtained by using a solution of 1 : 1 mixture of the two different simple complexes in 1 : 1 water–methanol.

Visible absorption spectra were scanned under identical conditions at 25° C using a SP 700 A Unicam recording spectrophotometer.

Results and Discussion

When the copper (II) complexes CuX_2 and CuY_2 are mixed together the following equilibrium is established.

$$CuX_2 + CuY_2 \rightleftharpoons 2 CuXY$$

with the disproportionation constant given by

$$K_{DXY} = \frac{[CuXY]^2}{[CuX_2] [CuY_2]}$$

From the e.s.r. spectra of the solutions, it could be seen that the line shapes were similar to those observed for single species. If more than one species were present in significant amounts the observed spectrum should have been an admixture of those for the simple complexes. In all the cases studied the line shapes of the spectra were different from the spectra of the simple complexes and the

TABLE I

Complex	λ_{max} nm	$g_{//}$	$g_\perp$	$\frac{A_{//}}{10^4 cm^{-1}}$	α^2
Cu (gly)$_2$	630	2·231	2·055	170	0 547
Cu (β-al6)$_2$	636	2·285	2·054	129	0·668
Cu (glygly)$_2$	640	2·214	2 049	184	0 497
Cu (gly) β(-ala)	637	2·229$_4$	2·052$_8$	167	0·537
Cu (gly) (glygly)	636	2 226	2 054	187	0 528
Cu (β-ala) (glygly)	636	2·215	2·052	176	0·502

recorded spectra were due to mixed-ligand complexes only. Results published earlier from this laboratory also show that mixed-ligand complexes in general are more stable than the simple complexes[6].

E.s.r. spectra of the complexes studied show the general pattern characteristic of tetragonal copper (II) complexes with an intense absorption at high field and two or three peaks of less intensity at low fields. $g_{//}$, $g_\perp$ and $A_{//}$ values were calculated from the low temperature spectra (77° K) and are given in Table I. $g_\perp$ values should be regarded as somewhat approximate since the spectra are not well resolved. At room temperature due to rapid rotations of the molecules anisotropic couplings are averaged out leaving only the isotropic ones, observed spectrum indicates average g value only. $g_{//}$ values for the mixed-ligand complexes are found to be in between the $g_{//}$ values of the simple complexes. In the case of Cu (glygly) (β-ala) complexes although $g_{//}$ value of the mixed-ligand complex is nearly equal to that of Cu(glygly)$_2$, $A_{//}$ value is in between those observed for the simple complexes. Orbital reduction factors α^2 were calculated using the simplified equation.

$$g_{//} = 2·0023\left(1 - \frac{4\alpha^2\lambda}{\Delta}\right)$$

where λ is the spin-orbit coupling constant[7]. α^2 values indicate that the bonding between copper and ligands is covalent in nature with delocalization of electron density into ligand orbitals.

From the e.s.r. data of the naturally occurring copper containing proteins, two types of molecules could be classified, one with considerably higher $A_{//}$ values than the other type[8]. It is believed that the site symmetry of copper ion in one case is nearly coplanar and in the other distorted tetrahedron[9]. Work is in progress to determine whether copper (II) ion could be forced into a tetrahedron

environment by employing high molecular weight peptides and other ligands.

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