Studies on Transition-metal–Peptide Complexes. Part 2.† Equilibrium Study of the Mixed Complexes of Copper(II) with Aliphatic Dipeptides and Amino-acids

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In a total of 39 equilibrium systems, the stability constants of mixed dipeptide–amino-acid complexes of copper(II) have been determined at 25 °C and ionic strength I = 0.2 mol dm^{-3} KCl. The dipeptides used are glycylglycine, glycyl-DL-α-alanine, and DL-α-alanyl-DL-α-alanine, and the amino-acids are glycine, α-alanine, α-aminobutyric acid, norvaline, β-alanine, serine, threonine, ornithine, lysine, asparagine, glutamine, aspartic acid, and glutamic acid. From the exceptionally high relative stabilities of the mixed-ligand complexes containing β-alanine and aspartic acid, it is concluded that in these complexes the amino-acids occupy one equatorial and one axial site in the co-ordination sphere of the copper(II).

THE study of mixed-ligand complexes is becoming increasingly more important in equilibrium chemistry, and is also relevant to investigations of the complexes of amino-acids and peptides.[1] However, of the copper(II)–dipeptide–amino-acid mixed complexes, only the copper(II)–glycylglycine–glycine system has been examined to date.[2] It is known from the available data that the properties of copper(II)–amino-acid complexes are affected in various ways by the length of the carbon chain in the ligand,[3] and by the nature and position of the third donor group.[4-8]

It is to be expected, therefore, that these structural factors will be reflected in the equilibrium conditions of the copper(II)–dipeptide–amino-acid mixed complexes. Accordingly, in the present work we set out to determine the stability constants of the mixed-ligand complexes in a total of 39 equilibrium systems involving Cu^{II}; the dipeptides were glycylglycine, glycyl-DL-α-alanine, and DL-α-alanyl-DL-α-alanine, and the amino-acids were glycine, α-alanine, α-aminobutyric acid, norvaline, β-alanine, serine, threonine, ornithine, lysine, asparagine, glutamine, aspartic acid, and glutamic acid. As regards the effects of the structures of the amino-acids on the equilibrium conditions of the mixed-ligand complexes, the series of measurements with the three dipeptides can be regarded as three parallel investigations.

EXPERIMENTAL

The amino acids and dipeptides used were purified by repeated recrystallization from water–alcohol. The concentrations of the stock solutions were checked by the method reported previously.[9]

Examinations by pH titrimetry were made at 25 °C with a Radiometer PHM 52 pH-meter, using G 202 C glass and K 401 calomel electrodes, on samples with an initial volume of 25 cm^3 at ionic strength I = 0.2 mol dm^{-3} KCl. The electrode system was calibrated *via* the method of Irving *et al.*[10] The dissociation constants of the ligands and the formation constants of the copper(II)–amino acid parent complexes were determined in solutions containing 0.006 and 0.004 mol dm^{-3} ligand and 0.002 mol dm^{-3} metal ion. These data were available in part from the literature and in part from our own earlier examinations. Repeated determinations were necessary so that auxiliary data obtained under completely identical conditions were available for the evaluation of the experimental results relating to the copper(II)–amino-acid–dipeptide mixed complexes. The values of the auxiliary constants are not reported in the present work.

In the case of the copper(II)–dipeptide–amino-acid mixed systems, four titrations were carried out under the concentration conditions shown in Table 1; 30—40 experimental points were utilized to calculate the formation constants. The calculations were made with the aid of the program described earlier.[11,12]

TABLE 1

Compositions (c/mol dm^{-3}) of solutions of the copper(II)–dipeptide–amino-acid system examined by pH titrimetry

Peptide	Amino-acid	Copper
0.005	0.005	0.002
0.005	0.002	0.002
0.002	0.005	0.002
0.002	0.002	0.002

RESULTS AND DISCUSSION

In the course of the evaluation of our experimental data it was observed that, if only formation of the mixed complex $[CuLBH_{-1}]$ ‡ was assumed, acceptable agreement could not be obtained between the constants calculated from the various experimental points. Here

† Part 1 is ref. 9.

‡ The charge of $[CuLBH_{-1}]$ is zero for the mixed complexes of diaminomonocarboxylic acids, −1 for monoaminomonocarboxylic acids, and −2 for monoaminodicarboxylic acids.

[1] 'Metal Ions in Biological Systems,' vol. 2, ed. H. Sigel, Marcell-Dekker, New York, 1973.
[2] R. P. Martin, L. Mosoni, and B. Sarkar, *J. Biol. Chem.*, 1971, **246**, 5944.
[3] A. Gergely, I. Sóvágó, I. Nagypál, and R. Király, *Inorg. Chim. Acta*, 1972, **6**, 435.
[4] E. W. Wilson, M. H. Kasperian, and R. B. Martin, *J. Amer. Chem. Soc.*, 1970, **92**, 5365.
[5] K. M. Wellmann, T. G. Mecca, W. Mungall, and C. R. Hare, *J. Amer. Chem. Soc.*, 1968, **90**, 805.
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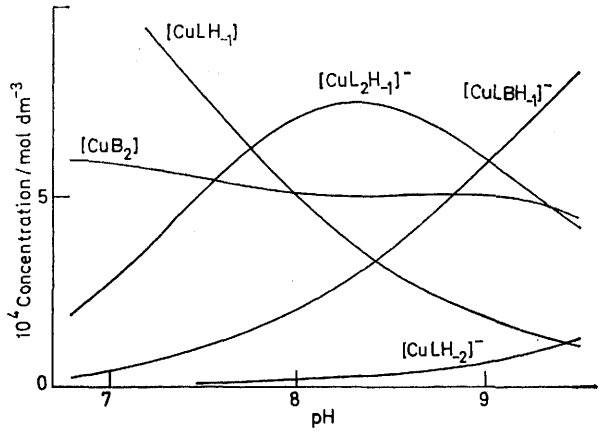
[8] A. Gergely, I. Nagypál, and E. Farkas, *J. Inorg. Nuclear Chem.*, 1975, **37**, 551.
[9] A. Gergely and I. Nagypál, preceding paper.
[10] H. Irving, M. G. Miles, and L. D. Pettit, *Analyt. Chim. Acta*, 1967, **38**, 475.
[11] I. Nagypál, *Acta Chim. Acad. Sci. Hung.*, 1974, **82**, 29.
[12] A. Gergely, I. Nagypál, and E. Farkas, *Acta Chim. Acad. Sci. Hung.*, 1974, **82**, 43.

and subsequently, L denotes the anionic form of the dipeptide and B the anionic form of the amino-acid. Accordingly, in addition to $[CuLBH_{-1}]$, the formation of the mixed complex [CuLB] was also assumed. The experimental data could then be well approximated to in each case, but, as regards the complexes [CuLB], the maximum concentrations of which were only 2—5%, the constants found cannot be regarded as reliable. Taking into account the formation of the complexes [CuLB] only makes the data relating to $[CuLBH_{-1}]$ more exact, and does not permit conclusions to be drawn about the [CuLB] species. Their log β values are therefore not reported, only an upper limit of



Concentration distribution of the complexes formed in the copper (II)-glycylalanine–glycine system in the range pH 7—9. $C_{GA} = C_{glycine} = 0.005$ mol dm^{-3}, $C_{Cu} = 0.002$ mol dm^{-3}, L = GA$^-$, B$^-$ = glycinate

log β < 12 being given. Exceptions are the systems containing aspartic acid where the complex [CuLB] is formed in essentially higher concentration.

The error in the data obtained for the formation constant of $[CuLBH_{-1}]$ can be estimated as ±0.1 log unit since, besides the direct experimental errors, this value also contains the errors of at least 11 previously determined formation constants, taken into consideration in the calculations.

To illustrate the equilibrium concentrations in the systems containing the dipeptides and amino-acids, the Figure presents the concentration distribution of the complexes formed in the copper(II)–glycyl-DL-α-alanine–glycine system as a function of pH. Formation of $[CuLBH_{-1}]$ begins at pH *ca.* 7, and with increasing pH its concentration reaches 50%. It is interesting that the concentration of the complex $[CuB_2]$ exhibits two maxima and one minimum as a function of pH. The unusual concentration distribution in this system is caused by the fact that increasing pH always increases the concentration of the $[OH]^-$ ligand, but in the case of the basic ligands conjugated to the weak acid the pH increase changes the concentration of the free ligand in accordance with a saturation curve. Unusual concentration distributions have also been observed in various equilibrium systems.[13-15] Certain possibilities for the occurrence of the unusual concentration distribution, and their interpretation, have been dealt with in detail recently.[16]

Table 2 gives the formation constants for the mixed complexes $[CuLBH_{-1}]$ and the equilibrium constants relating to the substitution processes (1). The following

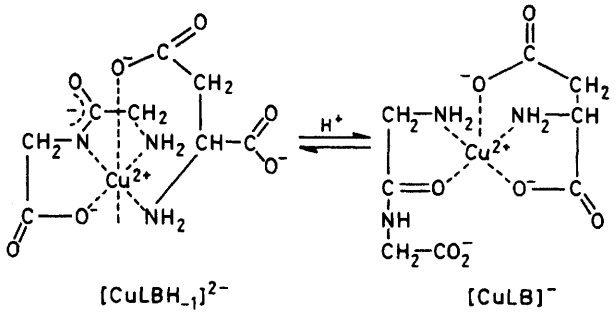
$$[CuL_2H_{-1}]^- + B^- \rightleftharpoons [CuLBH_{-1}]^- + L^- \quad (1)$$

important conclusions may be drawn from the tabulated data.

(*a*) For well known steric reasons, the copper(II) parent complex of β-alanine is substantially less stable than the complexes of α-amino-acids. Also, the stability of the mixed complex $[CuLBH_{-1}]$ is about the same as those of the α-amino-acid complexes. The phenomenon may be explained by the assumption that, similarly to the ' second ' peptide ligand in the complex $[CuL_2H_{-1}]^-$, the amino-acids occupy one equatorial and one axial co-ordination site [9] in their mixed complexes $[CuLBH_{-1}]$. In this structural arrangement the donor groups are separated by a greater distance than in the parent complexes of the amino-acids, where the equatorial co-ordination sites are occupied. Accordingly, the difference in stability between the five- and six-membered chelate rings may disappear; *i.e.* the stabilities of the mixed complexes of the α- and β-amino-acids may be the same.

(*b*) It can be seen from Table 2 that glycine forms essentially more stable mixed complexes than those of the other amino-acids. This phenomenon also can probably be explained by the absence of the alkyl chain.[17]

(*c*) Aspartic acid forms mixed complexes of exceptional stability with all the three dipeptides, attributed in



part to the fact that this ligand is capable of both glycine- and β-alanine-like co-ordination. From statistical considerations, a higher number of co-ordination possibilities is associated with a stability increase. For aspartic acid, assuming β-alanine-like equatorial–axial co-ordination, there is also a possibility of the α-CO_2^-

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[16] I. Nagypál and M. T. Beck, *Inorg. Chim. Acta*, 1975, **14**, 17.
[17] H. Irving and L. D. Pettit, *J. Chem. Soc.*, 1963, 1546.

TABLE 2

Formation constants of the copper(II)–dipeptide–amino-acid mixed complexes, and equilibrium constants of the substitution processes $[CuL_2H_{-1}]^- + B^- \rightleftharpoons [CuLBH_{-1}]^- + L^-$

Dipeptide (HL)	GG		GA		AA	
Amino-acid (HB)	$\log \beta_{CuLBH_{-1}}$ ±0.1	$\log K$ ±0.1	$\log \beta_{CuLBH_{-1}}$ ±0.1	$\log K$ ±0.1	$\log \beta_{CuLBH_{-1}}$ ±0.1	$\log K$ ±0.1
Glycine	5.29	0.83	5.43	0.80	5.26	1.13
α-Alanine	5.17	0.71	5.12	0.50	4.84	0.72
α-Aminobutyric acid	5.09	0.63	5.03	0.40	4.65	0.50
Norvaline	4.97	0.51	5.04	0.41	4.65	0.53
α-Alanine	4.96	0.50	5.02	0.39	4.74	0.60
Serine	4.94	0.48	5.07	0.44	4.86	0.73
Threonine	4.95	0.49	4.98	0.35	4.81	0.68
Ornithine	4.89	0.43	4.80	0.17	4.55	0.42
Lysine	4.96	0.50	5.09	0.45	4.72	0.54
Asparagine	4.92	0.46	4.93	0.31	4.45	0.32
Glutamine	4.65	0.19	4.69	0.06	4.32	0.12
Aspartic acid	5.73	1.27	5.55	0.93	5.16	1.02
Glutamic acid	5.07	0.61	5.14	0.51	4.67	0.54

group displacing the CO_2^- group of the peptide from the co-ordination sphere to a certain extent, with formation of the mixed complex [CuLB] as a result of the subsequent structural rearrangement. This may be the explanation why the complex [CuLB] is also formed in appreciable concentration (*ca.* 15—20%) in the case of aspartic acid. In the sequence glycylglycinate (GG^-), (GA^-), and alanylalaninate (AA^-), the logarithms of the formation constants of the complexes [CuLB] are **13.5**, **13.4**, and **12.7**, respectively.

(*d*) Based on the data relating to the mixed complexes of serine, threonine, ornithine, lysine, asparagine, and glutamic acid, the third potential donor groups do not influence the equilibrium conditions to any appreciable extent.

(*e*) Further investigations are necessary to provide an explanation as to why the stabilities of the mixed complexes of glutamine are exceptionally low.

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