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Weak Forces in Thermodynamic Stereoselectivity of Dipeptide Complex Formation in Aqueous Solution

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Weak Forces in Thermodynamic Stereoselectivity of Dipeptide Complex Formation in Aqueous Solution

The thermodynamic stereoselectivity of a wide series of dipeptide complexes with proton and copper (II) ions is examined. In particular, it is shown that non-covalent interactions are the driving forces of the chirality discrimination. Furthermore, the diagnostic character of ΔH° and ΔS° values is brought to light and compared with the spectroscopy approach for correlating the stereoselectivity with the structural properties of complexes.

Key Words: *non-covalent interactions, thermodynamic stereoselectivity, copper (II) complexes of diastereoisomeric dipeptides*

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The biological activity of a molecule often depends upon its stereochemistry, a fact well recognized for over a century. Carboxypeptidase is a zinc enzyme which only stereospecifically splits the terminal amino acid from a peptide in the L-form,[1] while chymotrypsin reacts efficiently with only one stereoisomer, the L-tryptophan methyl ester, to catalyze its hydrolysis.[2]

Notwithstanding the relevance of the above systems and other macromolecules in the stereochemical specificity of biological processes, accurate descriptions of the origins of stereoselectivity are rare. The complexity of natural systems may be the reason why attempts are often made to reach a better understanding of stereoselectivity by the investigation of models. Since chiral discrimination between macromolecules is a highly complex process and represents the outcome of a large number of simultaneously oc-

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curring molecular processes, the models allow easier quantitative identification of the nature and character of the forces determining a specific kind of biological recognition. Detailed understanding of such forces, sometimes called "secondary bonds," is of the greatest interest, since it promises to lead to a better insight into many life processes.

In this context, bearing in mind that the results obtained by the investigation of a model may only answer those questions which are already included in the model, it is the purpose of this Comment to review thermodynamic stereoselectivity in the complex formation of dipeptides.

The measurement of formation constants can be applied to the formation of labile complexes. Such labile complexes predominate in biological systems, and therefore thermodynamic stereoselectivity is the most important aspect, as kinetic stereoselectivity is for inert complexes.

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According to Pettit's report[3] that "potentiometric titrations, supported where possible by calorimetric studies, provide the more reliable quantitative results," mainly papers reporting a potentiometric and calorimetric approach have been reviewed.

$\Delta H°$ and $\Delta S°$ values have been used to understand the nature of the above-mentioned "secondary bonds"[4-6] that are weak forces, including hydrogen bonds, van der Waals, hydrophobic and electrostatic interactions, and to understand how their "weak" character makes them indispensable to chiral molecular recognition. Furthermore, being aware that many researchers lack confidence in obtaining structural information from thermodynamic parameters, and in order to achieve unambiguous results, spectroscopic measurements (UV, CD, NMR and e.p.r.) were carried out on the investigated systems by the devotees of thermodynamic stereoselectivity. Stereoselectivity is defined, within the context of transition metal chemistry, as the difference in the properties of molecular diastereoisomers. In particular, a dipeptide synthesized from optically pure amino acids can exist as four isomers, divided into two pairs. The LL and DD isomers are optically active or "pure" isomers, whereas the LD and DL isomers are meso or "mixed" isomers. The members of each pair are enantiomeric and the two pairs are diastereoisomeric. It has been shown that the formation constants of the enantiomeric dipeptides are indeed

identical as expected,[7] while proton and metal complexes of diastereoisomer dipeptides show, in general, thermodynamic stereoselectivity.[3]

PROTON COMPLEX FORMATION

Reported values of proton complex formation constants for diastereoisomeric dipeptides are presented in Tables I and II.

Stereoselective effects between the diastereoisomeric dipeptides are significant: log β values concerning the protonation of the amino group is higher for the D,L-peptide than for the L,L-isomer, while the opposite behavior is observed in the protonation of the carboxylate group. This trend is also observed in the diastereoisomer pairs of dipeptides containing other protonation sites (Table II) with the significant exception of histidyl–histidine systems.[17] The carboxylate protonation constant value of D-his-L-his is higher than that of L,L-isomer. A particular trend in the non-terminal protonation sites was not noted.

In general, stereoselectivity increases as the size of the side chain increases. The difference in stability ranges from 0.15 to 0.55 l.u. (logarithmic unity) on going from the ala-ala[3] couple to the tyr-trp one.[15] On the basis of the log β values only, thermodynamic stereoselectivity in proton complex formation can be explained in different ways. Due to the fact that molecular models show that the meso isomer can fold more easily than the pure isomer, Ellenbogen[9] attributed this stereoselectivity to differences in the electrostatic interaction. In the folded conformation of the zwitterion the oppositely charged NH_3^+ and CO_2^- groups are nearer in the case of the L,D isomer than in the case of the L,L one, which, consequently, is less stabilized than the meso diastereoisomer. Nakon and Angelici[7] interpreted the observed stereoselectivity by assuming that the dipeptides remained in the folded β-conformation in acid, neutral, and basic solutions. On the basis of this assumption, supported by experimental results,[18–20] it can be seen that protonation of the carboxyl group is electrostatically less stable in the L,D isomer than in the pure isomer due to the close proximity of the positively charged NH_3^+ group. For a similar reason protonation of the amine group is more stabilized in the

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TABLE I

Proton complex formation constants of some diastereoisomeric dipeptides at 25°C and $I = 0.1$ mol dm^{-3}

	$\log K(CO_2^-)$	$\log K(NH_2)$	Ref.
L-Ala-L-Ala	3.30, 3.31	8.17, 8.15	7, 8
D-Ala-D-Ala	3.30	8.14	9
L-Ala-D-Ala	3.12	8.30	7
D-Ala-L-Ala	3.15	8.28	8
L-Ala-L-Phe	3.25, 3.13	7.89, 7.93	8, 10
L-Ala-D-Phe	3.02, 2.95	8.08, 8.18	8, 10
L-Lys-L-Ala	3.22	7.62	9
L-Lys-D-Ala	3.00	7.74	9
L-Lys-L-Lys	3.01	7.53	9
L-Lys-D-Lys	2.85	7.53	9
D-Leu-D-Leu	3.52	7.89	7
L-Leu-L-Leu	3.45, 3.46	7.91, 7.91	7, 11
L-Leu-D-Leu	3.05, 3.17	8.20, 8.28	7, 11
D-Leu-L-Leu	3.13	8.25	7
L-Val-L-Phe	3.19, 3.20	7.89, 7.68	3, 12
L-Val-D-Phe	2.87, 2.92	8.24, 8.06	3, 12
L-Phe-L-Val	3.40	7.41	3
L-Phe-D-Val	3.09	7.77	3
L-Leu-L-Ala	3.36	8.08	3
L-Leu-D-Ala	2.98	8.17	3
L-Pro-L-Ala	3.27	8.98	3
L-Pro-D-Ala	3.15	9.09	3
L-Pro-L-Phe	3.40	8.70	3
L-Pro-D-Phe	2.91	9.08	3
L-Met-L-Val	3.43	7.45	3
L-Met-D-Val	3.07	7.69	3
L-Met-L-Phe	3.14	7.29	3
L-Met-D-Phe	2.87	7.72	3
L-Ala-L-Leu	3.34	8.02	10
D-Ala-L-Leu	3.12	8.24	10
L-Leu-L-Phe	3.18	7.69	10
L-Leu-D-Phe	2.89	8.15	10
L-Leu-L-Ileu	3.42	7.77	10
D-Leu-L-Ileu	3.05	8.08	10
L-Val-L-Val	3.39	7.97	13
L-Val-D-Val	3.04	8.22	13
L-Met-L-Met	3.22, 3.23	7.43, 7.39	13, 14
L-Met-D-Met	3.04	7.63	13
D-Met-L-Met	2.91	7.54	14

meso isomer. The presence of side chains can promote hydrophobic interaction between the two alkyl residues and the amide group. This "secondary" weak bond has the effect of bringing the charged

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TABLE II

Proton complex formation constants of some diastereoisomeric dipeptides at 25°C and $I = 0.1$ mol dm^{-3}

	$\log K(CO_2^-)$	$\log K(CO_2^-)$	$\log K(N_{Im})$	$\log K(N_{Im})$	$\log K(NH_2)$	$\log K(O^-)$	$\log K(O^-)$	Ref.
L-Tyr-L-Ala	3.378				7.506		9.975	15
L-Tyr-D-Ala	3.054				7.657		9.790	15
L-Tyr-L-Leu	3.394				7.413		10.112	16
L-Tyr-D-Leu	3.017				7.860		10.383	16
L-Leu-L-Tyr	3.263				7.840		10.179	16
D-Leu-L-Tyr	2.96				8.30		10.38	8
L-Tyr-L-Arg	3.087				7.239		9.820	15
L-Tyr-D-Arg	2.814				7.624		9.818	15
L-Tyr-L-Glu	3.166	4.477			7.685		10.093	15
L-Tyr-D-Glu	2.852	4.645			7.853		10.167	15
L-Tyr-L-His	2.680		6.645		7.631		10.001	15
L-Tyr-D-His	2.345		6.642		7.765		9.838	15
L-Tyr-L-Tyr	3.233				7.312	9.696	10.424	15
L-Tyr-D-Tyr	2.930				7.743	9.731	10.538	15
L-Tyr-L-Trp	3.510				7.367		9.910	15
L-Tyr-D-Trp	3.277				7.914		10.046	15
L-Pro-L-His	3.02			6.84	8.82			17
D-Pro-L-His	2.91			6.77	9.16			17
L-His-L-His	2.61		5.68	6.85	7.79			17
D-His-L-His	2.91		5.24	6.86	8.06			17

groups closer to each other in the meso isomer, thus increasing the stereoselectivity.

Kaneda and Martell[8] discussed stereoselectivity as a result of a conformational analysis carried out with the aid of molecular models, correlating different hydrophobic regions to a preferred cis configuration. Pettit and Hefford, in their review,[3] considering the explanations to date, list the different aspects that can contribute to the stereoselectivity and seem to incline toward conformational and steric origins of proton complex stereoselectivity.

$\Delta G°$, $\Delta H°$ and $\Delta S°$ values for the protonation of some dipeptides are reported in Table III. As can be seen, the protonation of the amine group is favored on enthalpy grounds, while that of the carboxylate only reveals a favorable entropy contribution. The overall negative $\Delta H°$ value of amine protonation has been ascribed to several factors[21]; in particular, there is an exothermic contribution due to the nitrogen bond formation that is greater than the endothermic desolvation contributions of both the amine group and the H_3O^+ ion. The positive contribution of $\Delta S°$ in the case of the carboxylate oxygen takes place due to the desolvation processes of the anion as well as that of H_3O^+ and from the consequent charge neutralization. In the case of the protonation of the amine group, the solvation process occurs more easily than that for the carboxylate and this accounts for its less favorable entropy contribution. Analogously, the several processes of desolvation and consequent cleavage of solvent bonds explain the resulting slight endothermic contribution of the carboxylate protonation. In this context, it was possible to interpret the differences in the thermodynamic parameters between the diastereoisomers of each complex, as well as the different behavior of the Ala-Ala, Ala-Leu and Met-Met systems with respect to the other dipeptides with branched alkyl side chains and aromatic residues. As regards the latter systems, it must be pointed out that the higher stability of the L,D derivative is due mainly to a more positive entropy contribution, while for the other dipeptides the order is reversed, i.e., "mixed" derivative is favored by a more negative enthalpy change. Since the β-conformation constrains the COO^- and NH_3^+ groups on the same side of the L,D-diastereoisomer molecules and then at a shorter end-to-end distance than the L,L-dipeptide, the degree of neutralization of the overall charge in L-Ala-D-Ala is greater

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TABLE III

Thermodynamic parameters of protonation of diastereoisomeric dipeptides at 25°C and $I = 0.1$ mol dm^{-3}

	$-\Delta G°$, kcal mol^{-1}		$-\Delta H°$, kcal mol^{-1}		$\Delta S°$, cal mol^{-1} deg^{-1}		
	NH_2	CO_2^-	NH_2	CO_2^-	NH_2	CO_2^-	Ref.
L-Ala-L-Ala	11.14	4.50	10.64	−0.26	1.7	15.9	21
L-Ala-D-Ala	11.34	4.34	10.26	−0.63	3.6	16.7	21
L-Ala-L-Leu	10.94	4.56	10.68	−0.24	0.9	16.1	10
D-Ala-L-Leu	11.25	4.26	10.58	−0.77	2.2	16.9	10
L-Leu-L-Ileu	10.60	4.66	10.69	−0.41	−0.3	17.0	12
D-Leu-L-Ileu	11.02	4.17	10.84	−0.72	0.6	16.4	12
L-Leu-L-Leu	10.78	4.71	10.21	−0.36	1.9	17.0	21
L-Leu-D-Leu	11.18	4.16	10.92	−0.77	0.9	16.5	21
L-Ala-L-Phe	10.82	4.28	10.51	−0.05	1.0	14.5	10
L-Ala-D-Phe	11.17	4.03	10.64	−0.39	1.8	14.6	10
L-Val-L-Phe	10.48	4.36	10.78	−0.02	−1.0	14.7	12
L-Val-D-Phe	11.00	3.98	10.89	−0.32	0.4	14.2	12
L-Leu-L-Phe	10.50	4.34	10.42	−0.08	0.3	14.3	10
L-Leu-D-Phe	11.11	3.94	11.0	−0.1	0.3	13.4	10
L-Leu-L-Tyr	10.68	4.41	10.43	0.0	1.0	14.6	21
D-Leu-L-Tyr	11.32	4.03	11.15	−0.1	0.6	13.9	21
L-Met-L-Met	10.05	4.39	10.64	−0.30	−2.0	16.0	14
D-Met-L-Met	10.26	3.96	10.43	−0.12	−0.5	13.1	14

than that occurring in the case of L-Ala-L-Ala peptide. 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 the cleavage of solvent bonds, which is not balanced by the $NH_3^+-COO^-$ electrostatic interaction. Thus the stereoselectivity here is due to a gain in entropy because of the conformation in the peptides. As regards the other dipeptides, it is possible to observe an enthalpy stabilization of the L,D- with respect to the L,L-derivative. Thus, one has to suppose that something else, besides electrostatic interaction, is occurring that can explain this difference. On the basis of a suggestion from Angelici *et al.*[7] it has been proposed that for this group of dipeptides a solvophobic[22] or, according to a more classical definition, a hydrophobic interaction[23,24] takes place in the "mixed" diastereoisomer, facilitated by the favorable conformation that placed the two side chains closer to each other in the L,D diastereoisomers (Fig. 1). Calorimetric studies on different systems have in fact shown that the solvophobic interaction is enthalpy favored.[25,26] Furthermore, the entropy driven stereoselectivity of amino group protonation is observed only for the dipeptides in which the side chains are small (Ala-Ala and Ala-Leu) and thus are not able to interact solvophobically with each other. The only exception among the systems reported in Table III is in the Met-Met dipeptide pair, but it can be explained with the major conformational freedom of this very long chain; that the elongation of the side chain does not involve enhancement of the solvophobic interaction has recently been reported in the literature.[27]

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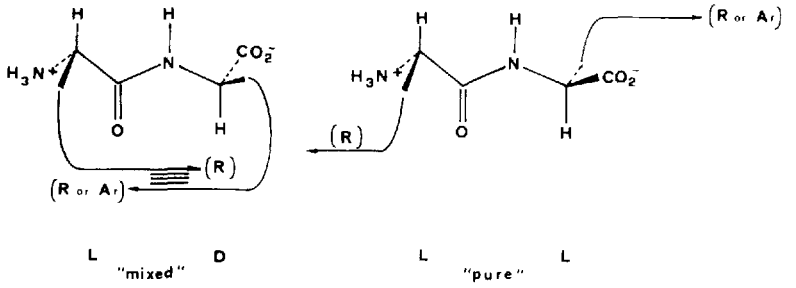

FIGURE 1 Tentative structures of L,L- and L,D-dipeptides in aqueous solution.

However, the difference observed in the thermodynamic parameters must be taken with care as a measure of the solvophobic effect on the amphiprotic species. In fact, this weak force takes place in all the protonation states of the "mixed" diastereoisomers, but becomes more effective and stronger for the amphiprotic species, owing to the electrostatic interaction, which should make the molecules more rigid and bring the side chains closer. Thus, in the protonation process there is only a variation in the extent of this interaction.

The ^{1}H NMR result,[12] summarized in Table IV, supported these suggestions. As expected, the deprotonation process causes a gradual downfield shift. This behavior shows no exception in those

TABLE IV

Chemical shifts (in Hz at 80 M Hz) of the methyl protons

Dipeptide	Cationic Species	Dipolar Species	Anionic Species
L-Ala-L-Ala	124.6[b]/116.8[c]	124.3[b]/108.8[c]	101.2[b]/108.2[c]
L-Ala-D-Ala	123.7[b]/116.7[c]	120.6[b]/108.3[c]	100.3[b]/107.5[c]
L-Ala-L-Leu	124.8[b]/76.3[c]	123.9[b]/73.6[c]	101.4[b]/73.4[c]
D-Ala-L-Leu	123.9[b]/75.2[c]	121.8[b]/74.1[c]	100.0[b]/73.5[c]
L-Leu-L-Ileu[d]	80.0	76.9	76.1
D-Leu-L-Ileu[d]	81.0	76.9	75.8
L-Leu-L-Leu[e]	78.8[b]/76.3[c]	77.7[b]/75.3[c]	70.6[b]/75.0[c]
L-Leu-D-Leu[e]	80.4[b]/75.1[c]	77.7[b]/74.1[c]	71.0[b]/74.0[c]
L-Ala-L-Phe	118.9	118.2	91.0
L-Ala-D-Phe	100.2	97.9	82.9
L-Val-L-Phe[e]	83.6	82.3	65.9
L-Val-D-Phe[e]	58.8	57.3	56.2
L-Leu-L-Phe[e]	72.9	71.7	67.6
L-Leu-D-Phe[e]	60.5	58.6	58.4
L-Leu-L-Tyr[e]	72.4	71.0	66.2
D-Leu-L-Tyr[e]	61.5	58.2	61.6

[a]Reference 12.
[b]Methyl close to the amino groups.
[c]Methyl close to the carboxylic groups
[d]The reported value refers to the leucine methyl doublet while both the nonequivalent methyls of isoleucine owing to their low intensity are not distinguishable in the spectra.
[e]The value is referred to the doublet of the two equivalent methyls.

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dipeptides the side chains of which are both alkyl residues. In the case of D,L-Leu-Tyr, however, the methyl chemical shift in the amphyprotic species is upfield even with respect to that in the anionic species. In order to minimize the protonation effect, the chemical shift differences of related methyl groups of corresponding species between the two diastereoisomers of the same dipeptide were calculated (Table V). These data show that, in all the dipeptides with an aromatic moiety, the differences are highest for the amphiprotic species. Furthermore, the chemical shifts observed for the "mixed" diastereoisomer are always upfield with respect to the corresponding "pure" dipeptide. This trend was rationalized as follows: (i) solvophobic interaction is effective in all the species of the "mixed" diastereoisomers with an aromatic moiety; (ii) the extent of this interaction varies in the different species, being greatest in the amphiprotic species and smallest in the anionic species. It is noteworthy that spectroscopic and thermodynamic data are quite consistent. Some differences can be explained bearing in mind that the electrostatic interaction also takes place in these "mixed" diastereoisomers, and therefore the thermodynamic parameters are the algebraic sum of different effects. In particular, as regards the enthalpy contribution, this corresponds to a negative

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TABLE V

Differences between the chemical shift (in Hz) of corresponding methyls of corresponding species of the two diastereoisomers of each dipeptide ($\delta_{\text{"pure"}} - \delta_{\text{"mixed"}}$)[a]

Dipeptide[b]	Cationic Species	Dipolar Species	Anionic Species
Ala-Ala	+0.9[c]/+0.1[d]	+3.7[c]/+0.5[d]	+0.9[c]/+0.7[d]
Ala-Leu	+0.9[c]/+1.1[d]	+2.1[c]/−0.5[d]	+1.4[c]/−0.1[d]
Leu-Ileu[e]	−0.2	0.0	+0.3
Leu-Leu	−1.6[c]/+1.2[d]	0.0[c]/+1.2[d]	−0.4[c]/+1.0[d]
Ala-Phe	+18.7	+20.3	+8.1
Val-Phe	+14.8	+25.0	+9.7
Leu-Phe	+12.4	+13.1	+9.2
Leu-Tyr	+10.9	+12.8	+4.6

[a]"Pure" = L,L; "mixed" = L, D or D, L.
[b]Reference 12.
[c]Methyls close to the amino groups.
[d]Methyls close to the carboxylic groups.
[e]The two equivalent methyls of leucine.

(favorable) contribution of the solvophobic interaction, which is not completely counterbalanced by the positive contribution of the electrostatic interaction, and should be considered larger than that present in the case of Ala-Ala and Ala-Leu (the solvophobic interaction itself makes the molecule shorter).

Summarizing, the results also show that the extent of solvophobic interaction is determined not only by the size but also by the shape, i.e., the kind of branching of the side chain, as can be seen by comparing the data of Leu-Leu with those of Leu-Ileu. Furthermore, other things being equal, the interaction between an alkyl and aryl group is more effective than an interaction between two alkyl groups.

METAL COMPLEX FORMATION

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The generalized overall formation reaction of metal(II) ions with peptide ligand is given in Eq. (1), where L is the negative species with peptide ligands.

$$mM^{2+} + lL + hH^{+} \rightleftharpoons [M_m(L_lH_h)]. \quad (1)$$

(Charges on the ligand and the metal(II) complexes are omitted for clarity in notation.) The stability constant is defined by Eq. (2).

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

In dipeptides with non-coordinating side chain residues, different stability constants have been found in the formation of peptide nitrogen deprotonated complex species MLH_{-1} (see Eq. (3)).

$$M^{2+} + L \overset{\beta_{11-1}}{\rightleftharpoons} [M(LH_{-1})] + H^{+}, \quad (3)$$

while for other species the published results cannot confirm the presence of stereoselectivity. This might be due to the fact that the ML, MLH_{-2}, ML_2H_{-1} and so on are minor species and their

stability constant values suffer from a high degree of uncertainty. Experimental limits for nickel(II) systems (tendency to form insoluble species at low pH) and the impossibility of obtaining unambiguous speciation for zinc(II) (tendency to form polynuclear and hydrolytic species) are likely to be the causes of the fact that the data reported in literature are pertinent nearly exclusively to copper(II) complexes (Table VI). In the case of dipeptides containing potential donor atoms in their chains, different stability constants have been found also for other mononuclear species as well as for dimer complexes (Table VII).

When only the log β values of all systems reported in Table VI are examined, it can be seen that it is not possible to identify a particular trend in the stereoselectivity of [Cu(L)] species, while the stability constant values pertinent to the $[Cu(LH_{-1})]$ complexes show that the L,L diastereoisomers form more stable metal-complexes than the D,L ones. Furthermore, the difference increases with the increase in length of the alkyl side chain residues.

Similarly, in some respects, to the discussion on the thermodynamic origin of the chelate effect, different conclusions have been drawn with respect to this stereoselectivity: i.e., some investigators have ascribed the effect to entropy loss, while others have suggested an entropy gain as the most important factor in contributing to the enhanced stability of copper(II) complexes with L,L dipeptides.[3] In particular, Kaneda and Martell[8] stated that a relatively more extensive hydrophobic region results in an unfavorable entropy contribution and in a relative lowering of stability. Nakon and Angelici,[7] 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.*,[16] 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 the opposite sides of the basal plane in the "mixed" complex. Previously, differences in steric interference between the peptide side chains and coordinated water had been claimed to account for the preferential formation of the optically homogeneous complex.[11]

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TABLE VI

Metal complex stability constants of some diastereoisomeric dipeptides at 25°C and $I = 0.1$ mol dm^{-3}

	$\log\beta_{110}$	$\log\beta_{11-1}$	pK^{H}_{CuL}	$\log\beta_{120}$	Ref.
Cu(II)					
L-Ala-L-Ala	5.54, 5.31		3.72, 3.58		7, 8
L-Ala-D-Ala	5.71	1.75	3.96		7
D-Ala-L-Ala	5.60	1.56	4.04		8
L-Ala-L-Phe	5.20, 5.35	1.76, 1.93	3.44, 3.42		7, 28
L-Ala-D-Phe	5.42, 5.18	1.49, 1.56	3.93, 3.62		7, 28
L-Leu-L-Leu	5.21, 5.24		3.88, 3.86		7, 11
L-Leu-D-Leu	5.48, 5.20		4.88, 4.57		7, 11
L-Val-L-Phe	5.35, 4.66	1.845, 1.71	3.50, 2.95		3, 28
L-Val-D-Phe	5.31, 4.82	1.395, 1.27	3.91, 3.55		3, 28
L-Phe-L-Val	4.65	1.155	3.45		3
L-Phe-D-Val	4.86	0.939	3.92		3
L-Leu-L-Ala	5.62	1.845	3.77		3
L-Leu-D-Ala	5.56	1.384	4.18		3
L-Pro-L-Ala	6.60	2.936	3.66		3
L-Pro-D-Ala	6.48	2.654	3.83		3
L-Pro-L-Phe	6.53	3.271	3.26		3
L-Pro-D-Phe	6.23	2.551	3.68		3
L-Leu-L-Ile	4.96	1.21	3.75		28
D-Leu-L-Ile	4.96	0.59	4.37		28
L-Leu-L-Phe	4.96	1.89	3.07		28
L-Leu-D-Phe	5.07	1.19	3.89		28
Ni(II)					
L-Ala-L-Ala	4.14		8.67	7.02	3
D-Ala-L-Ala	3.90		9.06	6.92	3
L-Val-L-Phe	3.19		8.50	5.39	3
L-Val-D-Phe	3.24		9.30	6.32	3
L-Phe-L-Val	2.77		8.65	5.21	3
L-Phe-D-Val	3.08		9.28	6.08	3
L-Leu-L-Ala	3.36		8.92	5.97	3
L-Leu-D-Ala	3.34		9.20	6.15	3
L-Pro-L-Ala	4.41		8.33	7.88	3
L-Pro-D-Ala	4.44		8.34	8.67	3
L-Pro-L-Phe	4.25		7.76	–	3
L-Pro-D-Phe	4.05		8.53	8.65	3
Zn(II)					
L-Ala-L-Ala	3.73			6.88	7
L-Ala-D-Ala	3.87			7.04	7
L-Ala-L-Phe	3.38			6.20	7
L-Ala-D-Phe	3.61			6.55	7

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TABLE VII

Logarithmic copper (II) complex stability constants of diastereoisomeric dipeptides containing potentially donor atoms side chains at 25°C and I = 0.1 mol dm^{-3}

Dipeptide	$log\beta_{112}$	$log\beta_{111}$	$log\beta_{110}$	$log\beta_{11-1}$	$log\beta_{11-2}$	$log\beta_{11-3}$	$log\beta_{220}$	$log\beta_{22-1}$	$log\beta_{22-2}$	Ref.
L-Tyr-L-Ala		15.04	11.623	2.67	−7.617				7.66	15
L-Tyr-D-Ala		14.70	11.217	2.11	−8.044				6.78	15
L-Tyr-L-Leu		15.04	11.177	1.73					6.88	16
L-Tyr-D-Leu		15.26	10.854	1.70					6.10	16
L-Tyr-L-Arg		14.38	11.335	2.58	−7.434				7.30	15
L-Tyr-D-Arg		14.52	10.849	1.94	−8.010				6.71	15
L-Tyr-L-Glu		16.615	11.824	2.255	−7.818			16.73	7.991	15
L-Tyr-D-Glu		16.431	11.716	2.29	−7.74			16.6	7.7	15
L-Tyr-L-Tyr	25.09	21.907	13.113	3.205	−7.360		28.94	19.57		15
L-Tyr-D-Tyr	25.37	21.715	12.898	2.914	−7.683		27.83	18.41		15
L-Tyr-L-Trp		15.570	11.724	2.793	−7.319	−19.44			9.277	15
L-Tyr-D-Trp		15.505	11.656	2.823	−7.325	19.06			8.08	15
L-Tyr-L-His	22.56	19.103	15.141	5.962	−4.387					15
L-Tyr-D-His		18.374	13.871	4.801	−5.218					15
L-His-L-His	19.22	15.77	11.10					19.15	12.70	17
D-His-L-His	19.56	16.20	12.00					20.2	14.08	17
L-Pro-L-His			10.05	5.43	−4.17	−15.02				17
D-Pro-L-His			9.89	5.17	−4.67	−16.11				17
L-Met-L-Phe			4.76	1.758						3
L-Met-D-Phe			4.93	1.294						3
L-Met-L-Val			4.96	1.161						3
L-Met-D-Val			5.01	0.788						3
L-Met-L-Met		8.82	5.07	1.70						14
D-Met-L-Met		9.11	5.12	1.43						14

Sigel and Martin[29] observed that upon deprotonation of the species formed by copper(II) ions and some dipeptides with two nonglycyl residues at pH = 4 (the coordination at the amide nitrogen occurs according to the equilibrium $[Cu(L)]^+ \rightleftharpoons [Cu(LH_{-1})] + H^+$) the corresponding equilibrium acidity constant pK^H_{CuL} is more acidic for the "pure" than for the "mixed" isomers. They suggested that hydrophobic interactions between side chains on the same side of the chelate plane in the LL complex provide a favorable effect compensating for the steric inhibition responsible for the high pK^H_{CuL} values found in other peptide systems such as Gly-Leu and Gly-ILeu.

Though complexes of dipeptides have been studied by means of several different experimental methods, all the above suggestions about the driving forces of thermodynamic stereoselectivity have been put forward on the basis of $\Delta G°$ values only. Table VIII shows the $\Delta H°$ and $\Delta S°$ in addition to the related $\Delta G°$ values of the main species $[Cu(LH_{-1})]$ of some diastereoisomers.[28] Comparison with the thermodynamic parameters pertinent to the copper(II) DL-Alanyl-DL-alanine species shows a similar trend in $\Delta H°$

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TABLE VIII

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	$-\Delta G°_{11-1}$, kcal mol^{-1}	$\Delta H°_{11-1}$ kcal mol^{-1}	$\Delta S°_{11-1}$ cal mol^{-1} deg^{-1}	Ref.
L-Ala-L-Ala	2.48	1.96	14.9	28
L-Ala-D-Ala	2.39	1.54	13.2	28
L-Ala-L-Phe	2.633	0.35	10.0	28
L-Ala-D-Phe	2.126	1.41	11.9	28
L-Val-L-Phe	2.326	0.61	9.8	28
L-Val-D-Phe	1.73	1.66	11.4	28
L-Leu-L-Phe	2.582	0.10	9.0	28
L-Leu-D-Phe	1.616	1.10	9.0	28
L-Leu-L-Tyr	2.41	2.0	14.8	28
L-Leu-D-Tyr	1.79	3.23	16.8	28
L-Leu-L-Ile	1.65	1.88	11.8	28
D-Leu-L-Ile	0.81	3.71	15.2	28
L-Leu-L-Leu	1.81	1.64	11.6	28
L-Leu-D-Leu	0.82	4.06	16.3	28

and ΔS° values. The species in the equilibrium $Cu^{2+} + L \rightleftharpoons [Cu(LH_{-1})] + H^+$ are entropically favored. The formation enthalpy reflects both the proton dissociation of peptide hydrogen and the new formation of bonds between copper(II) and the peptide and amine nitrogens, 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. Thus the resulting endothermic enthalpy changes are due to the prevalence of the deprotonation contribution in addition to the carboxylate bond formation with respect to the nitrogen bond formation. The above consideration also constitutes the basis for understanding the differences in ΔH° and ΔS° changes between the L,L and L,D diastereoisomers and among different dipeptide pairs. The enthalpy changes accompanying the formation of LL diastereoisomers of all dipeptides reported in Table VIII, except L-alanyl-L-alanine, are less positive than those associated with the corresponding LD species. This trend is opposite to that reported for the proton complex formation,[10,12,21] but it is understandable considering the set of donor atoms around the metal ion. In fact, the involvement of peptide nitrogen in the planar coordination geometry 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 (Fig. 2a).

In agreement with the rationalization of thermodynamic data pertinent to proton complex formation, weak solvophobic interaction is reflected in a gain of enthalpy contribution for the formation of a "pure" $[Cu(LH_{-1})]$ complex. The formation of "mixed" $[Cu(LH_{-1})]$ species involves, on the contrary, the elimination of the interaction present in the ligand before complex formation (Fig. 2b). The differences in ΔH° complexation values between the "pure" and the "mixed" isomers with aromatic residues are lower than those observed between the diastereoisomers of the dipeptides containing butyl side chains. This can probably be attributed to the fact that an interaction between alkyl groups and phenyl rings is less effective, owing to their spatial orientation, than the interaction between alkyl groups. It cannot be excluded that direct interaction between the d electrons of the metal ion

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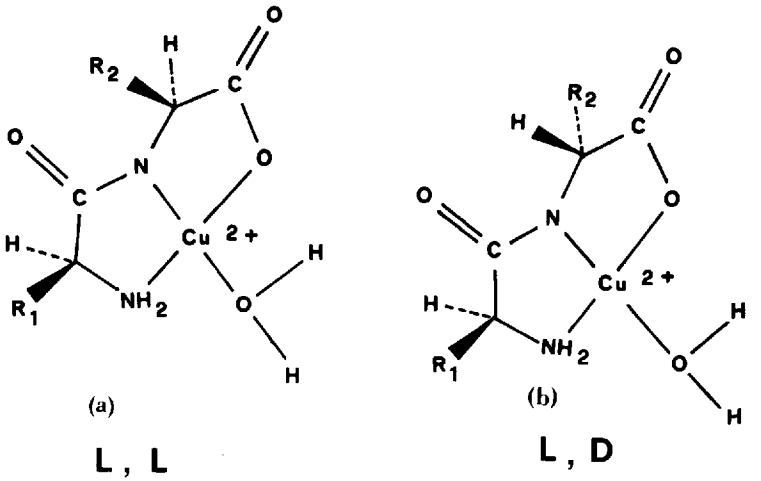

FIGURE 2 Hypothesized structure of [Cu(LH $_{-1}$)] species; (a) L,L- (b) L,D-dipeptide.

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and the π ring system, recently invoked,[28] decreases the solvophobic interaction between the two side chains.

Since $\Delta H°$ and $\Delta S°$ differences could also originate from a different number of solvent molecules in the first coordination sphere of the two diastereoisomers, auxiliary data is needed to exclude stereoselectivity due to different coordination numbers of the metal ion in the [Cu([LH$_{-1}$)] species. In order to obtain information about this aspect, EPR experiments have been carried out on some copper(II) complexes of diastereoisomeric dipeptides containing non-coordinating side chain groups.[28] As one can see in Table IX, the $g_{\|}$ values are characteristic of axial copper(II) complexes in tetragonally distorted octahedral, square-base pyramidal or square-planar stereochemistries, all copper(II) geometries being associated with a $d_{x^2-y^2}$ ground state. The $A_{\|}$ values are higher than those found in the case of copper(II)-bis(amino acidato) complexes which contain the same CuN_2O_2 chromophore.[30-32] These higher $A_{\|}$ 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 and, as a consequence, the metal ion should be considered to be essentially square-planar. In addition,

two trends can be observed in Table IX. First, when the size and type of R_1 and R_2 are varied, there is a slight decrease in $g_{\|}$ values and a small increase in $A_{\|}$ values from copper(II)-Ala-Ala to copper(II)-Val-Phe complexes. These shifts, parallelling those observed from the copper(II)Gly-Gly complex to Gly-Phe, have been ascribed to a probable substituent effect on the donor capabilities of both the two nitrogens and of the carboxylate oxygen coordinated to metal ion. There are also differences within each pair of copper(II) dipeptide complexes: $A_{\|}$ values are generally higher in the case of copper(II) complexes with LL dipeptides than those with LD dipeptides. In contrast, $g_{\|}$ is always slightly greater in the

TABLE IX

Spin-Hamiltonian parameters for copper (II) dipeptide complexes in water–methanol (95%–5%) mixtures at 150 K and room temperature

Ligand	$g_{\|}$	$A_{\|}$	$g_{\perp}$	$A_{\perp}$	g_{iso}	A_{iso}	$g_{\perp}$	$A_{\perp}$	Ref.
Ala-Ala									
LL	2.246	182	2.050	15	2.118	70.6	2.053	15	28
LD	2.246	182	2.050	15	2.118	70.6	2.053	15	28
Ala-Leu									
LL	2.248	183	2.046	15	2.117	72.5	2.052	17	28
LD	2.247	182	2.049	15	2.117	72.1	2.053	17	28
Leu-Leu									
LL	2.244	184	2.047	15	2.117	72.5	2.051	17	28
LD	2.245	178	2.049	15	2.117	71.3	2.051	18	28
Leu-Ile									
LL	2.243	186	2.046	15	2.117	71.8	2.051	15	28
DL	2.246	182	2.049	14	2.117	71.4	2.050	16	28
Ala-Phe									
LL	2.240	187	2.045	15	2.117	72.9	2.050	16	28
LD	2.243	183	2.043	15	2.117	72.3	2.051	17	28
Leu-Phe									
LL	2.237	187	2.045	15	2.117	72.5	2.050	15	28
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	28
LD	2.240	185	2.046	15	2.116	72.2	2.052	16	28
Val-Phe									
LL	2.239	187	2.045	15	2.116	72.1	2.051	15	28
LD	2.241	185	2.046	15	2.116	72.3	2.052	16	28
Gly-Gly	2.249	179	2.052	11	2.121	67.4	2.055	12	20
Gly-Leu	2.250	180	2.049	12	2.119	68.2	2.054	12	28
Gly-Phe	2.242	184	2.046	15	2.116	71.3	2.053	15	28

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case of the copper(II) complex with the LD isomer, even if little affected.

Considering the particular stereochemistry of each pair of LL and LD dipeptide complexes, it can 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 sites 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 the reported magnetic parameters, because more remarkable shifts in both $g_{\parallel}$ and $A_{\parallel}$ would have been expected by a coordination number ranging from 4 to 5 or 6.[33] Thus, to explain the higher $A_{\parallel}$ value of L,L dipeptide complexes with respect to corresponding LD isomer species, other factors have been invoked.[28] In particular, considering that in the $[Cu(LH_{-1})]$ species of LL diastereoisomeric molecules the side chains can interact above the plane of coordination, it has been suggested that, as a consequence of this weak 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 seem to reflect a small tetrahedral distortion, in agreement with Freeman's crystallographic work[34] that the basal plane formed by the dipeptide chelate group is distorted toward a tetrahedral situation. The assertion about the role of solvophobic forces is reinforced by the spectroscopic data collected in other solvents. In fact, when the proportion of the organic solvent was changed and the alkyl residues of the solvent could compete with the intramolecular solvophobic interaction of the side chains, it was found that the differences present in water tended to be minimized (see Tables IX and X). For instance, in an n-propyl alcohol–water mixture (80%–20%), the $A_{\parallel}$ differences fall within experimental error. Furthermore, it is remarkable that when one of the alkyl side chain groups is substituted with a phenyl or phenolic ring, the above-mentioned differences tend to become smaller. There must be another factor that plays a certain role in these complexes. Probably, the "stiffening" effect exerted by the side chain inter-

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TABLE X

EPR magnetic parameters of copper (II) dipeptide complexes in organic solvent–water (80%–20%) mixtures

Ligand		Methanol		Ethanol		n-Propyl Alcohol		Ref.
		$g_{\parallel}$	$A_{\parallel}$	$g_{\parallel}$	$A_{\parallel}$	$g_{\parallel}$	$A_{\parallel}$	
Ala-Ala	LL	2.247	183	2.247	183	2.246	183	28
	LD	2.247	183	2.247	183	2.246	183	28
Ala-Leu	LL	2.246	183	2.248	182	2.247	183	28
	LD	2.245	182	2.248	183	2.247	182	28
Leu-Leu	LL	2.244	185	2.249	183	2.247	183	28
	LD	2.245	181	2.248	180	2.247	182	28
Leu-Tyr	LL	2.237	188	2.243	186	2.245	185	28
	LD	2.240	186	2.246	184	2.244	184	28

actions 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) and the aromatic ring could account for this trend.[15]

Stereoselectivity in the copper(II) complex formation of dipeptide containing potentially coordinating side chain donor atoms has been mainly found in tyrosine derivatives (Table VII). The tyrosine-containing dipeptides, similarly to simple dipeptide, form a main species [Cu(L)] below pH 8. There is significant stereoselectivity in the 110 complexes (which correspond to the 11-1 complexes of simple dipeptides), the optically active species being the more stable. On the basis of this trend in the stability constants, it has been suggested[15] that positive stabilization in the "pure" complexes may be due to the weak interaction between the aryl-alkyl or aryl-aryl groups which are close together in the optically active complexes, but are on opposite sides of the molecule in the "mixed" complexes. In the pH range 8–10, a dimeric species $[Cu(LH_{-1})]_2$ was found. These species exhibit absorption and CD peaks at 380–400 nm at pH 8 ascribable to the charge transfer (CT) between Cu(II) and the phenolate group. The dimer formation by the peptides with a C-terminal aromatic ring is favored for the active peptide, especially with tryptophan. On the basis of molecular models, Pettit *et al.*[16] proposed an extended structure for the dimer (Fig. 3) where the stacking between the two bridging

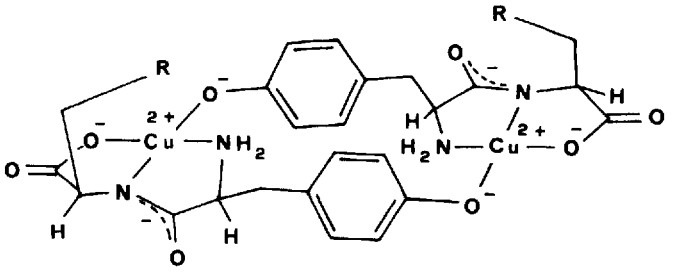

FIGURE 3 Probable extended structure of $[Cu_2(LH_{-1})_2]$ complex H_2L = tyrosylleucine.

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phenolate rings was considered to serve as a stabilizing factor. Yamauchi *et al.*[15] suggested a folded structure (Fig. 4) which may be stabilized by the weak non-covalent interaction between the two parallel aromatic rings and by additional stacking between closely disposed aromatic and/or pseudo-aromatic rings when R is equal to tyr or trp. Log K_d values (Table XI) pertinent to the following equilibrium:

$$2\,[Cu(LH_{-1})] \overset{K_d}{\rightleftharpoons} [Cu_2(L_2H_{-2})]$$

show for Tyr-Tyr and Tyr-Trp diastereoisomers that the active peptides are more stable than the meso ones. This can be explained by Yamauchi tentative structure[15] in which the C-terminal indole and phenol groups may stabilize the active (but not the meso) complexes by solvophobic interaction. The trend of log K_d values for the peptides with short aliphatic or charged groups is not straightforward; due to steric hindrance and/or electrostatic repulsion, active complexes might be destabilized according to the proposed structure (Fig. 4), but not the "mixed" complexes, where

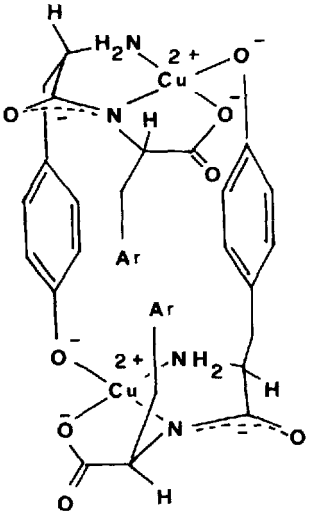

FIGURE 4 Hypothesized folded structure of copper(II)-Tyr-Tyr dymeric species.

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TABLE XI

Stability constants pertinent to $2[Cu(LH_{-1}] \rightleftharpoons [Cu_2(L_2H_{-2})]^2$ equilibrium of dipeptides at 25°C and I = 0.1 mol dm^{-3}

Dipeptide	log K_D	Ref.
L-Tyr-L-Ala	2.32	15
L-Tyr-D-Ala	2.56	15
L-Tyr-L-Arg	2.14	15
L-Tyr-D-Arg	2.83	15
L-Tyr-L-Glu	3.48	15
L-Tyr-D-Glu	3.12	15
L-Tyr-L-Tyr	2.72	15
L-Tyr-D-Tyr	2.03	15
L-Tyr-L-Trp	3.70	15
L-Tyr-D-Trp	2.44	15

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the side groups are directed outside of the domain produced by the folded dimeric core. CD magnitude anomaly has been used to reinforce the proposed structures.[15] For copper(II) and nickel(II) complexes of simple di- and tri-peptides, the CD magnitudes in the d-d region were found to be an additive function of the values of the complexes of the component amino acid residues.[29,35] Thus, the estimated magnitude $\Delta\varepsilon_{calcd}$ for the 1:1 copper(II) complex of a peptide X-Y is given by $\Delta\varepsilon_{calcd} = \Delta\varepsilon_{Cu(X\text{-}Gly)} + \Delta\varepsilon_{(Gly\text{-}X)}$ where $\Delta\varepsilon_{Cu(X\text{-}Gly)}$ and $\Delta\varepsilon_{Cu(Gl\text{-}Y)}$ denote the magnitudes exhibited by the 1:1 complexes of X-Gly and Gly-Y, respectively. Table XII shows CD magnitude additivity in the region 600–700 nm in 1:1 dipeptide systems at the dimeric species pH. The differences $\Delta\varepsilon - \Delta\varepsilon_{calcd}$ serve as a criterium of the magnitude additivity. While the additivity in the d-d region holds for the complexes of the dipeptides with a C-terminal aliphatic amino acid or a D-aromatic amino acid, a large deviation from $\Delta\varepsilon_{calcd}$ with sign inversion was found at pH 9.0–10.3, with the peak at 600–700 nm for the active complexes comprising a C-terminal aromatic amino acid. This diastereospecific magnitude anomaly has been ascribed to the dimers,[15] because it disappears at low concentration or in the presence of ammonia. The CD spectra in the d-d region observed for the Cu(II) dipeptide complexes reflect the peptide side chain conformation. In this context, the magnitude enhancement exhibited by Cu(II) and Ni(II)-

TABLE XII

CD magnitude additivity in the region 600–700 nm in 1:1 Cu(II)-dipeptide systems[a]

Dipeptide	pH	λ_{max}, nm	$\Delta\epsilon$	$\Delta\epsilon_{calcd}$	Δ	Sign Inversion
L-Tyr-L-Ala	6.4	680	−0.30	−0.19	0.11	no
	9.8	618	+0.06	+0.01	0.05	no
	11.2	595	+0.10	+0.09	0.01	no
L-Tyr-D-Ala	6.2	652	+0.60	+0.51	0.09	no
	9.4	670	+0.64	+0.64	0.00	no
	11.4	675	+0.59	+0.52	0.07	no
L-Tyr-L-Arg	5.6	655	−0.50	−0.25	0.25	no
	9.1	680	−0.31	−0.22	0.09	no
	11.3	680	−0.36	−0.25	0.11	no
L-Tyr-D-Arg	5.6	645	+0.69	+0.59	0.10	no
	9.0	662	+0.82	+0.71	0.11	no
	11.2	658	+0.74	+0.66	0.08	no
L-Tyr-L-Tyr	6.1	647	−0.66	−0.52	0.13	no
	9.0	683	+0.39	−0.38	0.77	yes
	10.3	683	+0.35	−0.42	0.77	yes
	11.5	678	−0.59	−0.47	0.12	no
L-Tyr-D-Tyr	6.3	635	+0.90	+0.83	0.07	no
	9.0	662	+0.79	+0.87	0.08	no
	10.1	665	+0.85	+0.88	0.03	no
	11.5	668	+0.94	+0.86	0.08	no
L-Tyr-L-Trp	6.6	636	−0.36	−0.40	0.04	no
	9.5	678	+0.78	0.40	1.18	yes
	11.0	680	+0.11	−0.29	0.40	yes
L-Tyr-D-Trp	6.6	634	+0.75	−0.55	0.20	no
	9.5	655	+0.91	−1.00	0.09	no
	11.0	663	+0.72	−0.69	0.03	no

[a]Ref. 15.

tetra and pentapeptide complexes[36] has been interpreted as due to intramolecular hydrogen bonding. For Tyr-Tyr and Tyr-Trp copper(II) complexes, the CD anomaly was ascribed to the conformational change of the C-terminal aromatic amino acid,[15] because this is more influential on the spectral pattern[35,37] and its aromatic side chain prefers to be located above the metal coordination plane as in the Pd(II)-phenylalanylphenilalaninate complex,[38] in solution, or in the copper(II) complexes of tyrosine,[39]

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glycyl-L-leucyl-L-tyrosine,[40] and glycyl-L-tryptophan,[41] in the solid state. Assuming that the active monomeric species has the side chain conformation reported in Fig. 5, it has been proposed that this conformation is distorted upon dimer formation by the bridging phenolate rings to make the side group lose access to the copper(II) coordination plane when the dimer has the structure reported in Fig. 4. No conformational change should occur with the meso complexes because of the outwardly directed side chain, thus giving an explanation for the diastereospecificity of the magnitude anomaly. The aromatic ring stacking in the active peptide complexes makes the side chain conformation more rigid, which is also compatible with the magnitude anomaly.

The spectroscopic approach together with potentiometric measurements were used in the study of the stereoselectivity of histidine containing dipeptides (Table VII). The spectroscopic properties (visible, c.d. and e.s.r.)[17] can identify the coordination mode. The reason for the stereoselectivity, explained by considering the preferred trans conformations of the peptide chains, is based only on the log β differences. In the case of copper(II) complexes with L-methionyl-L-methionine,[14] thermodynamic stereoselectivity was found in the formation of the amide-deprotonated complexes (Table XIII). In particular, the formation of the copper(II) complex with L,L dipeptide is enthalpically favored. Structural information obtained by the EPR spectra in solution (L,L-isomer: $g_{\parallel} = 2.243$, $A_{\parallel} = 0{,}0186\ cm^{-1}$, $g_{\perp} = 2.050$, $A_{\perp} = 0.0011\ cm^{-1}$, $g_{iso} = 2.119$,

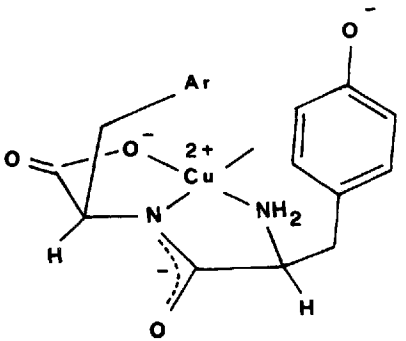

FIGURE 5 Tentative side chain conformation of monomeric copper(II) peptide complex.

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TABLE XIII

Thermodynamic parameters of copper (II) complex formation of diastereoisomeric methionyl-methionine dipeptides at 25°C and $I = 0.1$ mol dm^{-3}

Dipeptide	$-\Delta G^{\circ}_{11\text{-}1}$ kcal mol^{-1}	$\Delta H^{\circ}_{11\text{-}1}$ kcal mol^{-1}	$\Delta S^{\circ}_{11\text{-}1}$ cal mol^{-1} deg^{-1}	Ref.
L-Met-L-Met	2.32	1.90	14.3	14
D-Met-L-Met	1.95	3.60	18.8	14

$A_{iso} = 0{,}00714$ cm^{-1}; D,L-isomer: $g_{\parallel} = 2.240$, $A_{\parallel} = 0.0184$ cm^{-1}, $g_{\perp} = 2.049$, $A_{\perp} = 0.0011$ cm^{-1}, $g_{iso} = 2113$, $A_{iso} = 0{,}00703$ cm^{-1}) parallels the thermodynamic data: a slightly larger hyperfine coupling constant has been found in the case of the copper(II) complex with Met-Met.[14] Comparison with data pertinent dipeptides containing noncoordinating side chain groups (see Table IX) is evidence that the sulfur atom is not coordinated to the metal ion. The thermodynamic stereoselectivity of the amide deprotonated complex shows differences of 0.3 in log β and 1.7 kcal mol^{-1} in ΔH°, and has been attributed to the hydrophobic interaction between the residues of the side chains. This noncovalent "bonding," as stressed above, is possible only for the L,L-diastereoisomer where the side chains are on the same side of the coordination plane.

In conclusion, (a) thermodynamic stereoselectivity can be due to different kinds of non-covalent interactions and to the algebraic sum of their effects; (b) ΔH° and ΔS° values have proved diagnostic in evaluating the relative weight of these interactions; (c) enthalpy changes may also reflect the occurrence of solvophobic interactions[22] when the responsible groups are significantly far from each other, thus showing a "sensitivity" comparable to spectroscopic approaches (like NMR).

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