Non-covalent Interactions in Thermodynamic Stereoselectivity of Mixed-ligand Copper(II)–D- or L-Histidine Complexes with L-Amino Acids. A Possible Model of Metal Ion-assisted Molecular Recognition

Gianna Borghesani, Ferdinando Pulidori,* and Maurizio Remelli
Department of Chemistry, University of Ferrara, 44100 Ferrara, Italy
Roberto Purrello and Enrico Rizzarelli*
Department of Chemical Sciences, University of Catania, 95125 Catania, Italy

Formation constants of ternary complexes of copper(II), L/D-histidine and, in turn, glycine, L-alanine, L-valine, L-leucine, L-tryptophan, or L-phenylalanine have been determined potentiometrically at 25 °C and I = 0.1 mol dm^{-3} (KNO_3). In the case of amino acids with aromatic side chains the ternary complexes containing ligands of opposite chirality are more stable than those having ligands of the same chirality; the opposite is true for amino acids with aliphatic residues. Calorimetric measurements have been carried out to obtain the enthalpy and entropy values associated with complex formation. Copper(II)–histamine ternary systems with L-alanine or L-phenylalanine have also been investigated. Comparison of the thermodynamic parameters pertinent to formation of the histamine complexes with those of the analogous histidine complexes allows one to ascertain the number of donor atoms involved in the co-ordination to copper(II), in the histidine systems. The determination of ΔH° and ΔS° values renders easier understanding of the factors determining stereoselectivity in the above systems. The stereoselectivity may be explained in terms of non-covalent interactions between side-chain residues. The role played by the histidine carboxylate in the molecular recognition of amino acids is also discussed.

Biological systems show a high specificity in interaction between macromolecules. Receptors,[1] antibodies,[2] and enzymes[3,4] must all recognize their reaction partners, often in the presence of quite similar structures, before they are able to proceed with their functions. Furthermore, to obtain specific molecular recognition, extensive use is made of three-dimensional dissymmetric biomolecules; that is systems which are chiral. To analyse the structural features applied by nature to accomplish highly specific molecular recognition,[5] and to simulate these features by synthesis, organic chemists have synthesized an impressive variety of model systems,[6] leading to the development of a new branch of chemistry, biomimetic chemistry.[7–9] In this context new and revised concepts and approaches have been used. The chemist is now familiar with 'host–guest' relationships,[10–13] preorganized systems,[14] supramolecular species,[15] abiotic receptors,[16] *etc.*

In spite of the fascinating results obtained to date in synthesis and catalysis, no comparable success has been achieved in the quantitative elucidation, in classical chemical and physical terms, of the nature and character of the forces determining specific biological interactions using model systems. Several types of attractive forces between individual molecules can contribute to the interactions[17] occurring in biopolymer systems. These, usually called weak non-covalent bonds,[18] include Coulomb and van der Waals forces, hydrogen bonds, and hydrophobic interactions.

The contribution these forces make to the events allowing the mutual recognition of two molecular species and leading to a specific association appears extremely intriguing. The lack of progress can be explained first by the fact that this binding, a highly complex process, represents the outcome of a large number of simultaneously occurring molecular processes and secondly by the fact that efforts have mainly been focused on non-covalent bonds involving organic molecules.

In biological systems inorganic elements are essential and it has become increasingly evident that weak non-covalent interactions are often metal cation mediated.[19,20] The role of metal complexes in specific molecular recognition has been shown when the interactions involve nucleic acids and proteins.[21–24] Furthermore, in ligand-exchange chromatography, a chiral ligand bonded to the stationary phase and co-ordinated to a transition-metal ion has been used as a resolving agent for enantiomeric mixtures.[25,26] Recently, significant thermodynamic stereoselectivity in substitution labile complexes of metal ions has been found and attributed to the presence of non-covalent intramolecular interactions, steric factors involving different co-ordination geometry, or solvent interaction.[27] Usually these hypotheses have been put forward on the basis of the different stability constants between complexes with homochiral and heterochiral ligands,[28,29] c.d. (circular dichroism) spectral magnitude enhancements, and astatistical stability enhancement.[30] Studies concerning the stereoselectivity of copper(II) ternary complexes of D- or L-histidine and bidentate L-amino acids, bearing aliphatic or aromatic side chains, reveal two distinct trends, as indicated by their stability constants.[31,32] When the second amino acid contains an aromatic ring, *e.g.* L-phenylalanine or L-tryptophan, the *meso* ternary complex is significantly more stable than that containing ligands of the same chirality. With L-alanine, L-valine, and L-leucine, stereoselectivity is insignificant or tends to favour the optically active mixed complex.

The role of different non-covalent forces (electrostatic, hydrophobic, *etc.*) on the thermodynamic stereoselectivity of proton and metal complex formation involving biofunctional ligands has recently been elucidated on the basis of ΔH° and ΔS° values.[33–37] Extending the above approach, the present copper(II)–D- or L-histidine–L-amino acid systems were reviewed to bring to light the driving forces of chiral recognition and to elucidate the reasons for the two different trends by determining the ΔH° and ΔS° values of complex formation by direct calorimetry (25 °C and I = 0.1 mol dm^{-3} KNO_3) after carefully checking the stability constant data already available.

Table 1. Experimental details of potentiometric and calorimetric titrations at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)[a]

System	Analytical concentrations (mmol dm^{-3})				No. of titrations[b]	pH Range
	Cu^{II}	His	aa	hist		
Gly	—	—	5.0—12.0	—	8	2.0—11.0
Cu,Gly	3.5—5.0	—	10.0—11.0	—	4	2.5—6.0
Cu,Gly,L-His	6.0—8.0	7.0—7.5	7.5—8.5	—	6	3.0—5.5
Cu,Gly,D-His	6.0—8.0	7.0—7.5	7.5—8.5	—	6	3.0—5.5
Cu,L-Ala,L-His	5.0—7.0	5.9—7.9	7.6—9.2	—	3	3.3—7.0
Cu,L-Ala,D-His	5.0—7.0	4.7—6.3	7.2—10.2	—	4	3.3—7.0
Cu,L-Val,L-His	5.0	4.5—5.7	5.9—7.8	—	6	3.3—7.0
Cu,L-Val,D-His	5.0	4.6—5.7	6.0—7.9	—	8	3.3—7.0
Cu,L-Leu,L-His	5.0—7.0	4.0—5.5	4.1—8.4	—	13	3.6—4.0
Cu,L-Leu,D-His	5.0—7.0	4.0—5.9	5.7—8.3	—	13	2.8—6.4
Cu,L-Phe,L-His	5.0—7.0	4.0—6.2	6.1—9.2	—	4	2.9—7.0
Cu,L-Phe,D-His	5.0—7.0	4.0—6.0	5.8—9.1	—	5	2.9—7.0
Cu,L-Trp,L-His	5.0	3.8—4.5	5.5—6.6	—	9	2.9—6.0
Cu,L-Trp,D-His	5.0	4.0—4.5	5.5—6.6	—	8	2.9—6.0
Cu,L-Ala,hist	6.0—8.0	—	7.0—8.5	5.5—7.5	8	3.0—6.5
Cu,L-Phe,hist	3.0—8.0	—	7.0—8.5	5.5—7.5	8	3.0—6.5

[a] Titrant: 0.1 mol dm^{-3} NaOH. [b] 35—70 Points for each titration curve.

In addition, copper(II) complexes of histamine [4-(2′-aminoethyl)imidazole] with L-alanine or L-phenylalanine were also investigated under the same experimental conditions, in order to gain information on the set of donor atoms; in particular, to ascertain whether the carboxylic group of histidine is involved in complex formation or not.

Experimental

Materials.—L-Histidine (L-His), L-tryptophan (L-Trp), glycine (Gly), L-leucine (L-Leu) (Aldrich), D-histidine (D-His), L-valine (L-Val) (Sigma), L-alanine (L-Ala), L-phenylalanine (L-Phe) (Merck), and histamine (hist) (Fluka) were all high-purity products used without further purification. Their purity was checked by means of potentiometric titrations with standard NaOH solution and always proved higher than 99.8%. Polarimetric tests gave substantially identical results. Copper(II) nitrate was a Merck product 'reinst'. The concentration of stock solutions of this salt was determined by ethylenediaminetetraacetate titration. Stock solutions of HNO_3 and NaOH were made up from concentrated HNO_3 (Suprapur Merck) and from Normex C. Erba vials, respectively. Their concentrations were determined potentiometrically by titrating with tris(hydroxymethyl)methylaminomethane and potassium hydrogenphthalate, respectively. All solutions were prepared with CO_2-free freshly distilled (four times) water. The ionic strength was adjusted to 0.10 mol dm^{-3} by adding KNO_3 (Suprapur Merck). Grade A glassware was employed throughout.[3]

Procedure.—Potentiometric titrations were performed with the following equipment: (*i*) a Radiometer PHM-64 potentiometer (resolution 0.1 mV, accuracy 0.2 mV) provided with Metrohm EA 109H glass and Metrohm EA 404 saturated calomel electrodes; (*ii*) a Mettler DV10 motor burette (resolution 0.001 cm^3, accuracy 0.002 cm^3) provided with a DV205 dispenser unit (delivery volume 5 cm^3). The potentiometer and burette were interfaced with an IBM-PC computer with which the titrations were performed automatically. Constant-speed magnetic stirring was applied throughout. The temperature of the titration cell was kept at 25 ± 0.02 °C by means of a Haake F3C circulation thermostat. UPP grade nitrogen, previously saturated with water (0.1 mol dm^{-3} KNO_3), was bubbled through all the test solutions in order to maintain an inert atmosphere. The electrode couple was standardized on the pH = $-\log c_{H^+}$ scale by titrating HNO_3 (0.01—0.005 mol dm^{-3}) with standard KOH (0.1 mol dm^{-3}) at 25 °C and I = 0.1 mol dm^{-3} (KNO_3). Aliquots (20 cm^3) containing suitable amounts of copper(II), of HNO_3, and of the two ligands were titrated with standard KOH solutions until a precipitate or opalescence was just observed in the titration cell. Experimental details are reported in Table 1. The ΔH° and ΔS° values were determined by titration calorimetry with a Tronac model 450 isoperibol calorimeter equipped with a 25-cm^3 reaction vessel. The calorimetric measurements were carried out using a buffer solution of bidentate amino acids to titrate solutions containing the metal ion and the His ligand in a 1:1 ratio at the pH of the maximum degree of formation of the main complex species [Cu(HisO)(aaO)] (aa is the second amino acid). The ionic strength was maintained constant at I = 0.1 mol dm^{-3} by adding KNO_3. The ligand concentrations ranged from 0.005 to 0.01 mol dm^{-3}. For each system at least 120 experimental points were utilized to calculate the thermodynamic quantities. The reaction heats, corrected for the dilution heats determined by separate experiments, were calculated by considering the calorie as equivalent to 4.184 J. Experimental details are reported in Table 1. Other details were as previously reported.[3,8]

Calculations.—The calculations concerning the E° of the electrode system and the purity of the ligands were performed

Table 2. Thermodynamic parameters for proton and simple copper(II) complex formation of amino acids and histamine at 25 °C and I = 0.1 mol dm^{-3}, used in the calculation of ternary complex formation constants

Reaction	$-\Delta G^{\circ}$ (kcal mol^{-1})	$-\Delta H^{\circ}$ (kcal mol^{-1})	ΔS° (cal K^{-1} mol^{-1})
$Cu^{2+} + OH^- \rightleftharpoons [Cu(OH)]^+$	8.18[a]	5.0[a]	11
$2Cu^{2+} + 2OH^- \rightleftharpoons [Cu_2(OH)_2]^{2+}$	22.75[a]	8.5[a]	48
$3Cu^{2+} + 4OH^- \rightleftharpoons [Cu_3(OH)_4]^{2+}$	45.66[a]	28[a]	59
$H^+ + GlyO^- \rightleftharpoons Gly$	13.06[b]	10.2[c]	9.6
$2H^+ + GlyO^- \rightleftharpoons HGly^+$	16.28[b]	11.3[c]	16.7
$Cu^{2+} + GlyO^- \rightleftharpoons [Cu(GlyO)]^+$	11.06[b]	6.0[d]	17.0
$Cu^{2+} + 2GlyO^- \rightleftharpoons [Cu(GlyO)_2]$	20.39[b]	12.6[d]	26.1
$H^+ + AlaO^- \rightleftharpoons Ala$	13.32[e]	10.75[e]	8.6
$2H^+ + AlaO^- \rightleftharpoons HAla^+$	16.55[e]	11.5[e]	16.9
$Cu^{2+} + AlaO^- \rightleftharpoons [Cu(AlaO)]^+$	11.15[e]	5.47[e]	19.0
$Cu^{2+} + 2AlaO^- \rightleftharpoons [Cu(AlaO)_2]$	20.39[e]	12.0[e]	28.1
$H^+ + ValO^- \rightleftharpoons Val$	13.04[f]	10.99[c]	6.9
$Cu^{2+} + ValO^- \rightleftharpoons [Cu(ValO)]^+$	10.98[f]	5.56[c]	18.2
$Cu^{2+} + 2ValO^- \rightleftharpoons [Cu(ValO)_2]$	20.33[f]	11.78[c]	28.7
$H^+ + LeuO^- \rightleftharpoons Leu$	13.23[f]	10.8[g]	8.2
$2H^+ + LeuO^- \rightleftharpoons HLeu^+$	16.34[f]		
$Cu^{2+} + LeuO^- \rightleftharpoons [Cu(LeuO)]^+$	11.28[f]	5.6[g]	19.1
$Cu^{2+} + 2LeuO^- \rightleftharpoons [Cu(LeuO)_2]$	20.69[f]	11.5[g]	30.8
$H^+ + PheO^- \rightleftharpoons Phe$	12.39[h]	11.2[h]	3.7
$2H^+ + PheO^- \rightleftharpoons HPhe^+$	15.45[h]	12.7[h]	8.7
$Cu^{2+} + PheO^- \rightleftharpoons [Cu(PheO)]^+$	10.60[h]	5.5[h]	17
$Cu^{2+} + 2PheO^- \rightleftharpoons [Cu(PheO)_2]$	20.0[h]	12.5[h]	25
$H^+ + TrpO^- \rightleftharpoons Trp$	12.78[e]	10.68[e]	7.1
$2H^+ + TrpO^- \rightleftharpoons HTrp^+$	16.07[e]	11.8[e]	14.3
$Cu^{2+} + TrpO^- \rightleftharpoons [Cu(TrpO)]^+$	11.31[e]	5.86[e]	18.3
$Cu^{2+} + 2TrpO^- \rightleftharpoons [Cu(TrpO)_2]$	21.03[e]	13.4[e]	26.5
$H^+ + HisO^- \rightleftharpoons HHisO$	12.40[i]	10.53[i]	6.3
$2H^+ + HisO^- \rightleftharpoons H_2HisO^+$	20.63[i]	17.48[i]	10.6
$3H^+ + HisO^- \rightleftharpoons H_3HisO^{2+}$	23.1[i]	18.2[i]	16.4
$Cu^{2+} + HisO^- \rightleftharpoons [Cu(HisO)]^+$	13.85[i]	10.6[i]	10.9
$Cu^{2+} + HisO^- + H^+ \rightleftharpoons [Cu(HisO)H]^{2+}$	19.33[i]	13.8[i]	18.5
$Cu^{2+} + 2HisO^- \rightleftharpoons [Cu(HisO)_2]$	24.73[i]	19.6[i]	17.2
$Cu^{2+} + 2HisO^- + H^+ \rightleftharpoons [Cu(HisO)_2H]^+$	32.6[i]	25.4[i]	24
$Cu^{2+} + 2HisO^- + 2H^+ \rightleftharpoons [Cu(HisO)_2H_2]^{2+}$	37.0[i]	27[i]	34
$2Cu^{2+} + 2HisO^- \rightleftharpoons [Cu_2(HisO)_2H_{-2}] + 2H^+$	10.95[i]	3.6[i]	25
$Cu^{2+} + 2HisO^- \rightleftharpoons [Cu(HisO)_2H_{-1}]^- + H^+$	9.28[i]	9[i]	1
$H^+ + hist \rightleftharpoons Hhist^+$	13.36[i]	12.15[i]	4.1
$2H^+ + hist \rightleftharpoons H_2hist^{2+}$	21.64[i]	19.67[i]	6.6
$Cu^{2+} + hist \rightleftharpoons [Cu(hist)]^{2+}$	13.04[i]	12.1[i]	3.2
$Cu^{2+} + hist + H^+ \rightleftharpoons [Cu(hist)H]^{3+}$	17.53[i]	17.9[i]	−1
$Cu^{2+} + 2hist \rightleftharpoons [Cu(hist)_2]^{2+}$	21.98[i]	22.2[i]	−0.7
$Cu^{2+} + 2hist + H^+ \rightleftharpoons [Cu(hist)_2H]^{3+}$	29.8[i]	29.0[i]	3
$2Cu^{2+} + 2hist \rightleftharpoons [Cu_2(hist)_2H_{-2}] + 2H^+$	10.15[i]	8.8[i]	4
$Cu^{2+} + 2hist \rightleftharpoons [Cu(hist)_2H_{-1}]^+ + H^+$	7.34[i]	9.8[i]	−8

[a] G. Arena, R. Cali, E. Rizzarelli, and S. Sammartano, *Thermochim. Acta*, 1976, **16**, 315. [b] This work. [c] M. C. Lim and G. H. Nancollas, *Inorg. Chem.*, 1971, **10**, 1957. [d] T. P. I and G. H. Nancollas, *Inorg. Chem.*, 1972, **11**, 2414. [e] Ref. 47. [f] Ref. 32. [g] J. L. Meyer and J. E. Bauman, jun., *J. Chem. Eng. Data*, 1970, **15**, 404. [h] Ref. 48. [i] Ref. 49.

by the computer program ESAB,[39] which refines the parameters of an acid–base titration by using a non-linear least-squares method minimizing the function $U = \Sigma(V_{i,\text{exptl.}} - V_{i,\text{calc.}})^2$, and BEATRIX[40] based on the Gran method. The formation constants of the copper(II) complexes were calculated by means of the least-squares computer programs MINIQUAD[41] and SUPERQUAD,[42] which gave results in excellent agreement. To obtain the species distribution within the pH ranges explored the computer program DISDI[43] was used. This procedure allowed us to choose the 'best' conditions under which to run the calorimetric experiments. Complex formation heats were calculated by the least-squares computer program DOEC.[44] Throughout, errors are expressed as three times the standard deviation (3σ) where σ is the standard deviation between observed and calculated values of all points used to obtain the reported thermodynamic parameters. Table 2 lists the thermodynamic parameters of protonation and of simple complex formation for the ligands investigated which were used to obtain the ΔG°, ΔH°, and ΔS° values of mixed-ligand complexes.

Table 3. Thermodynamic parameters for the formation of copper(II) ternary complexes of L/D-histidine or histamine and some amino acids at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)[a]

Reaction	log β	$-\Delta G^{\circ}$ (kcal mol^{-1})	$-\Delta H^{\circ}$ (kcal mol^{-1})	ΔSfi (cal K^{-1} mol^{-1})
Cu^{2+} + GlyO$^-$ + L-HisO$^-$ ⇌ [Cu(GlyO)(L-HisO)]	17.66(2)	24.08(9)	15.65(3)	28.3(1)
Cu^{2+} + GlyO$^-$ + L-HisO$^-$ + H^+ ⇌ [Cu(GlyO)(L-HisO)H]$^+$	21.65(3)			
Cu^{2+} + GlyO$^-$ + D-HisO$^-$ ⇌ [Cu(GlyO)(D-HisO)]	17.66(2)	24.08(9)	15.64(3)	28.3(2)
Cu^{2+} + GlyO$^-$ + D-HisO$^-$ + H^+ ⇌ [Cu(GlyO)(D-HisO)H]$^+$	21.65(3)			
Cu^{2+} + L-AlaO$^-$ + L-HisO$^-$ ⇌ [Cu(L-AlaO)(L-HisO)]	17.80(2), 17.83,[b] 16.052[c]	24.27(9)	15.20(9)	30.4(6)
Cu^{2+} + L-AlaO$^-$ + L-HisO$^-$ + H^+ ⇌ [Cu(L-AlaO)(L-HisO)H]$^+$	21.88(3), 21.77[c]			
Cu^{2+} + L-AlaO$^-$ + D-HisO$^-$ ⇌ [Cu(L-AlaO)(D-HisO)]	17.76(2)	24.21(9)	15.13(6)	30.5(2)
Cu^{2+} + L-AlaO$^-$ + D-HisO$^-$ + H^+ ⇌ [Cu(L-AlaO)(D-HisO)H]$^+$	21.89(3)			
Cu^{2+} + L-ValO$^-$ + L-HisO$^-$ ⇌ [Cu(L-ValO)(L-HisO)]	17.92(3), 18.01,[b] 17.603[d]	24.43(4)	15.75(6)	29.1(2)
Cu^{2+} + L-ValO$^-$ + L-HisO$^-$ + H^+ ⇌ [Cu(L-ValO)(L-HisO)H]$^+$	21.51(3)			
Cu^{2+} + L-ValO$^-$ + D-HisO$^-$ ⇌ [Cu(L-ValO)(D-HisO)]	17.80(2), 17.546[d]	24.27(4)	15.29(3)	30.1(1)
Cu^{2+} + L-ValO$^-$ + D-HisO$^-$ + H^+ ⇌ [Cu(L-ValO)(D-HisO)H]$^+$	21.1(3)			
Cu^{2+} + L-LeuO$^-$ + L-HisO$^-$ ⇌ [Cu(L-LeuO)(L-HisO)]	17.79(1), 17.692[d]	24.26(2)	15.50(3)	29.4(1)
Cu^{2+} + L-LeuO$^-$ + L-HisO$^-$ + H^+ ⇌ [Cu(L-LeuO)(L-HisO)H]$^+$	21.4(1), 22.19[d]			
Cu^{2+} + L-LeuO$^-$ + D-HisO$^-$ ⇌ [Cu(L-LeuO)(D-HisO)]	17.74(1), 17.66[d]	24.18(2)	15.13(6)	30.4(2)
Cu^{2+} + L-LeuO$^-$ + D-HisO$^-$ + H^+ ⇌ [Cu(L-LeuO)(D-HisO)H]$^+$	21.3(1), 22.2[d]			
Cu^{2+} + L-PheO$^-$ + L-HisO$^-$ ⇌ [Cu(L-PheO)(L-HisO)]	17.53(1), 17.504[d]	23.90(2)	15.55(4)	28.0(1)
Cu^{2+} + L-PheO$^-$ + L-HisO$^-$ + H^+ ⇌ [Cu(L-PheO)(L-HisO)H]$^+$	21.52(3), 21.44[d]			
Cu^{2+} + L-PheO$^-$ + D-HisO$^-$ ⇌ [Cu(L-PheO)(D-HisO)]	17.70(1), 17.699[d]	24.14(2)	15.93(4)	27.5(1)
Cu^{2+} + L-PheO$^-$ + D-HisO$^-$ + H^+ ⇌ [Cu(L-PheO)(D-HisO)H]$^+$	21.55(6), 21.45[d]			
Cu^{2+} + L-TrpO$^-$ + L-HisO$^-$ ⇌ [Cu(L-TrpO)(L-HisO)]	18.29(1), 18.003[d]	24.94(2)	17.26(5)	25.8(2)
Cu^{2+} + L-TrpO$^-$ + L-HisO$^-$ + H^+ ⇌ [Cu(L-TrpO)(L-HisO)H]$^+$	21.83(6)			
Cu^{2+} + L-TrpO$^-$ + D-HisO$^-$ ⇌ [Cu(L-TrpO)(D-HisO)]	18.75(1), 18.475[d]	25.57(2)	18.68(6)	23.1(2)
Cu^{2+} + L-TrpO$^-$ + D-HisO$^-$ + H^+ ⇌ [Cu(L-TrpO)(D-HisO)H]$^+$	21.88(9)			
Cu^{2+} + L-AlaO$^-$ + hist ⇌ [Cu(hist)(L-AlaO)]$^+$	17.05(1)	23.25(2)	18.41(3)	16.2(1)
Cu^{2+} + L-PheO$^-$ + hist ⇌ [Cu(hist)(L-PheO)]$^+$	17.10(1)	23.32(3)	18.89(3)	14.8(1)

[a] 3σ in parentheses. [b] O. Yamauchi, T. Takaba, and T. Sakurai, *Bull. Chem. Soc. Jpn.*, 1980, **53**, 106. [c] Ref. 30. [d] Ref. 32.

Results and Discussion

The stability constants of the mixed-ligand complexes are reported in Table 3. These values show the same trends previously found although the differences with respect to those reported in the literature are slightly higher than the confidence limits. Thermodynamic stereoselectivity is present in the mixed neutral species; the protonated species show the same values of the formation constants. However, owing to the small percentages of formation of the latter species, we were able to determine the other thermodynamic parameters of the neutral species only.

The ΔG°, ΔH°, and ΔS° values pertinent to the equilibrium Cu^{2+} + HisO$^-$ + aaO$^-$ ⇌ [Cu(HisO)(aaO)] are reported in Table 3. The formation of ternary complexes is enthalpically and entropically favoured. The involvement of both nitrogen and oxygen atoms in the co-ordination can be inferred by comparing the data concerning the above equilibrium with the corresponding thermodynamic data for mixed-ligand complex formation with histamine (Table 3). The formation of the [Cu(hist)(AlaO)]$^+$ and [Cu(hist)(PheO)]$^+$ species is enthalpically and entropically favoured much more than that of the previously investigated simple [Cu(hist)]$^{2+}$ complex (see Table 2), where two nitrogen atoms are involved in co-ordination. The difference in enthalpy contribution between the mixed-ligand complexes and the [Cu(hist)]$^{2+}$ complex implies that one additional nitrogen is bonded in the mixed-ligand complex, since CO_2^--bonding is near zero or endothermic in ΔH°. The large difference in ΔS° between the two sets of systems cannot be due to nitrogen bonding, but to CO_2^--co-ordination. Therefore, these differences imply that the alaninate and phenylalaninate anions are co-ordinated in a bidentate fashion.

We may compare the co-ordination features of copper(II) mixed-ligand complexes of histamine presented above with those for HisO$^-$. Specifically, from a comparison of the ΔH° and ΔS° values for [Cu(AlaO)(HisO)] and [Cu(PheO)(HisO)] complexes (Table 3) with those obtained for the analogous histamine species (Table 3) it can be concluded that all the potential donor atoms are engaged in the co-ordination sphere of the copper(II) ion, as explained below. Although the histamine complexes are less stable than those of HisO, the change in enthalpy is more favourable for the former. The fact that histamine lacks a carboxylic group, where bonding involves an endothermic contribution, may account for the greater enthalpy contribution to bonding with histamine, as well as for the unfavourable entropic contribution due to the inability of histamine to neutralize the metal-ion charge. The differences in thermodynamic parameters are quite similar to those found between the simple [Cu(hist)]$^{2+}$ and [Cu(HisO)]$^+$ species in which the histidinate anion proved to be a tridentate ligand.[38] The tridentate co-ordination of His in ternary copper(II) complexes is also supported by the crystal and molecular structure of [Cu(HisO)(ThrO)] (Thr = threonine),[45] although it should be kept in mind that solution and solid-state structures must always be correlated with care.

The comparison of mixed-ligand copper(II) histamine and His complexes as presented above not only defines the co-ordination set of donor atoms but also allows one to show that

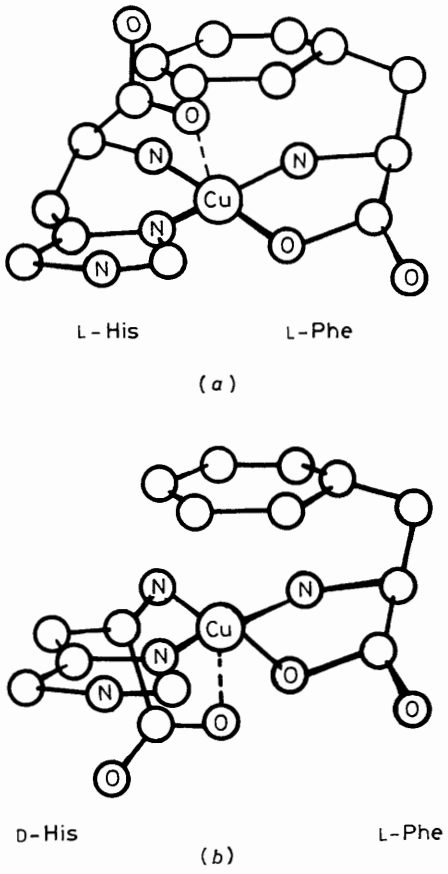

Figure 1. Copper(II) co-ordination mode in ternary complexes with L-histidine (*a*) or D-histidine (*b*) and L-phenylalanine, and hypothesized disposition of side-chain residues

the thermodynamic stereoselectivity is not due to a different metal-ion co-ordination number within the L- or D-His complexes. The differences in ΔH° and ΔS° values between L- and D-His complexes are too small to assume that in one case the copper is four- and in the other five-co-ordinated. It is interesting that the differences in enthalpy and entropy values for L- or D-His complex formation are of the same order as those found between $[Cu(hist)(AlaO)]^{+}$ and $[Cu(hist)(PheO)]^{+}$.

Recently, enhanced stability constants and increased asymmetry of co-ordinated amino acid side chains, determined by means of c.d. spectral magnitudes, have been found in mixed-ligand complexes of copper(II) with diamines and amino acids when both have aromatic rings.[46] This behaviour has been attributed to the formation of an intramolecular non-covalent bonding (stacking interaction) between the side chains of the L-amino acid and the diamine (diam) within the ternary copper(II) complex. In particular, the complexes $[Cu(diam)(aaO)]^{+}$ were stabilized with respect to $[Cu(en)(AlaO)]^{+}$ (en = ethylenediamine) in the order en $\approx$ *N*,*N*′-dibenzylethylenediamine < 1,2-diaminobenzene < histamine $\approx$ 2-aminomethylpyridine < 2,2′-bipyridine < 1,10-phenanthroline as diam and Ala $\approx$ Val < Phe < Tyr < Trp < 5-hydroxy-L-tryptophan as aa. These results may be coupled with those from previous calorimetric studies which showed that solvophobic interactions[17] are favoured on enthalpy grounds;[35,36,47–50] thus one may attribute the more exothermic contribution of the change in enthalpy accompanying the formation of the $[Cu(hist)(PheO)]^{+}$ complex with respect to $[Cu(hist)(AlaO)]^{+}$ to the solvophobic stacking interaction between the imidazole ring and the aromatic residue. The non-covalent interaction is absent in the Ala complex due to the shortness of the side chain. This interpretation opens the way to explaining the trend in thermodynamic stereoselectivity within the copper(II) histidinate complexes. Among the systems investigated, the complexes of D-His are more enthalpically favoured than those with L-His in the case of amino acids with aromatic or heteroaromatic side chains (Phe and Trp), while the opposite behaviour is found for those amino acids with aliphatic residues. It is worth noting that Gly shows the same thermodynamic parameters with L-or D-His, thus suggesting that the presence of a side chain is the decisive factor for the stereoselectivity in the other case.

Assuming that in solution the favoured structure of mixed-ligand copper(II) histidinate complexes is, as found in the solid state, *cis*,[45,51–54] we can see [Figure 1(*a*)] that the aromatic ring of L-Phe or the indole residue of L-Trp can interact with the imidazole group only in the case of D-His. This stacking interaction is not possible with L-His since the co-ordination of the carboxylate group repels the aromatic or heteroaromatic ring of the two amino acids [Figure 1(*b*)]. Consequently, the more exothermic contribution accompanying the formation of D-His complexes with respect to the analogous L-His species, in the case of L-amino acids with aromatic or pseudo-aromatic residues, is most likely due to the occurrence of a non-covalent interaction.

A comparison with the Gly systems in Table 3 indicates that even the small differences in the thermodynamic parameters found for the ternary complexes of the amino acids with aliphatic side chains are significant. In contrast to the results obtained for aromatic amino acids, the enthalpy change is more favourable for the L-His than for the D-His systems. This opposite trend can be explained by hypothesizing the existence of a hydrogen bond between one of the hydrogen atoms of the methyl group of Val or Leu and the oxygens of the carboxylate group of His, as shown in Figure 2. Such intramolecular bonding, previously suggested for organic molecules,[55] entailing a favourable enthalpic contribution to the free-energy variation is only possible for the L-His ternary complexes; in this case only, the carboxylate group and the side-chain residues are on the same side of the co-ordination plane [Figure 2(*a*)], whilst for the D-His systems they are on opposite sides [Figure 2(*b*)].

Conclusion

The tridentate co-ordination exhibited by the His ligand is the key to molecular recognition assisted by copper(II) ions and to the consequent thermodynamic stereoselectivity. The bonding of the carboxylate group in an axial position is the reason for the differences in stability between the L- and D-His copper(II) complexes with a given L-amino-acid. Although the solid-state structures of mixed-ligand copper(II) complexes with L-His and amino acids show a *cis* configuration, in solution and for bidentate amino acids the existence of an equilibrium between the *cis* and the *trans* configuration has been proposed.[56,57] It is reasonable to suppose that the presence of non-covalent forces favours the *cis* configuration. Furthermore, the results obtained reconfirm the diagnostic role of ΔH° and ΔS° values as far as the formation of weak interactions is concerned.[50] In addition, as already found for proton complex thermodynamic stereoselectivity of linear dipeptides,[33,34,37] the thermodynamic approach makes it possible to distinguish between the different weak forces (stacking and hydrogen bonding) which cause molecular recognition. Finally, the present results may be useful in achieving a rationale for chromatographic separation of amino acid enantiomeric mixtures using copper(II) His complexes.[25,58]

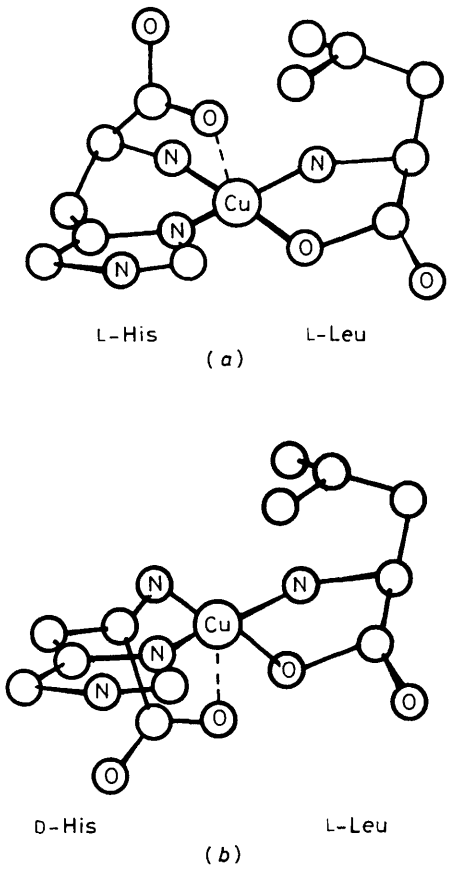

Figure 2. Copper(II) co-ordination mode in ternary complexes with L-histidine (*a*) or D-histidine (*b*) and L-leucine, and hypothesized disposition of side-chain residues

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