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Weak forces in the thermodynamic stereoselectivity of proton and copper(II) complexes with diastereoisomeric dipeptides containing aromatic side chains

Vincenzo Cucinotta [a], Giulia Grasso [b], Giuseppe Maccarrone [a], Lidia Mastruzzo [a], Giuseppe Pappalardo [b]

[a] *Dipartimento di Scienze Chimiche, Università di Catania, Catania, Italy*

[b] *Istituto per lo Studio delle Sostanze Naturali di Interesse, Alimentare e Chimico-Farmaceutico del C.N.R., Catania, Italy*

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Abstract

The origins of thermodynamic stereoselectivity in proton and copper(II) complexes of diastereoisomeric dipeptides with aromatic side chains were investigated. The use of a combined approach (potentiometric and calorimetric measurements together with spectroscopic studies) made it possible to examine the different forces that drive stereoselectivity. The aromatic side chains seem to assist proton complex formation (in different ways) in comparison with copper(II) complexation. This different role of side-chain residues is ascribed to a possible d–π interaction existing in both the copper(II) complexes with the two diastereoisomeric dipeptides.

Keywords: Thermodynamic stereoselectivity; Proton complexes; Copper complexes; Dipeptide complexes

1. Introduction

Stereoselectivity in proton and metal complex formation constitutes a special subset of molecular recognition that is due to extremely short-range forces, noncovalent or weak forces [1,2], including electrostatic interactions, hydrogen bonds, solvophobic [3] or, in more classic terms, hydrophobic interactions [4].

In particular, interest has been directed towards thermodynamic stereoselectivity in simple and mixed complexes of a number of bio-ligands [5–7]. Electrostatic and alkyl/alkyl or alkyl/aryl interactions have been put forward to explain the different stability found in proton or copper(II) complexes of diastereoisomeric linear dipeptides in aqueous solution [8]. In addition, the trend seen in spectroscopic results (NMR and electron paramagnetic resonance (EPR)) parallels the thermodynamic parameters [9,10]. The difference in stability constants was more evident in the copper(II) complexes of diastereoisomeric dipeptides with aliphatic side chains than in the analogous complexes of isomeric pairs with aromatic residues [10,11]. By contrast, in the case of proton complex formation, an increase in stereoselectivity was found for nearly all the systems with aromatic residues [9]. It was suggested that this different behaviour was due to the differential contribution of a d–π interaction between the metal ion and the aromatic ring, which counterbalances the favourable hydrophobic interaction present only in the complexes of L,L diastereoisomers [8]. In these complexes side-chain residues can interact, being on the same side of the coordination plane; hydrophobic interaction is not possible in the case of L,D dipeptide complexes, as the side chains are on opposite sides of the coordination plane. To obtain further evidence of the involvement of the above-mentioned d–π interaction, a new kind of weak force, we report here a detailed thermodynamic (potentiometric and calorimetric) and spectroscopic (NMR and EPR) investigation concerning proton and copper(II) complexes with L,L (pure) and L,D (mixed) diastereoisomers of alanyl-tryptophan, a peptide with a more extended aromatic residue than the dipeptides previously studied, where the side chain was a benzene ring.

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2. Experimental

2.1. Synthesis of L,D-alanyl-tryptophan

N-carbobenzoxy-L-alanine-4-nitrophenyl ester (2.9 mmol) was stepwise proportionately added to a solution of triethylamine (3.48 mmol) and D-tryptophan methyl ester hydrochloride (3.48 mmol) in methylene chloride (10 cm^3) in the presence of 1-hydroxybenzotriazole hydrate (hobt). The mixture was stirred at room temperature overnight, the solvent was evaporated off and the residue was solubilized in ethyl acetate. The reaction mixture was extracted with aqueous ammonia (1 mol dm^{-3}) and then with water until the rinse water was practically neutral. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The *N*-carbobenzoxy-L-alanine-D-tryptophanOMe obtained was dissolved in methanol, and NaOH (1 mol dm^{-3}) was added. After 5 h the solution was acidified with 10% citric acid and extracted with ethyl acetate. The *N*-carbobenzoxy-L-Ala-D-TrpOH was dissolved in ethanol (20 cm^3) and hydrogenolyzed over 10% palladium-on-charcoal catalyst (160 mg) with a slow stream of hydrogen until CO_2 generation ceased. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. Crystallization of the product from ethyl acetate/ethanol gave 0.9 g (75% yield) of colourless prisms with the following physical constants: m.p. 116.8 °C; $[\alpha]_D^{25}$ +15 (c 1, water); *m/z* 276 (M^+).

The dipeptide L-alanyl-L-tryptophan was purchased from Bachem (Switzerland) and was used as received.

2.2. Potentiometric measurements

Computer-controlled potentiometric titrations were performed using two distinct Metrohm digital pH meters (model 654) equipped with Metrohm 109 glass and Metrohm 404 saturated calomel electrodes. The titration cell was thermostatted at 25.0 ± 0.2 °C, and all solutions were kept under an atmosphere of nitrogen, which was bubbled through a solution of the same ionic strength and temperature as that of the solutions under study. The electrode couples were 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). $Cu(NO_3)_2$ was prepared from basic copper(II) carbonate by adding a slight excess of HNO_3. The concentrations of the stock solutions were determined by ethylenediamine tetraacetate titrations using murexide as an indicator [12]. Stock solutions of HNO_3 and KOH were made from concentrated HNO_3 (Suprapur Merck) and from Normex Carlos Erba vials, respectively, and their concentrations were determined potentiometrically by titrating with tris(hydroxymethyl)aminomethane and potassium hydrogenphthalate, respectively. The ionic strength was adjusted to 0.10 mol dm^{-3} by adding KNO_3 (Suprapur Merck).

Solution aliquots of 2.5 cm^3, containing different amounts of the dipeptide and HNO_3 or $Cu(NO_3)_2$, were titrated with standard KOH solutions; for the complexation the solutions contained the metal ion and the ligand in a 1:1 ratio. The analytical concentrations of peptides ranged from 0.004 to 0.006 mol dm^{-3}. The ionic strength was kept at 0.1 mol dm^{-3} by adding KNO_3.

2.3. Calorimetric measurements

The calorimetric data were obtained by titration calorimetry using an LKB 2277 thermal activity monitor equipped with a 2.5 cm^3 reaction Dewar flask. The calorimetric system was calibrated by titrating tris(hydroxymethyl)aminomethane with HCl in accordance with the method of Grenthe et al. [13]. The dipeptide heats of protonation and complexation were determined by titrating solutions of the ligands with standard HNO_3. For complexation the solutions contained the metal and the ligand in a 1:1 ratio. The dipeptide concentrations ranged from 0.004 to 0.006 mol dm^{-3}. Reaction heats, corrected for dilution heat, determined in separate experiments, are expressed in calories (1 cal = 4.184 J).

2.4. ^1H NMR measurements

The ^1H NMR spectra were obtained in D_2O at 25 °C at 200.13 MHz using a Bruker AC-200 spectrometer. Sample concentrations were close to 0.005 mol dm^{-3}. The D_2O solutions were adjusted to pD 11 by the addition of NaOD and were then titrated using DCl solution to obtain completely protonated and completely unprotonated forms of the peptides.

2.5. EPR 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-inch electromagnet. Quartz tubes were used for the frozen solution, while a Bruker quartz water solution cell was employed to collect room-temperature spectra. A low-temperature unit was used to achieve a temperature of 150 K. The microwave frequency was calibrated using powdered DPPH (1,1-diphenyl-2-picrylhydrazyl) samples ($g = 2.0036$), while the magnetic field was carefully measured during each spectrum scan by means of a Bruker ER 035M gaussmeter. Methanol was added to a water solution (0.005 mol dm^{-3} in $^{63}Cu(NO_3)_2$ and in dipeptide) at pH 6.0 to obtain a 95% water–5% methanol solution used to record the EPR spectra. The amount of methanol was a com-

promise between the desire to obtain well-resolved spectra and the need to not vary the proportion of water in favour of an organic solvent. Many spectra were also run on samples of the same complexes with various amounts (up to 80%) of organic solvent (methanol, ethanol, n-propyl alchol).

2.6. Calculations

Calculations to obtain the electrode system E^0 values, ligand purity and HNO_3 excess in the metal stock solutions were performed using the least-squares program ACBA [14]. The protonation and complexation constants were calculated using the computer program SUPERQUAD [15]. Protonation and complexation heats were calculated using the least-squares computer program DOEC [16]. 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.

3. Results and discussion

The protonation constants of the dipeptides investigated are listed in Table 1, together with previously reported values for similar molecules. The $\log K$ value for the protonation of the amino group is higher for L-Ala-D-Trp than for the L,L isomer, while the opposite is observed for the protonation of the carboxylate group. This is in accordance with the literature data for the other peptides. If we compare the differences in $\log K$ (NH_2) values between the diastereoisomeric pairs of Ala-Ala (0.13 l.u.), Ala-Phe (0.19 l.u.) and Ala-Trp (0.32 l.u.), we observe an increase in stereoselectivity upon increasing the size of the side chains. This can be explained by bearing in mind that in dipeptides where the aliphatic chains are short (Ala-Ala), the alkyl residues cannot interact with each other, while in dipeptides with an extended aromatic residue, there can be a solvophobic interaction with the alkyl residue, as shown by the NMR data summarized in Table 2. We focused on the chemical shifts of the methyl groups, to gain further information on the solvophobic interaction suggested by the thermodynamic data. The Ala-Trp protonation causes, as expected, a gradual downfield shift. The chemical shift observed for all the L-Ala-D-Trp diastereoisomer species is upfield compared with that for the corresponding L-Ala-L-Trp species. This trend can be ascribed to a solvophobic interaction in the 'mixed' dipeptide that causes the methyl protons to lie above the plane of the aromatic ring. These protons thus experience an upfield shift due to the ring-current effect [19].

The following discussion is based on the assumption that the dipeptides investigated are in a β-type conformation [20–23] in their acidic, neutral and basic species. In Table 3 we report the differences in the chemical shifts of the terminal methyl groups of the two diastereoisomer pairs in corresponding species. In doing so, we have used the 'pure' diastereoisomer data as a blank, assuming that the effect of protonation is analogous for the two diastereoisomers. It can be seen that in all the dipeptides with an aromatic moiety, the differences are highest for the amphiprotic species. Moreover, the Ala-Trp diastereoisomeric pairs show the largest differences, not only for the amphiprotic species, but also for the cationic and anionic species. This suggests that the solvophobic interaction is particularly strong in this system, owing to the larger size of the indole moiety compared with the phenyl moiety. Interestingly, the difference between the amphiprotic and the anionic species is smaller for Ala-Trp dipeptides (4.0 Hz) than for Ala-Phe dipeptides (12.2 Hz). Thus, while the absolute strength of the solvophobic interaction is larger for Ala-Trp, the increase in this interaction going from the anionic to the amphiprotic species is larger for Ala-Phe. In other words, while for Ala-Phe the contribution of the electrostatic interaction in assisting the solvophobic interaction is determinant, for Ala-Trp the interaction is so strong by itself that the contribution of the electrostatic interaction becomes less important.

The ΔG^0, ΔH^0 and ΔS^0 values for the two steps of protonation of the L-Ala-L-Trp and L-Ala-D-Trp diastereoisomers are reported in Table 4. The amino group protonation is favoured enthalpically, while the carboxyl group protonation is favoured entropically. In particular, the amino group protonation of L-Ala-D-Trp is more

Table 1
Cumulative protonation constants for diastereoisomeric dipeptides at 25 °C and $I=0.1$ mol dm^{-3} (KNO_3)

Dipeptide	$\log K$ (CO_2^-)	$\log K$ (NH_2)	Ref.
L-Ala-L-Ala	3.30	8.17	[17]
L-Ala-D-Ala	3.12	8.30	[18]
L-Ala-L-Phe	3.25	7.89	[17]
L-Ala-D-Phe	3.02	8.08	[17]
L-Ala-L-Trp	3.279(2)	7.880(1)	this work
L-Ala-D-Trp	3.106(3)	8.201(2)	this work

Table 2
Chemical shifts (in Hz) of the methyl protons

Dipeptide	Cationic	Dipolar	Anionic	Ref.
L-Ala-L-Ala	124.6 [a], 116.8 [b]	124.3 [a], 108.8 [b]	101.2 [a], 108.2 [b]	[9]
L-Ala-D-Ala	123.7 [a], 116.7 [b]	120.6 [a], 108.3 [b]	100.3 [a], 107.5 [b]	[9]
L-Ala-L-Phe	118.9	118.2	91.0	[9]
L-Ala-D-Phe	100.2	97.9	82.9	[9]
L-Ala-L-Trp	114.8	113.0	103.5	this work
L-Ala-D-Trp	89.0	83.12	77.4	this work

[a] Methyls close to the amino group.
[b] Methyls close to the carboxylic group.

Table 3
Differences between the chemical shifts (in Hz) of corresponding species of the two diastereoisomers of each dipeptide ($\delta_{pure} - \delta_{mixed}$)

Dipeptide	Cationic	Dipolar	Anionic	Ref.
Ala-Ala	0.9 [a], 0.1 [b]	3.7 [a], 0.5 [b]	0.9 [a], 0.7 [b]	[9]
Ala-Phe	18.7	20.3	8.1	[9]
Ala-Trp	25.76	30.08	26.08	this work

[a] Methyls close to the amino group.
[b] Methyls close to the carboxylic group.

Table 4
Thermodynamic parameters of protonation of diastereoisomeric dipeptides at 25 °C and $I = 0.1$ mol dm^{-3}

Ligand	$-\Delta G^0$ (kJ mol^{-1})		$-\Delta H^0$ (kJ mol^{-1})		ΔS^0 (J K^{-1} mol^{-1})		Ref.
	NH_2	CO_2^-	NH_2	CO_2^-	NH_2	CO_2^-	
L-Ala-L-Ala	46.61	18.82	44.51	−1.09	7.11	66.52	[24]
L-Ala-D-Ala	47.44	18.15	42.93	−2.63	15.06	69.87	[24]
L-Ala-L-Phe	45.27	17.90	43.97	−0.21	4.18	60.67	[24]
L-Ala-D-Phe	46.73	16.86	44.52	−1.63	7.53	61.09	[24]
L-Ala-L-Trp	44.94	18.70	41.6(3)	0.6(2)	10.88(8)	60.7(1)	this work
L-Ala-D-Trp	46.77	17.74	42.8(2)	−0.4(2)	12.97(8)	60.67(8)	this work

exothermic than that of the corresponding 'pure' diastereoisomer, and the carboxyl group protonation is more favoured entropically than that of the 'mixed' diastereoisomer; this is consistent with previously reported results for diastereoisomeric dipeptides [24]. From a quantitative point of view, the ΔH^0 difference between the two diastereoisomers (1.13 kJ mol^{-1}) is significantly larger than that for Ala-Phe (0.54 kJ mol^{-1}). We can explain this difference on the basis of conformational effects. Following the protonation of the amino group of the L,D diastereoisomers of Ala-Phe, the side chains must approach each other to strengthen the solvophobic interaction, thus causing a rigidity of the molecule with an associated endothermic effect. On the contrary, in Ala-Trp such an enthalpically unfavoured conformational variation does not occur, because, as shown by NMR results, the side chains are also close in the anionic species, and no increase in molecule rigidity occurs upon going from the anionic to the amphiprotic species.

The general overall formation reaction of copper(II) ions or protons with peptide ligands is given by Eq. (1):

$$m\mathrm{Cu}^{2+} + l\mathrm{L} + h\mathrm{H}^{+} \overset{\beta_{mlh}}{\rightleftharpoons} [\mathrm{Cu}_m\mathrm{L}_l\mathrm{H}_h] \quad (1)$$

where L is the negative species of the peptide ligands. Charges on the ligand and the copper(II) complexes are omitted for clarity of notation. The stability constant β_{mlh} is defined by Eq. (2):

$$\beta_{mlh} = \frac{[\mathrm{Cu}_m(\mathrm{L}_l\mathrm{H}_h)]}{[\mathrm{Cu}^{2+}]^m[\mathrm{L}]^l[\mathrm{H}]^h} \quad (2)$$

The equilibria necessary to fit the experimental titration curves for the solutions of copper(II) and peptide ligands under study are given by Eqs. (3)–(6):

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

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

Table 5
Cumulative association constants for copper(II) complexes with diastereoisomeric dipeptides at 25 °C and $I=0.1$ mol dm^{-3} (KNO_3)

Ligand	$\log \beta_{110}$	$\log \beta_{11-1}$	pK^{H}_{CuL} [a]	Ref.
L-Ala-L-Ala	5.54	1.82	3.72	[10]
L-Ala-D-Ala	5.71	1.75	3.96	[10]
L-Ala-L-Phe	5.20	1.76	3.44	[10]
L-Ala-D-Phe	5.42	1.49	3.93	[10]
L-Ala-L-Trp	5.16(5)	1.920(2)	3.24	this work
L-Ala-D-Trp	5.09(2)	1.734(1)	3.35	this work

[a] Constants concerning the equilibrium $CuL \rightleftharpoons CuLH_{-1} + H^+$.

Table 6
Thermodynamic functions for $[CuLH_{-1}]$ complex formation of copper(II) with diastereoisomeric dipeptides at 25 °C and $I=0.1$ mol dm^{-3} (KNO_3)

Ligand	$-\Delta G^0$ (kJ mol^{-1})	ΔH^0 (kJ mol^{-1})	ΔS^0 (J K^{-1} mol^{-1})	Ref.
L-Ala-L-Ala	10.38	8.2	62.3	[10]
L-Ala-D-Ala	10.00	6.4	55.2	[10]
L-Ala-L-Phe	9.87	1.4	41.8	[10]
L-Ala-D-Phe	8.87	5.9	49.8	[10]
L-Ala-L-Trp	11.30	3.3(2)	48.9(8)	this work
L-Ala-D-Trp	10.10	4.7(4)	49.4(1)	this work

Table 7
Spin-Hamiltonian parameters for $[CuLH_{-1}]$ complexes in a water–methanol (95%–5%) mixture at 150 K and room temperature

Ligand	$g_\parallel$	$A_\parallel$	Ref.
L-Ala-L-Ala	2.246	182	[10]
L-Ala-D-Ala	2.246	182	[10]
L-Ala-L-Phe	2.240	187	[10]
L-Ala-D-Phe	2.241	184	[10]
L-Ala-L-Trp	2.231(2)	184(1)	this work
L-Ala-D-Trp	2.234(1)	181(1)	this work

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

$$Cu^{2+} + 2L \overset{\beta_{12-1}}{\rightleftharpoons} [CuL_2H_{-1}] + H^+ \quad (6)$$

The formation percentages of the [CuLH] and $[CuL_2H_{-1}]$ species were always less than 5%; thus the formation constants of the main species only are listed in Table 5.

The values of the stability constants pertinent to the $[CuLH_{-1}]$ complexes show that the L-Ala-L-Trp diastereoisomer forms a more stable metal complex than the L,D isomer does. It can be seen that stereoselectivity is insignificant at low pH values, where the major species is [CuL], but appreciable at neutral pH (a region particularly important from a biological point of view), where the $[CuLH_{-1}]$ species predominates. For the proton complex formation stereoselectivity, the difference in $\log \beta_{11-1}$ values between the two diastereoisomeric copper(II) complexes with Ala-Trp is lower than that found for the analogous complexes with Ala-Phe.

A greater understanding of complexation effects can be obtained by inspecting the separate enthalpic and entropic contributions reported in Table 6. As observed for all the dipeptides already studied, except for the Ala-Ala pair, the L,L dipeptide is less unfavoured enthalpically in comparison with the corresponding L,D dipeptide, but the ΔH^0 difference between the two diastereoisomeric complexes is smaller than that found for the analogous species of Ala-Phe. The ΔS^0 changes accompanying the complex formation of the two diastereoisomers are nearly the same. The presence of a more extended aromatic system in the dipeptides seems to be responsible for the decrease in the stereoselectivity assisted by the metal ion.

To gain more information on the effects that give rise to the thermodynamic behaviour observed, and to ensure that these differences are not due to a different number of solvent molecules in the first coordination sphere of the two diastereoisomers, EPR experiments were also carried out. As one can see from Table 7, the spectra show $g_\parallel > g_\perp > 2.04$, values characteristic of axial copper(II) complexes in tetragonally distorted octahedral, square-base pyramidal or square-planar stereochemistries [25], all copper(II) geometries being associated with a $d_{x^2-y^2}$ ground state.

$A_\parallel$ values are always higher for copper(II) complexes with L,L dipeptides than for those with L,D dipeptides, whereas $g_\parallel$ values are always slightly higher in the case of the copper(II) complexes with the L,D isomer, even if affected very little. Considering that in the $[CuLH_{-1}]$

Table 8
EPR magnetic parameters of [$CuLH_{-1}$] complexes in organic solvent-water (80%–20%) mixtures

Ligand	Methanol		Ethanol		n-Propyl alcohol		Ref.
	$g_\parallel$	$A_\parallel$	$g_\parallel$	$A_\parallel$	$g_\parallel$	$A_\parallel$	
L-Ala-L-Ala	2.247	183	2.247	183	1.246	183	[10]
L-Ala-D-Ala	2.247	183	2.247	183	2.246	183	[10]
L-Ala-L-Leu	2.246	183	2.248	182	2.247	183	[10]
L-Ala-D-Leu	2.245	182	2.248	183	2.247	182	[10]
L-Leu-L-Leu	2.244	185	2.249	183	2.247	183	[10]
L-Leu-D-Leu	2.245	181	2.248	180	2.247	182	[10]
L-Ala-L-Trp	2.236(1)	187(1)	2.235(2)	183(1)	2.237(2)	182(1)	this work
L-Ala-D-Trp	2.241(1)	186(1)	2.236(1)	185(1)	2.236(1)	183(1)	this work

species of L,L diastereoisomers 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 in the basal plane [10]. The donor atoms of the dipeptide coordinated to a metal ion could thus achieve a quasi-ideal planar conformation. In contrast, where this interaction is not possible (for the L,D dipeptide complexes), the lower $A_\parallel$ values led us to think of either a small tetrahedral distortion [26] or a stronger interaction with apical solvent molecules. The above suggestion is 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 Table 8). As it is a well-known fact that hydrophobic interaction decreases with decreasing water percentage in the mixed solvent, this result seems to support the hypothesis that the small differences found within each pair of copper(II) complexes are to be ascribed to solvophobic forces. The thermodynamic and spectroscopic data show that stereoselectivity in proton-complex formation increases when the aliphatic side chain is substituted by an aromatic one [8,9,24], the effect becoming more evident when the dimensions of the aromatic ring are increased. This increase in the stereoselective effect seems not to occur in copper(II) complex formation. A sort of levelling effect is responsible for this decrease in stereoselectivity. A direct interaction between the d electrons of the metal ion and the π ring system of the coordinated ligand side chain has been suggested on the basis of spectroscopic data in solutions [27–30]. Two preliminary reports on a detailed examination of molecular orbitals have more recently demonstrated the formation of bonding orbitals between copper(II) and the six-membered ring of the phenyl residue in an optimized structure of [Cu(Leu-Phe)H_{-1}] complex [31] as well as between copper(II) and the five-membered ring of the indole residue in the [Cu(bpy)(L-Trp)H_2O]$^+$ species [32]. To rationalize these and previously reported thermodynamic and spectroscopic results, we are led to suppose that there is a similar interaction. In fact, the d–π interaction can assist metal complex formation with the L,D isomer, while the solvophobic interaction assists metal complex formation with the L,L dipeptide. In proton complex formation, where this d–π interaction does not exist, the stereoselectivity is due only to the noncovalent forces (electrostatic and solvophobic interactions). Thus, more extended aromatic side chains increase the difference in stability between the two diastereoisomeric proton complexes, as found by the combined thermodynamic and spectroscopic results.

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References

[1] E. Frieden, *J. Chem. Educ., 52* (1975) 754.
[2] J.D. Watson, N.H. Hopkins, J.W. Roberts, J. Argetsinger Steitz and A.M. Weiner, *Molecular Biology of the Gene*, Benjamin Cummings, Menlo Park, CA, 1987, Ch. 5.
[3] O. Sinanoglu, *Int. J. Quantum Chem., 18* (1980) 381.
[4] W. Kauzmann, *Adv. Protein Chem., 14* (1952) 34.
[5] L.D. Pettit and R.J.W. Hefford, *Met. Ions Biol. Syst., 9* (1979) 173.
[6] G. Borghesani, F. Pulidori, R. Purrello, M. Remelli and E. Rizzarelli, *J. Chem. Soc., Dalton Trans.*, (1990) 209.
[7] G. Arena, R.P. Bonomo, L. Casella, M. Gullotti, G. Impellizzeri, G. Maccarrone and E. Rizzarelli, *J. Chem. Soc., Dalton Trans.*, (1991) 3203.
[8] V. Cucinotta, R. Purrello and E. Rizzarelli, *Comments Inorg. Chem., 11* (1990) 85.
[9] R. Calì, V. Cucinotta, G. Impellizzeri, M.C. Maugeri and E. Rizzarelli, *Int. J. Peptide Protein Res., 32* (1988) 262.
[10] R.P. Bonomo, R. Calì, V. Cucinotta, G. Impellizzeri and E. Rizzarelli, *Inorg. Chem., 25* (1986) 10.

[11] R.P. Bonomo, G. Maccarrone, E. Rizzarelli and M. Vidali, *Inorg. Chem., 26* (1987) 2893.
[12] A. Flaschka, *EDTA Titrations*, Pergamon, London, 1959.
[13] E. Grenthe, H. Ots and O. Ginstrup, *Acta Chem. Scand., 24* (1970) 1607.
[14] G. Arena, E. Rizzarelli, S. Sammartano and C. Rigano, *Talanta, 26* (1979) 1.
[15] P. Gans, A. Sabatini and A. Vacca, *J. Chem. Soc., Dalton Trans.*, (1985) 1195.
[16] C. Rigano, E. Rizzarelli and S. Sammartano, *Thermochim. Acta, 33* (1979) 211.
[17] R. Nakon and R.J. Angelici, *J. Am. Chem. Soc., 96* (1974) 4178.
[18] A. Kaneda and A.E. Martell, *J. Am. Chem. Soc., 99* (1977) 1586.
[19] K.G. Wagner and R.J. Lawaczeck, *J. Magn. Reson., 8* (1972) 164.
[20] J. Beacham, V.T. Ivanov, G.W. Kenner and R.C. Sheppard, *J. Chem. Soc., Dalton Trans.*, (1965) 386.
[21] P.J. Flory and P.R. Schimmel, *J. Am. Chem. Soc., 89* (1967) 6807.
[22] S. Landè, *Biopolymers, 7* (1969) 879.
[23] R.U. Lemieux and M.A. Barton, *Can. J. Chem., 49* (1971) 767.
[24] G. Impellizzeri, R.P. Bonomo, R. Calì, V. Cucinotta and E. Rizzarelli, *Thermochim. Acta, 80* (1984) 275.
[25] B.J. Hathaway and D.E. Billing, *Coord. Chem. Rev., 5* (1970) 143.
[26] H.C. Freeman, in J. Peisach, P. Aisen and W.E. Blumberg (eds.), *The Biochemistry of Copper*, Academic Press, New York, 1966, p.77.
[27] W.L. Kwik, K.P. Ang and G.I. Chen, *J. Inorg. Nucl. Chem., 42* (1980) 303.
[28] P.I. Vestues and R.B. Martin, *J. Am. Chem. Soc., 102* (1980) 7906.
[29] S.H. Khim and R.B. Martin, *J. Am. Chem. Soc., 106* (1984) 1707.
[30] O. Yamauchi, K. Tsujide and A. Odani, *J. Am. Chem. Soc., 107* (1985) 659.
[31] G. Maccarrone, G. Nardin, L. Randaccio, E. Rizzarelli, M. Rosi, A. Sgamellotti and E. Zangrando, *Proc. Congresso Interdivisionale Società Chimica Italiana, Perugia, Italy, Oct. 7–11, 1989*, p. 491.
[32] H. Masuda, N. Fukushima, T. Sugimori and O. Yamauchi, *J. Inorg. Biochem., 51* (1993) 158.