Conformation and Solution Equilibria of Diastereoisomeric Dipeptides and Their Copper(II) and Nickel(II) Complexes[1,2]

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Abstract: The aqueous solution equilibria of diastereoisomeric dipeptides L-alanyl-L-alanine, D-alanyl-L-alanine, L-leucyl-L-tyrosine, and D-leucyl-L-tyrosine and their copper(II) and nickel(II) complexes have been studied by the potentiometric method. Considerable differences in the formation of the individual ionic species have been observed between the diastereoisomers of metal-free peptides and metal complexes. Conformational analyses for the ionic species present have been carried out with the aid of molecular models. Correlation of the stereochemical differences of the diastereoisomeric complexes with the differences in the corresponding equilibrium constants was employed to indicate the most probable conformations of individual species in aqueous solution. The hydrophobic nature of the side chains was interpreted as playing an important role in determining the conformations and equilibria of the diastereoisomeric complexes.

Ellengoben[3-6] showed that the proton association constants of lysyl and alanyl dipeptides consisting of two L-amino acids (abbreviated as L-L) are identical with those of the D-D isomer, and those of the D-L isomer are identical with those of the L-D isomer. However, the carboxyl proton association constants, K_2^H, of the D-L form were found to be smaller than those of the L-L, while the amino proton association constants, K_1^H, of the D-L isomer are larger than those of the L-L isomer.[3] This trend was confirmed by Kaneda and Martell[7] and by Nakon and Angelici[8] and most recently by Brookes and Pettit.[9]

Li et al.[10] observed that the values of β_1 ($[ML^+]/[M^{2+}][L^-]$) and β_2 ($[ML_2]/[M^{2+}][L^-]^2$) for leucyltyrosines and alanylalanines with cobalt(II), nickel(II), and zinc(II) ions are always larger for the D-L than for the L-L diastereoisomers.

While earlier equilibrium studies of metal peptide systems have been directed toward the determination of formation constants (β_1, β_2, etc.) not involving peptide proton dissociation, now it is known that the equilibria of copper(II)- and nickel(II)-polypeptide systems also involve proton dissociation from the amide group of the coordinated peptide linkages. Direct evidence of this was obtained from the infrared spectra for the complexes in aqueous solution[11-15] and from x-ray crystallographic studies for copper(II)-polyglycines in the solid state.[16-21] In recent years other examples of metal-peptide complex formation studies involving amide proton displacement have been provided.[7,8,22-28] The most recent work along these lines is that of Brookes and Pettit,[29,30] who examined the solution equilibria of several substituted L,L-dipeptides in the presence of copper(II) and nickel(II). A reexamination of the copper(II)- and nickel(II)-di-, tri-, and tetraglycine complexes by Kaneda and Martell[27] has revealed the presence of an additional species, the protonated complex $NiHL^{2+}$, at low pH in all nickel(II)-polyglycine systems.

It is the purpose of this paper to elucidate the most probable structure and conformation of each ionic species and to correlate the stereochemical predictions from the conformational analysis to the equilibrium constants determined for the diastereoisomeric dipeptide metal complexes in solution.

Experimental Section

Method. The potentiometric measurements employed have been described previously.[27] The ionic strength and temperature of all systems were maintained at 0.10 M with KNO_3 and 25 ± 0.02 °C, respectively. In this research pH is defined as the negative logarithm of the hydrogen ion concentration ($pH = -\log [H^+]$).

CD and ORD spectra were measured on a Jasco Model J-20 recording spectropolarimeter and the ultraviolet and visible spectra were recorded on a Cary Model 14 spectrophotometer.

Reagents. L-alanyl-L-alanine, L-leucyl-L-tyrosine, and D-leucyl-L-tyrosine were obtained from Nutritional Biochemical Corp. and D-alanyl-L-alanine was purchased from Cyclo Chemical Co. All reagents were of analytical grade, which were of the highest purity available. Before sample solutions were prepared the peptides were dried over phosphorus pentoxide in a vacuum desiccator for at least a day. All peptides were found to be at least 99.9% pure on the basis of potentiometric titrations with and without metal ions.

Results and Calculations

The equilibrium constants were determined from the potentiometric equilibrium data and were calculated using metal and ligand mass balance and the available acid concentration relationships (T_M, T_L, and T_H, respectively). All calculations were made using programs written in Fortran IV with the aid of an IBM 360-65 computer. The details of the calculations have been described elsewhere.[7,27] The proton association constants of the diastereoisomeric alanylalanines and leucyltyrosines are listed in Table I, together with those reported in the tables of critical stability constants published by Martell and Smith.[28]

Copper(II)- and Nickel(II)-Alanylalanine Systems. The complex equilibria for 1:1 and higher molar ratios of alanylalanine to copper(II) may be represented by eq 1-5

$$M^{2+} + L^- \rightleftharpoons ML^+ \quad (1)$$

$$MH_{-1}L + H^+ \rightleftharpoons ML^+ \quad (2)$$

$$MH_{-1}LOH^- + H^+ \rightleftharpoons MH_{-1}L \quad (3)$$

$$MH_{-1}L + L^- \rightleftharpoons M(H_{-1}L)L^- \quad (4)$$

$$MH_{-1}L + MH_{-1}LOH^- \rightleftharpoons (MH_{-1}L)_2OH^- \quad (5)$$

where $M = Cu^{2+}$ and $H_{-1}L^{2-}$ represent the coordinated anionic ligand from which the peptide proton has been displaced by the metal ion.

The formation of 1:2 complexes, such as CuL_2 and $Cu(H_{-1}L)_2^{2-}$, was not detected under the experimental conditions employed ($T_M = 1.00 \times 10^{-3}$ M, $T_L = 2.00 \times 10^{-3}$ M, and $\mu = 0.10$ M KNO_3) while a considerable amount of a different 1:2 complex, defined as $Cu(H_{-1}L)L^-$, was detected.

The equilibria involving 1:1 and 1:2 molar ratios of nickel(II) ion to alanylalanine may be represented by eq 1 and 2 and eq 6-8:

Table I. Proton Association Constants[a] of Diastereoisomeric Alanylalanines and Leucyltyrosines

Ligand	Log K_1^H	Log K_2^H	Log K_3^H
L-Ala-L-Ala	8.15[b] (8.05,[c] 8.17[d])	3.31[b] (3.20,[c] 3.30[d])	
D-Ala-L-Ala	8.28[b] (8.19,[c] 8.32[d])	3.15[b] (3.01,[c] 3.18[d])	
L-Leu-L-Tyr	10.08[b] (10.03[c])	7.73[b] (7.73,[c] 7.82[d])	3.20[b] (3.15,[c] 3.23[d])
D-Leu-L-Tyr	10.36[b] (10.30[c])	8.32[b] (8.29,[c] 8.30[d])	2.84[b] (2.92,[c] 2.96[d])

[a] $H_{n-1}L + H^+ \rightleftharpoons H_nL$ (K_n^H) (charges omitted for generality). [b] Present work, $T_L = 2.00 \times 10^{-3}$ M, $t = 25.00 \pm 0.02$ °C; $\mu = 0.10$ M (KNO_3), all constants are self-consistent to within ±0.01 log units. [c] Reference 28. [d] Reference 8.

Table II. Log of Equilibrium Constants of Cu(II)- and Ni(II)–Alanylalanine Complexes

Constant	Quotient	Defining eq	L-Ala-L-Ala		D-Ala-L-Ala	
			Cu^{2+}	Ni^{2+}	Cu^{2+}	Ni^{2+}
K_1	$\frac{[ML^+]}{[M^{2+}][L^-]}$	1	5.31[a] (5.54[d])	4.14	5.60[a] (5.71[d])	3.90[a]
K_{MHL}	$\frac{[MHL^{2+}]}{[M^{2+}][HL]}$	6		2.89[a]		2.04[a]
K_{MHL}^H	$\frac{[MHL^{2+}]}{[ML^+][H^+]}$	7		6.90[a]		6.42[a]
K_{1a}	$\frac{[ML^+]}{[MH_{-1}L][H^+]}$	2	3.58[a] (3.72[d])	8.67[c]	4.04[a] (3.96[d])	9.06[c]
K_{1b}	$\frac{[MH_{-1}L]}{[MH_{-1}LOH^-][H^+]}$	3	9.48[b]		9.45[b]	
$K_{2'}$	$\frac{[M(H_{-1}L)L^-]}{[MH_{-1}L][L^-]}$	4	2.96[b]		3.08[b]	
K_d	$\frac{[(MH_{-1}L)_2OH^-]}{[MH_{-1}L][MH_{-1}LOH^-]}$	5	2.36[b]		2.36[b]	
K_2	$\frac{[ML_2]}{[ML^+][L^-]}$	8		2.88[b]		3.02[b]

[a] Average deviation ±0.01. [b] Average deviation ±0.02. [c] Average deviation ±0.03. [d] Reference 8.

$$M^{2+} + HL^{\pm} \rightleftharpoons MHL^{2+} \quad (6)$$

$$ML^+ + H^+ \rightleftharpoons MHL^{2+} \quad (7)$$

$$ML^+ + L^- \rightleftharpoons ML_2 \quad (8)$$

In the computation it was necessary to include all these equilibria in order to produce the calculated curves which gave the closest possible fit to the experimental titration curves. The equilibrium constants describing the copper(II)- and nickel(II)-L-ala-L-ala and -D-ala-L-ala systems are listed in Table II.

Copper(II)- and Nickel(II)–Leucyltyrosine Systems. The aqueous equilibria of the Imslcopper(II)-leucyltyrosine (H_2L) systems may be represented by eq 9–13:

$$M^{2+} + HL^- \rightleftharpoons MHL^+ \quad (9)$$

$$M(H_{-1}HL) + H^+ \rightleftharpoons MHL^+ \quad (10)$$

$$MH_{-1}L^- + H^+ \rightleftharpoons M(H_{-1}HL) \quad (11)$$

$$MH_{-1}L(OH)^{2-} + H^+ \rightleftharpoons MH_{-1}L^- \quad (12)$$

$$MH_{-1}L^- + MH_{-1}L(OH)^{2-} \rightleftharpoons (MH_{-1}L)_2OH^{3-} \quad (13)$$

where M = Cu^{2+}. When M = Ni^{2+}, on the other hand, in addition to eq 9, 10, and 11, equilibrium 14 was found to be significant for the L-L isomer.

$$MHL^+ + HL^- \rightleftharpoons M(HL)_2 \quad (14)$$

The formation of the protonated species, NiH_2L^{2+}, analogous to equilibrium 6 of nickel(II)–alanylalanine systems could not be detected for the nickel(II)–leucyltyrosine systems. The 1:2 complex, $Ni(HL)_2$, of nickel(II)-L-Leu-L-Tyr system could not be detected, while the equilibrium corresponding to (11) could not be detected for nickel(II)-D-Leu-L-Tyr system. As a consequence, it was found that equilibria 9, 10, and 11 represent the Ni(II)-L-Leu-L-Tyr system, while (9), (10), and (14) describe the Ni(II)-D-Leu-L-Tyr system. The equilibrium constants of Ni(II)-L-Leu-L-Tyr and Ni(II)-D-Leu-L-Tyr are presented in Table III.

Ionic Species Distribution as a Function of −Log [H⁺]. Mass balance and proton balance equations were rearranged to calculate the free ligand concentration at each −log [H⁺] value. The resulting quadratic or cubic equations in free ligand concentration were solved by the application of the Newton-Raphson method.[31] The concentration of each ionic species was then calculated from the free ligand concentration with known equilibrium constants. The details of the calculation are given in a previous paper.[27]

An example of the results of such a calculation is given in Figure 1 for 1:1 Cu(II)-L-Ala-L-Ala and Figure 2 for Cu(II)-D-Ala-L-Ala. The species distribution diagrams for 1:2 Cu(II)-L-Ala-L-Ala and D-Ala-L-Ala, 1:1 Cu(II)-L-Leu-L-Tyr and -D-Leu-L-Tyr, 1:2 Ni(II)-L-Ala-L-Ala and -D-Ala-L-Ala, 1:1 Ni(II)-L-Leu-L-Tyr, and 1:2 Ni(II)-D-Leu-L-Tyr systems which were all obtained on the basis of the equilibrium constants shown in Tables I–III are presented in the dissertation of Kaneda.[7]

The important features to note in comparing Figures such as 1 and 2 are the small differences arising from different stereochemical interactions depending on the ligand enantiomer present. For example, upon careful scrutiny it becomes apparent that the species ML^+(D-L) appears at about twice

Table III. Log of Equilibrium Constants of Cu(II)- and Ni(II)–Leucyltyrosine Complexes

Constant	Quotient	Defining equation	L-Leu-L-Tyr		D-Leu-L-Tyr	
			Cu^{2+}	Ni^{2+}	Cu^{2+}	Ni^{2+}
K_{MHL}	$\frac{[MHL^+]}{[M^{2+}][HL^-]}$	9	5.19[a] (5.15[f])	3.28[b]	5.34[a] (5.40[f])	3.44[b]
K_{1a}'	$\frac{[MHL^+]}{[M(H_{-1}HL)][H^+]}$	10	3.27[a] (3.38[f])	8.06[c]	4.08[a] (4.07[f])	8.89[c]
K_{1a}^H	$\frac{[M(H_{-1}HL)]}{[MH_{-1}L^-][H^+]}$	11	9.04[b]	8.82[c]	9.09[a]	*e*
K_{1b}	$\frac{[MH_{-1}L^-]}{[MH_{-1}L(OH)^{2-}][H^+]}$	12	10.30[b]		10.33[b]	
K_d	$\frac{[(MH_{-1}L)_2OH^{3-}]}{[MH_{-1}L(OH)^{2-}][MH_{-1}L^-]}$	13	2.41[d]		2.42[d]	
K_2	$\frac{[M(HL)_2]}{[MHL^+][HL^-]}$	14		*e*		2.98[b]

[a] Average deviation is 0.01. [b] Average deviation is 0.02. [c] Average deviation is 0.03. [d] Average deviation is 0.1. [e] Could not be detected. [f] Reference 8.

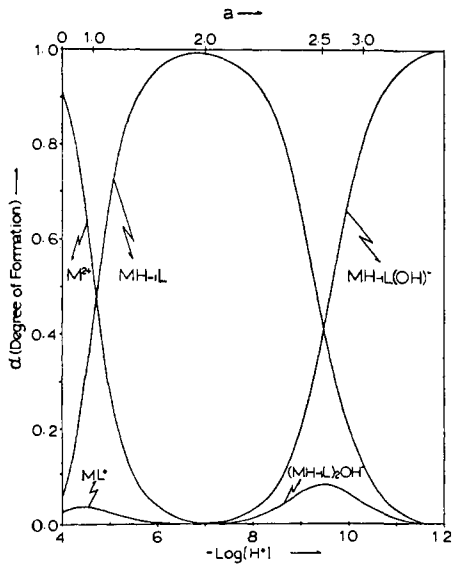


Figure 1. Ionic species distribution diagram of 1:1 Cu(II)-L-Ala-L-Ala system; $T_M = T_L = 2.00 \times 10^{-3}$ M; $t = 25.00$ °C; $\mu = 0.10$ M (KNO_3).

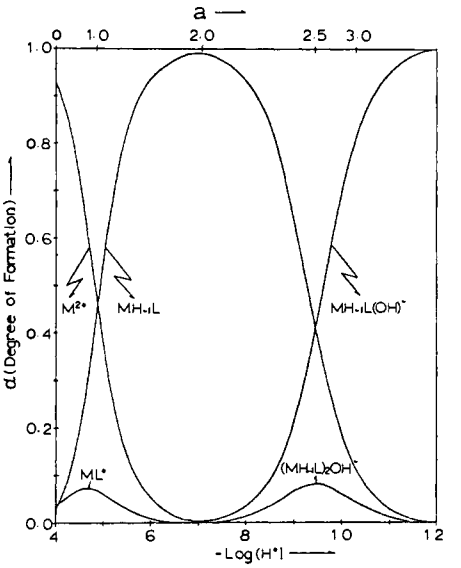


Figure 2. Ionic species distribution diagram of 1:1 Cu(II)-D-Ala-L-Ala system; $T_M = T_L = 2.00 \times 10^{-3}$ M; $t = 25.00$ °C; $\mu = 0.10$ M (KNO_3).

the concentration of ML^+(L-L) with the concomitant shift of the $MH_{-1}L$(L-L) distribution to lower pH values relative to that of $MH_{-1}L$(D-L) reflecting both K_1(L-L) < K_1(D-L) and K_{1a}(L-L) < K_{1a}(D-L). The former comparison reflects lesser stability of ML^+ (L-L species compared to D-L) whereas the latter reflects greater ease of formation of the peptide bound complex $MH_{-1}L$(D-L) over that of $MH_{-1}L$(L-L).

Although this kind of comparison is useful for orientation purposes and is rather accurate for systems involving relatively simple equilibria, ambiguities may arise where multiple (branched) equilibria involve a common species. In such cases, the actual values of the equilibrium constants probably represent a better comparison for the relative conformational energies involving optically active diastereomers.

Discussion

Proton Association Reactions and Conformations of Diastereoisomeric Alanylalanines and Leucyltyrosines. Potentiometric Data. Since in the case of alanylalanine the log K_1^H value of the D-L isomer is greater than that of the L-L isomer and the log K_2^H value of the L-L isomer is greater than that of the D-L isomer, proton association of the terminal amino nitrogen is favored for the D-L isomer over the L-L isomer while proton association on the terminal carboxylate is favored for the L-L isomer.

A similar trend in proton association was observed in the case of leucyltyrosine. This statement is based on the fact that log K_2^H value (proton association constant of the terminal amino nitrogen) of the D-L isomer is greater than that of the L-L isomer and log K_3^H value (proton association constant of the terminal carboxylate) of the L-L is greater than that of the D-L isomer.

The introduction of a large substituent in leucyltyrosine results in greater differences in the proton association constants of the leucyltyrosine diastereoisomers as compared to the alanylalanine diastereoisomers. The differences in the log K_2^H and log K_3^H of the D-L and L-L isomer for leucyltyrosine are 0.59 and 0.36, respectively, while the differences in the corresponding protonation constants for alanylalanine are 0.13 and 0.16 (in log units) as shown in Table I.

Although the present alanylalanine protonation constants are about 0.1 log units higher (see Table I) than those chosen in the standard compilation[28] there is closer agreement with a recent report by Nakon and Angelici.[8] This small discrepancy

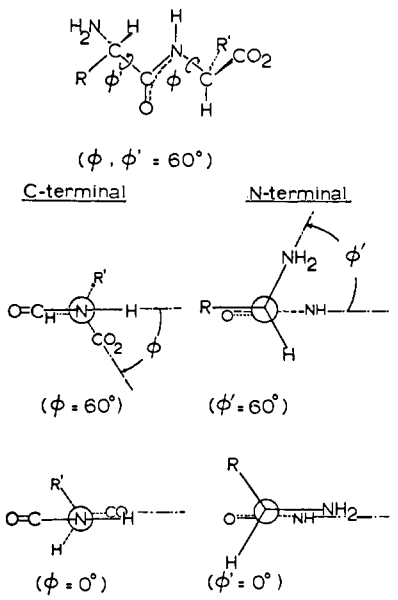


Figure 3. Rotational angles ϕ and ϕ' are defined as the dihedral angles with respect to rotation about the C_α-CO_2 and C_α-NH_2 bonds, measured from the peptide plane clockwise and counterclockwise, respectively. ϕ' corresponds to the notation of IUPAC-IUB[32] and is 180° off from the IUPAC convention.

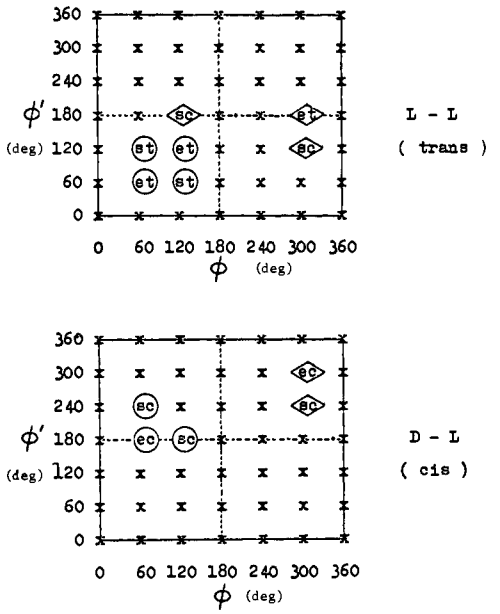


Figure 4. Steric hindrance of each conformation of the cationic species of alanylalanines, produced by rotating substituent groups about the N-C_α and C_α-CO bonds (ϕ and ϕ', respectively), was examined by constructing the molecular model (see Figure 3). X represents sterically not allowed forms, O sterically allowed forms, ◊ conformations with a small degree of steric hindrance; t, trans, c, cis, forms with respect to the relative configurations of the side chain "R" groups, s, staggered and e, eclipsed conformations. (The terminology for "staggered" and "eclipsed" is slightly different from standard practice because of the presence of the peptide linkage between the asymmetric carbons.)

may be due to lack of statistical sampling caused by a paucity of literature data.

The protonation constants for the leucyltyrosines are in somewhat better agreement with tabulated critical constants, as is shown in Table I; however, similar sampling considerations should also apply for these dipeptides. At any rate, the present values are offered as an improvement over the values previously reported.

Conformational Analysis. The present conformational analysis is based on the assumption that the following effects combine to determine the conformation of each ionic species: (1) coulombic attraction of the two oppositely charged terminal groups in the dipolar ionic species, (2) steric effects favoring certain rotational isomers, (3) entropy effects due to the presence and positioning of hydrophobic groups, and (4) the effects of hydrophilic groups. These four factors are expected to be of comparable importance in describing the conformation of the free ligand. For convenience, items (1), (2), and (4) were first selected for a preliminary determination of allowed conformations through the examination of molecular models. The present molecular model analysis may be represented using the definitions of the rotational angles shown in Figure 3, resulting in conformational maps for the cationic, dipolar, and anionic species in Figures 4, 5, and 6, respectively. The details of the molecular model analysis are described elsewhere.[7] The most favorable conformations thus predicted are shown in Table IV. The initial tentative conformations thus obtained were further refined on the basis of effect (3), the entropy effect of hydrophobic substituents.

It can be inferred from a molecular model analysis that due to the concentration of R groups on one side of the molecule the cis configuration possesses a more extensive hydrophobic region than does the trans form. The resulting organizing effect on the surrounding solvent molecules restricts the rotational and translational freedom of the water molecules and hence represents a lowering of entropy. It therefore follows that the entropy of the cis form in solution should be more negative than that of the trans species. Consequently, the change from cis to trans involves an entropy increase, indicating relatively higher stability of the latter.

As indicated by the probable conformations in Table IV, the

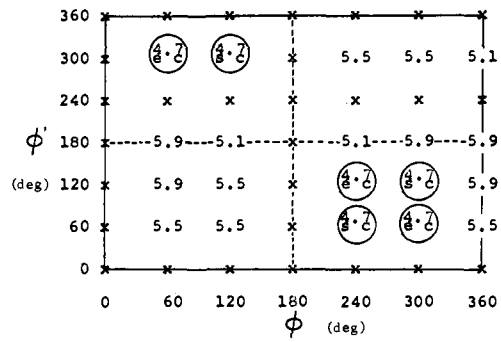
Table IV. The Relative Configuration of Side Chain Groups in Diastereoisomeric Alanylalanines[a]

	H_2L^+ [b]	$HL^\pm$ [b]	L^- [b]
L-Ala-L-Ala	Trans[c]	Cis[c,d]	Trans
D-Ala-L-Ala	Cis[d]	Trans	Cis[c,d]

[a] The most favorable conformers of each ionic species have been assigned tentatively assuming that the coulombic interaction and the steric requirements are dominant over the entropy effect of the hydrophobic side chain groups. A similar result[8] was obtained on the basis of coulombic effects alone. [b] H_2L^+, $HL^\pm$, and L^- represent the cationic, dipolar, and anionic species. [c] Trans and cis represent the relative configuration of the side chain groups of the most favorable conformers. [d] All cis configurations are somewhat distorted toward the trans orientation.

first proton association step, corresponding to K_1^H, of the L-L isomer involves a conversion in orientation from trans to cis, while that of the D-L isomer involves a cis to trans change. The second proton association step, K_2^H, of the L-L isomer involves a conversion from cis to trans while that of the D-L isomer involves a trans to cis conversion. From basic thermodynamic equilibrium relationships, it is obvious that at constant enthalpy an increase in entropy contributes to a higher equilibrium constant. As a consequence, assuming equivalent enthalpies of protonation, the first proton association of the D-L isomer is favored relative to that of the L-L isomer, while the second proton association of the L-L form is favored over that of the D-L isomer, in excellent agreement with the equilibrium data in Table I.

However, the actual conformation of each species should not be purely cis or trans. In fact, the entropy loss associated with the formation of the cis configuration should shift the conformational equilibrium in favor of the trans by sacrificing some of the enthalpy change involved in coulombic interaction



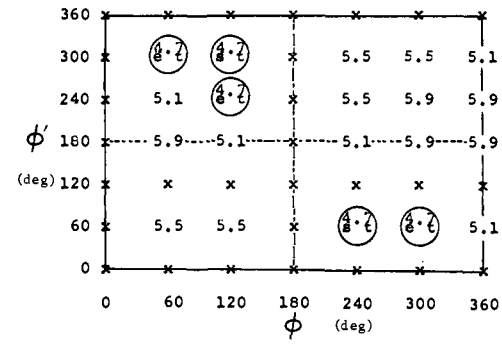
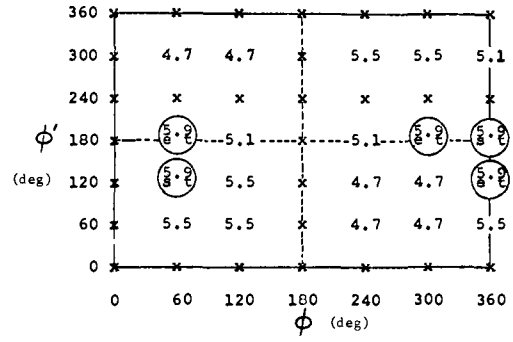


Figure 5. Conformational map of the dipolar species of alanylalanine. The numbers 4.7, 5.1, 5.5, and 5.9 represent the distance in ångström units between the carbon and nitrogen atoms of the negative carboxylate and positive amino groups, respectively. Other terms are defined in the caption of Figure 4.



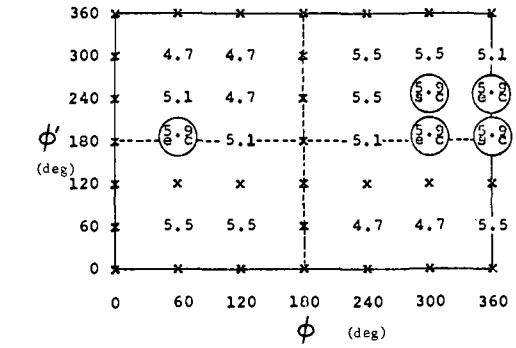


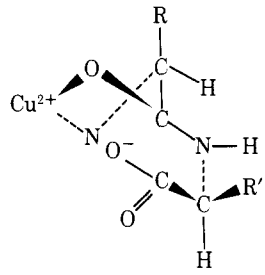
Figure 6. Conformational map of the anionic species of alanylalanine. Terms and legends are defined in captions of Figures 4 and 5.

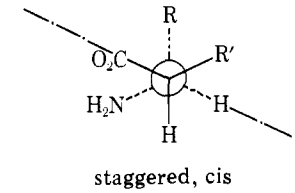
and steric hindrance. Thus it follows that the actual proton association equilibria should take into account the relative enthalpy changes of the L-L and D-L isomers and that the reasoning leading to the prediction of the cis configuration should allow for the formation of a certain amount of the trans form. Consequently, it may be shown from molecular models that the charge separation between the two terminal groups should be greater for the L-L than for the D-L isomer in the dipolar species.[33] The entropy and enthalpy changes involved in the proton association processes of diastereoisomeric forms of alanylalanine reported by Ellengoben[5] are in accord with these predictions.

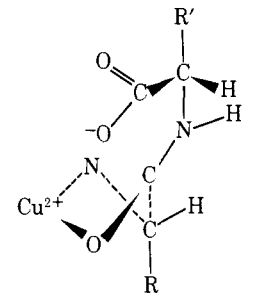
Aqueous Equilibria and Conformations of Copper(II)–Diastereoisomeric Alanylalanines. Potentiometric Data. The differences between the values of the equilibrium constants of the copper(II) complexes of diastereoisomeric alanylalanines, shown in Table II, lead to three conclusions. First, the formation of CuL^+ and $Cu(H_{-1}L)L^-$ from metal ion or complex and free ligand L^- are favored for the D-L isomer over the L-L isomer. Second, the formation of $CuH_{-1}L$ from CuL^+ (peptide proton dissociation) is favored for the L-L isomer over the D-L isomer. Finally, the formation of $CuH_{-1}LOH^-$ and $(CuH_{-1}L)_2OH^-$ seems to show no steric preference.

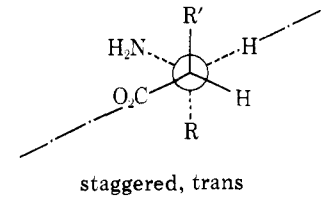
The aqueous equilibria of copper(II)–diastereoisomeric alanylalanine complexes may be visualized with the aid of the ionic species distribution diagrams, which lead to the same conclusions as above (see Figures 1 and 2).

Conformational Analysis. According to the present molecular model analysis of the copper(II)–alanylalanine complexes, the preferential ring conformation of CuL^+ in which the ligand is coordinated to the amino nitrogen and amide carbonyl oxygen, is always λ when the N-terminal amino acid residue is L (I) and δ when the N-terminal amino acid residue is D (II). This relationship is due to steric hindrance between the peptide





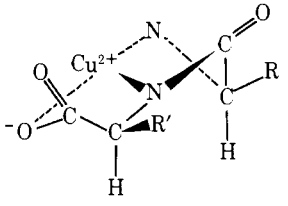




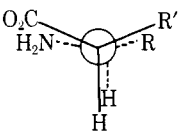
N–H bond and the R group of the N-terminal amino acid residue, since the peptide linkages should retain planarity, as was found in the CuL^+ complex of triglycine in the solid state.[17,18] Thus analyses with molecular models show that these preferential ring conformations, λ and δ for L- and D- (N-terminal amino acid residue) dipeptides, respectively, make the R group configuration always axial with respect to the metal plane. This is not the case with single copper(II) amino acid complexes[34] where the substituent may take either configuration. The coulombic attraction between the copper(II) ion and the carboxylate group may be expected to minimize

the distance between the two terminal groups. The favored conformations of the CuL^+ species are indicated by formulas I and II.

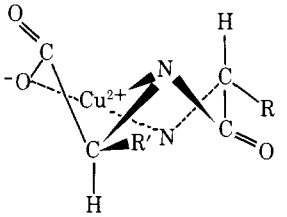
Since the amide proton is absent in the $CuH_{-1}L$ species, the main factor determining the ring conformation of this species is the steric repulsion between the R groups and the coordinated water molecule in the axial position of the metal ion. The ring conformation, therefore, will be restricted to the δ or λ conformation, in which the R groups are always equatorial. Thus the favored conformation of the L-L isomer of $CuH_{-1}L$ is (δ, δ) with respect to the ring conformation, equatorial-equatorial with respect to the conformation of R groups on the chelate rings, and eclipsed cis with respect to the relative configurations of R groups in the coordinated ligand. Similarly the conformation of the D-L isomer is λ, δ, equatorial-equatorial and staggered cis, respectively, as indicated by formulas III and IV. An alternative explanation of the relative stabilities



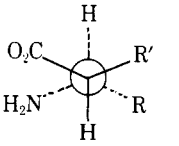
equatorial-equatorial, (δ, δ)
III(L-L)



eclipsed, cis



equatorial-equatorial, (λ, δ)
IV(D-L)



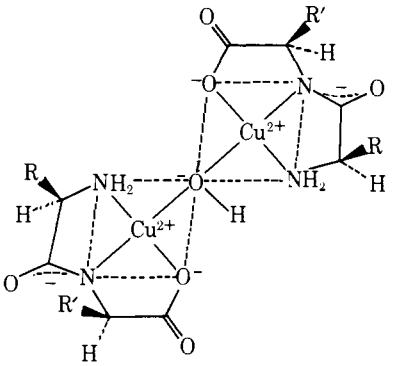
staggered, cis

of D-L and L,L isomers of $CuH_{-1}L$ has been offered[8] on the basis of possible differences in hydrophobic bonding.

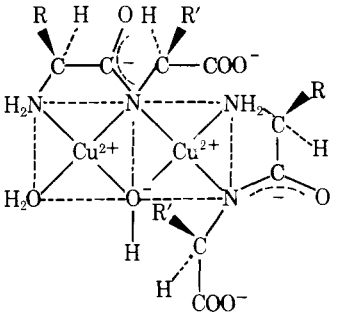
The conformational changes of the L-L isomer with respect to the relative position of R groups involve a trans to cis to cis conversion going from L^- to CuL^+ to $CuH_{-1}L$, while the conformational changes of the D-L isomer involve a cis to trans to cis sequence. Since a cis to trans conversion causes an increase in the entropy of the system while a trans to cis conversion decreases the entropy (vide supra), consideration of entropy changes in the conversion sequence leads to the prediction that the formation of CuL^+ is favored for the D-L isomer, while the formation of $CuH_{-1}L$ is favored for the L-L isomer, in excellent agreement with the relative values of the corresponding equilibrium constants obtained from the potentiometric study (Table II).

The formation of either $CuH_{-1}LOH^-$ or $(CuH_{-1}L)_2OH^-$ does not show a steric preference of the L-L relative to the D-L ligand. The formation of $CuH_{-1}LOH^-$ from the $CuH_{-1}L$ species involves simply the deprotonation of a coordinated water, a transformation which should not possess any appreciable steric preference. The lack of a steric effect in the formation of the dimeric $(CuH_{-1}L)_2OH^-$ species seems to suggest that the formation of the dimer does not involve change in the arrangement of the ligand donor groups around the central metal ion.

Koltun and Gurd[35] suggested a structure V with a monohydroxo bridge for the dimeric species $(CuH_{-1}L)_2OH^-$. A similar suggestion has been made more recently.[8] Since binuclear complexes of copper(II) in solution having only one bridging hydroxo group are not generally known, Kim and Martell[11,15] suggested the possibility that the coordinated

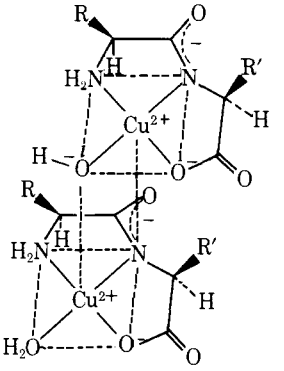


$(CuH_{-1}L)_2OH^-$
V

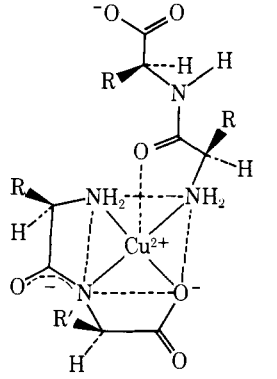


$(CuH_{-1}L)_2OH^-$
VI

negative peptide nitrogen may also serve as a bridging group, as indicated in VI. A careful study of the infrared data compiled from Kim's study[11,15] of copper(II)-diglycine complexes indicates that there is no significant free-carboxylate band in the region where the dimer should form. The ionic species distribution diagram of copper(II)-diglycine system[7] shows that the dimeric species is formed to a considerable extent, more than would be expected from the low intensity of the corresponding infrared band. Since VI would possess a strong carboxylate absorption band in the infrared, it is now suggested that the structure of the binuclear form resembles formula VII.



$(CuH_{-1}L)_2OH^-$
VII



$(CuH_{-1}L)L^-$
VIII

This seems to be a reasonable alternative since many five coordinated copper(II) complexes have recently been found in the solid state with this type of bonding.[16,19,21]

The formation of $Cu(H_{-1}L)L^-$ is favored for the D-L isomer

Table V. The Configurations of Side Chain Groups in Copper(II)- and Nickel(II)-Diastereoisomeric Alanylalanine Complexes[a]

		Copper(II) complexes		
		L^- [b]	CuL^+	$CuH_{-1}L$ [c]
L-Ala-L-Ala		Trans[d]	Cis[d,e]	Cis[e]
D-Ala-L-Ala		Cis[e]	Trans	Cis[e]
		Nickel(II) complexes		
	$HL^\pm$	$NiHL^{2+}$	NiL^+	$NiH_{-1}L$
L-Ala-L-Ala	Cis[e]	Trans	Cis[e]	Cis[e]
D-Ala-L-Ala	Trans	Cis[e]	Trans	Cis[e]

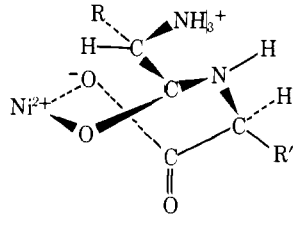
[a] The probable conformers of each ionic species have been assigned assuming that coulombic repulsions and steric requirements predominate over the entropy effect of the side chain groups. [b] L^- represents the peptide anion. [c] $H_{-1}L^{2-}$ is the coordinated peptide in which the peptide amide proton is dissociated and the amido nitrogen atom is bound to the metal ion. [d] Trans and cis represent the relative configurations of the R and R′ groups of the favored conformers. [e] This value may be distorted somewhat in favor of the trans form (see text).

over the L-L isomer, as indicated by log K_2' values in Table II. There are many conformations possible for this complex, but the increased formation for the D-L isomer may simply be due to the greater basicity of the anionic form of the D-L isomer (see Table I). The tentative suggested arrangement of the donor groups about the metal ion in this complex, VIII, is pyrimidal and thus similar to the geometry suggested for VII.

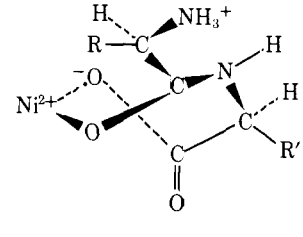
Aqueous Equilibria and Conformations of Diastereoisomeric Alanylalanine Complexes of Nickel(II). Potentiometric Data. It was found that the formation of only NiL_2 is favored for the D-L isomer while NiL^+, $NiHL^{2+}$, and $NiH_{-1}L$ forms are favored for the L-L isomer (see Table II).

The previous potentiometric equilibrium studies of the di-, tri-, and tetraglycine complexes of nickel(II) by Kaneda and Martell[27] clearly demonstrated the presence of protonated complexes at low pH. It was suggested that the structure of the protonated species involves a metal ion coordinated through the terminal carboxylate oxygen and the nearest peptide carbonyl oxygen forming a seven-membered chelate ring, with the terminal amino nitrogen remaining protonated.

Conformational Analysis. The most favorable conformations of $NiHL^{2+}$ are assigned to the trans (staggered) form for the L-L isomer and to the cis (staggered) form for the D-L isomer, as indicated in formulas IX and X, respectively. In the conformational analysis of these species it was assumed that coulombic repulsion maximizes the distance between the nickel(II) ion and the protonated terminal amino nitrogen. The conformations of the NiL^+ species may be assigned to be the same as those of CuL^+ species. The relative configurations of the R groups in each ionic species of the nickel(II)-alanylalanine complexes are indicated in Table V. The conformational variation of the L-L isomer in the equilibria of the acid form to the basic species involves conversion of cis $HL^\pm$ to trans $NiHL^\pm$ to cis NiL^+, while the D-L isomer undergoes a corresponding conversion of trans to cis to trans. Consideration of entropy changes that accompany these conformational variations leads to the prediction that the formation of $NiHL^{2+}$ is favored for the L-L isomer while the formation of NiL^+ is favored for the D-L isomer. These predictions based on the transformations as outlined in Table V are in excellent agreement with the potentiometric data shown in Table II.



staggered, trans
IX(L-L)



staggered, cis
X(D-L)

While the formation of the 1:2 complex, NiL_2, of the D-L isomer is favored over that of the L-L isomer, as shown by the log K_2 values in Table III, there seems to be very little steric preference of one conformer over the other. In fact the observed differences in stability may be attributed primarily to the fact that the peptide anion of the D-L isomer is more basic than that of the L-L isomer (Table I).

Proton dissociation from NiL^+ species may occur either from the coordinated water molecule or from the peptide amide hydrogen to give the formulas NiL(OH) and $NiH_{-1}L$. Both reactions would be expected to take place in about the same pH region. However, the log K_{1a} value of the L-L isomer is much less than that of the D-L isomer (Table II). If the formation of NiL(OH), in which one proton is dissociated from the coordinated water molecule, were actually occurring instead of the formation of $NiH_{-1}L$, in which the peptide proton is dissociated, this steric preference could not be explained. Thus the potentiometric equilibrium data require the formation of the $NiH_{-1}L$ species rather than the NiL(OH) form.

Since the structure of $NiH_{-1}L$ may be assumed to be quite similar to the structure of $CuH_{-1}L$, the deprotonation of NiL^+ to form $NiH_{-1}L$ must involve a cis-cis conversion for the L-L isomer and a trans-cis conversion for the D-L isomer. Therefore conformational analysis predicts a steric preference for the formation of the L-L isomer over the D-L form, as is observed potentiometrically. In this respect the copper(II)- and nickel(II)-alanylalanine systems are completely analogous.

Aqueous Equilibria and Conformations of Copper(II)- and Nickel(II)-Leucyltyrosines. Copper(II) Complexes. As seen in Table III, the formation of the 1:1 copper(II)-leucyltyrosine complex, $CuHL^+$, is favored for the D-L over the L-L isomer, while peptide proton dissociation is facilitated in the case of the L-L isomer. The log K_{1a} difference for the D-L and L-L isomers of alanylalanine is 0.45 while log K_{1a}' of the leucyltyrosine is 0.81 log unit. Thus, while the trends observed for the diastereoisomeric leucyltyrosine series are the same as those of the alanylalanine series, introduction of the bulkier side chain results in greater differences in the peptide proton dissociation constants for the diastereoisomers of leucyltyrosine than those observed for alanylalanine.

The formation of $Cu(H_{-1}L)^-$, $(CuH_{-1}L)_2OH^{3-}$, and $CuH_{-1}L(OH)^{2-}$ seems to have no stereoselectivity. Their equilibrium formation constants are essentially identical within experimental error. Since log K_{1a}^H values of copper(II)-leucyltyrosine series are of about one order of magnitude lower than log K_{1a}^H (Table I) values of the ligands, it is reasonable to conclude that the proton dissociation from $Cu(H_{-1}HL)$ (in which a peptide proton is dissociated) occurs from the tyrosine hydroxyl group to form $Cu(H_{-1}L)^-$ species, which would involve little stereoselectivity. The $Cu(H_{-1}L)^-$ species thus formed presumably combines with $CuH_{-1}L(OH)^{2-}$ species to form the dimeric form $(CuH_{-1}L)_2OH^{3-}$, while this equilibrium competes with the formation of $CuH_{-1}L(OH)^{2-}$ from $Cu(H_{-1}L)^-$ involving the dissociation of a coordinated water molecule. The log K_{1b} values of complexes of the L-L and of the D-L isomer are seen to be identical within experimental error.

The formation of $Cu(HH_{-1}L)(HL)^-$ in which the peptide nitrogen is bound to the metal ion in one of the coordinated ligands could not be detected. As shown in formula VIII, for

the corresponding 1:2 copper(II)-alanylalanine complex, the R group of the first coordinated ligand on the metal plane is relatively close to the R′ group of the second coordinated ligand on the metal axial position. The bulkier side chains of leucyltyrosine experience considerable steric hindrance with this arrangement according to the present molecular model analysis. It follows that the formation of the 1:2 copper(II)-leucyltyrosine complex may be prohibited because of this steric repulsion of the phenyl groups. If a 1:2 copper(II)-alanylalanine complex would be assumed to have a structure in which the second ligand were to coordinate simply through the terminal nitrogen without involvement of the peptide carbonyl oxygen, then it would be difficult to explain the lack of formation of $CuL(HL)^-$ species with leucyltyrosine ligands. The basicity of the amino nitrogen of the D-L isomer of leucyltyrosine is slightly greater than that of the corresponding alanylalanine (see Table I).

Nickel(II) Complexes. Unlike the nickel(II)-polyglycines[27] and the alanylalanine complexes described above, the formation of the analogous protonated species could not be detected in the nickel(II)-leucyltyrosine system. This result is probably due to the bulk of the isobutyl group of the leucyl residue and its steric effects in the expected structure of the protonated species NiH_2L^{2+}. As shown in formulas IX and X, for the corresponding nickel(II)-alanylalanine complex, the R group on the N-terminal amino acid residue is relatively close to the coordinated water molecule on the axial position of the metal ion. It follows that the steric hindrance between the coordinated water molecule and a sufficiently large moiety such as the isobutyl group may make the formation of the protonated species extremely difficult.

The potentiometric titration curves of the 1:1 and 1:2 nickel(II)-D-Leu-L-Tyr systems are quite similar to those of the corresponding nickel(II)-alanylalanine systems which have no inflection but extend up to $a = 1$. On the other hand, the titration curve of the 1:1 nickel(II)-L-Leu-L-Tyr system extends up to $a = 2$ without inflection. Therefore one more proton dissociation process is involved for the nickel(II)-L-Leu-L-Tyr system, as compared with the nickel(II)-D-Leu-L-Tyr and nickel(II)-alanylalanine systems.

Based upon these experimental results, and the appropriate equilibrium constants, the following conclusions may be deduced. In the case of the L-L isomer, one peptide proton is presumably dissociated from the 1:1 complex, $NiHL^+$, to form a $NiH(H_{-1})L$ species, which loses an additional proton from the tyrosine hydroxyl to form $Ni(H_{-1}L)$. The formation of the neutral 1:2 complex $Ni(HL)_2$ was not detected in this system. On the other hand, the 1:1 complex $NiHL^+$ of the D-L isomer may either dissociate the peptide proton of the coordinated ligand or a proton of the coordinated water molecule to form $Ni(HH_{-1}L)$ or $NiHL(OH)$. In this case, the formation of the 1:2 complex, $Ni(HL)_2$, was detected in appreciable concentration.

All observations for the nickel(II)-diastereoisomeric leucyltyrosine series showed the same trends as for nickel(II)-alanylalanine series with considerably enhanced effects resulting from the differences in steric effects reflecting the larger side chains in the diastereoisomeric complexes.

References and Notes

(1) Abstracted in part from a dissertation presented to the Faculty of Texas A&M University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, January, 1973.
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