are confident that a combined solvent/pressure dependence study, presently under way in our laboratories, will enable us to differentiate between these two possible transition states.

Preparation. To a slurry of $Nb_2Cl_6(THT)_3$ (0.66 g, 1 mmol) in 20 mL of THF at 0 °C was added 5 mL of concentrated HCl solution. When the mixture had turned green (usually after 0.5 h), it was evaporated to dryness. The solid residue was redissolved in 20 mL of water, affording a green stock solution of the Nb–aquo species, **3**. The addition of $K_2C_2O_4 \cdot H_2O$ (0.75 g, 5 mmol) precipitated a dark red solid, which was removed by filtration. The remaining red solution was divided in half, and one portion was layered with a 1:1 mixture of water and methanol containing $H_2NCH_2CH_2NH_2 \cdot 2HCl$ and the second with 1:3 water–methanol. After 3–4 weeks dark red needles of **1** and **2**, respectively, were obtained in estimated yields of 20–30%. An improved synthetic procedure is anticipated when we learn more about the nature and chemistry of **3**.

X-ray Crystallography. Single-crystal X-ray analyses of both salts have been carried out by standard procedures, which have been described in detail elsewhere.[9] Relevant crystallographic data are given in Table I.

In both cases the positions of the Nb atoms were derived from a three-dimensional Patterson function. The remaining non-hydrogen atoms were located and refined by a series of least-squares refinements and difference Fourier syntheses. An absorption correction by the method of Walker and Stuart[9c] was applied for **2** after isotropic refinement. Selected atoms in both compounds were assigned anisotropic displacement parameters (see Tables II and III) and the structures refined to convergence. In the case of **2** two water molecules were refined with fractional occupancy $^1/_2$ because of high displacement parameters of the corresponding oxygen atoms. There was also some residual electron density above 1 e $Å^{-3}$ remaining around the Nb atoms.

Tables of anisotropic displacement parameters, full listings of bond distances and angles, and listings of structure factors and relevant least-squares planes for both compounds are provided as a supplementary material.

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Supplementary Material Available: For the crystal structures of **1** and **2**, tables of complete bond distances and angles, anisotropic displacement parameters, least-squares planes for bases in the SA model and trapezoids in the DOD model (19 pages); for both structures, listings of observed and calculated structure factors (34 pages). Ordering information is given on any current masthead page.

(9) (a) Bino, A.; Cotton, F. A.; Fanwick, P. E. *Inorg. Chem.* **1979**, *18*, 3558. (b) Cotton, F. A.; Frenz, B. A.; Deganello, G.; Shaver, A. *J. Organomet. Chem.* **1973**, *50*, 227. (c) Walker, N.; Stuart, D. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1983**, *A39*, 158. (d) Calculations were done on a PDP-11/60 (RSX-11M V4.1) with SDP-PLUS and on a VAX-11/780 (VMS V4.2) with SDP/VAX V3.0.

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Copper(II) Complexes of Diastereoisomeric Methionylmethionines in Aqueous Solution. Favoring of the Amide-Deprotonated Complex in the L,L-Dipeptide without Sulfur Coordination

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A thermodynamic investigation has been carried out on copper(II) complexes with L-methionyl-L-methionine or D-methionyl-L-methionine at 25 °C and I = 0.1 mol dm^{-3} (KNO_3). Thermodynamic stereoselectivity has been found in the formation of the amide-deprotonated complexes. In particular, the formation of the copper(II) complex with the L,L-dipeptide is enthalpically favored. Structural information obtained by the EPR spectra in solution parallels the thermodynamic data: a slightly larger copper hyperfine coupling constant has been determined in the case of the copper(II) complex with L-methionyl-L-methionine. Comparison with data previously obtained for similar dipeptides containing noncoordinating side-chain groups evidences that the sulfur atom is not coordinated to the metal ion. The thermodynamic stereoselectivity of the amide-deprotonated complex differences of 0.3 in log β and 1.7 kcal mol^{-1} in $\Delta H°$ can be attributed to the hydrophobic interaction between the residues of side chains. This noncovalent "bonding" is possible only for the L,L-diastereoisomer where the side chains are on the same side of the coordination plane.

Recently, we have studied the formation, stability, and bonding details, in aqueous solution, of copper(II) coordination of some diastereoisomeric dipeptides containing noncoordinating side-chain groups.[1] By means of potentiometric and calorimetric measurements, evidence has been found of thermodynamic stereoselectivity in the formation of the amide-deprotonated complexes. If, on the basis of the EPR parameters, different coordination numbers are excluded when the metal ion is bound to the L,D- and L,L-isomers, the slightly larger stability of the copper(II) L,L-dipeptide complexes compared to that of the analogous L,D-dipeptide ones was attributed to the solvophobic interaction[2] between the side-chain groups. This noncovalent[3] "bonding" is possible only for the L,L-diastereoisomers, because the side chains are on the same side of the coordination plane.

Here we report the results of the thermodynamic studies for the complex formation of copper(II) L,L- or D,L-methionylmethionine peptides in aqueous solution. The aim of this work is to find unambiguous evidence of the possible involvement of sulfur atoms in the bonding to the metal ion and also to assess the role played by the thioether in the stability of the complexes. A small, but reproducible stereoselective effect has been observed in both free energy (0.67 kcal) and enthalpy (0.41 kcal) changes associated with the formation of the bis complexes Ni(D/L-Met)$_2$[4] (Met = methionine) and attributed to the tridentate binding of the amino acid, hypothesizing the existence of weak coordination between Ni^{2+} and the thioether sulfur atom in the Ni(D-Met)-(L-Met)complex.

As regards the ligands here investigated, if the sulfur is engaged in the coordination, two apical thioethereal bonds would be possible in the case of the L,D-isomer while one only would be possible in the case of the L,L-peptide. The resulting different coordination numbers of the metal ion could be the reason for the thermodynamic stereoselectivity. Thus, to obtain further information about

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(1) Bonomo, R. P.; Cali, R.; Cucinotta, V.; Impellizzeri, G.; Rizzarelli, E. *Inorg. Chem.* **1986**, *25*, 1641.
(2) Sinanoglu, O. *Molecular Interactions*; Ratajczak, H., Orville-Thomas, W. J., Eds.; Wiley: New York, 1982; Vol. 3, p 283.
(3) Frieden, E. *J. Chem. Educ.* **1975**, *52*, 754.
(4) Swash, J. L. M.; Pettit, L. D. *Inorg. Chim. Acta* **1979**, *19*, 19.

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the geometry and coordination numbers of these copper(II) complexes, we have also carried out EPR studies of the main species formed by the copper(II) with the above-cited ligands in addition to potentiometric and calorimetric investigations.

Experimental Section

Materials. The L-methionyl-L-methionine (Met-Met) and D-methionyl-L-methionine (D-Met-L-Met) were synthesized according to standard liquid-phase methods,[5] and with small differences in comparison with a previous preparation.[6] The starting materials were optically pure amino acids (Fluka). The amino groups were protected by synthesizing the *N*-tert-butoxycarbonyl derivatives and the amino acid residues were coupled by using the active-ester method. The active ester was prepared by using p-nitrophenol in the presence of dicyclohexylcarbodiimide. The N-protected active esters were then coupled with the second amino acid methyl ester in aqueous alkali ($NaHCO_3$ in a 1:1 dioxane–water mixture) to give a yellow oil. It was hydrolyzed with NaOH 1 N. The *tert*-butoxycarbonyl protecting group was removed with formic acid, and the "free" dipeptide was obtained by treatment with water followed by recrystallization from a water–methanol mixture.

Purity was checked by TLC using the following solvents: butanol–acetic acid–water (BAW) (3:1:1 v:v:v) and chloroform–methanol (9:1 v:v) as well as by elemental analysis. The water amount was determined by TG analysis. $Cu(NO_3)_2$ was prepared from basic copper(II) carbonate by adding a slight excess of HNO_3. The stock solution titer was determined by titration with EDTA.

Equilibria Measurements. Potentiometric titrations were carried out as previously reported.[1] The protonation constants were determined by titrating with KOH 25 mL (pH range 2.8–9) of aqueous dipeptide solution containing HNO_3 and KNO_3 (I = 0.1; 25 °C) at an acid to ligand ratio of 1.1:1. The ligand concentration ranged from 0.005 to 0.010 mol dm^{-3}. The conditions for the determination of the stability constants were the same as for the protonation constants, but KNO_3 was partly replaced by $Cu(NO_3)_2$. The titrations were carried out at a metal to ligand ratio of 1:1 and with a range of ligand concentrations between 0.003 and 0.008 mol dm^{-3} in the pH range 3.0–6.0. The titrant was KOH. All titrations were performed in triplicate. Other details were as previously reported.[7] $\Delta H°$ and $\Delta S°$ values were determined by calorimetric titration using a Tronac Model 450 isoperibol calorimeter equipped with a 25-mL reaction vessel. The calorimetric measurements were carried out by titrating with HNO_3 (0.2–0.4 mol dm^{-3}) solution containing the metal and the ligand in a 1:1 ratio at the pH of the maximum formation degree of the main species $Cu(H_{-1}L)$. The ionic strength was maintained constant at the value of I = 0.1 mol dm^{-3} by adding KNO_3. The ligand concentrations ranged from 0.005 to 0.010 mol dm^{-3}. Other experimental details were as previously reported.[1,7]

Spectroscopic Measurements. EPR spectra were measured with a conventional X-band spectrometer (Bruker Model 220D) at room temperature and at low temperature. The microwave frequency was calibrated with the use of powdered DPPH samples (g = 2.0036), while the magnetic field was carefully measured during any spectrum scan by means of a Bruker gaussmeter Type ER 035 M. Solutions containing 0.005 mol dm^{-3} of $^{63}Cu(NO_3)_2$ (in 95% water–5% methanol) and the dipeptide at pH 6.0 were used to record frozen-solution EPR spectra. Other details were as previously reported.[1]

Calculations. The calculations concerning the $E°$ of the electrode system, the purity of the ligands, and the HNO_3 excess amount in the metal ion stock solution were performed by the least-squares computer program ACBA.[8] The formation constants of the copper(II) complexes were calculated by the program MINIQUAD.[9] To obtain the species distribution with respect to the pH change (see calorimetric titration) we utilized the computer program DISDI.[10] The heats of complex formation were calculated by the program DOEC.[11] Throughout the paper, the errors are expressed as three times the standard deviations or as an uncertainty range (maximum deviation from the mean). In order to evaluate small differences among the magnetic parameters of these L,L- and D,L-dipeptide copper(II) complexes, isotopically pure ^{63}Cu was employed. Other details were as previously reported.[1,12,13]

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

dipeptide[a]	log β_{011}	log β_{021}	pK_{COOH}[b]	ref
L-Met-L-Met	7.389 (8), 7.43	10.617 (8), 10.65	3.228, 3.22	*c*, *d*
D-Met-L-Met	7.544 (7)	10.456 (9)	2.912	*c*
L-Met-D-Met	7.63	10.67	3.04	*d*
Sm-L-Cys-L-Met	7.03	10.21	3.18	*d*
Sm-L-Cys-D-Met	7.23	10.17	2.94	*d*
L-Met-Sm-L-Cys	7.40	10.38	2.98	*d*
D-Met-Sm-L-Cys	7.62	10.34	2.72	*d*
L-Met-L-Val	7.45	10.88	3.43	*e*
L-Met-D-Val	7.69	10.76	3.07	*e*
L-Ala-L-Ala	8.19	11.50	3.31	*f*
L-Ala-D-Ala	8.34	11.53	3.19	*f*
L-Ala-L-Leu	8.04	11.40	3.36	*g*
D-Ala-L-Leu	8.27	11.41	3.14	*g*
L-Leu-L-Ileu	7.79	11.21	3.42	*h*
D-Leu-L-Ileu	8.10	11.17	3.07	*h*
L-Leu-L-Leu	7.93	11.39	3.46	*f*
L-Leu-D-Leu	8.22	11.28	3.06	*f*

[a] Sm-Cys = *S*-methylcysteine; Val = valine; Ala = alanine, Leu = leucine; Ileu = isoleucine. [b] pK_{COOH} = log β_{021} − log β_{011}. [c] This work. [d] Reference 6. [e] Reference 14. [f] Reference 15. [g] Reference 16. [h] Reference 17.

Results

The generalized formation reaction of peptide ligands with proton and copper(II) is given in eq 1, where L is the negative

$$mCu^{2+} + hH + lL \rightleftharpoons^{\beta_{mhl}} Cu_m(H_hL_l) \quad (1)$$

species for the peptide ligands. Charges on the ligand and copper(II) complexes are omitted for clarity. The stability constant β_{mhl} is defined by eq 2. Analysis of the titration data for the

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

peptide ligands in the absence of copper(II) gives the association constants for amine and carboxylate protonation (eq 3 and 4).

$$H^+ + L \rightleftharpoons^{\beta_{011}} HL \quad (3)$$

$$2H^+ + L \rightleftharpoons^{\beta_{021}} H_2L \quad (4)$$

The values of the protonation constants determined in this study are given in Table I together with the corresponding values for similar dipeptides.

The equilibria needed to fit the experimental titration curves for the solutions of copper(II) and the peptide ligands under study are given by eq 5–7. In our experimental conditions, the major

$$Cu^{2+} + H^+ + L \rightleftharpoons^{\beta_{111}} Cu(HL) \quad (5)$$

$$Cu^{2+} + L \rightleftharpoons^{\beta_{101}} CuL \quad (6)$$

$$Cu^{2+} + L \rightleftharpoons^{\beta_{1-11}} Cu(H_{-1}L) + H^+ \quad (7)$$

species was the $Cu(H_{-1}L)$ complex; below pH 4 CuL and Cu(HL) complexes also form but in very small percentages. Other minor species $Cu(H_{-3}L_2)$ and $Cu(H_{-1}L_2)$ were also determined but with no significant increase in the goodness of the fit; thus, there was no evidence for their existence. $\Delta G°$, $\Delta H°$, and $\Delta S°$ values for

(5) Schussler, H; Zahn, H. *Chem. Ber.* **1962**, *95*, 1076.
(6) Lyons, A. Q.; Pettit, L. D. *J. Chem. Soc., Dalton. Trans.* **1984**, 2305.
(7) Amico, P.; Arena, G.; Daniele, P. G.; Ostacoli, G.; Rizzarelli, E.; Sammartano, S. *Inorg. Chem.* **1981**, *20*, 772.
(8) Arena, G.; Rizzarelli, E.; Sammartano, S.; Rigano, C. *Talanta* **1979**, *26*, 1.
(9) Gans, P.; Vacca, A.; Sabatini, A. *Inorg. Chim. Acta* **1976**, *18*, 237.
(10) Arena, G.; Rigano, C.; Sammartano, S. *Ann. Chim. (Rome)* **1978**, *68*, 693.
(11) Rigano, C.; Rizzarelli, E.; Sammartano, S. *Thermochim. Acta* **1979**, *33*, 211.
(12) Bonomo, R. P.; Riggi, F. *Lett. Nuovo Cimento Soc. Ital. Fis.* **1981**, *30*, 304.
(13) Bonomo, R. P.; Riggi, F. *Chem. Phys. Lett.* **1982**, *93*, 99.
(14) Pettit, L. D.; Hefford, R. J. W. *Metal Ions in Biological Systems*; Sigel, H., Ed.; Dekker: New York, 1979; Vol. 9.
(15) Impellizzeri, G.; Bonomo, R. P.; Cali, R.; Cucinotta, V.; Rizzarelli, E. *Thermochim. Acta* **1984**, *80*, 275.
(16) Impellizzeri, G.; Bonomo, R. P.; Cali, R.; Cucinotta, V.; Rizzarelli, E. *Thermochim. Acta* **1984**, *80*, 275.
(17) Impellizzeri, G.; Cali, R.; Cucinotta, V.; Rizzarelli, E., manuscript in preparation.

Table II. Thermodynamic Parameters of Proton Complex Formation of Diastereoisomeric Dipeptides at 25 °C and I = 0.1 mol dm^{-3} (KNO_3)

dipeptide	$-\Delta G°$ [a]		$-\Delta H°$ [a]		$\Delta S°$ [a]		ref
	NH_2	CO_2^-	NH_2	CO_2^-	NH_2	CO_2^-	
L-Met-L-Met	10.05 (1)	4.39 (1)	10.64 (9)	−0.30 (9)	−2.0 (3)	16.0 (9)	*b*
D-Met-L-Met	10.26 (1)	3.96 (1)	10.43 (8)	−0.12 (9)	−0.5 (3)	13.1 (3)	*b*
L-Ala-L-Ala	11.14	4.50	10.64	−0.26	1.7	15.9	*c*
L-Ala-D-Ala	11.34	4.34	10.26	−0.63	3.6	16.7	*c*
L-Ala-L-Leu	10.94	4.56	10.68	−0.24	0.9	16.1	*d*
D-Ala-L-Leu	11.25	4.26	10.58	−0.77	2.2	16.9	*d*
L-Leu-L-Ileu	10.60	4.66	10.69	−0.41	−0.3	17.0	*e*
D-Leu-L-Ileu	11.02	4.17	10.79	−0.88	0.8	16.9	*e*
L-Leu-L-Leu	10.78	4.71	10.21	−0.36	1.9	17.0	*c*
L-Leu-D-Leu	11.18	4.16	10.92	−0.77	0.9	16.5	*c*

[a] $\Delta G°$ and $\Delta H°$ in kcal mol^{-1}; $\Delta S°$ in cal mol^{-1} deg^{-1}. [b] This work. [c] Reference 15. [d] Reference 16. [e] Reference 17.

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

dipeptide	$\log \beta_{111}$	$\log \beta_{101}$	$\log \beta_{1\text{-}11}$	pK^{H}_{CuL} [a]	$\Delta G°_{1\text{-}11}$ [b]	$\Delta H°_{1\text{-}11}$ [b]	$\Delta S°_{1\text{-}11}$ [b]	ref
L-Met-L-Met	8.82 (6)	5.07 (6)	1.70 (1)	3.37	2.32 (2)	1.90 (9)	14.3 (3)	*c*
D-Met-L-Met	9.11 (9)	5.12 (6)	1.43 (1)	3.69	1.95 (2)	3.60 (6)	18.8 (2)	*c*
L-Ala-L-Ala		5.54	1.82	3.72	2.48	1.96	14.9	*d*
L-Ala-D-Ala		5.71	1.75	3.96	2.39	1.54	13.2	*d*
L-Leu-L-Ileu		4.96	1.21	3.75	1.65	1.88	11.8	*d*
D-Leu-L-Ileu		4.96	0.59	4.37	0.81	3.71	15.2	*d*
L-Leu-L-Leu		5.21	1.33	3.88	1.81	1.64	11.6	*d*
L-Leu-D-Leu		5.48	0.60	4.88	0.82	4.06	16.3	*d*
L-Met-L-Phe	...	4.76	1.76	3.00	...	...	...	*e*
L-Met-D-Phe	...	4.93	1.29	3.64	...	...	...	*e*
L-Met-L-Val	...	4.96	1.16	3.80	...	...	...	*e*
L-Met-D-Val	...	5.01	0.79	4.22	...	...	...	*e*

[a] For the reaction $CuL \rightleftarrows (CuLH_{-1}) + H^+$. [b] $\Delta G°$ and $\Delta H°$ in kcal mol^{-1}; $\Delta S°$ in cal mol^{-1} deg^{-1}. [c] This work. [d] Reference 1. [e] Reference 14.

the protonation and the formation of the main species $Cu(H_{-1}L)$ of the two diastereoisomers are also given in Tables II and III, respectively.

Discussion

Peptide Protonation. The values of proton complex formation constants for the ligand studied are in agreement with recent reported values.[6] Stereoselective effects between the two diastereoisomeric dipeptides are significant; the log β value concerning the protonation of the amine group is higher for the D,L-peptide than for the L,L-isomer, while the opposite behavior is observed in the protonation of the carboxylate group. The same trend was observed in the other diastereoisomeric pairs of dipeptide-containing sulfur atoms and in some couples of dipeptides containing amino acid residues of different chirality without thioether atoms (Table I). In general, the stereoselectivity increased as the size of the side chain increased. The difference in stability ranges from 0.15 to 0.4 l.u. (logarithmic unity) on going from the Ala-Ala couple to the Leu-Leu one. The difference between the log β values of protonation of D-Met-L-Met and L-Met-L-Met is similar to that found between L-Ala-D-Ala and L-Ala-L-Ala. On the basis of the $\Delta G°$ values only, the thermodynamic stereoselectivity in the proton complex formation of linear dipeptides has been attributed to the favorable folding of the L,D-dipeptide in the β-conformation (Figure 1).[18] Recently, we determined the $\Delta H°$ and $\Delta S°$ contributions to the $\Delta G°$ dipeptide protonation values[15-17] by calorimetric measurements and found that the protonation of the amine group of dipeptides is only enthalpy favored, while that of the carboxylate group reveals a favorable entropy contribution alone in accordance with that reported for amine and carboxylic ligands.[19,20] Although the thermodynamic data for the proton complex formation of methionine dipeptides follow the general trend, some points still need further clarification. In particular, the difference in the thermodynamic parameters between the diastereoisomers will be discussed. The larger amine protonation

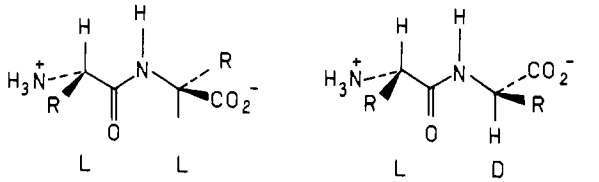

Figure 1. Conformations of neutral dipeptides: left, amino acids of same chirality (preferred conformation not charge stabilized); right, amino acids of opposite chirality (charge stabilized β conformation).

constant of D-Met-L-Met peptide in comparison with the L,L-isomer is due to less favorable $\Delta H°$ and less unfavorable $\Delta S°$ contributions. This behavior is similar to that shown by the Ala-Ala and Ala-Leu dipeptides but different from that of other dipeptides with branched-alkyl side chains (Table II). Since the β-conformation[21] constrains the COO^- and NH_3^+ groups on the same side of the L,D-diastereoisomer molecules (Figure 1), it has been possible to explain the thermodynamic quantities of Ala-Ala dipeptides as being only due to the stronger electrostatic interaction present in the L,D-diastereoisomers with respect to the L,L-diastereoisomers, since it was known that peptides containing some D-amino acids show a shorter end-to-end distance than do all L,L-peptides.[22-24] To explain the opposite behavior exhibited by the other dipeptides it was necessary to invoke a second interaction, namely a solvophobic[2] (or, according to more classical denomination, hydrophobic[25]) interaction between the side-chain groups, which in the L,D-diastereoisomers lie on the same side of the molecule. For these dipeptides, the difference in the thermodynamic parameters (in particular in $\Delta H°$ values between each pair of diastereoisomers) has been interpreted as resulting from the algebraic sum of the contribution due to the solvophobic interaction and the contribution of the opposite sign due to the electrostatic

(18) Ellenbogen, E. *J. Am. Chem. Soc.* **1956**, *78*, 369.
(19) Barone, V.; Barbucci, R.; Russo, N. *Gazz. Chim. Ital.* **1981**, *111*, 115.
(20) Arena, G.; Cali, R.; Rizzarelli, E.; Sammartano, S. *Transition Met. Chem. (Weinheim, Ger.)* **1978**, *3*, 147.
(21) Lande, S. *Biopolymers* **1969**, *7*, 879.
(22) Beacham, J.; Ivanov, V. T.; Kenner, G. W.; Sheppard, R. C. *J. Chem. Soc., Chem. Comm.* **1965**, 386.
(23) Flory, P. F;; Schimmel, P. R. *J. Am. Chem. Soc.* **1967**, *89*, 6807.
(24) Lemieux, R. U.; Barton, M. A. *Can. J. Chem.* **1971**, *49*, 767.
(25) Ben-Naim, A. *Hydrophobic Interactions*; Plenum: New York, 1980.

interaction.[16] As for Met-Met peptides, for which the stereoselectivity appears to be entropy driven, we can hypothesize that the solvophobic interaction between the two alkylic groups involves an exothermic enthalpy variation lower than the endothermic effect due to the electrostatic interaction.

Copper–Peptide Association. Cu(HL). Depending on the relative values of $\log \beta_{111}/\log \beta_{101}$ and $\log \beta_{1-11}$, the Cu(HL) species may be, in the 3–4 pH range, a nearly insignificant species or a minor component of the total copper(II) complexes, reaching 7% in the case of the L,D-isomer. On the basis of electronic absorption data and charge neutralization effects on the magnitude of pK'_{NH} ($pK'_{NH} = \log \beta_{111} - \log \beta_{101}$) for eq 8. Kaneda and Martell[26]

$$Cu(HL) \rightleftharpoons^{K'_{NH}} CuL + H^+ \qquad (8)$$

described the Cu(HL) structure as an amine-protonated species for peptide ligands that coordinate by means of the peptide C=O group and carboxylic oxygen. Our data, which parallel the trend in $\log \beta_{011}$, could be explained in the same way even if EPR and NMR data give evidence for interaction between the sulfur atom of methionine methyl ester and copper(II).[27,28]

CuL. Extensive studies[29,30] of CuL^+ complexes, where L is a peptide-containing glycyl residue, have shown that the ligand is almost certainly bound through the terminal amino group and the peptide oxygen atom. The possibility of additional apical coordination to the copper(II) of the sulfur and oxygen atoms of the side chains has been proposed for *S*-methylcysteinylglycine, serylglycine and threonylglycine.[31] The (α-alkyl-*S*/*O*-glycyl)-glycinate–copper(II) complexes were about 0.3–1.5 l.u. more stable than expected on the basis of the basicitiy of the terminal amino group of the dipeptides, showing that hydroxy and thioether groups substituted at the glycyl moiety enhance the complex stability and thus participate in complex formation. Also the values of the stability constants of (L,L-methionylmethioninato)- and (D,L-methionylmethioninato)copper(II) show this behavior as well as the analogous complexes of methionylvaline and methionylphenylalanine. It is noteworthy that the stereoselectivity in this species is insignificant and the difference in the stability constant values is lower than that already found in the amine protonation ones. This may be explained assuming that the apical coordination of the thioether atoms has a leveling effect on the thermodynamic parameters. Due to the low formation percentage we are not able to obtain reliable $\Delta H°$ and $\Delta S°$ values for the CuL and $Cu(LH)^+$ species, and thus we cannot give further support to previous suggestions.

$Cu(H_{-1}L)$. Our systems show significant stereoselectivity, the complex of L,L-dipeptide being more stable. The $\log \beta_{1-11}$ values are in the range found for dipeptides not containing chelating side-chain donor atoms. Comparison of the values for pK^{H}_{CuL} (i.e. ionization of the amide proton to form the major copper(II)–dipeptide species) shows that the complex Cu(Met-Met) is more acidic (3.37) than Cu(D-Met-L-Met) (3.69), but this trend parallels that found for other dipeptides with two non-glycyl residues (Table III). Thus, on the basis of $\Delta G°$ values, there is no evidence of sulfur involvement in the coordination sphere. $\Delta G°$, $\Delta H°$, and $\Delta S°$ values for the formation of $CuH_{-1}L$ of the diastereoisomeric dipeptides (Table III) show that the complex formation is entropically favored, due to the charge neutralization of the metal ion. According to the suggestion of Nancollas et al.,[32] the resulting endothermic enthalpy changes reflect the prevailing of the endothermic contributions due to the deprotonation of the peptide hydrogen and to the carboxylate bond formation over the exothermic contributions due to the peptide and amine nitrogen bond formation. Furthermore, the enthalpy and entropy changes of the copper(II) methionylmethionine complex formation give results similar (see Table III) to those pertinent to the same species of similar compounds wth noncoordinating side-chain groups (L,L- and D,L-Leu-Ileu peptides); this similarity confirms that the soft centers do not coordinate to copper(II) ion and, then, they are not responsible for the difference in the thermodynamic properties. Thus the thermodynamic stereoselectivity may be explained by invoking the same factors previously suggested for the dipeptides containing alkyl or aromatic residues.[1] In a manner different from the case of proton complex formation, the planar peptide backbone of the $Cu(H_{-1}L)$ requires the side-chain groups to be on the same side with respect to the coordination plane when, a L,L-dipeptide is considered (Figure 2a). This disposition of the amino acid residues favors their interaction in the complex of L,L-isomer, a solvophobic interaction that cannot exist in the complex of the D,L-isomer because the side chains are opposite each other with respect to the coordination plane (Figure 2b). In agreement with previous results, the solvophobic interaction reflects a gain of enthalpy contribution for the $Cu(H_{-1}L)$ complex of L,L-dipeptide and is the driving force of the thermodymanic stereoselectivity. (Probably also the sulfur atom can be involved in the solvophobic interaction).

In some cases the preferential formation of the optically homogeneous dipeptide complex has been attributed to differences in steric interference between the peptide side chains and coordinated water.[33]

The EPR parameters seem to exclude that the stereoselectivity can be due to a different coordination number achieved by the metal ion in the complexes of the two diastereoisomers. As one can see from Figure 3, the EPR spectra of the dipeptides show values of $g_\parallel > g_\perp > 2.04$, characteristic of axial copper(II)

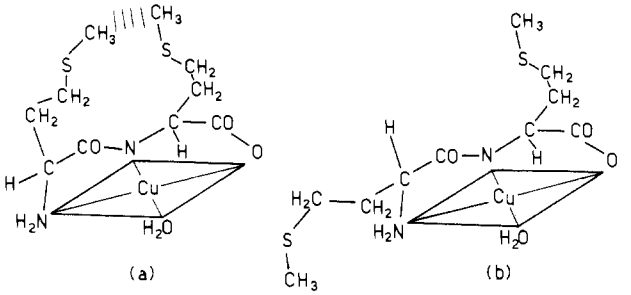

Figure 2. Hypothesized arrangements of side-chain groups in Cu(Met-Met) (a) and Cu(D-Met-L-Met) (b).

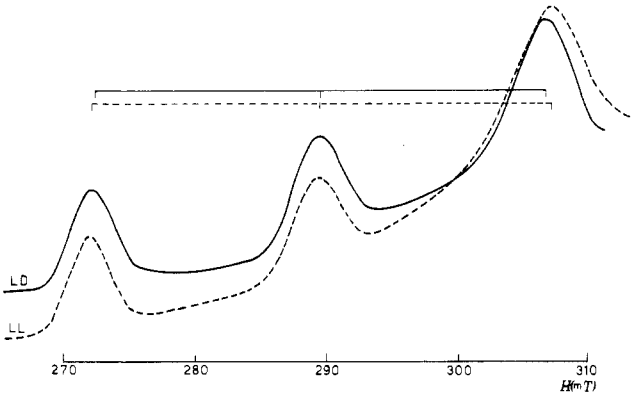

Figure 3. Parallel parts of the frozen solution due to $^{63}Cu(II)$–methionylmethionine complexes in water–methanol (95:5) at 150 K dotted line, L,L-isomer ($g_\parallel = 2.243$ (1), $A_\parallel = 0.00186$ (1) cm^{-1}, $g_\perp = 2.050$ (3), $A_\perp = 0.0011$ cm^{-1}, $g_{iso} = 2.119$ (1), $A_{iso} = 0.00714$ (5) cm^{-1}); solid line, D,L-isomers ($g_\parallel = 2.240$ (1), $A_\parallel = 0.0184$ (1) cm^{-1}, $g_\perp = 2.049$, $A_\perp = 0.0011$ cm^{-1}, $g_{iso} = 2.113$ (1), $A_{iso} = 0.00703$ (5) cm^{-1}).

(26) Kaneda, A.; Martell, A. E. *J. Coord. Chem.* **1975**, *4*, 137.
(27) Kozlowski, H.; Kovalik, T. *Inorg. Nucl. Chem. Lett.* **1978**, *14*, 201.
(28) Kozlowski, H.; Kovalik, T. *Inorg. Chim. Acta* **1979**, *34*, 1231.
(29) Freeman, H. C. *Adv. Protein Chem.* **1967**, *22*, 257.
(30) Freeman, H. C. *Inorganic Biochemistry*; Eichhorn, G. L., Ed.; Elsevier: New York, 1973; Chapter 4.
(31) Sigel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 385.
(32) Brunetti, A. P.; Lim, M. C.; Nancollas, G. H. *J. Am. Chem. Soc.* **1968**, *90*, 5120.
(33) Brookes, G.; Pettit, L. D. *J. Chem. Soc., Dalton Trans.* **1975**, 2302.
(34) Yokoi, H.; Sai, M.; Isobe, T.; Ohsawa, S. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 2189.

complexes in tetragonally distorted octahedral, square-base-pyramidal, or square-planar stereochemistries, all copper(II) geometries associated with a $d_{x^2-y^2}$ ground state. The $A_\parallel$ values are higher than those found in the copper bis(amino acidato) complexes that contain the CuN_2O_2 chromophore,[13,35] suggesting that a greater extent of the tetragonal elongation of the potential apical coordination sites is probably present in the copper(II) dipeptide systems. Furthermore, the EPR parameters show the same values found for $Cu(H_{-1}L)$ species of Leu-Ilue diasteroisomers,[1] suggesting that the sulfur atoms are not involved in the coordination. On the other hand the small difference in $A_\parallel$ values, giving evidence of stereoselectivity, cannot be attributed to a different interaction of the metal ion with the solvent or to its different geometries in the complexes of two diasteroisomers. The $A_\parallel$ data parallel those found in the case of the EPR study of the polycrystalline material obtained by doping $Zn(L\text{-Met})_2$,[36] which is isomorphous with the analogous copper bis complex. The molecular structure showed a trans arrangement of the two amino acidate molecules chelated to the copper atom. The coordination around the copper(II) atom is essentially square planar even if interactions with neighboring methionine oxygen atoms (2.751 or 2.676 Å) coming from carboxylate groups are important to build up the crystal packing. The $A_\parallel$ value of 182.5×10^{-3} cm^{-1} was obtained in situation like that in the copper(II) complex with the L,D-isomer. Thus, to explain the higher $A_\parallel$ value for the L,L-dipeptide complex than for the corresponding D,L-isomer species, other factors must be invoked. In the $Cu(H_{-1}L)$ with L,L-diastereoisomeric molecules the side chains can interact above the coordination plane; as a consequence of this solvophobic interaction, some "stiffening" may be experienced by the basal plane. This interaction could constrain the donor atoms of the dipeptide coordinated to the metal ion to achieve a quasi-ideal planar conformation. On the contrary, when this interaction is not possible (as for the D,L-dipeptide complex), the lower $A_\parallel$ value may be due to the presence of a small tetrahedral distortion.

This suggestion seems to be justified because it is well-known from Freeman's crystallographic work[37] that the basal plane formed by the dipeptide chelate group is distorted toward a tetrahedral situation. Thus, the thermodymamic stereoselectivity in copper(II) complex formation with the methionylmethionine peptide is due to the solvophobic forces and not to the bonding of sulfur atoms. If the thioethereal atoms were involved in the coordination to copper(II), the metal ion would have shown a different coordination number and geometry in the two diasteroisomeric complexes. The $A_\parallel$ values exclude this hypothesis.

As previously reported[1,14] the thermodymanic stereoselectivity is more evident on the basis of the enthalpy changes with respect to the free energy ones. The $\Delta H°$ value of the Cu(Met-Met) complex is nearly equal to the $\Delta H°$ of Cu(L-Leu-L-Ileu), showing that the elongation of the side chain does not involve an enhancement of the solvophobic interaction, such as recently noted by other authors.[38]

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(35) Szabo-Planke, T.; Horvárth, L. J. *Acta Chim. Hung.* **1983**, *114*, 15.
(36) Ou, C.; Powers, D. A.; Thich, J. A.; Felthouse, T. R.; Hendrickson, D. N.; Potenza, J. A.; Schugar, H. J. *Inorg. Chem.* **1978**, *17*, 34.
(37) Freman, H. C. *The Biochemistry of Copper*; Peisach, J., Aisen, P., Blumberg, W. E., Eds.; Academic: New York, 1966; p 77.
(38) Liang, G.; Tribolet, R.; Sigel, H. *Proceedings of the XXIV International Conference on Coordination Chemistry*; Athens, Greece, 1986; p 199.

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Pentacyanoferrate(II/III) Complexes of 2-Substituted Imidazoles and Imidazolates (R = CH_3, CHO, CO_2^-)

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$(CN)_5Fe^{III}L^{2-}$ and $(CN)_5Fe^{II}L^{3-}$ complexes have been studied with 2-substituted imidazoles (RimH; R = CH_3, CHO, CO_2^-). $2CO_2imH^-$ exhibits no complexation of $(CN)_5Fe^{3-}$, as shown by ^{13}C NMR with up to 10-fold excess ligand, but forms the Fe(III) derivative $(CN)_5Fe(2CO_2imH)^{3-}$. The formation constant of this species, $K_f \cong 35$ M^{-1}, is reduced by 9.7×10^3 relative to that for imH. The Fe(III) complexes exhibit LMCT transitions with the characteristic imidazole $d\pi \leftarrow (\pi_1)_L$ bands at 450, 460, 475, and 505 nm with R = CHO, CO_2^-, H, and CH_3, respectively, in concert with the σ_p substituent constants. Imidazoles are generally poor π acceptors, but 2CHOimH acts as a strong π-acceptor ligand toward $(CN)_5Fe^{3-}$; $(CN)_5Fe^{II}(2CHOimH)^{3-}$ exhibits an MLCT transition at 454 nm, similar to that for the pyrazine complex. The standard reduction potential for the $(CN)_5Fe(2CHOimH)^{2-/3-}$ complex is 0.42 V, ca. 0.06 V more favorable than $(CN)_5Fe(imH)^{2-/3-}$. The imidazolato forms of the Fe(III) complexes exhibit characteristic $d\pi \leftarrow (\pi_{2,n})_L$ transitions at 428, 438, and 448 nm for R = CHO, H, and CH_3, respectively, increasing in the order of the substituent's releasing influence. The imidazolates undergo solvent-assisted dissociation to form $(CN)_5FeOH^{3-}$ and the free ligand. The rate constant for dissociation of the $2CH_3im^-$ derivative is $k_d = (2.33 \pm 0.08) \times 10^{-3}$ s^{-1}, determined at 25.0 °C, 0.50 M OH^-, and $\mu = 1.00$ (NaCl), and $(1.9 \pm 0.3) \times 10^{-3}$ s^{-1}, at 0.10 M OH^- and $\mu = 1.00$. The steric enhancement for dissociation of $2CH_3im^-$ vs. im^- is a factor of ca. 8.9. The dissociation is prone to catalysis by traces of the Fe(II) complex. The catalysis may be blocked by the presence of excess $S_2O_8^{2-}$, but competitive ligand oxidation generates a mixture of $(CN)_5Fe^{III}(2CH_3imH)^{2-}$ and $(CN)_5Fe^{III}(2CO_2imH)^{3-}$. Dissociation of the imidazolato form of the latter is more rapid. The lability of $(CN)_5Fe^{III}(2CO_2imH)^{3-}$ was studied by using (dimethylamino)pyridine (dmapy) as a scavenger for $(CN)_5FeOH_2^{2-}$. dmapy substitutes on $(CN)_5FeOH_2^{2-}$ and the $Fe_2(CN)_{10}^{4-}$ dimer with rate constants of 12.2 M^{-1} s^{-1} and 2.0×10^{-3} s^{-1}, respectively. $(CN)_5Fe^{III}(2CO_2imH)^{3-}$ dissociates at least a power of 10 more slowly than the substitution of dmapy on $Fe_2(CN)_{10}^{4-}$. Both the free ligand $2CO_2imH^-$ and its Fe(III) complex $(CN)_5Fe(2CO_2imH)^{3-}$ react with H_2O_2 (but not $S_2O_8^{2-}$) within 24 h to form a new imidazole ligand and a $(CN)_5Fe(RimH)^{2-}$ complex. However, the imidazole products of the two processes are different, leading to what appears to be R = OH for the free ligand and R = $CH_2(OH)$ for the coordinated ligand.

Introduction

Dissociation of metal ion–imidazolate bonds may be important in the function of certain enzymes such as superoxide dismutase. Deprotonation of imidazole coordinated to $(CN)_5Fe^{2-}$ and dissociation of the imidazolato, Rim^-, ligand has been studied previously by Johnson et al.[1] with R = H. The reaction proceeds by a dissociative pathway in which the Fe(III)–imidazolate bond is very much stretched and weakened in the transition state (‡).[1] Rim^- is subsequently replaced by OH^- by two possible routes. One

(1) Johnson, C. R.; Shepherd, R. E.; Marr, B.; O'Donnell, S.; Dressick, W. *J. Am. Chem. Soc.* **1980**, *102*, 6227–6235.

0020-1669/87/1326-2897$01.50/0