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Complex Formation of Copper Ion with Aliphatic Dipeptides

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Formation constants for complexes between copper and a number of aliphatic dipeptides have been determined by potentiometric titrations. The formation of the complexes $[CuL_2H_2]$ and $[CuL_2]^{2-}$ ($L = {}^{+}H_3N\text{–}CHR\text{–}CO\text{–}NH\text{–}CHR'\text{–}CO_2^-$) could be observed and the possible structures of these species are discussed. Evidence for the dimeric species $[Cu_2L_2OH]^-$ postulated in former works was not found in any case. Complex distribution depending on pH and metal/peptide ratio is given for a series of dipeptides consisting of glycine, alanine, leucine and proline and influences of the side chains on this distribution are discussed.

Introduction

Interaction between proteins or peptides and transition metals play an important role in biochemistry and biology and have been studied extensively during the last two decades. Complexes of aminoacids and oligopeptides, for instance, are involved in the exchange and transport mechanism of trace metals in human body [1–3]. Oligopeptides have proved to be the most useful model compounds for such studies, since they are able to mimic to a great extent the metal binding site of much more complicated protein molecules [4]. For a less specific, but rather general study of the metal binding ability of peptides, even studies of dipeptides can supply much information.

Hence, such studies have been performed by various methods as circular dichroism [5–7], IR, UV [8, 9], ESR and NMR spectroscopy [10–14] or, as being used in this work, potentiometric titrations. With this method, glycylglycine [8, 15–18] and some other dipeptides [9, 19–22] have been studied extensively. Our investigations were carried out with most of the dipeptides consisting of glycine, alanine, leucine and proline. Such a series should give informations about the influence of the side chains on complex formation between the dipeptide and the copper ion, as well as about the dependence of formed complex species on the structure of the dipeptides.

Experimental

Materials

$CuCl_2 \cdot 2H_2O$ was dried at 130 °C until constant weight. The concentration of the copper stock solution was examined by complexometric titration. The dipeptides gly–gly, d,l-ala–gly*, d,l-leu–gly*, l-pro–gly, gly–d,l-ala*, gly–d,l-leu*, gly–l-pro, d,l-ala–d,l-ala*, d,l-ala–d,l-leu*, l-ala–l-pro, d,l-leu–d,l-leu* and l-pro–l-leu were obtained from Sigma Chemical Co., generally of Sigma analytical grade.

Physical Measurements

Copper complex formation constants were calculated from potentiometric titration curves of the dipeptides in absence and presence of copper. Changes in pH were followed using a combined glass electrode and a Schott pH-meter CG 803. Titrations were carried out at various metal/ligand ratios from 1:1 to 1:5. The concentration of copper chloride was $1.00 \cdot 10^{-3}$ *M* in all titrations. The systems were titrated with a 0.05 *M* NaOH solution. All investigations were carried out under nitrogen atmosphere at 20 °C and ionic strengh of 0.20 *M* KCl. For the calculation of the formation constants a Fortran computer program was used, inputting at least 200 experimental data per system. All computations were carried out at the CDC 3300 computer of the University of Innsbruck.

Approach used for the Simulation of the Titration Curves

Copper forms various 1:1 complexes. In one of them the peptide proton is retained. But only one 1:2 complex (CuL_2H^-) has been described so far, where one of these protons is not detached. For dipeptides not containing such a proton, however, another species (CuL_2H_2) was found. For that reason it was surprising that the other dipeptides should not form this or related species. Therefore our model contained all theoretically possible species of the 1:1 and 1:2

*These dipeptides were used in the d,l-form. The calculated formation constants are therefore mean values for all present stereoisomers.

TABLE I. Dissociation Constants for the Protolysis of Pure Peptides.

Dipeptide	pK_1 (Literature)	pK_1 (This work)	pK_2 (Literature)	pK_2 (This work)
gly–gly	−3.17[a] −3.19[b] −3.17[c] −3.18[d]	−3.18	8.13[a] 8.13[b] 8.15[c] 8.07[d]	8.25
gly–d,l-ala	−3.17[a]	−3.19	8.20[a]	8.40
gly–d,l-leu	–	−3.20	–	8.37
gly–l-pro	−2.97[b] −2.79[c] −2.85[e]	−2.93	8.48[b] 8.55[c] 8.56[e] 8.49[f]	8.77
d,l-ala–gly	−3.15[a]	−3.22	8.19[a]	8.33
d,l-leu–gly	–	−3.20	–	8.24
l-pro–gly	−3.19[b] −3.16[c]	−3.05	8.98[b] 8.97[c]	9.15
d,l-ala–d,l-ala	−3.08[a] −3.16[c]	−3.18	8.26[a] 8.33[c]	8.39
d,l-ala–d,l-leu	–	−3.15	–	8.32
l-ala–l-pro	–	−3.02	–	8.52
d,l-leu–d,l-leu	–	−3.20	–	8.34
l-pro–l-ala	–	−3.20	–	9.19
l-pro–l-leu	–	−3.21	–	9.16

[a]Ref. 22, 0.20 M KCl, 25 °C. [b]Ref. 9, 0.16 M KNO_3, 25 °C. [c]Ref. 20, 0.1 M $NaClO_4$, 25 °C. [d]Ref. 17, 0.10 M KNO_3, 25 °C. [e]Ref. 21, 0.10 M KNO_3, 25 °C. [f]Ref. 18, 0.1 M KNO_3, 25 °C.

complexes. If LH_2 denotes the zwitterionic dipeptide $^+H_3N\text{–}CHR\text{–}CO\text{–}NH\text{–}CHR'\text{–}CO_2^-$, the following possible reactions can be defined:

$$K_1 : LH_2 + H^+ \longrightarrow LH_3^+ \quad (1)$$
$$K_2 : LH_2 \longrightarrow LH^- + H^+ \quad (2)$$
$$K_3 : Cu^{2+} + LH_2 \longrightarrow CuLH^+ + H^+ \quad (3)$$
$$K_4 : Cu^{2+} + LH_2 \longrightarrow CuL + 2H^+ \quad (4)$$
$$K_5 : Cu^{2+} + LH_2 \longrightarrow CuLOH^- + 3H^+ \quad (5)$$
$$K_6 : Cu^{2+} + 2LH_2 \longrightarrow CuL_2H_2 + 2H^+ \quad (6)$$
$$K_7 : Cu^{2+} + 2LH_2 \longrightarrow CuL_2H^- + 3H^+ \quad (7)$$
$$K_8 : Cu^{2+} + 2LH_2 \longrightarrow CuL_2^{2-} + 4H^+ \quad (8)$$
$$K_9 : 2Cu^{2+} + 2LH_2 \longrightarrow Cu_2L_2OH^- + 5H^+ \quad (9)$$

Symbols:

$CuLH^+$: the peptide proton is not dissociated
CuL, $CuLOH^-$: the peptide proton is detached
CuL_2H_2 : both peptide protons are retained
CuL_2H : only one peptide proton is dissociated
CuL_2^{2-}, $Cu_2L_2OH^-$: both peptide protons are detached.

Results and Discussion

The dissociation constants for the protolysis of the pure peptides are given in Table I. Table II contains the complex formation constants with copper for all peptides being investigated, including a comparison with values published by other authors. These results will be discussed in the following two parts: the first one, with special regard to the 1:2 complexes and the dimeric species, being the most ambiguous ones in all previous investigations; the second one, with respect to the influence of structure, pH and concentration on the complex equilibria for all possible species.

The Species CuL_2H_2, CuL_2^{2-} and $Cu_2L_2OH^-$

Significance of the Complexes

At excess peptide concentration, the titration curves could be simulated omitting CuL_2H_2 and CuL_2^{2-} [22]. We found, however, using these species and omitting CuL and $CuLOH^-$ leads to almost the same result, indicating that these pairs of complexes can simply replace each other in the simulation. This procedure does not lead to satisfactory results, however, at a metal/peptide ratio of 1:1. Thus we had to find a simulation, which would hold for the whole concentration range. Including data of all titration curves (*i.e.* for metal/peptide ratios of 1:1 to 1:5) and in any case taking into account all species including CuL_2H_2 and CuL_2^{2-}, we could obtain a very satis-

TABLE II. Complex Formation Constants of Copper with Aliphatic Dipeptides.

	$CuLH^+$	CuL	$CuLOH^-$	CuL_2H_2	CuL_2H^-	CuL_2^{2-}
gly–gly						
pK_b (Lit.)	2.57[a]	6.80	16.17	–	11.80	–
	3.17[b]	7.07	16.44	–	12.13	–
	2.60[c]	6.59	–	–	–	–
	2.40[d]	6.61	15.85	–	11.84	–
pK_b[g]	2.46	6.79	16.27	5.56	11.64	21.76
ΔpK_b	0.40	0.05	0.10	0.65	0.10	0.18
gly–d,l-ala						
pK_b (Lit.)	2.44[a]	6.65	16.14	–	11.77	–
pK_b	2.61	6.85	16.38	5.42	11.88	23.14
ΔpK_b	0.34	0.07	0.09	0.46	0.09	0.36
gly–d,l-leu						
pK_b	2.44	7.30	16.75	5.73	12.03	22.92
ΔpK_b	0.12	0.04	0.13	0.19	0.13	0.22
gly–l-pro						
pK_b (Lit.)	2.05[b]			5.60[e]		
	2.05[c]			5.61[f]		
	2.11[d]					
pK_b	2.28			5.92		
ΔpK_b	0.13			0.15		
d,l-ala–gly						
pK_b (Lit.)	2.94[a]	6.84	16.35	–	12.43	–
pK_b	3.24	7.03	16.73	6.15	12.74	23.96
ΔpK_b	0.93	0.07	0.11	1.0	0.25	0.23
d,l-leu–gly						
pK_b	3.11	6.97	16.69	5.36	12.48	22.44
ΔpK_b	0.64	0.05	0.14	0.21	0.12	0.16
l-pro–gly						
pK_b (Lit.)	2.58[b]	6.53	15.87	–	11.55	–
	2.56[c]	6.32				
pK_b	3.18	6.52	15.95	4.75	12.27	22.87
ΔpK_b	0.38	0.03	0.09	0.24	0.13	0.18
d,l-ala–d,l-ala						
pK_b (Lit.)	2.93[a]	6.83	16.27	–	12.39	–
pK_b	2.78	6.65	16.07	5.92	11.89	21.90
ΔpK_b	0.38	0.04	0.09	0.63	0.08	0.12
d,l-ala–d,l-leu						
pK_b	3.28	7.27	16.78	5.42	12.54	23.18
ΔpK_b	0.53	0.04	0.08	0.19	0.11	0.50
l-ala–l-pro						
pK_b	2.66			6.68		
ΔpK_b	0.13			0.15		
d,l-leu–d,l-leu						
pK_b	–	7.42	17.00	4.97	12.20	22.70
ΔpK_b	–	0.06	0.11	0.22	0.17	0.27

(Continued overleaf)

TABLE II. *(Continued)*

l-pro–l-ala						
pK_b	2.87	6.30	16.06	4.45	12.06	23.38
ΔpK_b	0.33	0.05	0.11	0.29	0.10	0.15
l-pro–l-leu						
pK_b	–	6.95	16.78	5.83	12.62	–
ΔpK_b	–	0.08	0.12	1.0	0.15	–

[a,b,c,d,e,f] The notation is the same as used in Table I. [g] This work. [h] ΔpK_b serves as a measure for the significance of the constants. Changing the constant by ΔpK_b leads to an increase of $\sum_i (v_i^{theoret.} - v_i^{calc.})^2$ by a factor 2.

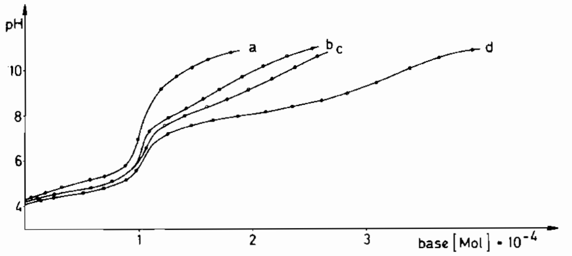

Fig. 1. Titration curves of the system copper–gly–d,l-leu at constant copper concentration ($1.00 \cdot 10^{-3}$ *M*). a: [d,l-leu–gly] = $1.09 \cdot 10^{-3}$ *M*; b: [d,l-leu–gly] = $2.32 \cdot 10^{-3}$ *M*; c: [d,l-leu–gly] = $2.88 \cdot 10^{-3}$ *M*; d: [d,l-leu–gly] = $5.06 \cdot 10^{-3}$ *M*. Full lines represent the experimental titration curves, dotted lines the calculated ones.

factory simulation (Fig. 1) with errors even reduced by 10–20% compared to former investigations. All species were found to exist at certain pH or concentration ranges exept $Cu_2L_2OH^-$ (eqn. 9), which seems not to be present to a significant extent at any pH or concentration. The fact, that in previous work this complex was sometimes found to contribute to the equilibria seems to be, therefore, an artefact due to the neglect of other important species or due to the consideration of a restricted concentration range only.

Structures of CuL_2H_2 and CuL_2^{2-}

Figure 2 shows the possible structures of CuL_2H_2 and CuL_2^{2-}. Rabin [23] postulated structure A, Nakao [24], according to X-ray investigations, structure B. There is some evidence, that structure B is more reasonable than A, since A allows only a monodentate coordination of one ligand, as for Cu^{2+} a strong axial coordination cannot be expected. Structure B, where both dipeptides form a five membered ring leading to chelation of the ion, should be much more stable. No structure has been proposed for the species CuL_2H_2 so far. $CuLH^+$ and CuL_2H_2 show a very similar chemical behaviour:

$$Cu^{2+} + LH_2 \longrightarrow CuLH^+ + H^+ \quad pK \simeq 2.5\text{–}3$$

$$CuLH^+ + {}^*LH_2 \longrightarrow CuL_2H_2 + H^+ \quad pK \simeq 3$$

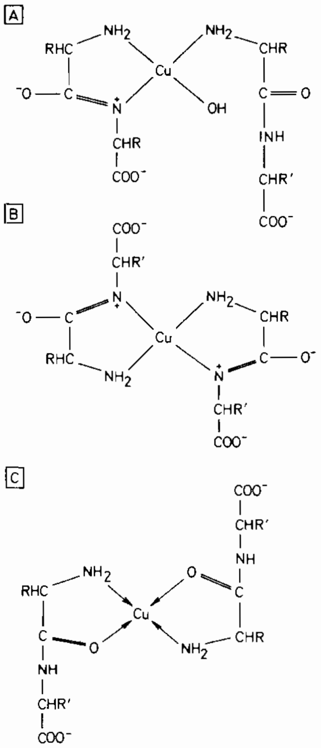

Fig. 2. A, B: Possible structures of the species CuL_2^{2-}. C: Postulated structure of the complex CuL_2H_2.

Both pK-values are of the same order of magnitude. This similarity in complex formation indicates, that the structures of the complexes should not be too different. ($^*LH_2$ is coordinated in the same way as LH_2 [21]). It seems reasonable, therefore, to assume a structure as given in Fig. 2C.

Influence of pH, Ligand Structure and Concentration on the Complex Equilibria

Species Distribution depending on Copper/Ligand Ratio and pH

Two species dominate the 1:1 system (Fig. 3). Between pH 6 and pH 11 only these two species are present. From pH 4 to pH 6 another complex ($CuLH^+$) is present, but only to a small extent. The

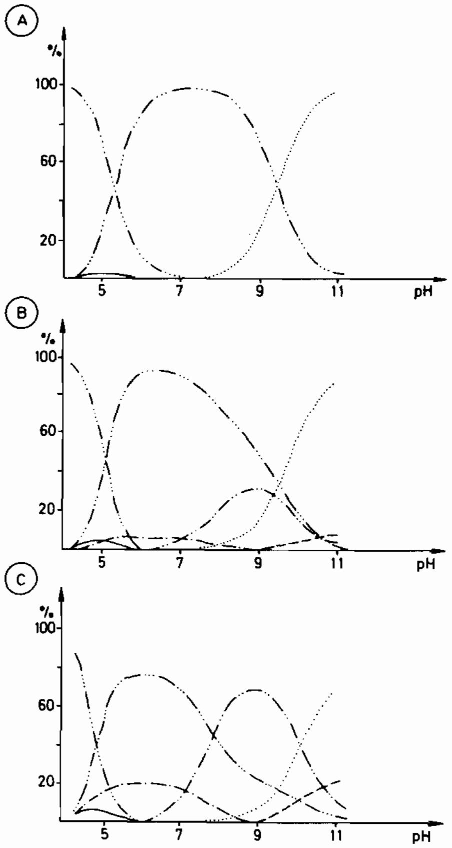

Fig. 3. Species distribution depending on copper/ligand ratio and pH at constant copper concentration ($1.00 \cdot 10^{-3}$ *M*) and varying peptide concentration (a: $1.00 \cdot 10^{-3}$ *M*, b: $2.00 \cdot 10^{-3}$ *M*, c: $5.00 \cdot 10^{-3}$ *M*). The dipeptide used was d,l-ala–d,l-leu. Concentration is given in percent of the total metal concentration. Notation: (———) $CuLH^+$; (– – –) CuL_2^{2-}; (•••••) $CuLOH^-$; (— • —) CuL_2H_2; (— •• —) CuL_2H^-; (— ••• —) CuL; (— •••• —) Cu^{2+}.

concentration distribution of this species strongly depends on the kind of dipeptide, as we shall discuss later. If the ligand concentration is increased, additional complexes are formed. CuL_2H_2 exists at pH 5 to pH 8. Between pH 7 and pH 11 the species CuL_2H^- is formed, having a maximum at pH 9. At the same pH the complex CuL_2^{2-} begins to form. Generally, increasing ligand concentration favours the formation of 1:2 complexes, but does not change the pH dependence of the formation of any of these complexes.

Influence of the Side Chains on the Concentration Distribution

Sigel [20] investigated the influence of the side chains on the stability of $CuLH^+$ and found that R′ showed no distinct effect; the complex formation is rather determined by pK_2 and R. According to Rabin [19, 23] a plot of pK_3 versus pK_2 leads to a straight line. In our work no linear relation between pK_2 and pK_3 was found. This is partly due to the fact that the species $CuLH^+$ is present at low concentrations only (Fig. 4). Thus pK_3 cannot be determined exactly. The influence of R, however, is in agreement with our work. The smaller R is, the higher is the concentration of $CuLH^+$. As can be seen from Fig. 4, all dipeptides with glycine as terminal group have relatively high maxima of $CuLH^+$. If the terminal group is leucine or proline, however, the species $CuLH^+$ can – in some cases – even be neglected. Besides the complex $CuLH^+$, only the formation of CuL_2H^- shows an obviously distinct dependence on the ligand in the way that the concentration maxima increase with increasing R′:

gly–d,l-leu > gly–d,l-ala ~ gly–gly
d,l-ala–d,l-ala ~ d,l-ala–d,l-leu > d,l-ala–gly

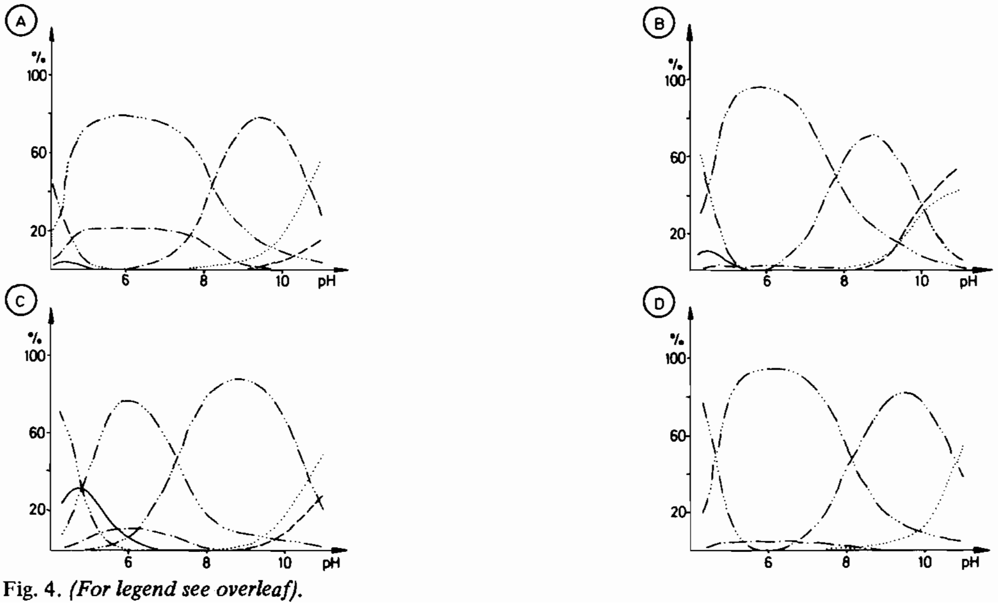

Fig. 4. *(For legend see overleaf).*

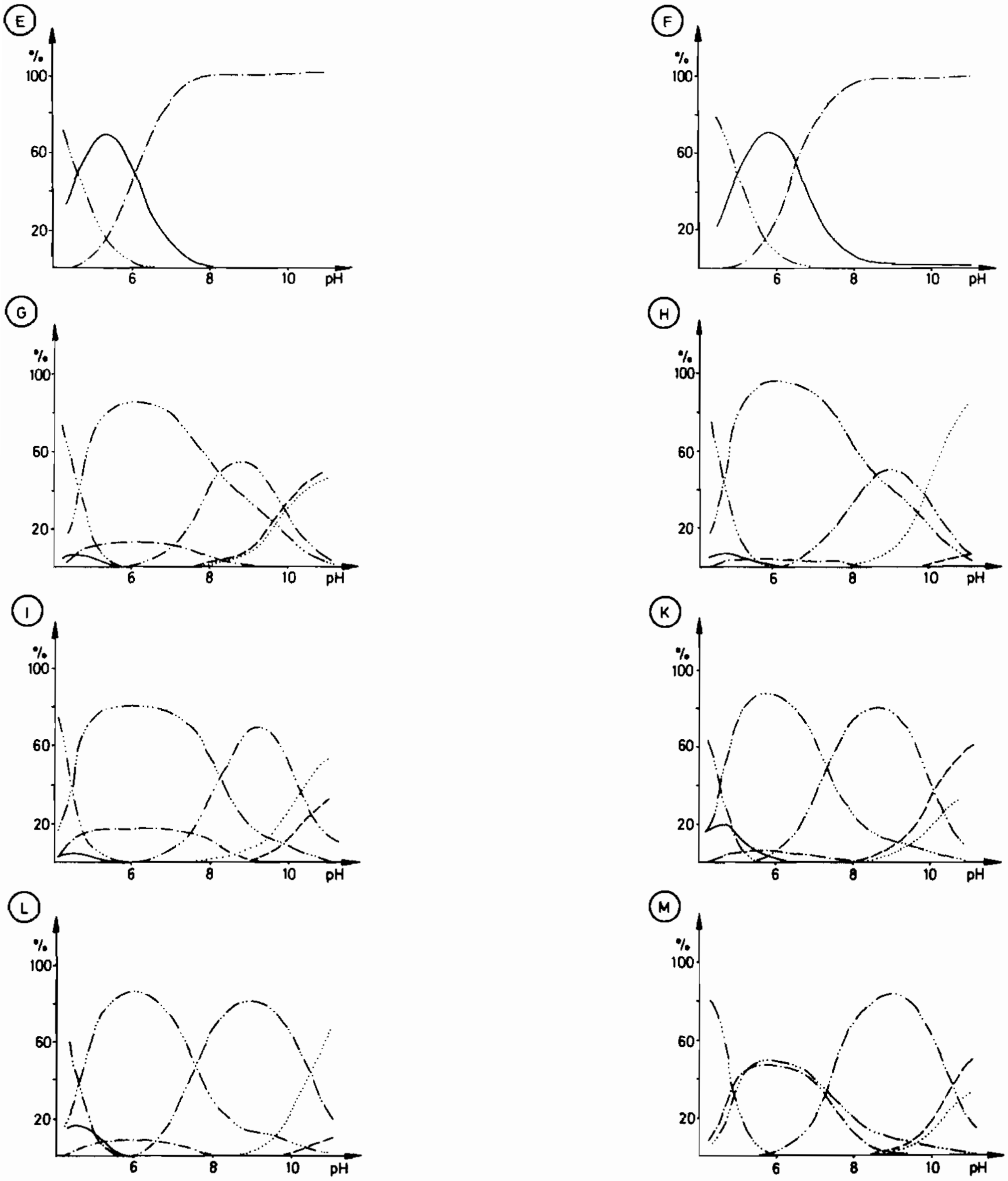

Fig. 4. Influence of the side chains on the concentration distribution of all species at metal/peptide ratio of 1:5. a) l-pro–l-ala; b) d,l-ala–d,l-ala, c) gly–d,l-leu, d) l-pro–l-leu, e) gly–l-pro, f) l-ala–l-pro, g) d,l-leu–gly, h) d,l-ala–gly, i) l-pro–gly, k) gly–gly, l) gly–d,l-ala, m) d,l-leu–d,l-leu. Notation is the same as used in Fig. 3.

d,l-leu–d,l-leu > d,l-leu–gly
l-pro–l-leu > l-pro–l-ala > l-pro–gly

No significant trend was found for the N-terminal side chain. The concentrations of the different species at physiological pH may be interesting in relation to biochemical systems. A summary of these data is thus given in Table III.

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References

1 S. Lau and B. Sarkar, *Can. J. Chem.*, *53*, 710 (1975).
2 B. Sarkar and Y. Wigfield, *Can. J. Biochem.*, *46*, 601 (1968).
3 B. Sarkar, *Can. J. Biochem.*, *48*, 1339 (1970).
4 B. Sarkar, in 'Metal–Ligand Interactions in Organic and Biochemistry', The Jerusalem Symposia on Quantum Chemistry and Biochemistry, IX, p. 193–228, D. Reidel ed., Dordrecht, Holland (1977).
5 G. F. Bryce *et al.*, *J. Biol. Chem.*, *241*, 1072 (1966).
6 G. F. Bryce *et al.*, *J. Biol. Chem.*, *241*, 1439 (1966).
7 J. M. Tsangaris, J. W. Chang and R. B. Martin, *J. Am. Chem. Soc.*, *91*, 726 (1969).
8 M. Kim and A. Martell, *Biochemistry*, *3*, 1170 (1964).

TABLE III. Concentrations of all Copper Complexes at pH 7.4 for Metal/Peptide Ratios of 1:1, 1:2 and 1:5 in Percent of Total Metal Concentration. Species below 1% were neglected.

Peptide	CuL (1:1)[a]	CuL	CuL_2H_2 (1:2)[a]	CuL_2H^-	CuL	CuL_2H_2 (1:5)[a]	CuL_2H^-
gly–gly	99	79	1	19	47	2	50
gly–d,l-ala	99	83	2	15	54	5	41
gly–d,l-leu	98	74	2	23	39	5	56
d,l-ala–gly	99	95	1	4	83	2	14
d,l-ala–d,l-ala	99	88	–	10	66	1	32
d,l-ala–d,l-leu	99	86	5	9	60	14	26
d,l-leu–gly	99	91	3	6	72	10	18
d,l-leu–d,l-leu	98	69	12	18	32	27	40
l-pro–gly	99	91	5	4	72	16	12
l-pro–l-ala	99	90	6	4	70	18	11
l-pro–l-leu	99	94	1	5	80	4	16

[a]Metal/peptide ratio.

9 W. L. Koltun, M. Fried and F. Gurd, *J. Am. Chem. Soc., 82,* 233 (1960).
10 B. Jezowska-Trzebiatowska, *Bull. Acad. Pol. Sci., Ser. Sci. Chim., 22,* 499 (1974).
11 D. C. Gould and H. S. Mason, *Biochemistry, 6,* 801 (1967).
12 T. H. Crawford and J. O. Dalton, *Arch. Biochem. Biophys., 131,* 123 (1969).
13 M. Sheinblatt, *Magn. Resonance Biol. Syst. Proc. Int. Conf. 2nd,* 41–4, Stockholm (1966).
14 M. K. Kim and A. E. Martell, *J. Am. Chem. Soc., 91,* 872 (1969).
15 R. F. Pasternack and H. Sigel, *J. Am. Chem. Soc., 94,* 8031 (1972).
16 M. Doran, S. Chaberek and A. Martell, *J. Am. Chem. Soc., 86,* 2129 (1964).
17 A. Kaneda and A. Martell, *J. Coord. Chem., 4,* 137 (1975).
18 A. Brunetti *et al., J. Solution Chem., 1,* 153 (1972).
19 S. Datta and R. Rabin, *Trans. Faraday Soc., 52,* 1123 (1956).
20 H. Sigel, *Inorg. Chem., 14,* 1535 (1975).
21 G. Brookes and L. Pettit, *J. Chem. Soc. Dalton Trans.,* 2106 (1975).
22 I. Nagypal and A. Gergely, *J. Chem. Soc. Dalton Trans., 11,* 1104 (1977).
23 R. Rabin, *Trans. Faraday Soc., 52,* 1130 (1956).
24 Y. Nakao, K. Sakurai and A. Nakahara, *Bull. Chem. Soc. Japan, 39,* 1608 (1966).