DALTON

Ultraviolet–circular dichroism spectra for structural analysis of copper(II) complexes with aliphatic and aromatic ligands in aqueous solution

Pier G. Daniele,* Enrico Prenesti and Giorgio Ostacoli

Dipartimento di Chimica Analitica dell'Università, Via Pietro Giuria 5, 10125 Torino, Italy

An attempt has been made to identify the characteristic positions, and their eventual displacements with changing co-ordination, of the charge-transfer bands involving donor groups located on molecules of biological interest [L-malic acid, *N*-acetyl-L-aspartic acid, (1*R*,2*R*)-cyclohexane-1,2-diamine, L-alanyl-L-alanine, L-γ-glutamyl-L-ε-lysine, *N*-acetyl-L-histidine, β-alanyl-L-histidine and *N*-benzoylglycyl-L-histidyl-L-leucine] such as carboxylate, alcoholate, amine, deprotonated peptide and imidazole. Information about the species formed in solution was obtained by means of pH-metric readings while ultraviolet–circular dichroism spectra were recorded, at fixed pH values, 298 K and $I = 0.1$ mol dm^{-3}, for the proton–ligand and proton–copper(II)–ligand systems, in order to evaluate a spectrum for each complex formed in solution. Intraligand and charge-transfer bands were assigned for each spectrum with the aim of relating spectral features to the structure of the species formed in solution.

Ultraviolet–circular dichroism (UV/CD) spectra have been employed for the determination of the α-helix content of proteins,[1] of the influence on ligands of metal ions[2] and for structural definition in micellar aggregates of a series of chiral surfactants.[3] When metal–ligand interactions are investigated the nature of the donor groups involved in the co-ordination sphere of a given metal ion might be detected. Both visible and ultraviolet–circular dichroism spectra can provide very useful structural information for metal complexes in solution. The results obtained by means of the two techniques are complementary; nevertheless from UV/CD spectra it is also possible to study complex formation (*i*) with colourless metal ions,[4] (*ii*) with metal ions having low molar absorption in the visible, such as octahedral complexes of nickel(II), (*iii*) between chiral anionic ligands (such as anions of carboxylic acids) and cationic ligands (such as protonated amines), and (*iv*) in real samples of biological or environmental interest (by using suitable techniques for chemical separation and for data elaboration).

The UV/CD spectra obtained from several systems containing copper(II) and simple model ligands should allow us to propose a correlation between spectral features and complex structures, as the spectrum of each species in solution (including the free and protonated forms of the pro-ligand) can be calculated from experimental UV/CD data recorded on solutions of different known compositions and from known values of formation constants. It is necessary to distinguish between intraligand (i.l.) bands, which can be modified, in intensity, sign and position, as a consequence of co-ordination and charge-transfer (c.t.) bands, which should be attributed to the donor groups.

In the literature there are many articles in which UV/CD spectra are used for structural definitions in solution but the attributions are directly based on experimental spectra, without taking into account either the superposition of the different metal complexes at a given pH (often only the structures of the predominant species in solution are discussed) or the contribution of the differently protonated forms of the pro-ligand and, moreover, with the exception of metal complexes with amino acids[2,5–8] there are no systematic studies on model ligands.

The aim of this study was to identify the characteristic positions, and their eventual displacements with changing co-ordination, of bands due to charge transfer between copper(II) and donor groups located on molecules of biological interest such as carboxylate, alcoholate, amine, deprotonated peptide and imidazole groups. As ligands we chose L-malic acid, *N*-acetyl-L-aspartic acid, (1*R*,2*R*)-cyclohexane-1,2-diamine, L-alanyl-L-alanine, L-γ-glutamyl-L-ε-lysine, *N*-acetyl-L-histidine, L-carnosine (β-alanyl-L-histidine) and hippuroyl-L-histidyl-L-leucine (*N*-benzoylglycyl-L-histidyl-L-leucine). For each of the systems studied information about the species formed in solution was obtained by means of pH-metric readings while UV/CD spectra were recorded, during alkalimetric titrations at 298 K and $I = 0.1$ mol dm^{-3}, for the proton–ligand and –copper(II)–ligand systems, at varying pH, in order to evaluate UV/CD spectra for the different complexes with the proton and copper(II).

Experimental

Chemicals

L-Malic acid (H_2mal) was obtained from Merck, *N*-acetyl-L-aspartic acid (MeCO-Asp), (1*R*,2*R*)-*trans*-cyclohexane-1,2-diamine (chxn) and L-carnosine (β-Ala-His) from Fluka, L-alanyl-L-alanine (Ala-Ala) and *N*-acetyl-L-histidine (MeCO-His) from Sigma, L-γ-glutamyl-L-ε-lysine (γ-Glu-ε-Lys) and hippuroyl-L-histidyl-L-leucine (PhCO-Gly-His-Leu) from Bachem Feinchemikalien. All were employed without further purification; their purity (≥99%) was checked by potentiometric titrations.[9] Copper(II) chloride stock solution was standardized by means of complexometric titrations with ethylenedinitrilotetraacetate (edta), in the presence of the metallochromic indicator murexide. All the solutions were prepared by using deionized and twice distilled water. The ionic strength was adjusted to 0.1 mol dm^{-3} by addition of KCl.

Electromotive force measurements

Potentiometric measurements were performed at 298 K and ionic strength $I = 0.1$ mol dm^{-3} with a Metrohm E-605 potentiometer equipped with a combined glass electrode. The couple was calibrated in $-\log[H_3O^+]$ units (pH) by employing alkalimetric titrations of hydrochloric acid with standard, carbonate-free, potassium hydroxide carried out in a stream of purified nitrogen. Temperature control was achieved by means of a thermocryostat (model D1-G Haake). The ionic strength of the calibrating solutions was the same as that of the

solutions being examined. The concentration range examined was from 2 to 6 mmol dm^{-3} for c_L (c means analytical concentration); within this range, usually employed in potentiometry, with aliphatic compounds it is possible to record significant UV/CD spectra until 200 nm, avoiding excessive voltage values in the photomultiplier tube used as a detector while with the aromatic compounds it is necessary to stop measurements at about 230 nm. The metal to pro-ligand ratios were 1 : 1 for H_2mal, Ala-Ala, MeCO-Asp and PhCO-Gly-His-Leu, from 1 : 1 to 1 : 2 for chxn, from 1 : 1 to 2 : 1 for γ-Glu-ε-Lys and β-Ala-His and from 1 : 1 to 1 : 10 for MeCO-His.

Circular dichroism measurements

For ultraviolet–circular dichroism a model J-600 JASCO spectropolarimeter was employed, from 200 to 400 nm (optical path 0.100 cm) under the same experimental conditions as for the potentiometric measurements. The solution being examined was transferred from the potentiometric to an optical cell using a peristaltic pump. The metal concentration and the metal to pro-ligand ratios were the same as in the potentiometric determinations.

Data analysis and calculations

The stability constants of the complexes are expressed by the general formula $\beta_{pqr} = [Cu_pL_qH_r]/[Cu]^p[L]^q[H]^r$; their values were refined by means of the STACO program,[9] which minimizes the error-squares sum for electromotive force values and takes into account eventual variations of ionic strength among and/or during titrations. Circular dichroism data were analysed by means of the least-squares computer program MOLEX,[10] which calculates the values of molecular ellipticities, $[\theta_\lambda]$,[11] by using experimental spectra, analytical concentrations of the reagents and the proposed chemical model (stoichiometric coefficients and known stability constants of all complexes) as input. After the calculation of the species distribution, CD spectra were estimated for each complex formed in solution, assuming only the additivity of the ellipticity in the investigated concentration range. No assumptions on the shape of the curves nor on the nature of electronic transitions are taken into account by the program. In all calculations the hydrolysis of copper(II)[12,13] ion was considered.

Results and Discussion

Potentiometric measurements

Experimental potentiometric data for the proton–copper(II)–L-malic acid system previously obtained[14] have been re-evaluated, together with more recent measurements, by the STACO program; both the speciation and the formation constant values agree well with previous results.[14] The proton–copper(II)–L-γ-glutamyl-L-ε-lysine, –copper(II)–L-carnosine and –copper(II)–hippuroyl-L-histidyl-L-leucine systems were recently investigated by means of potentiometric and visible absorption techniques.[15–17] The other systems were entirely studied in this work. Table 1 lists the values of the formation constants (as log β_{pqr}), at 298 K and I = 0.1 mol dm^{-3} (KCl), for the proton and copper(II) complexes (the protonation constants corresponding to peptide hydrogens are not reported since in the absence of metal ions, up to pH 10.5, the dissociation of peptide hydrogen can be considered negligible).

Proton–copper(II)–L-malic acid. The UV/CD spectra (Ψ *vs.* λ) for the free pro-ligand were first recorded at pH 2.5–8. With increasing pH the ellipticity (Ψ in mdegrees) rises regularly and λ_{max} is gradually shifted from 215 to 205 nm. A UV/CD spectrum was then calculated ($\Delta\varepsilon$ *vs.* λ) for the different protonated forms of the pro-ligand: H_2L (λ_{max} = 215), HL^- (208) and L^{2-} (205 nm). For all the complexes this band is due to an n ⟶ π* transition in the carbonyl chromophore.[18] The UV/CD spectra were recorded on solutions containing copper(II), at the same pH as above. For pH < 3 only a modification of the i.l. bands is observed; while, with increasing pH, three c.t. bands appear, at λ_{max} 255–265 (pH > 3.5), ≈285 (≈4) and 305–310 nm (>4.5). A UV/CD spectrum was then calculated for each complex with copper(II) [Table 1 lists the calculated $\Delta\varepsilon_{max}$ values for each complex with the proton or copper(II)]. For $[Cu_2L_2H_{-2}]^{2-}$ the bands at 210 and 230 nm are due to i.l. transitions in the carbonyl chromophore of the π ⟶ π* and n ⟶ π* type, respectively.[2,19] The band at 262 nm is very probably $CO_2^- \rightarrow Cu^{II}$ c.t. The band at 310 nm can be assigned to charge transfer from the alcoholate group to copper(II) just as a band at 300 nm has been attributed[20] to c.t. from a deprotonated hydroxyl group of the ribose moiety of ATP to Cu^{II}. As regards [CuL] the band at 205 nm is due to a π ⟶ π* i.l. transition. That at 240 nm is located between the positions of the i.l. n ⟶ π* and of c.t. $CO_2^- \rightarrow Cu^{II}$ transitions and it very probably contains contributions from both. Comparison with the corresponding succinate complex (log β_{110} = 2.85[21]) shows that the stability with L-malate is significantly higher (log β_{110} = 3.67); this difference is probably due to the presence of an alcohol group in L-malic acid and, consequently, to the two five- and six-membered chelate rings which may form instead of one with seven members. The weak band at ≈285 nm, recorded without any superposition with other bands, might be assigned to charge transfer from the alcohol group to the copper(II) ion. [It has been suggested[22] that a band at 275 nm, recorded at pH 7 for the copper(II)–D-tyrosine system, is due to an i.l. transition with an $OH \rightarrow Cu^{II}$ charge-transfer component.] Other evidence for the participation, even if weak, of an alcohol group in co-ordination can be found from the UV/CD spectrum of the copper(II)–*N*-acetyl-L-aspartate system, for which no c.t. band has been recorded: it seems that the formation of chelate rings of suitable dimensions may be a condition for the appearance of the c.t. $CO_2^- \rightarrow Cu^{II}$ band in a UV/CD spectrum. The UV/CD spectrum calculated for $[Cu_2L_2H_{-1}]^-$ shows i.l. π ⟶ π* (215), c.t. $O^- \rightarrow Cu^{II}$ (305) and c.t. $OH \rightarrow Cu^{II}$ bands (285 nm); the peak at 245 nm might be attributed to the superposition of the c.t. $CO_2^- \rightarrow Cu^{II}$ and i.l. n ⟶ π* bands. In the dimer $[Cu_2L_2H_{-2}]^{2-}$ two equivalent copper(II) ions, each co-ordinated by two carboxylate and one alcoholate group, are present, while in $[Cu_2L_2H_{-1}]^-$ the two copper(II) ions cannot be equivalent and the band at 285 nm suggests co-ordination by the alcoholic hydroxyl group as well. As for $[Cu(HL)]^+$, the value of log K calculated for reaction $Cu^{2+} + HL^- \rightleftharpoons [Cu(HL)]^+$ (6.78 − 4.64 = 2.14) might suggest the formation of a copper(II)–monocarboxylate bond and the involvement of the undissociated alcohol group in co-ordination (at 298 K and I = 0.1 mol dm^{-3}, log K = 1.83 and 2.55[23] for the formation of the corresponding acetate and lactate complexes, respectively). The band at 205 and the shoulder at 235 nm are due to i.l. transitions in the carbonyl chromophore of the π ⟶ π* and n ⟶ π* type, respectively.[2,19,24] The wide band at 255 nm is due to a c.t. transition of the carboxylate group to the copper(II) ion and probably includes the weak contribution from the $OH \rightarrow Cu^{II}$ c.t.; its sign is opposite that for the dimeric complexes, probably as a consequence of a great modification in the ligand conformation.

Proton–copper(II)–(1*R*,2*R*)-cyclohexane-1,2-diamine. Until pH 7 the CD signal recorded for the free pro-ligand (titrated at 3 ≤ pH ≤ 10.5) was not distinguishable from that of KCl. A significant UV/CD spectrum was obtained only for the neutral amine: λ_{max} < 200 nm, $\Delta\varepsilon_{200}$ = 2.21 $dm^3\ mol^{-1}\ cm^{-1}$. Probably λ_{max} is shifted towards shorter wavelengths with increasing protonation. The UV/CD spectra were recorded on solutions containing copper(II) at 3.5 ≤ pH ≤ 10.5; a band at 245 nm appears, its ellipticity increasing regularly with pH.

Table 1 Calculated values of proton and copper(II) complex-formation constants (log β_{pqr}) and UV/CD spectra (i.l. and c.t. bands), at 298 K and I = 0.1 mol dm^{-3}. The probable error in $\Delta\varepsilon_{max}$ values ranges between ± 2 and ± 10%, according to the extent of formation of each complex

Pro-ligand	Complex	*pqr*	log β_{pqr} [a]	λ_{max}/nm ($\Delta\varepsilon_{max}$/dm^3 mol^{-1} cm^{-1})
mal	L^{2-}	—	—	205 (2.21)
	HL^-	011	4.64(1)	208 (1.42)
	H_2L	012	7.88(1)	215 (0.92)
	$[Cu(HL)]^+$	111	6.78(5)	205 (−1.08), 235 (sh), 255 (0.27)
	$[CuL]$	110	3.67(2)	205 (−2.94), 240 (0.25), 285 (0.07)
	$[Cu_2L_2H_{-1}]^-$	22-1	5.15(6)	215 (−4.49), 245 (−4.13), 285 (0.20), 305 (−0.24)
	$[Cu_2L_2H_{-2}]^{2-}$	22-2	0.99(4)	210 (−1.60), 230 (0.50), 262 (−1.28), 310 (0.14)
chxn	L	—	—	< 200
	HL^+	011	9.72(2)	—
	H_2L^{2+}	012	16.23(3)	—
	$[CuL]^{2+}$	110	11.07(2)	245 (1.35)
	$[CuL_2]^{2+}$	120	20.68(4)	245 (3.85)
Ala-Ala	L^-	—	—	< 200
	HL	011	8.09(1)	< 200, 225 (0.22)
	H_2L^+	012	11.39(1)	212 (2.59)
	$[CuL]^+$	110	5.31(5)	—
	$[CuLH_{-1}]$	11-1	1.74(1)	212 (3.44), 230 (−1.08), 260 (−1.04), 310 (0.21)
	$[CuLH_{-2}]^-$	11-2	−7.66(2)	209 (3.57), 230 (1.30), 245 (−1.78), 300 (0.24)
	$[Cu_2L_2H_{-3}]^-$	22-3	−3.74(5)	212 (11.54), 233 (−4.05), 255 (−3.05), 305 (0.65)
γ-Glu-ε-lys	L^{2-}	—	— [b]	210 (1.21)
	HL^-	011	9.71	206 (1.46)
	H_2L	012	18.57	< 200
	H_3L^+	013	21.13	205 (2.17)
	H_4L^{2+}	014	22.91	—
	$[Cu(HL)]^+$	111	17.59	250 (−0.68)
	$[CuL]$	110	13.73	210 (−1.45), 233 (4.33), 255 (3.06)
	$[CuLH_{-1}]^-$	11-1	2.36	208 (−0.88), 235 (3.88), 255 (2.70)
	$[Cu_2L]^{2+}$	210	15.68	210 (1.54), 235 (−3.64), 245 (−3.78)
MeCO-His	L^-	—	—	210 (3.75)
	HL	011	7.01(1)	212 (1.50)
	H_2L^+	012	9.89(1)	210 (2.00), 235 (−0.17)
	$[CuL]^+$	110	4.24(1)	242 (0.05), 285 (−0.09)
	$[CuL_2]$	120	7.63(2)	245 (0.48), 300 (−0.15)
	$[CuL_3]^-$	130	10.10(5)	242 (0.55), 292 (−0.05)
β-Ala-His	L^-	—	— [c]	212 (2.93)
	HL	011	9.372	212 (3.35)
	H_2L^+	012	16.146	215 (1.46)
	H_3L^{2+}	013	18.740	< 200
	$[Cu(HL)]^{2+}$	111	13.30	—
	$[CuL]^+$	110	8.47	262 (−1.56), 315 (0.34)
	$[CuLH_{-1}]$	11-1	2.44	250 (0.62), 295 (−0.24), 323 (0.20)
	$[Cu_2L_2H_{-2}]$	22-2	8.35	258 (3.03), 295 (−1.65), 342 (0.15)
	$[Cu_2LH_{-1}]^{2+}$	21-1	5.37	265 (−1.10), 310 (0.25), 330 (0.15)
PhCO-Gly-His-Leu	L^-	—	— [d]	235 (2.53), 275 (−0.10)
	HL	011	6.60	< 230
	H_2L^+	012	16.35	238 (−0.79)
	$[CuL]^+$	110	3.95	—
	$[CuLH_{-1}]$	11-1	−2.3	—
	$[CuLH_{-2}]^-$	11-2	−9.86	242 (0.73), 268 (0.41), 305 (−0.50), 338 (0.05)
	$[CuLH_{-3}]^{2-}$	11-3	−19.60	248 (4.25), 303 (0.77), 345 (−2.24)

[a] The errors in parentheses are ± 3σ in the last significant digit. [b] Ref. 15. [c] Ref. 16. [d] Ref. 17.

No increase in CD signal was observed at pH > 7. A UV/CD spectrum was then calculated for each copper(II) complex. Both for $[CuL]^{2+}$ and $[CuL_2]^{2+}$ there is a band at 245 nm, which is due to $NH_2 \rightarrow Cu^{II}$ c.t. The spectral position of this band is in fairly good agreement with that (233 nm) identified by Bunel *et al.*[25] for the copper(II)-L-propane-1,2-diamine system.

Proton–copper(II)–L-alanyl-L-alanine. The UV/CD spectra for the free pro-ligand were first recorded at 3 ⩽ pH ⩽ 9 (hydrolysis of the peptide occurs at more basic pH values). For all the complexes with the proton it was possible to calculate a UV/CD spectrum (Table 1). The UV/CD spectra were recorded for solutions containing copper(II) at 4 ⩽ pH ⩽ 10. At pH > 5 four bands were present at about 210, 230, 255 and 305 nm. A UV/CD spectrum was then calculated for each complex with copper(II) except $[CuL]^+$, because the amount formed in solution is too low. The spectra calculated for $[CuLH_{-1}]$, $[CuLH_{-2}]^-$ and $[Cu_2L_2H_{-3}]^-$ are qualitatively similar: very probably the same donor groups are present in all the complexes. Moreover in the spectra for $[CuLH_{-1}]$ and $[CuLH_{-2}]^-$ also the values of $\Delta\varepsilon_{max}$ are very similar for all the bands, suggesting the same structure for the two complexes; the log K^H value for the reaction $[CuLH_{-1}] \rightleftharpoons [CuLH_{-2}]^- + H^+$, *i.e.* −1.74 − 7.66 = −9.40, indicates a probable hydrolysis. For all the complexes the band at 210 nm is due to i.l. transitions, of the $\pi \longrightarrow \pi^*$ type, in the carbonyl chromophore and the band at 305 nm is the c.t. from the deprotonated peptide group[2,19,24] to Cu^{II}. Based on the results obtained, and in agreement with those obtained from many amino acids,[2,5,6] the band at 230 nm should be due to i.l. transitions in the carbonyl chromophore of the $n \longrightarrow \pi^*$ type, while that at 255 nm should be a c.t. to copper(II) due to the co-ordination of both amino and carboxylate groups, since the position of the c.t. band for bis(L-amino acidato)copper(II) complexes may vary,[6] for different L-amino acids, from 240 to 260 nm and the i.l. band due to $n \longrightarrow \pi^*$ is probably masked by the c.t. band.[2,6]

Proton–copper(II)–L-γ-glutamyl-L-ε-lysine. The above results, obtained on aliphatic model ligands, have been applied to the copper(II) species formed by a particular dipeptide in which two α-amino acidic groups are present, located at a long distance from each other. As a consequence of this structure, the involvement of the deprotonated peptide group in the co-ordination does not seem to be favoured. All the results obtained by using other experimental techniques[15] indicated no interaction between copper(II) and deprotonated peptide nitrogen. The UV/CD spectra should give useful information about the reliability of the structures previously[15] proposed.

The spectra of the free pro-ligand were first recorded at $2.5 \leqslant pH \leqslant 10.5$. Owing to its low extent of formation it was not possible to calculate the spectrum for the H_4L^{2+} species; for all the other complexes with the proton a UV/CD spectrum was calculated (Table 1). The UV/CD spectra were then recorded for solutions containing copper(II) at $2.5 \leqslant pH \leqslant 10$ (Fig. 1). Until pH 3 a weak negative CD signal at about 255 nm is observed (together with a positive i.l. band at $\lambda_{max} < 200$ nm); at pH > 4 three bands, the ellipticities of which increase regularly with pH, are present at about 210 (negative), 235 (positive) and 255 nm (positive). The spectra calculated for [CuL] and $[CuLH_{-1}]^-$ (Fig. 2) are nearly the same as regards sign, intensity and position of the peaks. The bands at 205 and 235 nm are due to i.l. transitions of the $\pi \longrightarrow \pi^*$ and $n \longrightarrow \pi^*$ type, respectively, that at 255 nm to the c.t. from the amino and carboxylate groups to Cu^{II}. The absence of a band at about 305 nm [see also the copper(II)–Ala-Ala system] in both spectra shows that the peptide group does not participate in co-ordination in the two complexes. The two species have the same structure, involving the two α-amino acid residues; $[CuLH_{-1}]^-$ is very probably formed by a hydrolysis reaction. In the spectra calculated for $[Cu(HL)]^+$ (Fig. 2) there is a weak c.t. band (250 nm) involving only one α-amino acid residue, which probably contains a contribution from an i.l. band of the $n \longrightarrow \pi^*$ type. The UV/CD spectrum calculated for the binuclear species $[Cu_2L]^{2+}$ (Fig. 2) mirrors those

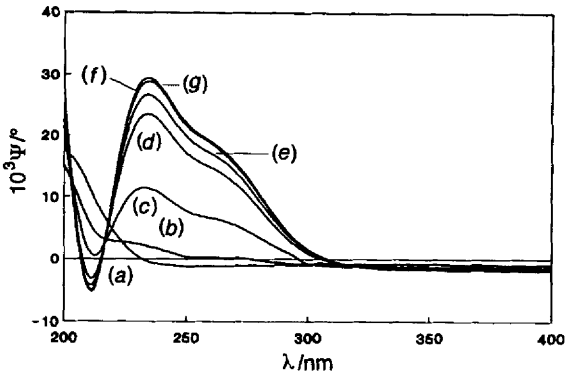

Fig. 1 The UV/CD spectra for the copper(II)–γ-Glu-ε-Lys system recorded on a solution containing $c_{Cu} = 2.00$ and $c_L = 2.05$ mmol dm^{-3} at different pH: (*a*) 2.83, (*b*) 3.60, (*c*) 4.15, (*d*) 4.83, (*e*) 5.20, (*f*) 6.81 and (*g*) 9.56

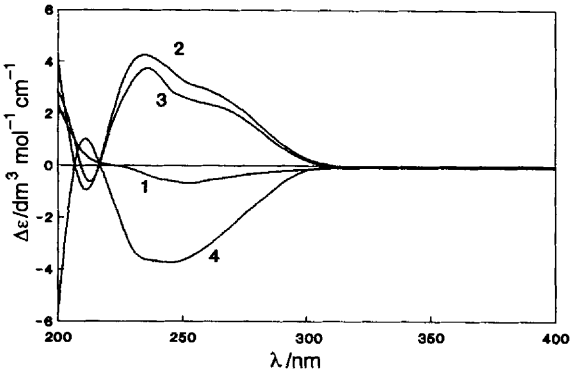

Fig. 2 The UV/CD spectra calculated for each copper(II) complex of γ-Glu-ε-Lys: 1, $[Cu(HL)]^+$; 2, [CuL]; 3, $[CuLH_{-1}]^-$; 4, $[Cu_2L]^{2+}$

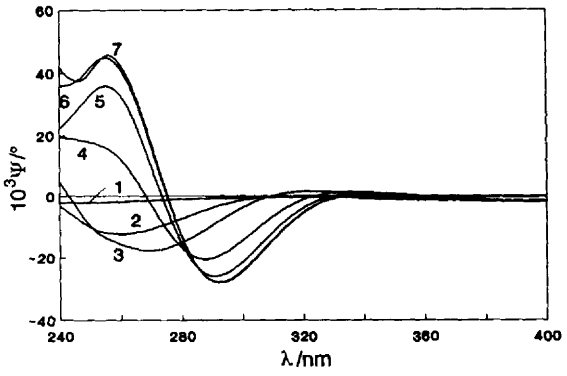

Fig. 4 The UV/CD spectra for the copper(II)–β-Ala-His system recorded on a solution containing c_{Cu} = 12.00 and c_L = 12.30 mmol dm^{-3} at different pH values: (1) 3.63, (2) 4.70, (3) 5.20, (4) 5.70, (5) 6.22, (6) 6.98 and (7) 7.66

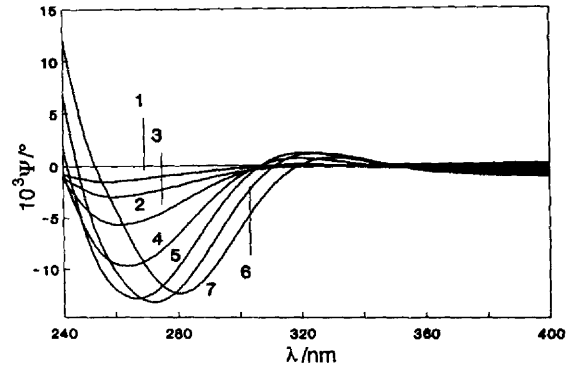

Fig. 5 The UV/CD spectra for the copper(II)–β-Ala-His system recorded on a solution containing c_{Cu} = 12.00 and c_L = 6.00 mmol dm^{-3} at different pH values: (1) 4.03, (2) 4.33, (3) 4.59, (4) 4.84, (5) 5.08, (6) 5.34 and (7) 5.50

increases, an important bathochromic shift, from 285 to 300 nm, also occurs for the π_1 band. From $[CuL_2]$ to $[CuL_3]^-$ no relevant modifications occur in the π_2 band, while significant hypsochromic (from 300 to 292 nm) and hypochromic effects are recorded for the π_1 band. The intensity of the charge transfer at 240–245 nm (π_2) increases with the number of nitrogen donors, although the increment is very low for the third donor; from the π_1 band (285–300 nm) it is evident that charge transfer is favoured in $[CuL_2]$ with respect to $[CuL]^+$.

Proton–copper(II)–L-carnosine. The UV/CD spectra for the free pro-ligand were first recorded, in the 200–400 nm spectral range, at 2.8 ≤ pH ≤ 9. A spectrum for each complex of β-Ala-His with the proton was calculated (Table 1). As for MeCO-His, a band can be assigned to the n ⟶ π* transition of the carbonyl chromophore with a minor contribution from the π ⟶ π* transition of the imidazole ring. As regards the copper(II)-containing system, when $c_{Cu}:c_L$ = 1:1, up to pH ≈ 5 two bands appear at about 265 and 315 nm while at pH > 5 there are three bands at 260, 295 and 340 nm (Fig. 4); on the other hand, when $c_{Cu}:c_L$ > 1:1(pH < 5.7) there are two bands at 260–280 and 310 nm with a shoulder at ≈330 nm (Fig. 5). As regards the $[CuL]^+$ complex we previously proposed [16] a structure in which copper(II) is co-ordinated by terminal amino and dissociated peptide groups; in calculated UV/CD spectra (Fig. 6) the band at 262 nm can be assigned to the c.t. from NH_2 to Cu^{II}, while that at 315 nm is due to c.t. from N^- to copper(II). For the $[Cu_2LH_{-1}]^{2+}$ complex we proposed [16] a similar structure with the second copper(II) ion co-ordinated to the imidazole pyridine nitrogen. In the spectrum calculated for this complex (Fig. 7) the bands have nearly the same intensity and position as those calculated for $[CuL]^+$ (Table 1), with a shoulder at ≈330 nm assignable to the π_1 component of

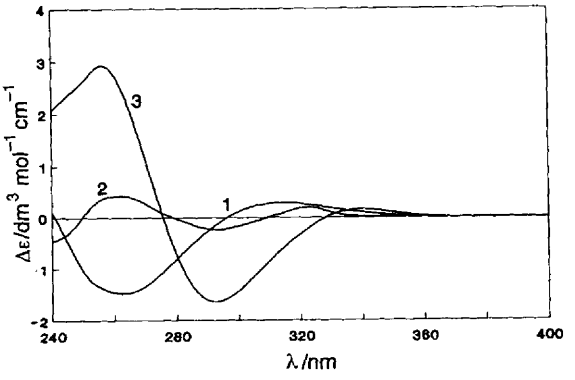

Fig. 6 The UV/CD spectra calculated for some copper(II) complexes of β-Ala-His: (1) $[CuL]^+$, (2) $[CuLH_{-1}]$ and (3) $[Cu_2L_2H_{-2}]$

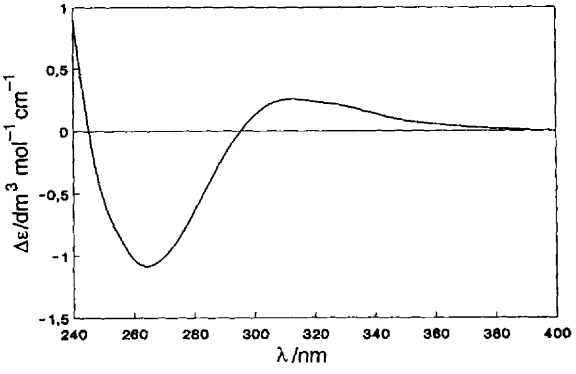

Fig. 7 The UV/CD spectrum calculated for the binuclear copper(II) complex $[Cu_2LH_{-1}]^{2+}$ of β-Ala-His

the imidazole, in agreement with literature data.[28,29] In the spectrum of the dimer $[Cu_2L_2H_{-2}]$ (Fig. 6) three bands appear at 258, 295 and 342 nm. For this complex we previously proposed [16] a structure containing two equivalent copper(II) ions, each bound to amino, dissociated peptide, imidazole pyridine nitrogen and carboxylate groups, while that proposed [16] for the corresponding monomeric complex $[CuLH_{-1}]$ involves the same donor groups, with the exclusion of carboxylate (three CD bands at 250, 295 and 323 nm, Fig. 6). For both the monomer and dimer the bands at 250 or 258 nm can be assigned to the c.t. from NH_2 to copper(II) and from π_2 imidazole to copper(II), while those at 323 or 342 nm are due to the π_1 components of the imidazole. The band at 295 nm can be assigned to the c.t. from N^- to copper(II). Table 2 lists $\Delta\varepsilon_{max}$ values for the $N^-\rightarrow Cu^{II}$ c.t. band calculated for a series of complexes. The value of $\Delta\varepsilon/n$ is fairly constant where n is the number of negatively charged peptide nitrogens involved in co-ordination. The exception of the L-carnosine dimer ($\Delta\varepsilon_{295}/n$ = −0.82 dm^3 mol^{-1} cm^{-1}) may be explained by considering that in the dimer also the contribution from the $CO_2^-\rightarrow Cu^{II}$ c.t. must be taken into account. As for $[Cu(HL)]^{2+}$, in which the Cu^{II} is bound only through the pyridine nitrogen of the imidazole ring,[16] it was not possible to calculate a significant UV/CD spectrum probably owing to the too low intensity of the c.t. bands corresponding to the copper(II)–imidazole interaction.

Proton–copper(II)–hippuroyl-L-histidyl-L-leucine. The UV/CD spectra for the free pro-ligand were first recorded, in the 230–400 nm range, at 2.5 ≤ pH ≤ 10. A spectrum for each protonated form was calculated (Table 1). The band at around 240 nm can be assigned to the n ⟶ π* transition of the carbonyl chromophore (with a possible minor contribution from the π ⟶ π* transition of the imidazole ring); the red shift observed with respect to MeCO-His or β-Ala-His (≈15 nm) is probably due to the conjugation of the phenyl with the

Table 2 Values of $\Delta\varepsilon_{max}$ for the $N^-\rightarrow Cu^{II}$ c.t. band calculated for a series of complexes

Pro-ligand	Complex	n[a]	λ_{max}/nm	$\Delta\varepsilon_{max}$/dm^3 mol^{-1} cm^{-1}	$(\Delta\varepsilon_{max}/n)$/dm^3 mol^{-1} cm^{-1}
Ala-Ala	$[CuLH_{-1}]$	1	310	0.21	0.21
	$[CuLH_{-2}]^-$	1	300	0.24	0.24
	$[Cu_2L_2H_{-3}]^-$	2	305	0.65	0.32
PhCO-Gly-His-Leu	$[CuLH_{-2}]^-$	2	305	−0.50	−0.25
	$[CuLH_{-3}]^{2-}$	3	303	0.77	0.26
Ala-Ala-Asp-Ala[b]	$[CuLH_{-2}]^{2-}$	2	306	0.59	0.29
β-Ala-His	$[CuL]^+$	1	315	0.34	0.34
	$[Cu_2LH_{-1}]^{2+}$	1	310	0.25	0.25
	$[CuLH_{-1}]$	1	295	−0.24	−0.24
	$[Cu_2L_2H_{-2}]$	2	295	−1.65	−0.82

[a] Number of deprotonated peptide groups involved in co-ordination. [b] Ref. 30.

Table 3 Ultraviolet–circular dichroism spectral map: positions of the bands of the pro-ligand and of copper(II) complexes (i.l. and c.t.) due to chromophores located on molecules of biological interest

	λ_{max}/nm	
Chromophore	pro-ligand, i.l.	c.t. to Cu^{II}
NH_2	< 200 (amine in pro-ligand)	233[a]–245
CO_2^-	205–215 (n ⟶ π*) (carbonyl in pro-ligand) 205 (π ⟶ π*), 230–235 (n ⟶ π*) (i.l. of carbonyl in complexes)	255–265
OH	182[b] (n ⟶ σ*) (hydroxyl chromophore in the free ligand)	≈285
O^-		300–310
N^-	≈210 (n ⟶ π*) (i.l. of amide)	295–315
Imidazole	205–210[c]	220[c] 245–260 (π_2) 280–345 (π_1)
Phenyl	220[d] (L_a) 270–275 (L_b)	—

[a] Ref. 25. [b] Ref. 11. [c] Ref. 26. [d] Ref. 2.

Table 4 Spectral position of the c.t. band due to the π_1 component of imidazole (N^π) for several copper(II) complexes

		Donor group		
Pro-ligand	Complex	Type	Number	λ_{max}/nm
MeCO-His	$[CuL]^+$	N^π	1	285
	$[CuL_2]$	$2N^\pi$	2	300
	$[CuL_3]^-$	$3N^\pi$	3	292
β-Ala-His	$[CuLH_{-1}]$	N^π, NH_2, N^-	3	323
	$[Cu_2LH_{-1}]^{2+}$	N^π, NH_2, N^-	3	330
PhCO-Gly-His-Leu	$[CuLH_{-2}]^-$	N^π, $2N^-$	3	338
	$[CuLH_{-3}]^{2-}$	N^π, $3N^-$	4	345
β-Ala-His	$[Cu_2L_2H_{-2}]$	N^π, NH_2, N^-, CO_2^-	4	342

amide group. The weak band at 275 nm can be assigned to the aromatic transition (L_b) of the phenyl ring.[2,28] In the experimental spectra recorded for the copper(II)-containing system, in the 230–400 nm interval, three bands at about 250, 305 and 345 nm appear. Before precipitation it is difficult to distinguish between the UV/CD signal recorded in the absence and in the presence of the metal ion; hence it was not possible to calculate a significant UV/CD spectrum for $[CuL]^+$ [the intensity of the c.t. bands due to the copper(II)–imidazole bond is very low, *cf.* MeCO-His and β-Ala-His systems]. It was not possible to calculate a UV/CD spectrum for $[CuLH_{-1}]$ because of the small amount formed. For the $[CuLH_{-2}]^-$ complex we previously proposed[17] a structure in which the copper(II) ion is co-ordinated by three nitrogen donors (imidazole, deprotonated peptide and amide). In the UV/CD spectra the positive band at 242 nm is probably due to the i.l. transition of the carbonyl chromophore (positive band at 235 nm for the free pro-ligand) with a contribution from the π_2 component of the imidazole; the band at 268 nm can be assigned, as for the free pro-ligand, to an i.l. transition in the phenyl residue. The bands at 305 and 338 nm are due to c.t. from N^- and the π_1 component of imidazole, respectively. Owing to the break-point action of the L-histidyl residue, the most probable structure[17] for $[CuLH_{-3}]^{2-}$ should involve copper(II) co-ordinated as in $[CuLH_{-2}]^-$ in the equatorial plane with the deprotonated peptide nitrogen of the L-leucyl residue in an axial position. In the UV/CD spectrum calculated for this complex three bands appear in about the same positions as those calculated for $[CuLH_{-2}]^-$, at 248, 303 and 345 nm. The weak i.l. band due to the phenyl residue is probably masked by the strong band at 248 nm ($\Delta\varepsilon_{max}$ = 4.25 dm^3 mol^{-1} cm^{-1}), whilst the corresponding c.t. bands have opposite signs for the two species. This fact might exclude a simple hydrolysis reaction, from $[CuLH_{-2}]^-$ to $[CuLH_{-3}]^{2-}$, and should indicate a significant modification in the ligand conformation, as a consequence of the axial co-ordination by the N^- group of the L-leucyl residue.

Conclusion

The results obtained are summarized in Table 3, which contains an ultraviolet–circular dichroism spectral map of the positions of the charge-transfer bands involving donor groups located on molecules of biological interest. The following conclusions can be drawn. (*i*) In the series $\lambda_{max}(O^-, N^-) > \lambda_{max}(CO_2^-) \geqslant \lambda_{max}(NH_2)$ charge transfer is more easily promoted by strongly basic alcholate or deprotonated peptide groups than by carboxylate, the charge of which is stabilized by resonance and amino groups. (*ii*) In the different complexes two or more contributions from the n ⟶ π* i.l. band and from the amino, π_2 imidazole or carboxylate c.t. bands might be superimposed. (*iii*) As regards imidazole participation in copper(II) co-ordination, the π_1 component should be more important than the π_2 since our results show no spectral superimposition among this and other c.t. bands, even though the latter is usually more intense. (*iv*) The spectral position of the π_1 component of imidazole (c.t. π_1 imidazole⟶Cu^{II}) seems to be dependent (Table 4) on the total number of donor groups involved in the metal co-ordination sphere, being red shifted with increase in this number. (*v*) The $N^-\rightarrow Cu^{II}$ c.t. band is characterized by a quite well defined position together with low intensity: these characteristics should allow one to identify eventual superpositions (Table 2).

References

1 T. Yamamoto, M. Tasumi and M. Tanokura, *Can. J. Chem.*, 1991, **69**, 1679.
2 J. M. Tsangaris, J. W. Chang and R. B. Martin, *J. Am. Chem. Soc.*, 1969, **91**, 726.
3 M. Shinitzky and R. Haimovitz, *J. Am. Chem. Soc.*, 1993, **115**, 12545.

4 C. Garcia-Echeverria, F. Albericio, E. Giralt and M. Pons, *J. Am. Chem. Soc.*, 1993, **115**, 11663.
5 K. M. Wellman, S. Bogdansky, W. Mungall, T. G. Mecca and C. R. Hare, *Tetrahedron Lett.*, 1967, **37**, 3607.
6 C. Ibarra, R. Soto, L. Adan, A. Decinti and S. Bunel, *Inorg. Chim. Acta*, 1972, **6**, 601.
7 C. V. Phan, L. Tosi and A. Garnier, *J. Inorg. Nucl. Chem.*, 1975, **37**, 2385.
8 S. Bunel, L. Gil and H. Bobadilla, *J. Inorg. Nucl. Chem.*, 1977, **39**, 365.
9 C. De Stefano, P. Mineo, C. Rigano and S. Sammartano, *Ann. Chim. (Rome)*, 1993, **83**, 243.
10 A. Sabatini and A. Vacca, personal communication.
11 E. Charney, *The Molecular Basis of Optical Activity*, R. E. Krieger Publishing Company, Malabar, FL, 1985.
12 G. Arena, R. Calì, E. Rizzarelli and S. Sammartano, *Thermochim. Acta*, 1976, **16**, 315.
13 C. E. Baes and R. E. Mesmer, *The Hydrolysis of Cations*, Wiley, New York, 1976.
14 G. Ostacoli, P. G. Daniele and A. Vanni, *Ann. Chim. (Rome)*, 1975, **65**, 197.
15 P. G. Daniele, E. Prenesti and G. Ostacoli, *J. Inorg. Biochem.*, 1996, **61**, 165.
16 P. G. Daniele, E. Prenesti, V. Zelano and G. Ostacoli, *Spectrochim. Acta, Part A*, 1993, **49**, 1299.
17 P. G. Daniele, E. Prenesti, R. Aigotti and G. Ostacoli, *J. Inorg. Biochem.*, 1995, **58**, 139.
18 D. N. Kirk, *Tetrahedron*, 1986, **42**, 777.
19 G. Formicka-Kozlowska, H. Kozlowski, M. Bezer, L. D. Pettit, G. Kupryszewski and J. Przybylski, *Inorg. Chim. Acta*, 1981, **56**, 79.
20 M. Gabriel, D. Larcher, C. Thirion, J. Torreilles and A. Crastes de Paulet, *Inorg. Chim. Acta*, 1977, **24**, 187.
21 P. G. Daniele, G. Ostacoli and A. Vanni, *Atti. Accad. Sci. Torino*, 1974–1975, **109**, 547.
22 A. Garnier-Suillerot, J. P. Albertini, A. Collet, L. Faury, J. M. Pastor and L. Tosi, *J. Chem. Soc., Dalton Trans.*, 1981, 2544.
23 A. E. Martell and R. M. Smith, *Critical Stability Constants*, Plenum, New York, 1974, vol. 1.
24 H. Sigel and R. B. Martin, *Chem. Rev.*, 1982, **82**, 385.
25 S. Bunel, C. Ibarra, M. Rodriguez, A. Urbina and C. A. Bunton, *J. Inorg. Nucl. Chem.*, 1981, **43**, 971.
26 T. G. Fawcett, E. E. Bernanducci, K. Krogh-Jespersen and H. J. Schugar, *J. Am. Chem. Soc.*, 1980, **102**, 2598.
27 P. E. Grebow and T. M. Hooker, jun., *Biopolymers*, 1975, **14**, 871.
28 K. Gerega, H. Kozlowski, E. Masiukiewicz, L. D. Pettit, S. Pyburn and B. Rzeszotararska, *J. Inorg. Biochem.*, 1988, **33**, 11.
29 C. E. Livera, L. D. Pettit, M. Bataille, B. Perly, H. Kozlowski and B. Radomska, *J. Chem. Soc., Dalton Trans.*, 1987, 661.
30 B. Decock-Reverend, A. Lebkiri, C. Livera and L. D. Pettit, *Inorg. Chim. Acta*, 1986, **124**, L19.

Received 12*th February* 1996; *Paper* 6/00987E