Institute of Inorganic Analytical Chemistry,
L. Kossuth University, Debrecen, Hungary

Equilibrium Relations of Alpha-aminoacid Mixed Complexes of Transition Metal Ions

A. Gergely,* I. Sóvágó, I. Nagypál, and R. Király

Received, September 9, 1971

pH-metric equilibrium studies were carried out on the parent complexes and, in every possible pairing, the mixed complexes of glycine, alanine, alpha-aminobutyric acid, norvaline, serine, threonine, phenylalanine and tyrosine with copper(II). In the case of nickel(II) the above ligands were again used, and in addition norleucine. Studies on the cobalt(II) parent and mixed complexes were made with the ligands glycine, norvaline, phenylalanine and tyrosine.

The stabilities of the mixed complexes of the ratio 1:1:1 correspond generally to the statistical case. The little stability increase observed mainly in the case of copper(II)-aliphatic-aromatic aminoacid mixed complexes was interpreted by back-coordination. Mixed complexes of copper(II) containing glycine and another aliphatic aminoacid are less stable than those formed by other kinds of aliphatic aminoacids.

Introduction

The equilibrium relations of the transition metal aminoacid complexes have been dealt with by a number of authors during the last twenty years.[1] From among the aromatic aminoacids, the thermodynamic relations of the copper(II) and nickel(II) complexes of phenylalanine have been studied in recent years by *Izatt* et al.[2] and *Anderson* et al.[3,4] *Letter* and *Bauman*[5] extended these studies to the copper(II) and nickel(II) complexes with tyrosine. The possibility of back-coordination was suggested by *Izatt* et al.[2] in connection with the copper(II) –phenylalanine system. This assumption was confirmed with high probability by our own calorimetric studies[6] relating to the complexes of phenylalanine and tyrosine with other metals of the first transition series.

The investigation of the equilibrium relations of the aminoacid mixed complexes is of greater importance than for the parent complexes. Such studies began fairly recently. Thus, *Martin* and *Paris*[7,8] obtained stability constants larger than the statistical values for the glycine, alanine and tyrosine mixed complexes of copper(II). However, these authors did not touch upon the interpretation of this phenomenon. *Petit-Ramel* and *Paris*[9-11] likewise restrict themselves, with regard to the copper(II) –glycine –valine and the copper(II) –proline–valine systems, simply to the statement that the mixed complexes are more stable than the parent complexes. From a study of the ethylenediamine, serine and histamine mixed complexes of copper(II) and nickel(II), *Perrin* et al. [12,13] found that the formation is more favoured than the statistical case, and that the effects are more significant in the copper(II) mixed complexes (primarily for steric reasons) than in the nickel(II) complexes. In connection with the mixed complexes of nickel(II) with the ligands glycine, alanine and diglycine, *Martin* and *Mosoni*[14] similarly found recently that the stabilities of the mixed complexes exceed those of the parent complexes. At the same time, it is clear from their results that the stabilization is less in the case of nickel(II)–glycine–diglycine than for nickel(II)–glycine–alanine.

The factors determining the stabilities of mixed complexes are interpreted in various ways.[15,16] In addition, as regards the aminoacid complexes there are still not sufficient equilibrium data available for adequate conclusions to be drawn on a sound basis. At the same time, the small effects which may be expected require that experimental data obtained simultaneously and under the same conditions be available.

In accordance with the above, the aim of this work is the study of the equilibrium relations of copper(II)-nickel(II)-and cobalt(II)-aminoacid-aminoacid systems.

---

* The adress of the author to whom correspondence and proofs are to be sent: Dr. Arthur Gergely, Institute of Inorganic and Analytical Chemistry, Debrecen 10., Hungary.

(1) L. G. Sillén and A. E. Martell: Stability Constants of Metal-Ion Complexes, Special Publication No. 17. The Chemical Society, London 1964.

(2) R. M. Izatt, J. W. Warthall and K. P. Anderson: *J. Phys. Chem.*, 65, 1914 (1961).

(3) K. P. Anderson, W. O. Greenhalgh and R. M. Izatt: *Inorg. Chem.*, 5, 2106 (1966).

(4) K. P. Anderson, W. O. Greenhalgh and E. A. Butler: *Inorg. Chem.*, 5, 1056 (1967).

(5) J. E. Letter, Jr. and J. E. Bauman, Jr.: *J. Amer. Chem. Soc.*, 92, 443 (1970).

(6) A. Gergely, I. Nagypàl and B. Király: *Acta Chim. Acad. Sci. Hung.*, *68*, 285 (1971).

(7) R. P. Martin and R. A. Paris: *Compt. Rend.*, *257*, 3932 (1963).

(8) R. P. Martin and R. A. Paris: *Compt. Rend.*, *258*, 3038 (1964).

(9) M. Petit-Ramel and M. R. Paris: *Compt. Rend.*, *266*, 1368 (1968).

(10) M. Petit-Ramel and M. R. Paris: *Bull. Soc. Chim. France*, *1968*, 2791.

(11) M. Petit-Ramel and M. R. Paris: *Bull. Soc. Chim. France*, *1969*, 3070.

(12) D. D. Perrin, I. G. Sayce and V. C. Sharma: *J. Chem. Soc.* (A) *1967*, 1755.

(13) D. D. Perrin and V. S. Sharma: *J. Chem. Soc.*, (A), *1968*, 446.

(14) R. P. Martin and L. Mosoni: *Bull. Soc. Chim. France*, *1970*, 2917.

(15) Y. Marcus: *Coord. Chem. Rev.*, *4*, 273 (1969).

(16) M. T. Beck: Chemistry of Complex Equilibria. Van Nostrand-Reinhold, London, 1970.

Our study extended to the following aminoacids: glycine, alanine, α-aminobutyric acid, norvaline, norleucine, serine, threonine, phenylalanine and tyrosine.

In the cases of copper(II) and nickel(II) the measurements were made with the above ligands in all possible pairings. Considering the greater uncertainty of the constants for cobalt(II), however, the study was carried out only with glycine, norvaline, phenylalanine and tyrosine.

Experimental Section

Reanal chemicals were used for the experiments. The aminoacids were purified by recrystallization from alcohol-water mixtures. The concentrations of the copper(II)-chloride, nickel(II)-chloride and cobalt(II)-chloride solutions were checked gravimetrically via the oxine complexes.

Although the majority of the protonation constants of the aminoacids and the stability constants of the parent complexes are available,[1-6] these were redetermined here because of the small effects involved.

The volume of each of the samples prepared for the titrations was 25 ml. The concentrations of the copper(II), nickel(II) and cobalt(II) were in all cases 2×10^{-3} *M*, while the concentrations of the aminoacids in the study of the parent complexes were 4.0×10^{-3} *M*; in the mixed complexes the concentration of each of the two aminoacids was 2.0×10^{-3} *M*. The ionic strength was adjusted to 0.05 *M* with KCl solution. The 0.1 *M* NaOH solution used for the titration was carbonate-free and was kept under nitrogen. All the measurements were carried out at 25 ± 0.1 °C.

For the measurement of pH a RADIOMETER PHM-4 instrument was used, with a G-200B glass and a K-100 calomel electrode. The potassium hydrogen phthalate used for the calibration was purified by repeated recrystallization. A 0.05 *M* solution was prepared from this, the pH of which at 25 °C was 4.008[17]. The method suggested by *Irving* et al.[18] was used to eliminate the error arising from the difference in the diffusion potential-differences, and simultaneously to convert the hydrogen ion activities to concentrations. With each of the metal ions the measurements were carried out in three parallel titration series. 20-25 experimental points were obtained for the copper(II) complexes, and 10-15 for the nickel(II) and cobalt(II) complexes.

Calculations

The protonation constants and the stability constants of the parent complexes were calculated on the basis of the formation function, applying the least squares principle. To ensure that only complexes of the types MA and MA_2 were formed, the experimental range applied was selected with the help of the curve reduction method.[19] Accordingly, the experimental points used in the calculations lay in the range $0.4 < \bar{n} < 1.5$ for the copper(II) complexes, and $0.3 < \bar{n} < 1.15$ for the cobalt(II) and nickel(II) complexes.

The stability constants of the mixed complexes, defined by equation (1) were calculated by the method

$$\beta_{111}=\frac{[MAB]}{[M][A][B]} \tag{1}$$

proposed by *Näsänen* and *Koskinen*.[20]

The stability constant corresponding to the statistical value was obtained by taking into consideration the constants for the parent complexes and also the statistical factor:

$$\log\beta_{111}{}^{*}=\tfrac{1}{2}\log\beta_{12A}+\tfrac{1}{2}\log\beta_{12B}+\log2 \tag{2}$$

From a comparison of the constant calculated according to eqn. (2) with the experimentally obtained value, we obtained the value corrected by the statistical factor; this was denoted by

$$\Delta\log\beta_{111}=\log\beta_{111}-\log\beta_{111}{}^{*} \tag{3}$$

Thus, the value defined by eqn. (3) expresses the increased or the decreased stability of the mixed complex relative to the parent complex.

The following defined value is also used[21] to characterize the stability relations of the ligands bound in the mixed complexes:

$$\Delta\log K=\log K^{MA}_{MAB}-\log K^{M}_{MB}=\log K^{MB}_{MAB}-\log K^{M}_{MA} \tag{4}$$

$$K^{A}_{MA}=\frac{[MA]}{[M][A]} \tag{5}$$

$$K^{MA}_{MAB}=\frac{[MAB]}{[MA][B]} \tag{6}$$

The following relation exists between the Δ log K of eqn. (4) and the stability constants of the parent and mixed complexes:

$$\Delta\log K=\log\beta_{111}-\log K^{M}_{MA}-\log K^{M}_{MB} \tag{7}$$

All the calculations were carried out with an ODRA 1013 digital computer with programmes written in MOST-1 autocode. The relative error for the proton and parent complexes, calculated from the scatter of the experimental points, was about ±0.01 log K.

Results and Discussion

The values of the stability constants of the proton complexes and of the copper(II), nickel(II) and cobalt-

---

[17] R. G. Bates: Determination of pH. Theory and Practice. John Willey and Sons, Inc., New York, London, Sydney, 1964.

[18] H. M. Irving, M. G. Miles and L. D. Pettit: *Anal. Chim. Acta*, *38*, 475 (1967).

[19] I. Nagypál, A. Gergely and P. Jékel: *J. Inorg. Nucl. Chem.*, *31*, 3447 (1969).

[20] R. Näsänen and H. Koskinen: *Soumen Kemistilehti B.*, *40*, 23 (1967).

[21] P. R. Huber, R. Griesser, B. Prijs and H. Sigel: *European J. Biochem.*, *10*, 238 (1969).

Table I. Protonation constants of the aminoacids at 25°C and I=0,05 *M*.

aminoacid	pK_1	pK_2
glycine	2.33	9.61
alanine	2.33	9.73
α-amino-butyric acid	2.30	9.66
norvaline	2.31	9.68
norleucine	2.30	9.70
serine	2.17	9.08
threonine	2.17	8.98
phenylalanine	2.20	9.11
tyrosine	2.22	9.07

Table II. Stability constants of the copper(II)-aminoacid parent complexes at 25°C and I=0.05 *M*.

aminoacid	$\log K_1$	$\log K_2$	$\log K_1/K_2$	pK_2—$\log K_1$	pK_2—$\log K_2$
glycine	8.22	6.89	1.33	1.39	2.72
alanine	8.18	6.81	1.37	1.55	2.92
α-amino butyric acid	8.13	6.80	1.33	1.53	2.86
norvaline	8.17	6.87	1.30	1.51	2.81
serine	7.93	6.64	1.29	1.15	2.44
threonine	8.03	6.74	1.29	0.95	2.24
phenylalanine	7.86	6.91	0.95	1.25	2.20
tyrosine	7.81	6.93	0.88	1.26	2.14

Table III. Stability constants of the nickel(II)-aminoacid parent complexes at 25°C and I=0.05 *M*.

aminoacid	$\log K_1$	$\log K_2$	$\log K_1/K_2$	pK_2—$\log K_1$	pK_2—$\log K_2$
glycine	5.80	4.85	0.95	3.80	4.75
alanine	5.48	4.53	0.95	4.25	5.20
α-amino-butyric acid	5.38	4.42	0.96	4.28	5.24
norvaline	5.42	4.45	0.97	4.26	5.23
norleucine	5.43	4.45	0.98	4.27	5.25
serine	5.43	4.53	0.90	3.66	4.56
threonine	5.50	4.58	0.92	3.48	4.40
phenylalanine	5.15	4.44	0.71	3.96	4.67
tyrosine	5.10	4.36	0.74	3.98	4.72

Table IV. Stability constants of the cobalt(II)-aminoacid parent complexes at 25°C and I = 0.05 *M*.

aminoacid	$\log K_1$	$\log K_2$	$\log K_1/K_2$	pK_2—$\log K_1$	pK_2—$\log K_2$
glycine	4.70	3.88	0.82	4.90	5.72
norvaline	4.29	3.52	0.77	5.48	6.15
phenylalanine	4.05	3.51	0.54	5.05	5.59
tyrosine	3.87	3.65	0.22	5.21	5.43

(II) parent complexes are given in Tables I-IV, together with the pK_2 — $\log K_n$ and the $\log K_1/K_2$ values.*

Taking into account the differences in the ionic strength, the stability data in Tables I-IV agree well with the recently published literature data wich can be classified as accurate.[1-6,22]

In connection with the protonation constants of the aminoacids, it can be stated on the basis of Table I that a certain periodicity can be observed in the pK_2 values of the aliphatic aminoacids, although with a decreasing tendency. Thus, the proton complexes of the aminoacids containing odd numbers of carbon atoms are more stable than those containing even numbers. This experimental fact is in agreement with the similar behavior characteristic of the aliphatic carboxylic acids. This observation no longer appears in relation to norvaline-norleucine, which can probably be explained by the increasing electron-donating effect of the alkyl chain. In accordance with expectations, the pK_2 values of phenylalanine, tyrosine, serine and threonine are smaller by 0.6 — 0.7 pK than those of the others.

The following emerge from the stability data in Tables II-IV for the aminoacid parent complexes of the copper (II), nickel (II) and cobalt (II):

1. The periodicity in the pK_2 values of the aliphatic aminoacids is not fully expressed in the stability constants of the parent complexes. Considering the pK_2 — $\log K_n$ values, the complexes of glycine prove to be the most stable, while in the cases of all three metal ions the stabilities of the other aliphatic aminoacid complexes have almost the same values. This can presumably be explained by steric reasons.[23]

2. A comparison of the stability constants of the serine and threonine complexes with the similar data for the complexes of alanine and α-aminobutyric acid, which do not contain alcoholic hydroxy groups, reveals a stability increase. Considering the thermodynamic data too, this was interpreted in an earlier

---

* Measurements were not made for the copper(II)-norleucine system because of the formation of a precipitate.

(22) A. Gergely, B. Király, I. Nagypál and J. Mojzes: *Acta Chim. Acad. Sci. Hung.*, *67*, 133 (1971).

(23) H. Irving and L. D. Pettit: *J. Chem. Soc.*, *1963*, 1546.

(24) A. Gergely, J. Mojes and Zs. Kassai-Bazsa: *J. Inorg. Nucl. Chem.*, in press.

Table V. Stability constants of the copper(II)-aminoacid mixed complexes at 25°C and I = 0.05 *M*.

tyrosine	phenyl-alanine	threonine	serine	norvaline	α-amino butyric acid	alanine	ligand A ligand B
15.24	15.30	15.24	15.10	15.35	15.28	15.36	glycine
15.23	15.24	15.23	15.12	15.33	15.27	—	alanine
15.18	15.21	15.16	15.06	15.30	—	—	α-amino butyric acid
15.32	15.29	15.22	15.13	—	—	—	norvaline
14.96	15.00	14.95	—	—	—	—	serine
15.06	15.09	—	—	—	—	—	threonine
15.08	—	—	—	—	—	—	phenylalanine

Table VI. Stabilization effects of the copper(II)-aminoacid mixed complexes at 25°C and I = 0.05 *M*.

	tyrosine	phenyl-alanine	threonine	serine	norvaline	α-amino butyric acid	alanine	ligand A ligand B
$-\Delta \log K$	0.79	0.78	1.01	1.05	1.04	1.07	1.04	glycine
$\Delta \log \beta_{111}$	0.01	0.06	0.00	−0.04	−0.03	−0.04	0.01	
$-\Delta \log K$	0.76	0.80	0.98	0.99	1.02	1.04	—	alanine
$\Delta \log \beta_{111}$	0.06	0.06	0.05	0.04	0.01	0.01	—	
$-\Delta \log K$	0.76	0.78	1.00	1.00	1.00	—	—	α-amino-butyric acid
$\Delta \log \beta_{111}$	0.04	0.06	0.01	0.01	0.01	—	—	
$-\Delta \log K$	0.66	0.74	0.98	0.97	—	—	—	norvaline
$\Delta \log \beta_{111}$	0.13	0.08	0.01	0.02	—	—	—	
$-\Delta \log K$	0.78	0.79	1.01	—	—	—	—	serine
$\Delta \log \beta_{111}$	0.00	0.03	−0.02	—	—	—	—	
$-\Delta \log K$	0.78	0.80	—	—	—	—	—	threonine
$\Delta \log \beta_{111}$	0.00	0.02	—	—	—	—	—	
$-\Delta \log K$	0.59	—	—	—	—	—	—	phenylalanine
$\Delta \log \beta_{111}$	0.02	—	—	—	—	—	—	

Table VII. Stability constants of the nickel(II)-aminoacid mixed complexes at 25°C and I = 0.05 *M*.

tyrosine	phenyl-alanine	threonine	serine	norleucine	norvaline	α-amino butyric acid	alanine	ligand A ligand B
10.37	10.43	10.70	10.63	10.62	10.66	10.59	10.72	glycine
10.10	10.11	10.37	10.29	10.26	10.26	10.24	—	alanine
10.02	10.02	10.27	10.21	10.18	10.21	—	—	α-amino-butyric acid
10.05	10.07	10.29	10.27	10.22	—	—	—	norvaline
10.08	10.07	10.30	10.21	—	—	—	—	norleucine
10.03	10.07	10.34	—	—	—	—	—	serine
10.14	10.09	—	—	—	—	—	—	threonine
9.86	—	—	—	—	—	—	—	phenylalanine

paper[24] by the participation of the non-dissociated alcoholic hydroxy groups in the bonding.

3. The parent complexes of phenylalanine and tyrosine are more stable than the complexes of the aliphatic aminoacids. The stability increase is generally more pronounced for the MA_2-type complexes. As has already been referred to,[6] this phenomenon was interpreted by the possibility of back-coordination.

The log β_{111}, $-\Delta$ log K and $\Delta \log \beta_{111}$ values relating to the aminoacid-aminoacid mixed complexes are given in Tables V-IX.

From a comparison of the results of the three measurement series carried out with each of the metal ions studied it could be established that the error of the data in the Tables was ± 0.02 — 0.04 log K for the copper(II) and nickel(II) mixed complexes, and somewhat larger for the cobalt(II) mixed complexes.

The following conclusions can be drawn from the data in Tables V-IX:

1. The stabilities of the copper(II)-aliphatic-aliphatic aminoacid mixed complexes (see Table V and VI) correspond approximately to the statistical case. If, however, one of the ligands is glycine, then a destabilization results. (A similar result was reached by *Martin* and *Paris*[8] for the copper(II)-glycine-alanine system). At the same time, the complexes of glycine are the most stable of the parent complexes (see Table II). This difference in the stabilities can probably be explained in that, as a result of the presence of the B ligand in the glycine-other alpha-aminoacid complexes a certain distortion of the bond angles and a stretching of the bond distances occur, similarly as in the MB_2 parent complexes.[23]

2. In the case of all three metal ions the aliphatic-aromatic aminoacid mixed complexes are more stable than the parent complexes. This stability increase is first of all characteristic for copper(II), but considering the — Δ log K values to some extent also valid for the complexes of nickel(II) and cobalt(II). In

Table VIII. Stabilization effects of the nickel(II)-aminoacid mixed complexes at 25°C and I = 0.05 *M*.

	tyrosine	phenyl-alanine	threonine	serine	norleucine	norvaline	α-amino butyric acid	alanine	ligand A ligand B
— Δ log K	0.53	0.52	0.60	0.60	0.61	0.56	0.59	0.56	glycine
Δ log β_{III}	0.01	0.01	0.03	0.02	0.04	0.08	0.06	0.09	
— Δ log K	0.48	0.52	0.61	0.62	0.65	0.64	0.62	—	alanine
Δ log β_{III}	0.07	0.01	0.04	0.02	0.02	0.02	0.05	—	
— Δ log K	0.46	0.51	0.61	0.60	0.63	0.59	—	—	α-amino-butyric acid
Δ log β_{III}	0.09	0.03	0.04	0.04	0.05	0.08	—	—	
— Δ log K	0.47	0.50	0.63	0.58	0.63	—	—	—	norvaline
Δ log β_{III}	0.08	0.03	0.02	0.06	0.05	—	—	—	
— Δ log K	0.45	0.51	0.63	0.65	—	—	—	—	norleucine
Δ log β_{III}	0.10	0.03	0.02	0.00	—	—	—	—	
— Δ log K	0.50	0.51	0.59	—	—	—	—	—	serine
Δ log β_{III}	0.02	0.00	0.03	—	—	—	—	—	
— Δ log K	0.51	0.51	—	—	—	—	—	—	threonine
Δ log β_{III}	0.03	0.00	—	—	—	—	—	—	
— Δ log K	0.39	—	—	—	—	—	—	—	phenylalanine
Δ log β_{III}	0.03	—	—	—	—	—	—	—	

Table IX. Stability constants and stabilization effects of the cobalt(II)-aminoacid mixed complexes at 25°C and I = 0.05 *M*

ligand A	ligand B	—Δ log K	Δ log β_{III}	log β_{III}
glycine	norvaline	0.48	0.02	8.51
glycine	phenylalanine	0.40	—0.02	8.35
glycine	tyrosine	0.27	—0.05	8.30
norvaline	phenylalanine	0.30	0.05	8.02
norvaline	tyrosine	0.16	0.05	8.03
phenylalanine	tyrosine	0.10	—0.01	7.84

addition, it is more pronounced in the mixed complexes formed with tyrosine than in those containing phenylalanine.

The probable explanation of the above phenomena, at least as regards copper(II) where the data are more clear-cut may be the electron-attraction of the aromatic systems. As a result of this the stability-increasing effect of the higher electron density on the nitrogen donor atoms of the aliphatic aminoacids can assert itself in these mixed complexes. This explanation also seems to be supported by the fact that if the aliphatic aminoacid is serine or threonine then because of the electron-attraction of the alcoholic hydroxy groups stabilization scarcely appears, or even not at all.

3. According to the data of Table VII and VIII the stabilities of the nickel(II)-aliphatic-aliphatic aminoacid mixed complexes correspond in general to the statistical values. Nevertheless, from a comparison with the corresponding copper(II) complexes it is clear that the stabilization of the mixed complexes of nickel(II) containing glycine are greater than those of the copper(II) complexes. The differences between the stabilities of copper(II) and nickel(II) mixed complexes may probably be explained by the same steric reasons as in the case of the parent complexes.