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Copper(II) and Zinc(II) Binding of Optically Active Dipeptides[1]

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Abstract: Equilibrium constants are reported for the protonation and metal ion coordination of L,L ("pure") and D,L ("mixed") diastereomers of the dipeptides Ala-Ala, Ala-Phe, Leu-Leu, and Leu-Tyr. The higher pK_a values of the NH_2 group and the lower pK_a values of the CO_2^- group in the "mixed" as compared to the "pure" diastereomers can be explained by assuming the dipeptides have a predominantly β-conformation under all pH conditions. Solutions of Cu(II) and the dipeptides (HL) form the same general types of complexes previously reported for Gly-Gly: $Cu^{2+} + L^- \rightleftharpoons CuL^+$, K_1; $CuL^+ \rightleftharpoons Cu(H_{-1}L) + H^+$, K_{1a}; $Cu(H_{-1}L) + OH^- \rightleftharpoons Cu(H_{-1}L)(OH)^-$, K_{OH}. The K_1 values for "mixed" dipeptides are larger than for their "pure" diastereomers. This trend is also observed with Zn(II) and can again be rationalized by assuming a β-conformation for the uncomplexed dipeptide anion (L^-). Amide deprotonation of CuL^+ complexes of "pure" dipeptides occurs with K_{1a} values up to ten times larger than for the corresponding complexes of the "mixed" dipeptides. This type of preferential complexation of "pure" dipeptides can be explained by noting that the two nonpolar side chains of the "pure" dipeptide in $Cu(H_{-1}L)$ complexes are on the same side of the coordination plane, whereas they are on opposite sides in those of the "mixed" dipeptides. Hydrophobic bonding of the side chains with the amide group and with each other is suggested as the reason for the higher stability of the $Cu(H_{-1}L)$ complexes of the "pure" dipeptides. Hydroxo formation constants (K_{OH}) are the same for complexes of both "pure" and "mixed" dipeptides; this is expected because of the very similar structures of the $Cu(H_{-1}L)$ and $Cu(H_{-1}L)(OH)^-$ complexes.

The diverse functions of transition metal ions in biological systems depend in part upon the nature of the biological ligands which bind them. The particular importance of peptide binding to metal ions may be inferred from the large number of studies that have been carried out on metal–peptide complexes.[3,4] Yet little is known about metal ion binding of peptides having differing chiral centers. In the past, we examined the stereoselective binding of optically active amino acids by metal complexes bearing optically active ligands.[5] In this paper, we extend these studies to the stereoselective binding of optically active dipeptides by Cu(II) and Zn(II).

Metal ion binding of dipeptides (HL) in aqueous solution has been shown[3,4,6] to involve one or more of the following equilibria

$$M^{2+} + L^- \overset{K_1}{\rightleftharpoons} ML^+ \quad (1)$$

$$ML^+ + L^- \overset{K_2}{\rightleftharpoons} ML_2 \quad (2)$$

$$ML^+ \overset{K_{1a}}{\rightleftharpoons} M(H_{-1}L) + H^+ \quad (3)$$

Using optically active dipeptides, Li, *et al.*,[7] found that K_1 is larger for L,D-Ala-Ala (alanylalanine) than for L,L-Ala-Ala with Co(II). Likewise, K_1 was larger for D,L-Leu-Tyr (leucyltyrosine) than for L,L-Leu-Tyr with Co(II), Ni(II), and Zn(II). In contrast, Karczynski and Kupryszewski[8–10] found that the L,L diastereomers of both Leu-Tyr and Leu-Ala exhibited higher K_1 values than did their L,D isomers with Cu(II), Ni(II), and Zn(II). Since these authors were apparently unaware of the importance of the amide deprotonation equilibrium (eq 3) in the Cu(II) complexation reactions, it appears that their K_1 values are in error. After our studies were completed, a report by Kaneda (with A. E. Martell)[11] came to our attention. They found that K_1 values for the reaction of Cu(II) and Ni(II) with Ala-Ala and Leu-Tyr were larger for the L,D diastereomers than for the L,L derivatives. Values of K_{1a}, however, were larger for L,L dipeptides. Our interest in these equilibria was to examine the factors which give rise to the stereoselectivity of Cu(II) and Zn(II) in binding L,L and L,D dipeptides.

Experimental Section

Reagents. The D,L, D,D, and L,L isomers of leucylleucine (Leu-Leu) were purchased from Research Plus Laboratories, while the L,D isomer was purchased from Sigma Chemical Co. The L,L and

(1) Presented by R. N. at the 166th National Meeting of the American Chemical Society, Chicago, Ill., Aug 1973.
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Table I. Protonation[a] and Cu(II)–Dipeptide Formation Constants for Different Optical Isomers of Leucylleucine[b]

Isomer	log K_1'	log K_2'	log K_1	log K_{1a}	log K_{OH}	log K_D
L,L-	7.91 ± 0.01	3.45 ± 0.01	5.21 ± 0.01	−3.88 ± 0.01	4.31 ± 0.01	2.42 ± 0.01
D,D-	7.91 ± 0.01	3.43 ± 0.01	5.20 ± 0.02	−3.90 ± 0.02	4.32 ± 0.01	2.44 ± 0.01
L,D-	8.20 ± 0.01	3.05 ± 0.01	5.48 ± 0.02	−4.88 ± 0.02	4.32 ± 0.02	2.43 ± 0.02
D,L-	8.21 ± 0.01	3.07 ± 0.01	5.45 ± 0.02	−4.89 ± 0.02	4.33 ± 0.01	2.45 ± 0.01

[a] K_1' refers to the NH_3^+ group. K_2' refers to the CO_2H group. [b] At 25.0° and 0.10 M (KNO_3) ionic strength.

Table II. Protonation and Metal Ion Formation Constants for Different Diastereomers of Dipeptides[a]

Dipeptide	pK_a		Cu(II)		Zn(II)	
	NH_3^+	CO_2H	log K_1	log K_{1a}	log K_1	log K_2
L,L-Ala-Ala	8.17 ± 0.01	3.30 ± 0.02	5.54 ± 0.01	−3.72 ± 0.01	3.73 ± 0.02	3.15 ± 0.02
L,D-Ala-Ala	8.32 ± 0.01	3.18 ± 0.02	5.71 ± 0.02	−3.96 ± 0.02	3.87 ± 0.01	3.17 ± 0.02
L,L-Ala-Phe	7.87 ± 0.01	3.25 ± 0.02	5.20 ± 0.02	−3.44 ± 0.02	3.38 ± 0.01	2.82 ± 0.03
L,D-Ala-Phe	8.08 ± 0.01	3.02 ± 0.01	5.42 ± 0.02	−3.93 ± 0.02	3.61 ± 0.02	2.94 ± 0.02
L,L-Leu-Leu	7.91 ± 0.01	3.45 ± 0.01	5.21 ± 0.01	−3.88 ± 0.01		
L,D-Leu-Leu	8.20 ± 0.01	3.05 ± 0.01	5.48 ± 0.02	−4.88 ± 0.02		
L,L-Leu-Tyr[b]	7.82 ± 0.01	3.23 ± 0.02	5.15 ± 0.02	−3.38 ± 0.02	3.36 ± 0.01	2.96 ± 0.01
D,L-Leu-Tyr[c]	8.30 ± 0.01	2.96 ± 0.02	5.40 ± 0.02	−4.09 ± 0.02	3.89 ± 0.02	3.20 ± 0.02

[a] At 25.0° and 0.10 M (KNO_3) ionic strength. [b] Phenolic OH pK_a is 10.15. [c] Phenolic OH pK_a is 10.38.

L,D isomers of alanylphenylalanine (Ala-Phe) were purchased from Fox Chemical Co. The above peptides were used without further purification. The L,L and L,D isomers of alanylalanine (Ala-Ala) from Fox Chemical Co. and D,L- and L,L-leucyltyrosine (Leu-Tyr) from Nutritional Biochemicals Corp. were recrystallized from methanol by the addition of ethyl acetate. Solutions of the dipeptides were standardized by potentiometric titration.

Baker Analyzed Reagent Grade $Cu(NO_3)_2 \cdot 3H_2O$ and $Zn(NO_3)_2$ $6H_2O$ were used in the preparations of metal ion solutions which were standardized by passing them through a Dowex 50W-X8 strongly acidic cation exchange resin.[12] The effluent acid solutions were titrated with standardized NaOH.

Potentiometric Measurements. Potentiometric titrations were carried out in a double-walled cell of 50 ml capacity. A glass electrode used with a Corning Digital 112 Research Model pH meter was used to determine hydrogen ion concentrations. The electrode was calibrated in terms of $-\log [H^+]$ (pH_c) according to the procedure of Rajan and Martell[13] using standard solutions of HCl, acetic acid, and NaOH. The reaction solutions were maintained at 25.00 ± 0.05° by circulating thermostated water through the outer jacket of the cell. In addition to the glass and calomel electrodes, a microburet delivery tube and an N_2 inlet tube were inserted through the cell cover. The ionic strength of all solutions was maintained at 0.10 M by addition of 1.0 M KNO_3, and Cu(II) and dipeptide concentrations were each 5×10^{-3} M. The solutions were stirred by a magnetic stirring bar. All titrations were performed in triplicate.

Perchloric acid (5.0×10^{-3} M) was added to dipeptide solutions in order to determine the CO_2^- protonation constants, K_2' or K_3'. All calculations were carried out on an IBM 360-65 digital computer using data from 20 to 80% of the desired buffer zone.

Visible spectra of 1.0×10^{-2} and 5.0×10^{-3} M solutions of 1:1 Cu(II) to dipeptide were obtained on a Beckman DB-G grating spectrophotometer at 25.0°, and solutions had an ionic strength of 0.10 M (KNO_3).

Results

Values of the protonation constants (K_1', K_2', and K_3') were calculated according to Bjerrum's method[14] and are summarized in Tables I and II. As shown in Table I, the "pure" enantiomers, L,L- and D,D-Leu-Leu, have the same protonation constants. This is also true for the "mixed" L,D- and D,L-Leu-Leu enantiomers. Since enantiomers are required to have the same protonation constants, these comparisions indicate the high precision of the results. Also our results agree well with previously reported pK_a values[6,11] for diastereomers of Ala-Ala and Leu-Tyr.

Titration curves of 1:1 Cu(II) to dipeptide solutions consist of a low pH buffer zone terminated by a sharp inflection at $a = 2$ mol of base per mole of ligand; this is followed by a high pH buffer region. Data from $a = 0.5$ to 1.5 in the low pH buffer zone indicate that reactions 1 and 3 predominate in this region, as Kim and Martell[6] had previously found for 1:1 Cu(II) to glycylglycine solutions. Martell and Kaneda[11] recently reported these equilibria also for the diastereomers of Ala-Ala and Leu-Tyr. Values of K_1 and K_{1a} (Tables I and II) were calculated using an iterative program using charge and mass balance equations derived by Kim and Martell.[6]

$$K_1 = (C_L - B \cdot AL)(A \cdot D - B)/ (D \cdot B \cdot AL)[D(CS + 2T_M) - T_M] \quad (4)$$

where C_L = total dipeptide concentration, T_M = total Cu(II) concentration, $CS = [OH^-] - [H^+] - [Na^+]$, $A = 3K_1'K_2'[H^+]^2 + 2K_1'[H^+] + 1$, $B = K_1'K_2'[H^+]^2 + K_1'[H^+]$, $D = 1 + K_{1a}/[H^+]$, and $AL = [D(CS + 2T_M) - T_M]/(A \cdot D + B)$.

The high pH buffer zone ($a = 2.0$–2.5) data for Cu(II) and Leu-Leu again follow previous studies[7,11] involving glycylglycine and Ala-Ala and Leu-Tyr. They indicate the formation of hydroxo complexes and hydroxo-bridged dimers according to eq 5 and 6. By combining

$$Cu(H_{-1}L) + OH^- \overset{K_{OH}}{\rightleftharpoons} Cu(H_{-1}L)(OH)^- \quad (5)$$

$$Cu(H_{-1}L)(OH)^- + Cu(H_{-1}L) \overset{K_D}{\rightleftharpoons} Cu_2(H_{-1}L)_2(OH)^- \quad (6)$$

appropriate mass and charge balance equations for these equilibria, the following relationship was derived.

$$K_D = \{[-CS - 2T_M - K_{OH}[OH^-]F/C]C^2\}/ [F^2K_{OH}[OH^-]] \quad (7)$$

where $F = 5T_M + 2CS$, $C = 1 - K_{OH}[OH^-]$, and T_M and CS are as defined above.

Data from titrations of solutions containing 2.5×10^{-3} M Zn(II) and 5.0×10^{-3} M dipeptide indicate

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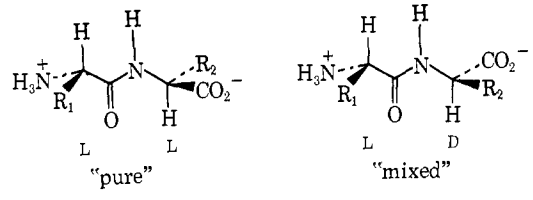

Figure 1. β Conformation of dipeptides.

the formation of ZnL^+ and ZnL_2 according to eq 1 and 2. The K_1 and K_2 values (Table II) associated with these equilibria were calculated using Bjerrum's method.[14]

The λ_{max} and ϵ_{max} values of the $Cu(H_{-1}L)$ complexes of Ala-Ala and Leu-Tyr are given in Table III. They

Table III. Visible Spectra of $Cu(H_{-1}L)$ Complexes[a]

L	λ_{max}, nm	ϵ_{max}, M^{-1} cm^{-1}
L,L-Ala-Ala	612	85
L,D-Ala-Ala	615	83
L,L-Leu-Tyr	613	90
D,L-Leu-Tyr	615	93

[a] At a = 2 mol of base per mole of ligand.

show that complexes of different diastereomers have the same uv–visible spectra within experimental error. Similar results were found previously for complexes of Cu(II) with diastereomers of Leu-Ala and Leu-Phe.[10]

Discussion

Protonation Constants. Since the L,L- and D,D-Leu-Leu isomers are enantiomers, their protonation constants should be identical, as found experimentally (Table I). Likewise the L,D- and D,L-Leu-Leu enantiomers have the same protonation constants. Ellenbogen[15,16] had previously verified this for the isomers of Ala-Ala.

On the other hand, the "pure" (L,L or D,D) Leu-Leu isomers are diastereomers of the "mixed" (L,D or D,L) derivatives and their protonation constants should be different. This is observed for Leu-Leu (Table I) as well as for the other dipeptides (Table II). For all dipeptides, the pK_a value of the CO_2H group of the "mixed" dipeptide is lower than that for the "pure;" this means that protonation of the CO_2^- group of the zwitterionic peptide is less favorable for the "mixed" isomer. In contrast, the pK_a of the amino group of the "mixed" dipeptide is higher than that for the "pure;" this means that protonation of the NH_2 group of the anionic form of the dipeptide is more favorable for the "mixed" isomer. The same trends in pK_a values for these groups were observed previously[3,11,15,16] on a smaller sampling of dipeptides. The trends were explained in terms of folding and unfolding of the dipeptide upon successive protonation. This folding motion involved rotation around the peptide N-alkyl C bond which would allow a relatively close approach of the NH_3^+ and CO_2^- groups in the zwitterionic form and unfolding or repulsion in the completely protonated or unprotonated forms. The relative positions of the bulky side-chain R groups determined the ease with which the folding and unfolding occurred. While these structural changes correctly account for the observed pK_a trends, there is little independent evidence to suggest that folding and unfolding accompanies protonation of the $-NH_2$ and CO_2^- groups.

The problem of peptide conformations has been discussed,[17,18] yet not until recently was there much experimental evidence to support any particular structures. However, Lemieux and Barton[19] have recently suggested on the basis of nmr studies of Ala-Phe and Phe-Ala that the same conformation of these dipeptides predominates in acidic, basic and neutral solutions, and this so-called β-type conformation is that shown in Figure 1. Dielectric constant measurements of solutions of peptide diastereomers also support this conformation.[20] The protonation constants can also be interpreted in terms of the β conformation. As shown in Figure 1, the NH_3^+ and CO_2^- groups in the "pure" diastereomers are on opposite sides of the plane defined by the planar amide bond, while they are on the same side of that plane and therefore closer to each other in the "mixed" diastereomers. Thus protonation of the CO_2^- group in zwitterionic dipeptides is electrostatically (because of the nearby NH_3^+ group) less favorable in the "mixed" isomer than in the "pure." On the other hand, deprotonation of the NH_3^+ group in the zwitterions is less favorable (because of the nearby CO_2^- group) in the "mixed" isomer than in the "pure." Thus both trends can be interpreted in terms of the β conformation and may be said to support that conformation.

It might be noted that the difference in pK_a values between the "pure" and "mixed" diastereomers increases with the dipeptide in the order Ala-Ala < Ala-Phe < Leu-Leu $\leq$ Leu-Tyr. This order suggests that bulky α substituents enhance pK_a differences between the diastereomers. In the "mixed" isomer, the R_1 and R_2 α substituents lie on the same side of the molecule and could attract each other in a hydrophobic region above the planar amide group. Lemieux and Barton[19] have interpreted results of their nmr studies of Ala-Phe and Phe-Ala to indicate that the side-chain benzyl group extends over the amide group in both dipeptides and that this configuration is stabilized by a hydrophobic interaction between the phenyl and the amide groups. If it is assumed that larger side chains promote hydrophobic bonding of the R_1 and R_2 groups with the amide group as well as with each other, this attraction may compress the molecule and also bring the NH_3^+ and CO_2^- groups closer to each other in the "mixed" isomer. A closer approach of these charged groups would cause the pK_a value for the NH_3^+ group to increase relative to the "pure" isomer and the pK_a for the CO_2^- group to decrease. Although hydrophobic bonding[21,22] and its effect on the structure of the water solvent[23] have been investigated in a variety of chemical

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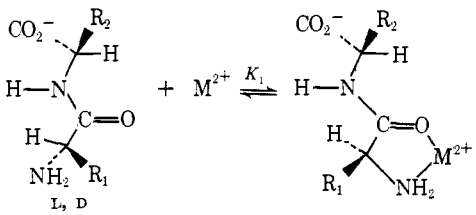
systems, little is known about its importance to the conformations of dipeptides in aqueous solution. Until more is known, our use of it to account for the observed trends in pK_a values must be regarded as tentative.

Bidentate Coordination of Dipeptides by Cu(II) and Zn(II). In the low pH region, Cu(II) and Zn(II) react with dipeptide anions (L^-) to form ML^+ according to eq 1. Based on extensive studies[3,24] of CuL^+ complexes, where L is a peptide containing glycyl residues, the ligand is almost certainly bound through the terminal amino group and the peptide oxygen atom, as shown in eq 8. While the solution structure of ZnL^+ is not



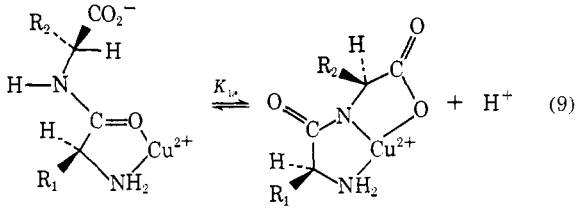
(8)

known unequivocally, X-ray studies[3,24] of Zn(II) complexes of di- and tripeptides of glycine show the same type of coordination. For this reason and because the trends in K_1 are so similar for Cu(II) and Zn(II), we believe the metal ion coordination for both CuL^+ and ZnL^+ is that shown in eq 8. It should be noted, however, that the conformation around the amide N to alkyl C bond is not known in these complexes.

Values of K_1 for equilibrium 8 with Cu(II) and Zn(II) are larger in all cases for the "mixed" diastereomers than for the "pure." The differences are not large but of the same general magnitude as was observed for the NH_3^+ pK_a values. If one assumes the β conformation for the free dipeptide (as in eq 8), essentially the same explanation that was used to account for the larger NH_3^+ pK_a value for the "mixed" isomer can be applied to the values of K_1. That is, in the L,D form of L^-, the negative and repulsive CO_2^- and NH_2 groups are on the same side of the molecule. This repulsion would provide a stronger driving force for complexation with the positive M^{2+} than it would in the L,L (or "pure") isomer where the CO_2^- and NH_2 groups would be on opposite sides of the molecule (Figure 1). While this explanation correctly accounts for the observed trends, it does not take into account any differences in stability of the product ML^+ whose conformation in solution is unknown.

For Zn(II), we also measured equilibrium constants (K_2) for the bidentate coordination of a second dipeptide (L^-) ligand according to eq 2. If the conformation of the free dipeptide (L^-) is of primary importance to the values of K_2 as it was for K_1, the K_2 values should also be larger for the "mixed" diastereomers than for the "pure." This is true, but the differences are smaller than for the K_1 values indicating that the diastereomeric dipeptide ligand present in the ZnL^+ complex somewhat reduces the stereoselectivity of the metal ion for the "mixed" isomer as compared to the stereoselectivity of the free Zn(II).

Tridentate Coordination of Dipeptides by Cu(II). At higher pH, the bidentate CuL^+ complex undergoes amide proton ionization and rearrangement (eq 3) to a tridentate chelate complex, $Cu(H_{-1}L)$. The structure of the product $Cu(H_{-1}L)$ complex, where $H_{-1}L$ is deprotonated glycylglycine, has been unambiguously established[3] as that shown in eq 9. The planar peptide



(9)

backbone in the product $Cu(H_{-1}L)$ complex requires R_1 and R_2 to be on the same side of that plane when a "pure" dipeptide (L,L as in eq 9) is used. With a "mixed" dipeptide, R_1 and R_2 are on opposite sides of the plane.

Equilibrium constants (K_{1a}) for reaction 9 show (Table II) that proton ionization is more favorable for the "pure" diastereomers as compared to their "mixed" analogs. Also the difference in K_{1a} for these isomers increases as the bulkiness of R_1 and R_2 increases. The greatest difference occurs for the Leu-Leu complexes where the K_{1a} value for Cu(L,L-Leu-Leu)$^+$ is ten times greater than that for Cu(L,D-Leu-Leu)$^+$. These very large differences in K_{1a} could be attributed either to differences in structures of the reactants, the products, or both. While little detail is known about the structures of the reactants, their stabilities seem not to be significantly different as indicated by the relatively small differences in the K_1 values for the "pure" and "mixed" isomers.

Thus it appears that the structure of the $Cu(H_{-1}L)$ product is the origin of the favored proton ionization of the "pure" dipeptide complex. Since R_1 and R_2 are on the same side of the plane of the complex in the "pure" complex, this arrangement must be more stable than that where R_1 and R_2 are on opposite sides of the plane. The most probable explanation for this enhanced stabilization is hydrophobic bonding[21,22] of R_1 and R_2 to the amide group as well as to each other in complexes of the "pure" dipeptides. This "internal micelle" would also modify the solvent structure[23] to give perhaps a more favorable complex–water interaction than is possible with the complex of the "mixed" dipeptide. This is basically the same type of interaction that was used earlier to account for the increasing differences in the pK_a values of the NH_3^+ and CO_2^- groups in the "pure" and "mixed" isomers as the size of R_1 and R_2 increased. It might be noted, however, that the difference in stabilities of the diastereomeric $Cu(H_{-1}L)$ complexes is not manifest in their visible spectra (Table III), which are the same within experimental error.

Values of K_{OH} and K_D. Unlike the protonation constants, K_1, and K_{1a}, the values of K_{OH} (eq 5) and K_D (eq 6) do not depend upon the diastereomeric nature of the dipeptide ligand. The values of K_{OH} for $Cu(H_{-1}L)$ complexes of all four isomers of Leu-Leu are the same. This is not surprising since the OH group in the product $Cu(H_{-1}L)(OH)^-$ is presumably[6] located in the remaining square planar coordination site (eq 9) at a considerable distance from the chiral centers. It also suggests that the geometry of the deprotonated dipeptide ligand does not change during this reaction.

The structure of the product $Cu_2(H_{-1}L)_2(OH)^-$ in the

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dimerization reaction (eq 6) is uncertain, but bridged structures containing one OH^- bridge and another containing one OH^- and one deprotonated amide nitrogen bridge have been proposed[6] for the analogous glycylglycine complex, $Cu_2(H_{-1}Glygly)_2(OH)^-$. Since K_D for the formation of these dimers is the same (Table I) for all isomers of Leu-Leu, it appears that there is relatively little peptide rearrangement during dimer formation and also there is probably little contact between the two peptide ligands in the dimer. Thus the structure with one OH^- group bridging the two $Cu(H_{-1}L)$ residues *via* the Cu(II) atoms appears to be the most probable.

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Heuristic Pattern Recognition Analysis of Carbon-13 Nuclear Magnetic Resonance Spectra

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Abstract: The first application of linear discriminant function analysis to experimental proton noise-decoupled ^{13}C high-resolution nuclear magnetic resonance spectra is reported. Results of various preprocessing methods are discussed and the implications with respect to the usefulness of the present approach for structural elucidation problems are considered. It is shown that the analysis of nmr data *via* a learning machine approach is comparable in efficacy to previous studies where mass and infrared spectral data were interpreted in the same way for the purpose of answering structural questions.

Since the use of the heuristic pattern recognition technique called the "learning machine"[1] method was introduced to chemical data analysis by Isenhour and coworkers,[2] its feasibility as a general approach to the interpretation of masses of experimental data has been studied extensively.[3-6] Among the structural elucidation techniques which have been examined most in this way are mass spectrometry[7-9] and infrared spectrometry.[10,11] We report here the first application of linear discriminant function analysis to natural abundance noise-decoupled ^{13}C nuclear magnetic resonance data. Roberts has suggested that the enormous sensitivity of ^{13}C chemical shifts to structural changes should make this technique a far more useful tool for the investigation of structure than proton nmr.[12] Because of the availability of instrumentation for relatively routine determination of high-resolution natural abundance ^{13}C nmr spectra, it seems imperative that rapid effective means of interpreting such data be developed.

In this paper, an entirely new approach to interpretation of ^{13}C nmr spectra, proceeding directly from spectrum to structural information and circumventing the detailed assignment of chemical shifts and coupling constants, is outlined.

Experimental Section

Data Base. As a data base for the study we have used a recently published collection of ^{13}C nmr spectra containing a total of 500 spectra measured on two different instruments and in eight different spectral solvents.[13] Chemical shifts were referenced to tetramethylsilane and, for the most part, covered a range of 200 ppm. Eighty of the spectra were obtained in the continuous-wave mode, the remainder were determined using Fourier transform operation. Intensities were digitized manually and added to the original structure-coded, peak frequency list contained in Johnson and Jankowski's collection.[13]

Computation Method. Binary pattern classification using a simple error correction feedback method[5] and various preprocessing methods was employed to analyze the coded spectral data. Programs were written in Fortran IV, using algorithms described below, and all computations were carried out using an IBM 360/65 computer. A typical computation including preprocessing, feature selection and development of a final weighting vector required between 1 and 3 min of central processor time.

Results and Discussion

Briefly, the analytical approach is to represent the ^{13}C nmr spectra as points in pattern space and then to find hyperplanes (linear discriminant functions) which separate them into binary subsets. Such decision surfaces may be developed for any desired binary choice (*e.g.*,

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