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Copper(II) Complexes of Diastereoisomeric Dipeptides in Aqueous Solution. Effect of Side-Chain Groups on the Thermodynamic Stereoselectivity

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A thermodynamic investigation has been carried out on a series of copper(II) complexes of diastereoisomeric couples of dipeptides at 25 °C and I = 0.1 mol dm^{-3} (KNO_3) in aqueous solution. Evidence has been found of thermodynamic stereoselectivity in the formation of the amide deprotonated complexes, with enthalpically favored formation of the complexes of the LL diastereoisomers. The results are discussed with reference to hydrophobic effects and to the structural information obtained by EPR spectra in solution.

Introduction

In a recent review describing the thermodynamic stereoselectivity in metal complexes of amino acids and dipeptides, Pettit et al.[1] reported the higher stabilities of copper(II) complexes of optically active or "pure" deprotonated dipeptides with noncoordinating side chains (LL or DD diastereoisomers) over those of their "mixed" counterparts (LD or DL diastereoisomers). Sigel and Martin[2] earlier observed that upon deprotonation of the species formed between copper(II) ion and some dipeptides with two nonglycyl residue at pH 4 (the coordination at the amide nitrogen occurs according to the equilibrium $[CuL]^+ \rightleftarrows [Cu(LH_{-1})] + H^+$) the corresponding equilibrium acidity constant pK^H_{CuL} is more acidic for the "pure" than for the "mixed" isomers. They suggested that hydrophobic interactions[3] between side chains on the same side of the chelate plane in the LL complex provide a favorable effect compensating for steric inhibition responsible for the high pK^H_{CuL} values found in other peptide systems such as Gly-Leu and Gly-Ile (Gly = glycine, Leu = L-leucine, and Ile = L-isoleucine).

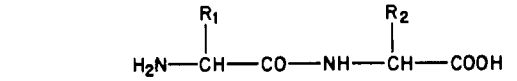
Similar, in some aspects, to the discussion on the thermodynamic origin of the chelate effect,[4] different conclusions have been drawn with respect to the thermodynamic stereoselectivity; i.e., some investigators have ascribed the effect to the entropy loss, while others have suggested an entropy gain as the most important factor in contributing to the enhanced stabilities of copper(II) complexes with LL dipeptides.[5-8]

In particular, Kaneda and Martell[5] stated that a relatively more extensive hydrophobic region results in an unfavorable entropy contribution and in a relative lowering of stability. Nakon and Angelici,[6] on the other hand, suggested that by creating an internal micelle, with a large hydrophobic region, it is possible to decrease an energetically unfavorable solvent–complex interface, leading to the formation of a more stable complex.

Pettit et al.,[7] in agreement with Nakon and Angelici's point of view, hypothesized that the positive stabilization found in the optically active complex could be the result of the hydrophobic interaction between the two side chains, which are close together in the optically active complex but are on opposite sides of the basal plane in the "mixed" complex.

Previously, for similar systems, differences in steric interference between the peptide side chains and coordinated water have been claimed to account for preferential formation of the optically homogeneous complex.[8] While complexes of dipeptides have been studied by using several different experimental methods, all the above suggestions about the driving forces of thermodynamic stereoselectivity have been put forward on the basis of ΔG^0 values only.

Chart I



R_1	R_2	abbrev
-H	-H	Gly-Gly
-H	$-CH_2-CH(CH_3)_2$	Gly-Leu
-H	$-CH_2-C_6H_5$	Gly-Phe
$-CH_3$	$-CH_3$	Ala-Ala
$-CH_3$	$-CH_2-CH(CH_3)_2$	Ala-Leu
$-CH_2-CH(CH_3)_2$	$-CH_2-CH(CH_3)_2$	Leu-Leu
$-CH_2-CH(CH_3)_2$	$-CH(CH_3)-CH_2-CH_3$	Leu-Ile
$-CH_3$	$-CH_2-C_6H_5$	Ala-Phe
$-CH_2-CH(CH_3)_2$	$-CH_2-C_6H_5$	Leu-Phe
$-CH_2-CH(CH_3)_2$	$-CH_2-C_6H_4-OH$	Leu-Tyr
$-CH(CH_3)_2$	$-CH_2-C_6H_5$	Val-Phe

One of the purposes of this study is to determine whether the stereoselectivity effect found in peptide complexes of copper(II) is the result of either enthalpic or entropic stabilization factors or a combination of both. This was accomplished by comparing log K, ΔH^0, and ΔS^0 values for the reaction of this metal with LL alanylalanine (Ala-Ala), alanylphenylalanine (Ala-Phe), leucylleucine (Leu-Leu), leucylphenylalanine (Leu-Phe), leucyltyrosine (Leu-Tyr), leucylisoleucine (Leu-Ile), and valylphenylalanine (Val-Phe) with the corresponding values for the complexes of LD or DL isomers (see Chart I). Such experiments can help in understanding the role played by the noncovalent interaction[9] on the thermodynamic stereoselectivity of copper(II) complexation with this class of biofunctional ligands and give some information about the stereoselectivity effect by itself.

Recently, we have shown that ΔH^0 and ΔS^0 values can be used to recognize the presence of intraligand solvophobic[10] (according to more classical denomination hydrophobic or stacking[3]) interactions between two aromatic or heteroaromatic groups of bio-

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functional molecules coordinated to metal ions.[11-13]

The thermodynamic approach has also been used in order to evaluate the role played by electrostatic and solvophobic interactions, respectively, in the stereoselectivity of the proton complex formation of the LL dipeptides with respect to its LD diastereoisomers.[14,15]

Extending the above-mentioned approach to the copper(II) complex formation of diastereoisomeric dipeptides, we thought that the comparison between the thermodynamic parameters concerning the LL or LD isomers would be meaningful if the nature of the final complexes with "pure" or "mixed" dipeptidic ligands is similar; namely, the number of solvent molecules in the primary coordination sphere (bound to metal) is the same in both complexes.

To obtain information about the geometry and the coordination number of the investigated complexes and, then, to distinguish between noncovalent contributions[9] and solvation effects, we have also carried out EPR studies on the main species formed by the copper(II) ion with the above-cited ligands and other dipeptides such as glycylglycine (Gly-Gly), glycyl-L-leucine (Gly-Leu), glycyl-L-phenylalanine (Gly-Phe), L-alanyl-L-leucine, and L-alanyl-D-leucine (Ala-Leu). The usefulness of the contemporary thermodynamic and spectroscopic approach was shown for copper(II) mixed-complex formation.[16-19]

Experimental Section

Materials. Dipeptides L-alanyl-L-phenylalanine, L-alanyl-L-leucine, L-leucyl-L-leucine, L-leucyl-D-leucine, glycylglycine, glycyl-L-phenylalanine, glycyl-L-leucine, and D-alanyl-L-leucine were purchased from Sigma (Munich, FRG) and L-alanyl-L-alanine, L-alanyl-D-alanine, L-leucyl-L-tyrosine, L-leucyl-D-tyrosine, L-valyl-L-phenylalanine, and L-leucyl-L-phenylalanine from Serva (Heidelberg, FRG). L-Alanyl-D-phenylalanine, L-leucyl-D-phenylalanine, L-leucyl-L-isoleucine, D-leucyl-L-isoleucine, and L-valyl-D-phenylalanine were synthesized by us as reported elsewhere.[20] Before sample solutions were prepared, all peptides were dried over phosphorus pentoxide in a vacuum desiccator for at least 1 day. Nevertheless, owing to their hygroscopicity, during the solution preparation, dipeptides absorbed an indeterminable water amount. In order to check their water content, the ACBA program was used (see Calculations). $Cu(NO_3)_2$ was prepared from basic copper(II) carbonate by adding a slight HNO_3 excess. The stock solution titer was determined by titration with EDTA.

Procedure. Potentiometric measurements were carried out by using an Orion 801 A meter equipped with an E1L glass and an Ingold saturated calomel electrode, the potentiometer being connected with an Amel timer-printer (Model 882) controlling the addition of the titrant (KOH) delivered from an Amel digital dispenser (Model 232) and the titration being performed automatically. The electrode couple was standardized on the pH $\equiv -\log C_{H^+}$ scale by titrating HNO_3 (0.01–0.005 mol dm^{-3}) with KOH at 25 °C and I = 0.1 mol dm^{-3} (KNO_3). Titrations were carried out at a metal to ligand ratio of 1:1 and with a range of ligand concentrations between 0.002 and 0.008 mol dm^{-3}, in the pH range 3.0–6.0. All titrations were performed in triplicate. Other details were as previously reported.[21] log K values for Ala-Ala, Leu-Leu, and Leu-Tyr systems are those determined by Angelici et al.[6] under the same experimental conditions. ΔH^0 and ΔS^0 values were determined by titration calorimetry with a Tronac Model 450 isoperibol calorimeter equipped with a 25-mL reaction vessel. The calorimetric measurements were carried out by titrating with HNO_3 (0.2–0.4 mol dm^{-3}) solutions containing the metal and the ligand in a 1:1 ratio at the pH of the maximum formation degree of the main complex species [$CuLH_{-1}$]. The ionic strength was maintained constant at the value of 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 150 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 unit equivalent to 4.184 J. Other experimental details were as previously reported.[21]

Spectroscopic Measurements. EPR spectra were measured with a conventional X-band spectrometer (Bruker Model 220 D) operating at 9.3–9.5 GHz and using 100-kHz field modulation and a 10-in. electromagnet. Quartz tubes were used for frozen solutions, while a Bruker quartz water solution cell was employed to collect room-temperature spectra. A low-temperature unit was used to achieve the temperature of 150 K. The microwave frequency was calibrated with the use of powdered DPPH samples (g = 2.0036), while the magnetic field was carefully measured during any spectrum scan by means of a Bruker gauss meter, type ER 035 M.

Solutions 0.005 mol dm^{-3} in $^{63}Cu^{II}(NO_3)_2$ (in 95% water–5% methanol) and the dipeptide at pH 6.0 were used to record the EPR spectra. The amount of methanol was a compromise between the purpose of obtaining well-resolved spectra and the necessity of not varying the proportion of water in favor of an organic solvent. Many spectra were also run on samples of the same complexes with the amount of organic solvent (methanol, ethanol, n-propyl alcohol) varying up to 80%.

Calculations. The calculations concerning the E° of the electrode system, the purity of the ligands, and the HNO_3 excess amount in the metal ion stock solutions were performed by the least-squares computer program ACBA,[22] which refines the parameters of an acid–base titration by using a nonlinear least-squares method minimizing the function $U = \sum(v - v_{calcd})^2$. The calculations concerning the formation constants of the copper(II) complexes were performed by the least-squares computer program MINIQUAD.[23] To obtain the species distribution with respect to the pH change (see discussion on calorimetric titrations), we utilized the computer program DISDI.[24] The heats of the complex formations were calculated by the least-squares computer program DOEC.[25]

Throughout the paper, the errors are expressed as 3 times the standard deviation or as uncertainty ranges (maximum deviations from the mean).

In Table I the thermodynamic parameters of protonations of dipeptides elsewhere reported[14,15,20] are listed.

Table I. Thermodynamic Parameters of Proton Complex Formation of Diastereoisomeric Dipeptides at 25 °C and I = 0.10 mol dm^{-3} (KNO_3)

ligand	$-\Delta G^0$, kcal mol^{-1}		$-\Delta H^0$, kcal mol^{-1}		ΔS^0, cal mol^{-1} deg^{-1}		ref
	NH_2	CO_2^-	NH_2	CO_2^-	NH_2	CO_2^-	
L-Ala-L-Ala	11.14	4.50	10.64	−0.26	1.7	15.9	*a*
L-Ala-D-Ala	11.34	4.34	10.26	−0.63	3.6	16.7	*a*
L-Leu-L-Leu	10.78	4.71	10.21	−0.36	1.9	17.0	*a*
L-Leu-D-Leu	11.18	4.16	10.92	−0.77	0.9	16.5	*a*
L-Leu-L-Ile	10.60	4.66	10.69	−0.41	−0.3	17.0	*c*
D-Leu-L-Ile	11.02	4.17	10.84	−0.72	0.6	16.4	*c*
L-Ala-L-Phe	10.82	4.28	10.51	−0.05	1.0	14.5	*b*
L-Ala-D-Phe	11.17	4.03	10.64	−0.39	1.8	14.6	*b*
L-Val-L-Phe	10.48	4.36	10.78	−0.02	−1.0	14.7	*c*
L-Val-D-Phe	11.00	3.93	10.89	−0.32	0.4	14.2	*c*
L-Leu-L-Phe	10.50	4.34	10.42	−0.08	0.3	14.3	*b*
L-Leu-D-Phe	11.11	3.94	11.0	−0.1	0.3	13.4	*b*
L-Leu-L-Tyr	10.68	4.41	10.43	0.0	1.0	14.6	*a*
L-Leu-D-Tyr	11.32	4.03	11.15	−0.1	0.6	13.9	*a*

[a] Reference 14. [b] Reference 15. [c] Reference 20.

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Table II. Spin-Hamiltonian Parameters for Copper(II) Dipeptide Complexes in Water–Methanol (95%–5%) Mixtures at 150 K and Room Temperature[d]

ligand		$g_\parallel$ (±0.001)	$A_\parallel$ (±1)	$g_\perp$ (±0.002)[a]	$A_\perp$ (±2)[a]	g_{iso} (±0.001)	A_{iso} (±0.5)	$g_\perp$ (±0.005)[b]	$A_\perp$ (±2)[c]
Ala-Ala	LL	2.246	182	2.050	15	2.118	70.6	2.053	15
	LD	2.246	182	2.050	15	2.118	70.6	2.053	15
Ala-Leu	LL	2.248	183	2.046	15	2.117	72.5	2.052	17
	LD	2.247	18	2.049	15	2.117	72.1	2.053	17
Leu–Leu	LL	2.244	184	2.047	15	2.117	72.5	2.051	17
	LD	2.245	178	2.049	15	2.117	71.3	2.051	18
Leu-Ile	LL	2.243	186	2.046	15	2.117	71.8	2.051	15
	DL	2.246	182	2.049	14	2.117	71.4	2.050	16
Ala-Phe	LL	2.240	187	2.045	15	2.117	72.9	2.050	16
	LD	2.243	183	2.043	15	2.117	72.3	2.051	17
Leu-Phe	LL	2.237	187	2.045	15	2.117	72.5	2.050	15
	LD	2.241	184	2.044	15	2.117	72.3	2.051	16
Leu-Tyr	LL	2.238	187	2.045	15	2.116	72.4	2.052	15
	LD	2.240	185	2.046	15	2.116	72.2	2.052	16
Val-Phe	LL	2.239	187	2.045	15	2.116	72.1	2.051	15
	LD	2.241	185	2.046	15	2.116	72.3	2.052	16
Gly-Gly		2.249	179	2.052	11	2.121	67.4	2.055	12
Gly-Leu		2.250	180	2.049	12	2.119	68.2	2.054	12
Gly-Phe		2.242	184	2.046	15	2.116	71.3	2.053	15

[a] $g_\perp$ and $A_\perp$ values calculated by means of the extra-peak field. [b] $g_\perp$ calculated in the usual way by taking the field at half of the peak-to-peak distance. [c] $A_\perp$ calculated by making use of the relation $A_{iso} = {}^1/_3(A_\parallel + 2A_\perp)$. [d] All hyperfine coupling constants are expressed in units of 10^4 cm^{-1}. Errors are given as confidence limits for $P = 0.01$ (Hellbronner, E. *J. Chem. Educ.* **1979**, *56*, 240).

In order to evaluate small differences among the magnetic parameters of these LL- and LD-dipeptide–copper(II) complexes, isotopically pure ^{63}Cu was employed. None of these complexes showed parallel nitrogen superhyperfine patterns even if two nitrogens were surely involved in the first coordination sphere of these complexes. $g_\parallel$ and $A_\parallel$ values were taken directly from the experimental spectra recorded in an enlarged scale (generally 400 G; a ratio of 1.07 G mm^{-1} allows determination of the position of an extremum with a confidence limit of less than ±1 G). The perpendicular part was very often complicated by the appearence of somewhat well resolved nitrogen hyperfine structure. $g_\perp$ and $A_\perp$ values were then calculated by exploiting the presence of an overshoot in all the spectra as explained in two previous papers.[26,27]

Table II summarizes the spin-Hamiltonian parameters found for these complexes. $g_\perp$ values calculated in the usual way and $A_\perp$ values calculated from the relation $A_\perp = (3A_{iso} - A_\parallel)/2$ were added for a comparison.

Results and Discussion

The generalized overall formation reaction of copper(II) ions or protons with peptide ligands is given in eq 1, where L is the negative species of the peptide ligands. Charges on the ligand

$$mCu^{2+} + lL + hH^+ \overset{\beta_{mlh}}{\rightleftharpoons} [Cu_m(L_lH_h)] \quad (1)$$

and the copper(II) complexes are omitted for clarity in notation. The stability constant β_{mlh} is defined by eq 2. The equilibria

$$\beta_{mlh} = \frac{[Cu_m(L_lH_h)]}{[Cu^{2+}]^m[L]^l[H]^h} \quad (2)$$

needed to fit the experimental titration curves for solutions of copper(II) and peptide ligands under study are given by eq 3–6.

$$Cu^{2+} + L + H^+ \overset{\beta_{111}}{\rightleftharpoons} [Cu(LH)] \quad (3)$$

$$Cu^{2+} + L \overset{\beta_{110}}{\rightleftharpoons} [Cu(L)] \quad (4)$$

$$Cu^{2+} + L \overset{\beta_{11-1}}{\rightleftharpoons} [Cu(LH_{-1})] + H^+ \quad (5)$$

$$Cu^{2+} + 2L \overset{\beta_{12-1}}{\rightleftharpoons} [Cu(L_2H_{-1})] + H^+ \quad (6)$$

The formation percentages of the species [Cu(LH)] and [Cu(L$_2$H$_{-1}$)] were less than 5%; thus, the formation constants of only the main species are listed in Table III.

Table III. Cumulative Association Constants for Copper(II) Diastereoisomeric Dipeptides at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)

	log β_{110}	log β_{11-1}	pK^H_{CuL}[a]	ref
L-Ala-L-Ala	5.54	1.82	3.72	*b*
L-Ala-D-Ala	5.71	1.75	3.96	*b*
L-Leu-L-Ile	4.96 (6)	1.208 (9)	3.75	*c*
D-Leu-L-Ile	4.96 (6)	0.593 (9)	4.37	*c*
L-Leu-L-Leu	5.21	1.33	3.88	*b*
L-Leu-D-Leu	5.48	0.60	4.88	*b*
L-Ala-L-Phe	5.20, 5.35 (6)	1.76, 1.931 (6)	3.44, 3.42	*b*, *c*
L-Ala-D-Phe	5.42, 5.18 (3)	1.49, 1.559 (3)	3.93, 3.62	*b*, *c*
L-Val-L-Phe	4.66 (6)	1.706 (3)	2.95	*c*
L-Val-D-Phe	4.82 (9)	1.270 (9)	3.55	*c*
L-Leu-L-Tyr	5.15	1.77	3.38	*b*
L-Leu-D-Tyr	5.40	1.31	4.09	*b*
L-Leu-L-Phe	4.96 (6)	1.894 (3)	3.07	*c*
L-Leu-D-Phe	5.07 (9)	1.185 (6)	3.89	*c*

[a] For the equilibrium

$$CuL \overset{pK^H_{CuL}}{\rightleftharpoons} Cu(LH_{-1}) + H^+$$

[b] Reference 6. [c] This work.

While no difference from literature data[6] was found in the protonation constants for Ala-Ala, Leu-Leu, and Leu-Tyr systems and in the pertinent formation constants for copper(II) complexes, in the case of the copper(II) Ala-Phe complexes our values are different from those previously reported by other workers,[6] as already found in the case of the related protonation constants,[15] probably due to the different degrees of purity of LD isomers. Purity limits of some commercial dipeptides have been indicated by Katz[28] and verified by us as well. On the other hand, different stability constants have been found by Pettit et al.[1] with respect to those reported by Nakon et al.[6] In the case of Leu-Phe, Leu-Ile, and Val-Phe complexes of copper(II) our data are the first published results.

When only the log β values of all systems reported in Table III are examined, it can be seen that stereoselectivity is insignificant at low pH values where the major species is [CuL] but

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Table IV. Thermodynamic Functions for the Complex Formation of Copper(II) with Diastereoisomeric Dipeptides at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)

ligand	$-\Delta G^0_{11-1}$, kcal mol^{-1}	ΔH^0_{11-1}, kcal mol^{-1}	ΔS^0_{11-1}, cal mol^{-1} deg^{-1}
L-Ala-L-Ala	2.48	1.96 (6)	14.9 (2)
L-Ala-D-Ala	2.39	1.54 (5)	13.2 (2)
L-Ala-L-Phe	2.633 (8)	0.35 (6)	10.0 (2)
L-Ala-D-Phe	2.126 (4)	1.41 (8)	11.9 (3)
L-Val-L-Phe	2.326 (4)	0.61 (9)	9.8 (3)
L-Val-D-Phe	1.73 (1)	1.66 (9)	11.4 (3)
L-Leu-L-Phe	2.582 (4)	0.10 (8)	9.0 (3)
L-Leu-D-Phe	1.616 (8)	1.10 (8)	9.0 (3)
L-Leu-L-Tyr	2.41	2.0 (1)	14.8 (4)
L-Leu-D-Tyr	1.79	3.23 (6)	16.8 (2)
L-Leu-L-Ile	1.65 (1)	1.88 (9)	11.8 (3)
D-Leu-L-Ile	0.81 (1)	3.71 (8)	15.2 (3)
L-Leu-L-Leu	1.81	1.64 (3)	11.6 (1)
L-Leu-D-Leu	0.82	4.06 (4)	16.3 (2)

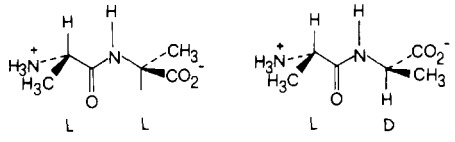

Figure 1. β conformation of L-alanyl-L-alanine and L-alanyl-D-alanine.

is important in the biological pH region, where the [$CuLH_{-1}$] species predominates. When the length of alkyl side chain increases, the difference between the log K values of deprotonated species also increases.

ΔG^0, ΔH^0, and ΔS^0 values for the formation of main species [$CuLH_{-1}$] of different diastereoisomers are given in Table IV. These data constitute the first report on copper(II) complexes with LL and LD diastereoisomeric dipeptides. Comparison with the thermodynamic parameters pertinent to the copper(II) DL-alanyl-DL-alanine species shows a similar trend in ΔH^0 and ΔS^0 values.[29] The species in the equilibrium $Cu^{2+} + L^- \rightleftarrows [CuLH_{-1}] + H^+$ are entropically favored.

According to the suggestions of Nancollas et al.,[30] the enthalpy of formation reflects both the proton dissociation of peptidic hydrogen and the new formation of bonds between copper(II), the nitrogen atom of the peptide group, and the carboxyl oxygen atom. Although there are no enthalpy data available for the dissociation of the hydrogen from an unbound peptide group, the value would be expected to be even more endothermic than the enthalpy changes accompanying the proton dissociation from the zwitterions (see Table I).

In conclusion, the resulting endothermic enthalpy changes are due to the prevailing of deprotonation contribution in addition to the carboxylate bond formation with respect to the nitrogen bond formation. But, if the above consideration explains the general trend of thermodynamic values, the differences in ΔH^0 and ΔS^0 changes between the LL and LD diastereoisomers and among different dipeptide pairs can be used to draw out further information about the thermodynamic stereoselectivity.

In particular, the enthalpy changes accompanying the formation of LL diastereoisomers of all dipeptides reported in Table IV, except L-alanyl-L-alanine, are less positive than those associated with the corresponding LD species.

The following discussion about the thermodynamic parameters is based on the assumption that the investigated dipeptides form complexes in a β-type conformation (see Figure 1) in their acidic, neutral, and basic species. This assumption, justified on the grounds of NMR results[20,33] (there is also other evidence[31,32]),

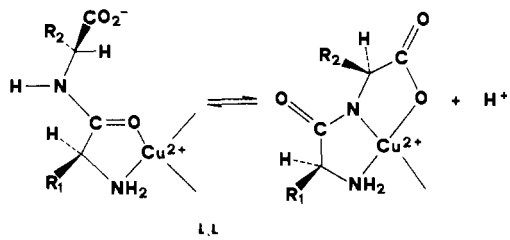

Figure 2. Scheme proposing the conformational changes of the LL dipeptide on going from the [Cu(L)] to the [$Cu(LH_{-1})$] complexes, because of the deprotonation of the peptide nitrogen.

has the consequence that the LD dipeptide presents a shorter end-to-end distance than the LL one and, moreover, the LD diastereoisomer side chains lie on the same side of the molecule, whereas they are on opposite sides when one considers the LL isomer. Bearing in mind the above-mentioned structural features, we interpreted the differences found in the thermodynamic protonation data reported in Table I.[14,15] In particular, in the case of LD Ala-Ala, the protonation of the amine group is favored by the electrostatic interaction between the NH_3^+ and COO^- groups (see Figure 1). Consequently, the degree of neutralization of the overall charge is greater than that occuring in the case of the LL derivative. The greater desolvation of the protonated amine group gives rise not only to a more positive entropy contribution but also to a lower enthalpy change due to cleavage of solvent bonds, which is not balanced by the NH_3^+–COO^- electrostatic interaction. Thus in this case the stereoselectivity is due to a gain in entropy because of the conformation in these peptides.

As regards the other dipeptides, unlike the case for the Ala-Ala system, we can observe an enthalpy stabilization of the LD with respect to the LL derivative. We consider that the greater enthalpy stabilization is due to the presence of a solvophobic interaction in the LD derivative. In fact, in the "mixed" isomer the large side chains of this dipeptide lie on the same side of the molecule and therefore may interact with each other and with the amide group, unlike in the "pure" isomer, where they point in opposite directions.

Calorimetric studies[11-13] have shown that solvophobic interactions are favored on enthalpy grounds. Hence for the dipeptides with large side-chain groups, the stereoselectivity is probably due not only to the favorable electrostatic interaction, such as is found in the Ala-Ala system, but also to the presence of this "secondary bond".

The occurrence of such solvophobic interactions in the LD dipeptides, possible in all protonation states, becomes more effective and stronger in the ampholytic state, owing to the electrostatic interaction that should make the molecule more rigid and the side-chain groups closer.

Consequently, in the "mixed" isomer the neutral species will be stabilized, favoring the protonation of the amine group and disfavoring that of the carboxylate group compared with the case for the "pure" isomer. The nonrelevant exothermic difference between the pairs of diastereoisomers results from the negative enthalpy contribution of the solvophobic interaction, which is partially counterbalanced by the positive contribution of the electrostatic interaction and which must be considered larger than that present in the case of L-alanyl-D-alanine.

In contrast, in the case of the copper(II) complex formation, the planar peptide backbone of the [$Cu(LH_{-1})$] species requires the side-chain groups to be on the same side with respect to the coordination plane, when an LL dipeptide is considered (see Figure 2). In agreement with previous results, the above solvophobic interaction is reflected in a gain of enthalpy contribution for the formation of a "pure" [$CuLH_{-1}$] complex, the maximum enthalpy change being in the case of the species containing Leu-Leu. The differences in ΔH^0 complexation values between the "pure" and the "mixed" isomers with phenyl groups are lower than those observed between the diastereoisomers of the dipeptides containing

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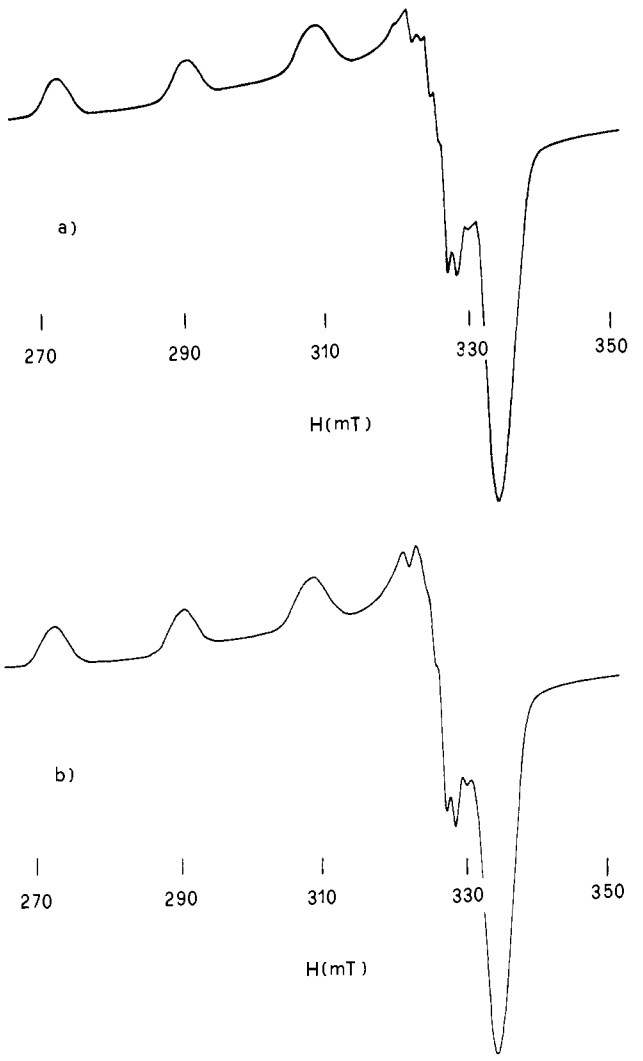

Figure 3. Frozen-solution EPR spectra of $^{63}Cu^{II}$–valylphenylalanine complexes in water–methanol mixtures (95%–5%) at 150 K: (a) LL dipeptide; (b) LD dipeptide.

butyl side chains and seem to indicate a minor contribution of the solvophobic interaction in the phenyl-containing dipeptides.

Probably this can be attributed to the fact that an interaction between alkyl groups and phenyl rings is less effective, owing to their spatial bearing, than the interaction between alkyl groups. We cannot exclude the possibility that a direct interaction occurring between the d electrons of the metal ion and the π ring system, recently invoked,[34-37] could decrease the solvophobic interaction between the two side chains.

In order to obtain major information about this problem, EPR experiments have been carried out. In particular, the EPR spectra would answer the question whether the complexes with different pairs of dipeptides, namely the predominant species $[CuLH_{-1}]$, have the same number of solvent molecules in the first coordination sphere.

Figure 3 shows EPR frozen-solution spectra taken at 150 K of copper(II) complexes with LL and LD Val-Phe. EPR spectra of the other systems are quite similar and are typical of copper(II) ions in axial environments. Figure 4 shows the parallel parts in an enlarged scale of the EPR spectra of copper(II) complexes with LL and LD Leu-Leu.

As one can see from inspection of Table II, the spectra show $g_\parallel \geq g_\perp \geq 2.04$, characteristic of axial copper(II) complexes in

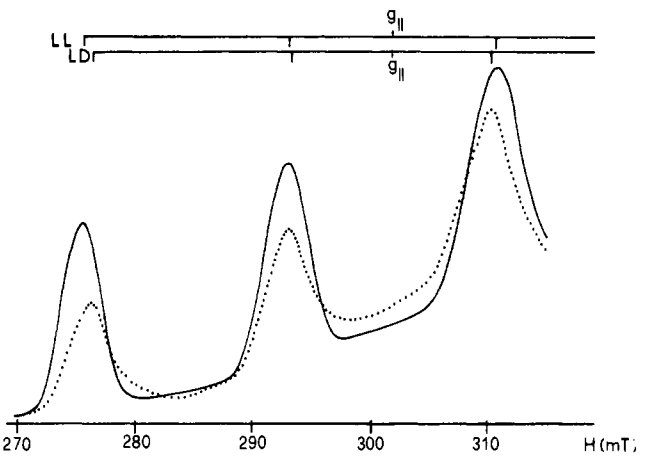

Figure 4. Parallel parts of the frozen-solution spectra due to $^{63}Cu^{II}$–leucylleucine complexes in water–methanol mixtures (95%–5%) at 150 K: (—) LL dipeptide; (···) LD dipeptide. Experimental conditions are as follows: microwave frequency, 9.485 GHz; microwave power, 15 mW; modulation frequency, 100 kHz; modulation intensity, 8 G; time constant, 0.5 s; scan time, 500 s.

tetragonally distorted octahedral, square-base pyramidal, or square-planar stereochemistries,[38] all copper(II) geometries associated with a $d_{x^2-y^2}$ ground state. Moreover, all the parallel values of copper(II) hyperfine coupling constants are in the range 0.0180–0.0190 cm^{-1}. These $A_\parallel$ values are higher than those found in the case of copper(II) bis(amino acidato) complexes which contain the same CuN_2O_2 chromophore; in water–glycerine mixture mean values of 0.0159 (2) cm^{-1} [39] and in water–methanol 0.0177 (2) cm^{-1} [40,41] are the $A_\parallel$ values reported in the literature. These higher $A_\parallel$ values support the idea that a greater extent of the tetragonal elongation of the two apical water molecules is probably present in the copper(II) dipeptide system. In other words, we would stress that the interaction of copper(II) ions with apical solvent molecules is certainly smaller than that occurring for similar systems. In consequence we will consider the copper(II) ion to be essentially in a square-planar situation. In Table II two trends can be observed. First, when the size and the type of R_1 and R_2 are varied, there is a slight decrease in $g_\parallel$ values and small increase in $A_\parallel$ values on going from copper(II) Ala-Ala to copper(II) Val-Phe complexes. These shifts parallel those observed on going from the copper(II) Gly-Gly complex to that with Gly-Phe. Hence, they can be ascribed to a probable substituent effect on the donor capabilities of two nitrogens and of the carboxylate oxygen linked to copper(II) ion. Second, there are also differences within each pair of copper(II) dipeptide complexes that are more difficult to rationalize. In fact $g_\parallel$ is not much affected even if it is always slightly greater in the case of the copper(II) complex with the LD isomer. In contrast, $A_\parallel$ values are generally higher in the case of copper(II) complexes with LL dipeptides than those with LD dipeptides. The largest difference (0.006 cm^{-1}) is reached in the case of copper(II) with LL or LD Leu-Leu.

Considering the particular stereochemistry of each pair of LL and LD dipeptide complexes, it could be supposed that in the former case, in which the side-chain groups are on the same side, the opposite coordination site would be approachable by a solvent molecule. In contrast, for the latter, in which these groups are on opposite sides with respect to the ideal basal plane, either a square plane or an elongated octahedron would have been the probable geometry of these copper(II) complexes. None of these assumptions can be justified on the basis of our experimental results, because more remarkable shifts in both $g_\parallel$ and $A_\parallel$ would

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Table V. EPR Magnetic Parameters of Copper(II) Dipeptide Complexes in Organic Solvent–Water (80%–20%) Mixtures

ligand		methanol		ethanol		*n*-propyl alcohol	
		$g_\parallel$	$A_\parallel$[a]	$g_\parallel$	$A_\parallel$[a]	$g_\parallel$	$A_\parallel$[a]
Ala-Ala	LL	2.247	183	2.247	183	2.246	183
	LD	2.247	183	2.247	183	2.246	183
Ala-Leu	LL	2.246	183	2.248	182	2.247	183
	LD	2.245	182	2.248	183	2.247	182
Leu-Leu	LL	2.244	185	2.249	183	2.247	183
	LD	2.245	181	2.248	180	2.247	182
Leu-Tyr	LL	2.237	188	2.243	186	2.245	185
	LD	2.240	186	2.246	184	2.244	184

[a] Hyperfine coupling constants expressed in units of 10^4 cm^{-1}.

have been expected by a coordination number ranging from 4 to 5 or 6.[42]

Then, to explain the higher $A_\parallel$ value of LL dipeptide complexes with respect to corresponding LD isomer species, other factors must be invoked. Bearing in mind that in the $[CuLH_{-1}]$ species of LL diastereoisomeric molecules the side chains can interact above the plane of coordination, one can expect that, as a consequence of this solvophobic interaction, a certain constraint is experienced by the basal plane. Thus, the donor atoms of dipeptide coordinated to metal ion could achieve a quasi-ideal planar conformation. In contrast, where this interaction is not possible (for the LD dipeptide complexes), the lower $A_\parallel$ values led us to think of either a small tetrahedral distortion or a stronger interaction with apical solvent molecules. We favor the former hypothesis, since it is well-known from Freeman's crystallographic work[43] that the basal plane formed by the dipeptide chelate group is distorted toward a tetrahedral situation. The above suggestion seems to be reinforced by the spectroscopic data collected in other solvents. In fact, when the proportion of the organic solvent was changed, it was found that the differences present in water tend to be minimized (see Tables II and V). For instance, in an *n*-propyl alcohol–water mixture (80%–20%) the $A_\parallel$ differences fall within the experimental error. Being well-known that the hydrophobic interaction decreases when the water percentage in the mixed solvent decreases, this last result seems to support that the small differences found within each pair of copper(II) complexes are to be ascribed to solvophobic forces only.

Furthermore, it is remarkable that when one of the alkyl side-chain groups is substituted with a phenyl or a phenolic ring, the above-mentioned differences tend to become smaller. There must be another factor that could play a certain role in these complexes. Other workers have carried out careful EPR studies on the copper(II) di- and tripeptide systems.[44-46] In particular the work of Sportelli et al.[46] was devoted to the investigation of the aromatic group influence on the spectral features of these complexes. Nevertheless, none of them have tried to give evidence to eventual hydrophobic interactions. Recently, a paper by Yamauchi et al.[37] postulated a copper(II)–aromatic ring interaction above the coordination plane of monomeric complexes with some tyrosine-containing dipeptides.

The question that arises in a complete rationalization of EPR data is as follows: why do the differences found in the case of copper(II) complexes with LL and LD Leu-Leu or LL and LD Leu-Ile appear to be less evident in the case of those with LL and LD Ala-Phe, LL and LD Leu-Tyr, LL and LD Leu-Phe, and LL and LD Val-Phe? Probably the "stiffening" effect exerted by the side-chain interactions occurring in the LL complex is, at least partly, counterbalanced by a similar effect taking place in the LD complexes with dipeptides having aromatic groups. A possible d–π interaction between the copper(II) ion and the aromatic ring can account for this trend. This is the only assumption that makes us able to rationalize the different trends along this series of copper(II) dipeptide complexes.

In conclusion, the thermodynamic and spectroscopic data led us to think that the stereoselectivity of the complex formation of this class of compounds with copper(II) is due to the solvophobic forces and that a certain role is also played by the interaction between the d electrons of the metal ion with the π systems of the side-chain groups.

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