Thermodynamic Stereoselectivity assisted by Weak Interactions in Metal Complexes. Copper(II) Ternary Complexes of Cyclo-L-histidyl-L-histidine and L- or D-Amino Acids in Aqueous Solution

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The thermodynamic stereoselectivity and spectroscopic characteristics of copper(II) ternary complexes with L- or D-amino acids (Xaa) and cyclo-L-histidyl-L-histidine, cyclo(-His-His-), have been investigated. Ternary complex formation with L-amino acids is more enthalpy and less entropy favoured than that of the analogous D-amino acids. For aromatic amino acids these differences increase going from phenylalanine to tryptophan, suggesting involvement of aromatic ring-stacking interactions between one of the dipeptide imidazoles and the Xaa aromatic side chain in the mixed-ligand complexes. Enthalpy changes are the driving force for the thermodynamic stereoselectivity. A detailed analysis of the CD spectra of these ternary systems resulted in a correlation between $\Delta(\Delta\varepsilon)$, the difference between the molar CD coefficients at the wavelength where it is maximized, and the ΔG° and ΔH° values. On the basis of EPR spectra, it is excluded that the differences between the mixed-ligand complexes with L- or D-amino acids are due to a different degree of solvation. It is proposed that hydrophobic and ring-stacking interactions, responsible for stereoselectivity, are favoured by a certain degree of tetrahedral distortion of the tetragonal copper(II) chromophore formed by the two imidazole nitrogen donors of cyclo(-His-His-) and the nitrogen and carboxylate oxygen donors of the L- or D-amino acids.

It is well known that stereochemical features are often the driving forces of the specific chemical and biological activity of a molecule. Despite the large involvement of chiral recognition in pharmaceutical[1,2] and biochemical fields,[3,4] bioorganic chemistry[5] as well as in separation technology,[6,7] it has been affirmed that 'accurate descriptions of the origins of stereo and enantioselectivity are rare.'[8] From an inspection of the literature it seems that the more common explanation of the discriminating ability of a given enantiomeric species with respect to the other enantiomer is based on the 'three-point-bonding' theory.[9] This theory, proposed to explain the enantiospecific nature of enzymatic reactions, requires a minimum of three simultaneous interactions between the two partners. These forces, usually called non-covalent interactions[10] or secondary bonds,[11] include Coulomb and van der Waals forces, hydrogen bond and solvophobic[12] or, in more classical terms, hydrophobic interactions.[13] They have been invoked to explain chiral recognition *via* host–guest and donor–acceptor complexes of organic molecules.[14–18]

Chirality in chemistry is not simply confined to the asymmetric tetrahedral carbon atoms. In the 1950s Dwyer[19] looked for differential biological activity between isomers of the tris(1,10-phenanthroline)metal complexes which, more recently, have been used to examine structures and local conformations along the DNA helix.[20] In binding to β-DNA, only the D isomer may intercalate easily due to non-covalent interactions. Furthermore, equilibrium dialysis studies revealed that human serum albumin (hsa) has a distinct preference for binding the racemic isomer of Fe^{III} with *N*,*N*′-ethylenebis[(5-bromo-2-hydroxyphenyl)glycinate] at the high-affinity specific site of bilirubin IX a.[21] Even when the conformation of the bound bilirubin is yet not known despite extensive studies, the above-cited work,[21] showing the stereoselectivity of binding, suggested that the unique structure of the racemic iron(III) complex may be similar to that of the hsa-bound bilirubin. As a consequence, the use of rigid metal complexes, involving the formation of secondary bonds, allows one to explore the shape of a protein binding site. In addition, Davankov *et al.*[22] succeeded in the separation of up to seven racemic amino acids into enantiomers using a novel chiral phase (reversed-phase packing coated with *N*-alkyl-L-hydroxyproline) and an eluent containing copper(II) acetate. The enantioselectivity is assumed to be caused by a three-site sorbate–sorbent interaction, involving both bidentate co-ordination of two amino acids to the copper(II) ion and hydrophobic interactions between hydrocarbon side chains of amino acids and the alkyl groups of the support.[22] However, despite the fact that chemists more frequently use chiral complexes which show stereoselectivity due to different non-covalent interactions, such as stacking, hydrophobic, *etc.*, few reports have tackled the problem of the energetics involved in such interactions.[23] Only recently, the role of different non-covalent forces in the thermodynamic stereoselectivity of proton and metal complex formation of biofunctional ligands has been elucidated on the basis of ΔH° and ΔS° values.[24–29] More recently the determination of all thermodynamic parameters concerning the stereoselectivity of copper(II) ternary complexes with D- or L-histidine and bidentate L-amino acids, bearing aliphatic or aromatic side chains, provided evidence for the factors determining the selective metal ion-assisted molecular recognition of the amino acids.[30] These factors were both non-covalent interactions between side-chain residues and the co-ordination of the

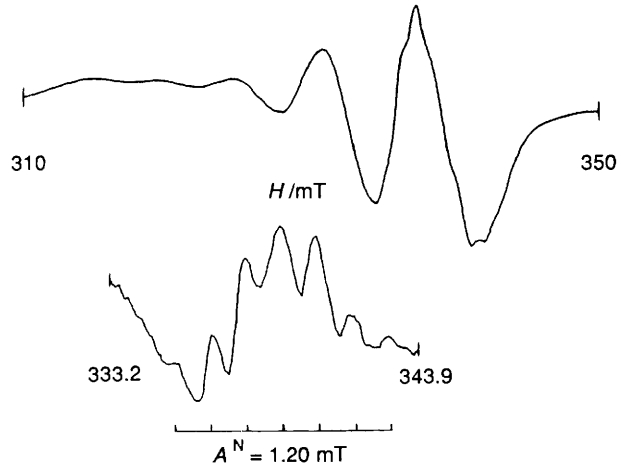

Fig. 1 Room-temperature EPR spectrum of an aqueous solution containing the [Cu{cyclo(-His-His-)}(D-AlaO)]$^+$ species at pH 5.5, the inset showing the second derivative of the highest-field feature

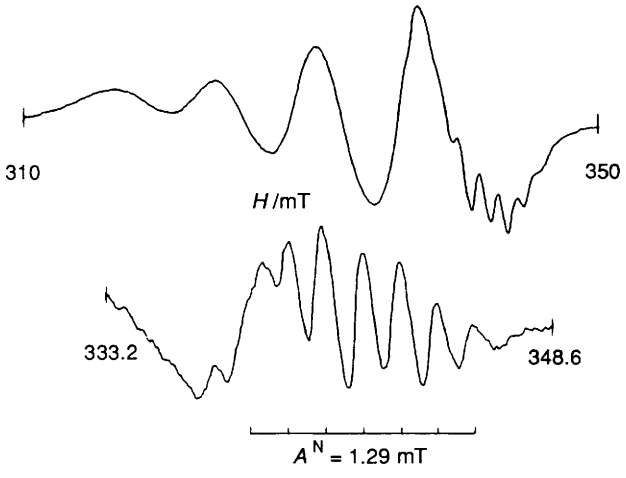

Fig. 2 Room-temperature EPR spectrum of an aqueous solution containing Cu^{2+}:cyclo(-His-His-):D-AlaO$^-$ in the ratio 1:1.2:1 at pH 7.5. The system contains the two species [Cu{cyclo(-His-His-)}(D-AlaO)]$^+$ and [Cu{cyclo(-His-His-)}(D-AlaO)H_{-1}]. The highest-field features are also shown in the second derivative mode on an enlarged scale

histidine carboxylate group. Extending this approach, we now report a study of the thermodynamic stereoselectivity in systems containing the copper(II) ion, the L- or D-amino acids (Xaa), and cyclo-L-histidyl-L-histidine, cyclo(-His-His-). The aim of this work is the study of the chiral discrimination factors, which could be due to interactions between the side-chain groups and/or to the different degree of solvent–metal ion interaction. Potentiometric and calorimetric measurements have been carried out at 25 °C and I = 0.1 mol dm^{-3} (KNO_3). Further information on these complexes has been obtained by EPR and circular dichroism (CD) spectroscopic measurements.

Experimental

Chemicals.—The dipeptide cyclo(-His-His-) was synthesised by cyclization of L-histidine methyl ester dihydrochloride in MeOH at 37 °C as previously reported.[31,32] The amino acids (Aldrich) were all high-purity products used without further purification. Their purity was checked by means of potentiometric titrations with standard KOH solution and always proved to be higher than 99.8%. Polarimetric tests gave substantially identical results. Stock solutions of $Cu(NO_3)_2$, HNO_3 and KOH were prepared and standarized as previously reported.[32] All solutions were prepared with CO_2-free freshly twice-distilled water. The ionic strength was adjusted to 0.1 mol dm^{-3} by adding KNO_3 (Suprapur Merck). Grade A glassware was employed throughout.

Table 1 Spin-Hamiltonian parameters for copper(II) mixed-ligand complexes with cyclo-L-histidyl-L-histidine and D- or L-amino acids in water–methanol (95:5) at 150 K

Complex species	$g_{\parallel}$ (±0.001)	$A_{\parallel}$ (±1)*	$g_{\perp}$ (±0.003)	$A_{\perp}$ (±2)*
[Cu{cyclo(-His-His-)}(XaaO)]$^+$				
L-AlaO$^-$	2.256	187	2.049	17
D-AlaO$^-$	2.255	187	2.049	17
L-LeuO$^-$	2.255	188	2.051	17
D-LeuO$^-$	2.256	188	2.052	17
L-PheO$^-$	2.254	188	2.053	17
D-PheO$^-$	2.254	189	2.052	16
L-TrpO$^-$	2.252	188	2.048	16
D-TrpO$^-$	2.252	187	2.051	16
[Cu{cyclo(-His-His-)}(XaaO)H_{-1}]				
L-AlaO$^-$	2.231	194	2.048	17
D-AlaO$^-$	2.230	195	2.047	16

* Hyperfine coupling constants are expressed in 10^4 cm^{-1}.

Electromotive Force Measurements.—Details of potentiometric measurements were previously described.[30,33] The electrode couple was standarized by titrating HNO_3 (5–10 mmol dm^{-3}) with KOH (100 mmol dm^{-3}) at 25 °C and I = 0.1 mol dm^{-3} (KNO_3). At least five independent potentiometric titrations have been performed for each system in which the ratio Cu^{2+}:cyclo(-His-His-):Xaa was 1:1:1.

Calorimetric Measurements.—Details of the calorimetric titrations are as previously reported.[30–33] The formation heats of mixed species were determined by using a buffer solution of amino acids to titrate solutions containing the metal ion and the dipeptide ligand in a 1:1 ratio at the pH of the maximum degree of formation of the main complex species [Cu{cyclo(-His-His-)}(XaaO)]$^+$. The ionic strength was maintained constant at I = 0.1 mol dm^{-3} by adding KNO_3. The ligand concentrations ranged from 0.003 to 0.01 mol dm^{-3}. For each system at least 150 experimental points were utilized to calculate the thermodynamic parameters.

EPR Spectra.—The EPR spectra were recorded with a Bruker X-band spectrometer (type ER 200 D) both at room and low temperature. A Bruker flat quartz cell was employed at room temperature in aqueous solution. Mixed-ligand complexes were prepared by mixing aqueous solutions of cyclo(-His-His-), the pertinent L- or D-amino acid and $^{63}Cu(NO_3)_2$ in the ratio 1.2:1:1. The copper(II) ion concentration was 5 mmol dm^{-3}. Potassium hydroxide was added to adjust the pH to 5.5 in the case of the 1:1:1:0 species and, in some cases, to *ca.* 7–8 in order to force the formation of the 1:1:1:−1 species. Figs. 1 and 2 show room-temperature EPR spectra of the Cu–cyclo(-His-His-)–D-Ala system at pH 5.5 and 7.5, respectively.

Spin-Hamiltonian parameters directly obtained from the frozen-solution spectra are reported in Table 1. In order to increase the resolution, 5% methanol was added to the aqueous solutions. Frozen-solution EPR spectra were recorded at 150 K. Other details were as previously reported.[34]

Electronic and CD Spectra.—Electronic and CD spectra were recorded on a Perkin-Elmer lambda 5 spectrophotometer and on a JASCO J-500 C dichrograph, respectively. Calibration of the CD instrument was performed with a solution of isoandrosterone in dioxane ($\Delta\varepsilon$ = 3.31 cm^3 mol^{-1} cm^{-1} at 304

Table 2 Thermodynamic parameters for the formation of 1:1:1:0 species in the systems containing copper(II), cyclo-L-histidyl-L-histidine and some amino acids at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)

Ligand	log β	$-\Delta G^{\circ}$/ kJ mol^{-1}	$-\Delta H^{\circ}$/ kJ mol^{-1}	ΔS°/ J K^{-1} mol^{-1}
L-AlaO$^-$	13.52(1)	77.15(4)	64.4(4)	43(1)
D-AlaO$^-$	13.50(2)	77.03(8)	64.0(4)	44(1)
L-ValO$^-$	13.56(2)	77.40(8)	67.2(2)	34.3(6)
D-ValO$^-$	13.52(2)	77.15(8)	65.7(2)	38.5(6)
L-LeuO$^-$	13.62(2)	77.74(8)	67.0(2)	36.0(6)
D-LeuO$^-$	13.63(2)	77.78(8)	66.9(2)	36.8(6)
L-PheO$^-$	13.50(1)	77.07(4)	69.6(2)	25.1(6)
D-PheO$^-$	13.32(2)	76.02(8)	67.6(2)	28.4(6)
L-TyrO$^-$	13.65(1)	77.90(4)	—	—
D-TyrO$^-$	13.48(2)	76.94(8)	—	—
L-TrpO$^-$	14.62(1)	83.43(4)	71.9(2)	38.5(6)
D-TrpO$^-$	14.30(1)	81.59(4)	69.4(2)	40.6(6)

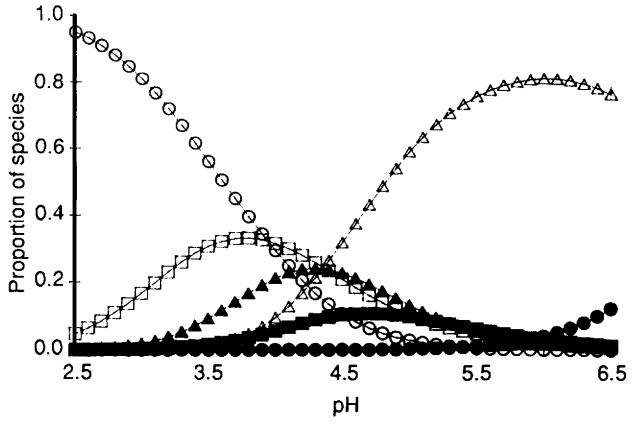

Fig. 3 Species distribution diagram obtained by potentiometric measurements on the Cu–cyclo(-His-His-)–D-TrpO$^-$ system at 25 °C at I = 0.1 mol dm^{-3}(KNO_3):[Cu^{2+}] = [cyclo(-His-His-)] = [D-TrpO$^-$] = 5 mmol dm^{-3}; M: cyclo(-His-His-)] : Xaa:H = 1:0:0:0 (free metal ion) (○) 1:0:1:0 (□), 1:1:1:0 (△), 1:1:1:1 (▲), 1:1:1:−1 (●) and 1:1:0:0 (■)

nm). The optical absorption and circular dichroism spectra were recorded at room temperature on freshly prepared aqueous solutions of the binary and ternary systems. The spectral range between 220 and 780 nm was covered with the use of quartz cells of various path length so that dilution of the solution was not required. Results are reported in terms of ε (molar absorption coefficient) and Δε (molar CD coefficient) in dm^3 mol^{-1} cm^{-1}.

Calculations.—Calculations of electrode system E° values, ligand purities, protonation constants and HNO_3 excess in the metal-ion stock solutions were performed by the least-squares computer program ACBA.[35] The formation constants of the copper complexes were calculated by the least-squares program SUPERQUAD.[36] The species distribution as a function of the pH was obtained by using the computer program DISDI.[37] The heats of complexation were calculated by the least-squares computer program DOEC.[38] Errors are expressed as three times the standard deviation. The thermodynamic data concerning the proton and the simple complex formation have been previously reported.[30–32]

Results and Discussion

The reaction of cyclo(-His-His-) (L) with copper(II) and the amino acids is represented in equation (1), where L′ is the anionic form of an amino acid with charges omitted for simplicity, and the stability constant $\beta_{mll'h}$ is defined by equation (2).

$$m\mathrm{Cu} + l\mathrm{L} + l'\mathrm{L}' + h\mathrm{H} \rightleftharpoons \mathrm{Cu}_m\mathrm{L}_l\mathrm{L}'_{l'}\mathrm{H}_h \quad (1)$$

$$\beta_{mll'h} = [\mathrm{Cu}_m(\mathrm{L}_l\mathrm{L}'_{l'}\mathrm{H}_h)]/[\mathrm{Cu}]^m[\mathrm{L}]^l[\mathrm{L}']^{l'}[\mathrm{H}]^h \quad (2)$$

In the pH ranges explored in the present work [Cu{cyclo(-His-His-)}(XaaO)]$^+$ is the main species. Two other species have been identified, [Cu{cyclo(-His-His-)}(XaaO)H]$^{2+}$ below pH 4 and [Cu{cyclo(-His-His-)](XaaO)H$_{-1}$] above pH 7. However, owing to the small amounts of these complexes we were not able to determine their stability constants (see Fig. 3). The stability constants of the mixed-ligand complexes are reported in Table 2. These values reveal two distinct trends. When the amino acid contains an aromatic residue, the ternary complexes of the L enantiomer are significantly more stable than those of the D. In the alanine-, valine- and leucine-containing complexes, stereoselectivity seems insignificant or absent. The log β, ΔG°, ΔH° and ΔS° values reported in Table 2 are pertinent to the equilibrium (3). The formation of ternary

$$\mathrm{Cu}^{2+} + \text{cyclo(-His-His-)} + \mathrm{XaaO}^- \rightleftharpoons [\mathrm{Cu}\{\text{cyclo(-His-His-)}\}(\mathrm{XaaO})]^+ \quad (3)$$

complexes is enthalpically and entropically favoured. The involvement of all potential donor atoms (three nitrogens and one oxygen) in the co-ordination sphere of the copper(II) ion can be inferred by comparing the data for equilibrium (3) with those for copper(II)–histamine–amino acids previously determined[30] (Table 3) in which the metal ion is co-ordinated to three nitrogen and one oxygen donor atom. The formation of histamine mixed-ligand complexes is enthalpy and entropy favoured. Even if the ΔH° and ΔS° values are more favourable than those of the analogous cyclo(-His-His-) complexes, this behaviour does not imply that all the potential donor atoms are engaged in the co-ordination sphere of the copper(II) ion in the dipeptide complexes. The difference (see Table 3) between the mixed-ligand complexes reflects that found between the simple complexes of the two amine ligands.[32,33] Comparison of the enthalpy and entropy values associated with the formation of ternary complexes of D- with those of L-amino acids, shows that, except for AlaO$^-$ and LeuO$^-$ systems, the ternary copper(II) complexes with L-amino acid complexes are more enthalpy and less entropy favoured. The difference increases on going from phenylalanine to tryptophan suggesting an effect depending on the characteristics of side-chain residues. Previously, it has been shown for simple and ternary complexes that the stacking interaction between non-co-ordinating side-chain groups is enthalpy favoured and entropy disfavoured[27–30,39–41] and that the effect increases with increasing size of the side-chain residues.[30] On this basis, these thermodynamic parameters provide evidence not only for the set of donor atoms actually involved in the bonding but also suggest that the presence of significant stereoselectivity in ternary complexes of copper(II) with cyclo(-His-His-) and aromatic amino acids is due to the stacking interaction between the imidazole and the aromatic or pseudo-aromatic rings of the two ligands. Furthermore, the enthalpy change is the driving force for the observed thermodynamic stereoselectivity. As regards the copper(II) ternary complexes with aliphatic amino acids, the ΔH° and ΔS° values seem to indicate that a stereoselective effect is also present in the valinate systems due to the weak interactions between the aliphatic side-chain residue of the L-amino acid and the pseudo-aromatic ring of cyclo(-His-His-) ligand. Assuming that the methyl group of the L-alaninate ligand is too small to interact with the pseudo-aromatic residues of the dipeptide, these thermodynamic parameters do not suggest an unambiguous explanation for the different behaviour of the L-leucinate complex with respect to the L-valinate one. Tentatively, different interactions with the solvent

Table 3 Thermodynamic parameters for the formation of some copper(II) simple and mixed-ligand complexes of histamine (hist) and cyclo-L-histidyl-L-histidine, previously determined at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)

Reaction	$-\Delta G^{\circ}$/ kJ mol^{-1}	$-\Delta H^{\circ}$/ kJ mol^{-1}	$-\Delta S^{\circ}$/ J K^{-1} mol^{-1}	Ref.
Cu^{2+} + hist + L-AlaO$^-$ $\rightleftharpoons$ [Cu(hist)(L-AlaO)]$^+$	97.28	77.02	67.8	30
Cu^{2+} + hist + L-PheO$^-$ $\rightleftharpoons$ [Cu(hist)(L-PheO)]$^+$	97.57	79.03	61.9	30
Cu^{2+} + hist $\rightleftharpoons$ [Cu(hist)]$^{2+}$	54.56	50.6	13.4	33
Cu^{2+} + cyclo(-His-His-) $\rightleftharpoons$ [Cu{cyclo(-His-His-)}]$^{2+}$	34.30	41.34	−23.8	32

Table 4 Electronic and CD spectral data [λ_{max}/nm (ε or Δε/dm^3 mol^{-1} cm^{-1})] for the systems Cu^{2+}:cyclo(-His-His-) 1:1 and 1:2

System	UV/VIS	CD
Cu:cyclo(-His-His-) 1:1[a]		
pH 4.95	250 (sh) (420), 290 (sh) (150), 685 (30)	255 (sh) (+1.46), 312 (−0.56), 603 (−0.26), 755 (+0.25)
pH 6.95	250 (sh) (2500), 290 (sh) (1250), 665 (105), 750 (sh) (100)	256 (−2.98), 308 (−4.34), 600 (−0.91), 760 (+1.52)
Cu:cyclo(-His-His-) 1:2[b]		
pH 4.68	255 (sh) (425), 300 (sh) (110), 350 (sh) (40), 750 (sh), (35)	255 (sh) (+1.75), 316 (−0.30), 622 (−0.24), 750[c] (+0.15)
pH 5.73	255 (sh) (1750), 290 (sh) (725), 630 (sh) (70), 732 (80)	255 (sh) (−1.27), 310 (−2.80), 603 (−0.70), 770 (+0.98)

[a] $[Cu^{2+}] = 6 \times 10^{-3}$ mol dm^{-3}. [b] $[Cu^{2+}] = 4 \times 10^{-3}$ mol dm^{-3}. [c] Maximum too close or beyond the range covered by the instrument; Δε refers to the λ indicated.

could be invoked. That is, the enthalpy-favoured and entropy disfavoured contributions due to non-covalent interaction could be more or less counterbalanced by a more positive ΔS° and less negative ΔH° value due to a desolvation effect of the water bound to the copper(II) ion, caused by the more encumbering residue present in the leucinate ligand.

The EPR measurements were carried out specifically to answer the question of whether different interactions with the solvent were present in the series of these copper(II) complexes. Room-temperature EPR spectra of all the mixed-ligand are identical. The highest-field feature is well resolved showing superhyperfine (s.h.f.) structure, superimposed on the copper line, due to the interaction of the odd electron with the nitrogen nuclei present in the co-ordination sphere. As shown by Fig. 1, seven superhyperfine lines correspond to the interaction of the copper(II) odd electron with three nitrogen donor atoms. The s.h.f. structure is not very symmetric probably owing to the different nature of these donors: two nitrogen atoms from imidazole residues and one from the NH_2 group of the amino acid. This result is quite important because it allows us to ascertain that three of the equatorial positions in the copper(II) polyhedron are surely occupied by nitrogen atoms. The spin-Hamiltonian parameters reported in Table 1 are characteristic of tetragonally elongated octahedral copper(II) complexes with a $d_{x^2-y^2}$ ground state, the other polyhedron sites being occupied by the amino acidate oxygen and water molecule oxygens. The only point which needs to be better clarified is whether the carboxylate oxygen of the amino acidate molecule is co-ordinated in an apical or equatorial position. Experiments in which successive amounts of free imidazole were added to aqueous solutions of the mixed-ligand complexes up to the ratio imidazole:complex = 4:1 did not produce any shift in the magnetic parameters or increase in the number of superhyperfine lines as a consequence of possible substitution of the water oxygen, involved in the equatorial plane bonding, with the added imidazole nitrogen. Hence, we conclude that the co-ordination sphere of these mixed-ligand complexes is formed by two imidazole nitrogen donors of the cyclo(-His-His-) ligand, one amine nitrogen and one carboxylate oxygen donor from the amino acid molecule, all bound to the copper(II) ion in the equatorial plane. Fig. 2 shows one room-temperature EPR spectrum of the system Cu–cyclo(-His-His-)–D-AlaO when the pH of the aqueous solution is raised to 7.5. The spectral pattern is complicated by the presence not only of the species determined at pH 5, but also an additional species which gives an absorption trace at slightly different values of the magnetic field. The second-derivative EPR spectrum shows the composite s.h.f. pattern due to the two species. Nevertheless, owing to slightly different nitrogen coupling constants [A^N_{iso} (1:1:1:0) = 1.20, (1:1:1:−1) = 1.29 mT] it is possible to recognize another seven-line pattern. This result is an indication of the displacement of one of the imidazole groups and the consequent co-ordination by one deprotonated nitrogen of the diketopiperazine ring. The number of nitrogen donors bound to copper(II) ion in the equatorial plane does not change and, hence, the s.h.f. pattern of seven lines is preserved. The shifts in the magnetic parameters, reported in Table 1 for the 1:1:1:−1 species when compared with the pertinent 1:1:1:0, are ascribable to the formation of a stronger Cu–N bond. Also in the deprotonated ternary species with aromatic and aliphatic amino acids, the magnetic parameters of the L form are equal to those of the D form. On the basis of these EPR results we can exclude that the observed stereoselectivity is due to a different degree of interaction of the metal ion with the solvent in the L- and D-enantiomer complexes.

To obtain further information on tyrosine systems (the low solubility of the amino acid prevents direct calorimetric measurements) and to support ΔH° and ΔS°, indirect indication that non-covalent interactions are responsible for stereoselectivity, electronic and CD spectra were recorded. In order to analyse the CD spectra of copper(II) mixed-ligand complexes of the type [Cu{cyclo(-His-His-)}(XaaO)]$^+$, it is useful to consider the CD contribution expected for the simple complexes [Cu(XaaO)]$^+$ and [Cu{cyclo(-His-His)}]$^{2+}$, according to the distribution data obtained by means of the DISDI program.[37] In both systems (see Table 4) the visible CD spectrum contains a two-signed curve, with a negative maximum near 600 nm and a positive maximum above 750 nm, and the near-UV spectrum presents a CD minimum near 310 nm and a positive shoulder near 250 nm. The lack of significant contribution to the above CD spectra by other species that may be present in low amount in the two Cu^{2+}–cyclo(-His-His-) systems was demonstrated by recording spectra at different pH. The dimeric species [Cu_2{cyclo(-His-His-)}$_2H_{-2}$]$^{2+}$, containing deprotonated diketopiperazine rings,[42] is the most abundant species in the 1:1 Cu^{2+}:cyclo(-His-His-) system above pH 6, while the four-co-ordinated [Cu{cyclo-(-His-His-)}$_2$]$^{2+}$ predominates at a 1:2 ratio at neutral pH.[32] The CD spectra of these species are quite similar to each other and display much more intense optical activity than does [Cu{cyclo(-His-His-)}]$^{2+}$ (Table 4). Although the CD extrema occur at about the same wavelengths, in all the three systems there is a distinct feature that differentiates the CD spectra of [Cu_2{cyclo(-His-His-)}$_2H_{-2}$]$^{2+}$ and [Cu{cyclo(-His-His-)}$_2$]$^{2+}$ from that of [Cu{cyclo(-His-

Table 5 Electronic and CD spectral data [λ_{max}/nm (ε or Δε/dm^3 mol^{-1} cm^{-1})] for the ternary system Cu^{2+}:cyclo(-His-His-):$XaaO^-$ (6 × 10^{-3} mol dm^{-3})

XaaO	pH	UV–VIS	CD
L-AlaO$^-$	5.85	250 (sh) (3200) 630 (70) 800 (sh) (35)	250 (sh) (+1.00), 265 (sh) (−0.30), 308 (−1.25), 590 (−0.32), 750* (+0.48)
D-AlaO$^-$	5.85	250 (sh) (3200) 630 (70) 800 (sh) (35)	250 (sh) (+1.88), 308 (−1.20), 590 (−0.30), 750* (+0.47)
L-LeuO$^-$	5.93	250 (sh) (2850) 625 (70) 800 (sh) (35)	250 (sh) (+0.88), 265 (sh) (−0.45), 308 (−1.25), 590 (−0.30), 750* (+0.45)
D-LeuO$^-$	5.90	250 (sh) (2850) 630 (65) 800 (sh) (35)	250 (sh) (+1.94), 310 (−1.22), 590 (−0.31), 750* (+0.48)
L-ValO$^-$	5.91	250 (sh) (3500) 625 (70) 800 (sh) (30)	250 (sh) (+0.75), 265 (sh) (−0.70), 305 (−1.25), 585 (−0.36), 750* (+0.42)
D-ValO$^-$	5.90	250 (sh) (3300) 625 (70) 800 (sh) (35)	250 (sh) (+2.31), 310 (−1.20), 585 (−0.25), 750* (+0.45)
L-PheO$^-$	5.90	250 (sh) (3300) 625 (65) 800 (sh) (30)	250 (sh) (+3.55), 308 (−0.64), 585 (−0.44), 750* (+0.08)
D-PheO$^-$	5.85	250 (sh) (3100) 630 (70) 800 (sh) (40)	265 (−0.25), 312 (−0.81), 595 (−0.07), 750 (+0.32)
L-TyrO$^-$	5.85	250 (sh) (3400) 275 (sh) (2400) 630 (60) 800 (sh) (25)	250 (sh) (+3.60), 280 (sh) (+0.15), 308 (−0.86), 595 (−0.44), 750* (+0.23)
D-TyrO$^-$	5.93	250 (sh) (3400) 275 (sh) (2600) 630 (60) 800 (sh) (25)	258 (sh) (−1.60), 310 (−0.65), 575 (sh) (+0.20), 670 (+0.34)
L-TrpO$^-$	5.91	270 (5800) 277 (5700) 286 (4600) 625 (60) 800 (sh) (25)	250 (sh) (+7.17), 270 (+1.52), 275 (sh) (+0.91), 286 (sh) (+0.08) 292 (−0.31), 314 (−0.60), 576 (+0.50), 750* (+0.10)
D-TrpO	5.83	270 (6100) 277 (5900) 625 (60) 800 (sh) (25)	247 (−4.80), 270 (sh) (−2.00), 275 (sh) (−1.80), 286 (sh) (−1.41), 315 (sh) (−0.70), 570 (sh) (+0.35), 645 (+0.44)

* Maximum too close or beyond the range covered by the instrument; Δε refers at the λ indicated.

Table 6 Absolute value of Δ(Δε) between the CD Spectra of the couples [Cu{cyclo(-His-His-)}(L-XaaO)]$^+$ and [Cu{cyclo(-His-His-)}-(D-XaaO)]$^+$

XaaO$^-$	λ/nm	Δ(Δε)/dm^3 mol^{-1} cm^{-1}
AlaO$^-$	590	0
LeuO$^-$	590	0
ValO$^-$	590	0.12
PheO$^-$	590	0.36
TyrO$^-$	600	0.67
TrpO$^-$	600	0.86

His-)}]$^{2+}$ and thus it excludes their contribution to the CD spectra of the latter species, *i.e.* the sign of the CD band near 255 nm is positive for [Cu{cyclo(-His-His-)}]$^{2+}$ and negative for both former species.

Following Schugar's[43] analysis of the electronic spectra of various copper(II)–imidazole chromophores, the CD bands near 260 and 310 nm can be attributed to charge-transfer transitions from the two highest-occupied orbitals of π symmetry of the imidazole ligands to the copper(II) d vacancy and have been observed in the spectra of various binary and ternary systems containing Cu^{II} and L-histidine.[44] The visible and UV bands derived from aliphatic amino acids can be rationalized on the basis of a previous analysis.[45,46] They are somewhat red-shifted for the complexes with aromatic amino acids, particularly when one considers Trp, for which the CD activity extends to above 300 nm, because of the presence of aromatic ring transitions (data not shown). It is important to note that, in general, the CD activity of the species [Cu(XaaO)]$^+$ is weaker than that attributed to [Cu{cyclo(-His-His-)}]$^{2+}$. The absorption and CD spectral data of the ternary systems Cu^{2+}:cyclo(-His-His-):$XaaO^-$ 1:1:1 are summarized in Table 5. For the mixed-ligand complexes containing aliphatic amino acids the shape of the CD curve is clearly independent of the absolute configuration of the amino acids and follows the pattern described above for [Cu{cyclo(-His-His-)}]$^{2+}$. The spectra of [Cu{cyclo(-His-His-)}(L-AlaO)]$^+$, [Cu{cyclo(-His-His-)}(D-AlaO)]$^+$, [Cu{cyclo(-His-His-)}(L-LeuO)]$^+$ and [Cu{cyclo(-His-His-)}(D-LeuO)]$^+$ are all practically superimposable in the visible region and it is only in the near-UV region, between 220 and 300 nm, that the CD surves of the diastereoisomeric couples with the same amino acid residue are slightly different. The spectra of the complexes containing L-amino acids display a negative shoulder near 260 nm which is missing from those of their D-Xaa counterparts. For the mixed-ligand complexes containing valine residues the CD spectral differences between the L and D forms are more marked and can be observed also in the visible region. For the mixed-ligand complexes derived from aromatic amino acids the CD spectra are remarkably different for each diastereoisomeric couple [Cy{cyclo(-His-His-)}(L-XaaO)]$^+$ and [Cu{cyclo(-His-His-)}(D-XaaO)]$^+$. Apart from the CD band near 310 nm, which is scarcely affected by the absolute configuration of the amino acid and the nature of the side chain, the rest of the CD spectrum becomes progressively simplified in the series Phe, Tyr, Trp, since single bands grow in near 250 and 600 nm and the systems [Cu{cyclo(-His-His-)}(L-XaaO]$^+$ and [Cu{cyclo(-His-His-)}(D-XaaO]$^+$ tend to display mirror-image behaviour. The pattern of the signs of the CD bands near 250 and 600 nm becomes similar to that of the corresponding [Cu(L-XaaO)]$^+$ and [Cu(D-XaaO)]$^+$ species but the CD intensities are so magnified that it can be excluded that the CD features exhibited by the mixed-ligand complexes are simply determined by the [Cu(XaaO)]$^+$ residues.

If we take Δ(Δε), the difference between the molar CD coefficients at the wavelengths where it is maximized, as a parameter to differentiate between the CD curves of the diastereoisomeric [Cu{cyclo(-His-His-)}(L-XaaO)]$^+$ and [Cu{cyclo(-His-His-)}(D-XaaO)]$^+$ species, it can be seen from the data in Table 6 that such differences increase with the steric encumbrance of the amino acid side chain, with clear amplification of the effect for the aromatic amino acids. The increase in Δ(Δε) parallels the trend of increasing differences in the ΔG° and ΔH° values of the different enantiomers of the mixed-ligand complexes and, therefore, the progressive differentiation in the CD spectra of the diastereoisomeric couples is related to some stereoselective interaction between the co-ordinated ligands. Since such interactions involve the amino acid side chains they can only be of non-covalent nature, *i.e.* hydrophobic or aromatic ring stacking with the imidazole nuclei of the cyclodipeptide. The effect of hydrophobic interactions in ternary complexes is weak but it has been evidenced in copper(II) systems containing aliphatic and aromatic amino acids,[47] while stacking between the imidazole ring of histidine and the side chain of aromatic amino acids has been characterized in systems of the type [Cu(HisO)(XaaO)].[30,48] In other ternary copper(II) complexes containing large heterocyclic ligands such as 1,10-phenanthroline and amino acids the effects of hydrophobic[49] and ring-stacking interactions[50] are obviously more dramatic.

It is expected that ligand–ligand interactions in the ternary complexes restrict the conformational mobility of the participating groups. Some evidence for the relative immobilization of

the groups involved in the present systems is provided by the CD spectra of [Cu{cyclo(-His-His-)}(L-TrpO)]$^+$ and [Cu{cyclo(-His-His-)}(D-TrpO)]$^+$ in the near-UV region, where the well resolved vibrational fine structure between 270 and 300 nm indicates restricted mobility of the indole nucleus {such structure is absent, for instance, from the spectra of [Cu(TrpO)]$^+$}.[51] In the other systems, as usual, this kind of evidence is not available but even though there are no specific features that can be attributed to direct ligand–ligand interactions we still associate the growth of significant optical activity in the near-UV region with the relative immobilization of the ligands in the mixed-ligand complexes. This situation can reduce the motional averaging of the couplings involving the transition moments associated with the ligand-to-metal charge-transfer of aromatic ring transitions which are reponsible for the absorption in the near-UV region and thus lead to an enhancement of the net optical activity within the electronic bands. The opposite sign of the CD activity observed for the diastereoisomeric couples [Cu{cyclo(-His-His-)}(L-XaaO)]$^+$ and [Cu{cyclo(-His-His-)}(D-XaaO)]$^+$ depends on the different relative orientation assumed by the interacting transition vectors in the two systems.

Conclusion

Inspection of molecular models shows that stereoselective non-covalent interactions between the side chains of amino acids and the imidazole nuclei of cyclo(-His-His-) may occur if only a certain tetrahedral distortion of the copper(II) equatorial plane is allowed and/or the donor groups of the amino acid bind the copper(II) ion involving an equatorial and an apical position. However, EPR results exclude this latter hypothesis.

From the X-ray structural study of the crystallized complex [Cu{cyclo(-His-His-)}$_2$]$^{2+}$, the angle formed by the two planes containing the copper atom and the pairs of imidazole nitrogen atoms was found to be 29°.[52] If such a non-coplanar arrangement of donor groups is also kept by the cyclo(-His-His-) ligand in these ternary complexes, the stacking interaction of the imidazole aromatic ring could be more specific with one of the two enantiomers. The experimental results support this hypothesis, because this kind of interaction is more efficient when a L enantiomer is considered. This different spatial and dissymmetric disposition of the two imidazole heterocyclic groups is probably the cause of the restricted mobility of the indole nucleus for the tryptophanate systems as indicated by the CD spectra. Also in the case of valinate mixed-ligand complexes, spectral and thermodynamic data support this conclusion.

As regards the absence of stereoselectivity in the leucinate ternary system, a different disposition of the side chain must be taken into account. The longer chain of leucine could exhibit a minor interaction with the cyclo(-His-His-) imidazole residues, the C–H group pointing towards these heteroaromatic groups. This geometrical disposition has been already found for leucinate in other systems in which the valinate side chain gave rise to a solvophobic interaction.[53]

Summing up our results we can assert that: (*i*) the thermodynamic parameters associated with the molecular recognition, assisted by the copper(II) ion, of amino acids by a cyclopeptide parallel the spectral CD data; (*ii*) non-covalent interactions determine the stereoselectivity; (*iii*) the driving force of the thermodynamic discrimination is enthalpic in origin, reflecting a geometrical control due to the disposition of the side chain non-co-ordinating groups.

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