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Chelation of Some Bivalent Metal Ions with Alanine and Phenylalanine*

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The interaction of *D*, *L*, or *racemic* forms of alanine and phenylalanine with some bipositive metal ions (Cd, Cu, Ni, Pb and Zn) has been studied. The proton dissociation constants of the ligands and stability constants of the complex species formed have been determined. No stereospecific effects have been observed, *i.e.* no significant differences have been found between the stability constants of the corresponding MD or ML, and MD_2, ML_2 or MLD complexes in aqueous solution at 20°C and an ionic strength of 0.37 ($NaNO_3$). The MLD complex is considered as being the predominant species in the system consisting of metal ions and a racemic ligand.

Interest in the stability of complexes with optically active ligands and the existing disagreement in opinion about the possibility of there being differences in the stability constants of complexes containing enantiomeric *D* and *L* or racemic *DL* ligands caused us to investigate the systems consisting of some bivalent metal ions and stereoisomeric forms of several amino acids.

In a previous paper by the present authors[1] the equilibria in solutions of a metal ion and a racemic ligand were considered theoretically. The conclusions made in that paper can be particularly useful if the complexes formed have a square-planar configuration. Then the species represented by formulae ML_2 and MD_2 are optical antipodes, while the mixed complex species (represented by MLD) is assymetrical and it can be assigned two structures which are mirror images. Thus, the total number of complex species reduces to four (ML_2, MD_2, d-MLD, and l-MLD) the number of determinable stability constants being three (K_2^{LL}, K_2^{DD} and K_2^{LD}). The last constant refers then to the sum of the concentrations of both isomers of MLD. It seems that in a more general case, when the ML_2 and MD_2 species can be assymmetrical, it is not possible to give a coherent account with regard to the stability of the stereoisomeric complex species formed, because the number of such species becomes too large.

In an attempt to gain experimental support for the above theoretical considerations, the stability constants of the chelates formed by alanine and phenylalanine with bivalent ions of cadmium, copper, nickel, lead and zinc have been determined by a potentiometric titration method, all stereoisomeric forms of the ligands being investigated. Copper(II) is known to have a tendency

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to form square-planar complexes and therefore this ion was of particular interest in the present work. The other metals were investigated because only few attempts appear to have been made to study their complexes with alanine and phenylalanine at 20°C.

EXPERIMENTAL

Materials

Alanine and phenylalanine were supplied by Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. Metal nitrates and other chemicals used were of analytical reagent quality and were used without further purification.

Metal ion solutions (0.01 *M*) were obtained by dissolving metal nitrates in 0.37 *M* $NaNO_3$ and were standardized by complexometric titration according to Schwarzenbach[2]. Their titre was checked from time to time by the same procedure. The probable error of such titrations was estimated to be about 0.2%.

Amino acid solutions were prepared by dissolving the required quantity of the respective acid in 0.1 *M* nitric acid (with the same salt background) and diluting to 0.01 *M* with 0.37 *M* $NaNO_3$. The final concentration of nitric acid amounted to approximately 0.011 *M*.

The titrant solution (0.4 *M* NaOH with the same salt background) was prepared by a procedure similar to that described by Pregl[3].

Method

Acid dissociation constants as well as stability constants of metal chelates were determined by a potentiometric titration method. The titration curves were recorded with a »Radiometer« automatic titration apparatus *(Titrigraph SBR 2c* and *Titrator TTT 1c)* equipped with a glass electrode G 202 B for use in highly alkaline solutions and a saturated calomel electrode K 4312 which was separated from the solution by a porous ceramic pin liquid junction. Before and after each experiment the electrodes were standardized using two standard buffer solutions, 0.05 *M* potassium biphthalate and 0.01 *M* borax (*p*H 4.00 and 9.23 respectively)[4]. The standardization of the titrant solution was performed each day against a 0.02 *M* potassium biphthalate solution.

For the determination of the *p*K values 15 ml. of amino acid solution were titrated, followed immediately by 15 ml. of »blank« (nitric acid of exactly the same concentration as before and with the same salt background). Stability constants were determined by a similar procedure, recording the titration curves of the ligand in the presence and absence of metal ions. The ligand to metal concentration ratio was adjusted as close as possible to 2 : 1, the initial ligand concentration being 0.00667 *M*, and metal concentration 0.00333 *M*. An inert atmosphere of CO_2-free nitrogen gas was maintained inside the titration cell by bubbling through purified nitrogen gas, which also supplemented the stirring. The titration cell and its contents were kept at 20.0 ± 0.1°C.

Calculations

The formation curves for "proton complexes" were calculated by comparing the titraton curves of the ligand and that of the "blank" solution. The formation curves for metal chelates were calculated by a procedure similar to that described by Irving and Rossotti[5] and Rossotti and Rossotti[6].

Depending on the range covered by the formation curve, stability constants were calculated by the "correction term" method[6,7] if $\bar{n}$ reached 1.5 or by a least-squares method for cadmium, lead and zinc complexes where $\bar{n}$ failed to reach 1.5 because of hydrolysis. It was found not to be necessary to calculate the stability constants of the protonated complex species. For the computations we have developed a programme for use with a *Zuse Z 23 V* digital electronic computor.

The method of calculation outlined above yielded stoichiometric stability constants of the metal complexes investigated whereas the proton dissociation constants are the so called Brönsted constants. Such constants are useful intermediates in the computation of stability constants.

RESULTS

The proton dissociation constants of the ligands were determined in solutions of *L*-(+)-alanine and *L*-(—)-phenylalanine respectively. Their *p*K values, given as the mean of five independent measurements, are shown in Table 1. pK_1 and pK_2 were reproducible within ±0.04 and ±0.06 *log* units, respectively.

TABLE 1

Dissociation Constants of Ligands.
Valid for 20⁰C and $\mu = 0.37$ ($NaNO_3$)

Ligand	pK_1	pK_2
Alanine	2.54	9.93
Phenylalanine	2.41	9.35

The stability constants determined in the present work are given in Tables 2 and 3.

TABLE 2

Stability Constants of Alaninato Complexes.
Valid for 20⁰C and $\mu = 0.37$ ($NaNO_3$)

Metal	Enantiomeric form	*log* K_1	*log* K_1K_2
Cd^{+2}	*L*	4.09	7.40
	D	4.00	7.40
	LD	3.98	7.40
Cu^{+2}	*L*	8.16	14.98
	D	8.14	15.00
	LD	8.20	15.04
Ni^{+2}	*L*	5.23	9.91
	D	5.22	9.87
	LD	5.21	9.96
Pb^{+2}	*L*	4.20	9.34
	D	4.16	9.40
	LD	4.08	9.42
Zn^{+2}	*L*	4.63	~ 9
	D	4.62	~ 9
	LD	4.65	~ 9

The stability constants of copper and nickel complexes were reproducible to within ±0.05 *log* units whereas the stability constants of chelates of other metals are somewhat less precise because the *p*H range in which these complexes do exist is rather narrow, and therefore the data obtained are less reliable. This particularly applies to β_2 values. However, most of these stability constants could be reproduced to within ±0.1 *log* units.

DISCUSSION

Comparison of data obtained in the present work with the values found in the literature is rather difficult. Many papers[8–18] dealing with the stabilities of alaninato and phenylalaninato complexes have been published, and it can be said generally that almost all authors working in this field disagree to some

TABLE 3

Stability Constants of Phenylalaninato Complexes.
Valid for 20°C and $\mu = 0.37$ ($NaNO_3$)

Metal	Enantiomeric form	*log* K_1	*log* K_1K_2
Cd^{+2}	*L*	3.90	6.73
	D	3.87	6.72
	LD	3.84	6.75
Cu^{+2}	*L*	7.93	15.10
	D	8.00	15.08
	LD	8.03	15.14
Ni^{+2}	*L*	5.23	9.72
	D	5.24	9.70
	LD	5.19	9.66
Pb^{+2}	*L*	4.02	8.86
	D	3.99	8.88
	LD	4.03	8.79
Zn^{+2}	*L*	4.43	~ 8.5
	D	4.43	~ 8.5
	LD	4.41	~ 8.5

extent. Special difficulty in comparing results obtained in this work with data previously reported has been found due to different experimental conditions used (ionic strength and temperature), so that the constants quoted are not strictly comparable. However, it is obvious that the substantial differences observed are far too great to be explained solely by these different experimental conditions.

pK *values*

pK_1 of alanine is remarkably higher than any value reported previously, while pK_2 is in good accordance with almost all values given by other authors, the differences being compatible with those reported in other studies where the same technique has been applied. For phenylalanine the agreement is even better.

Alaninato complexes

Stability constants of alaninato complexes of nickel(II), lead(II) and zinc (II) as determined in this work are somewhat lower than any cited in the literature[8–18], and the stability constants for copper and cadmium complexes fall into the range of the previously reported values, agreeing reasonably well with those obtained by Curchod[13], Martin[12], Maley and Mellor[8], and Irving and Pettit[14].

Phenylalaninato complexes

The data for phenylalaninato complexes previously reported in other studies[8, 10, 11, 13] are less numerous than those for alaninato complexes; all the same certain comparison may be made. Generally, our K_1 values are somewhat higher than the majority of the published data, except those for copper and cadmium for which values higher than ours can be found. The β_2 values obtained here however, are rather low in comparison with those previously reported, with the exception of that for copper. As far as we are aware, the stability constants of lead(II) phenylalaninato complexes have not yet been published.

Stereochemical considerations

As already mentioned, the stability data of copper complexes with optical isomers of alanine and phenylalanine are of some stereochemical interest. The stabilities of the complexes with enantiomeric forms of the ligands studied here are essentially equal, as are also the apparent stability constants determined when using the racemic form. For such a system an equation deduced formerly by us[1] can be applied and the stability constant of the mixed species can be calculated:

$$\beta^{LD}_{2,app.} = \frac{2\beta_2 + \beta_2^{LD}}{4} \tag{1}$$

$\beta^{LD}_{2,app.}$ being the apparent stability constant determined when the racemic form of the ligand is used and β_2 the stability constant of an enantiomeric complex species. These constants are cumulative stability constants and the subscript denotes the degree of complexity of the respective species.

By applying the above expression to the stability data for copper the results given in Table 4 have been computed:

TABLE 4

System	$log\ \beta_2$	$log\ \beta^{LD}_{2,app.}$	$log\ \beta^{LD}_2$
Cu^{+2} + alanine	15.0	15.0	15.3
Cu^{+2} + phenylalanine	15.1	15.1	15.4

From the β_2^{LD} values given in Table 4 K_2^{LD} have been computed using for K_1 the mean of the values obtained in the experiments with *D*, *L* and *DL* alanine or phenylalanine respectively. K_2^{LD} obtained in this way is twice greater than that computed from a similar equation deduced by Bennett[19]. This inconsistency, however, is only apparent because Bennett's constant has a different physical meaning. It is defined as

$${}^{B}K_2 = \frac{[MLD]}{2\,[ML]\,[D]} = \frac{[MLD]}{2\,[MD]\,[L]}, \tag{2}$$

and therefore refers to the total *MLD* concentration, irrespective of the way in which it is formed. Our constant defined as

$$K_2^{LD} = \frac{[MLD]}{[ML]\,[D]} = \frac{[MLD]}{[MD]\,[L]} \tag{3}$$

is a more fundamental one, taking into account only one of two possible (and equivalent) ways of forming the *MLD* species.

It can be concluded that, for the systems under consideration, the concentration of mixed species (*MLD*) is equal to the sum of concentrations of

»pure« (ML_2 or MD_2) species. Thus, the mixed species is the predominant species in the solution. This conclusion is in accordance with results obtained by Ritsma and collaborators[20] for Cu(II) complexes with aspartic acid, asparagine, glutamic acid and glutamine.

The results obtained with cadmium, lead(II), nickel and zinc are quite analogous. Because in these cases no square-planar complexes are formed, the systems are rather complicated, so the present data are not sufficient to characterize these systems in more detail. The same would apply to the results of Ritsma *et al.*[20] obtained with nickel and cobalt(II) and the mentioned ligands. However, it seems highly probable that there are no stereospecific effects in these systems.

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IZVOD

Helati dvovalentnih iona nekih metala s alaninom i fenilalaninom

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Studirana je interakcija desnih (*D*), lijevih (*L*) i racemičnih oblika alanina i fenilalanina s dvovalentnim ionima bakra, kadmija, nikla, olova i cinka. Određene

su konstante kiselinske disocijacije liganada kao i konstante stabilnosti nastalih kompleksnih vrsta. Nisu opaženi nikakvi stereospecifični efekti, tj. nije nađena razlika između stabilnosti *MD* i *ML*, dotično MD_2, ML_2 i *MLD* kompleksa. Izmjerene konstante vrijede kod temperature 20°C i ionske jakosti 0,37 ($NaNO_3$). Kompleks *MLD* je dominantna kompleksna vrsta u sistemima koji sadrže ione metala i racemični oblik liganda.

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