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The Complex Formation of Cadmium(II) and Mercury(II) with Aliphatic Dipeptides

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Stability constants for complexes between cadmium(II) and mercury(II) and a series of dipeptides consisting of glycine, alanine, leucine and proline have been determined by the pH-titration method. Influences of the side chains on the stability of the complexes are discussed. Cadmium(II) forms 1:1 complexes of the type $CdLH^+$ ($LH_2 = H_3\overset{+}{N}-CHR-CO-NH-CHR'-COO^-$), as well as 1:2 complexes of the type CdL_2H_2. Mercury(II) also forms 1:1 complexes of the type $HgLH^+$; there is, however, only a small tendency for the formation of HgL_2H_2. Possible structures are postulated according to the results of PMR studies on glycylglycine in water in the presence of $CdCl_2$. The PMR spectra suggest that complex species of the type $CdLH_2^{2+}$ may exist; difficult to detect by the pH-titration method. The complex formation of cadmium(II) is compared with that of mercury(II).

Introduction

Although the toxicity of both metals has been known for a long time, scientists have not been able to explain the effects of cadmium and mercury on the human body in detail, to a large extent because of the difficulties associated with investigating metal–protein complexes. For this reason short linear peptides are frequently used as model substances in attempts to understand the metal–protein interaction. Aliphatic dipeptides have 3 possible binding abilities: the N-terminal amino group, the C-terminal carboxyl group, and one peptide bond. The complex formation of Cd(II) with various dipeptides has been studied and a great variety of methods has been applied [1–6]. In contrast to this, the complex formation of Hg(II) with dipeptides has not been investigated frequently [7].

Experimental

Chemicals

The concentrations of the cadmium chloride stock solution and the sublimate stock solution were determined by complexometric titration. The dipeptides listed in Table I were obtained from Sigma Chemical Co. and used without further purification.

TABLE I. Dissociation Constants for the Protolysis of Dipeptides. pK1, pK2 notation see text; superfixes correspond to references.

Dipeptide	$pK1^8$	$pK2^8$	$pK1^{Lit.}$	$pK2^{Lit.}$
gly-gly	−3.18	8.25	−3.17[10]	8.13[10]
			−3.23[11]	8.19[11]
			−3.19[12]	8.13[12]
gly-d,l-ala	−3.19	8.40	−3.17[10]	8.20[10]
			−3.15[11]	8.23[11]
gly-d,l-leu	−3.20	8.37	−3.28[11]	8.23[11]
gly-l-pro	−2.93	8.77	−2.97[12]	8.48[12]
d,l-ala-gly	−3.22	8.33	–	8.27[11]
			−3.15[10]	8.19[10]
d,l-leu-gly	−3.20	8.24	−3.28[11]	8.07[11]
l-pro-gly	−3.05	9.15	−3.19[12]	8.98[12]
			−3.16[13]	8.97[13]
d,l-ala-d,l-ala	−3.18	8.39	−3.08[10]	8.26[10]
d,l-ala-d,l-leu	−3.15	8.36[+]	–	8.32[8]
l-pro-l-ala	−3.20	9.19	–	–
l-pro-l-leu	−3.21	9.16	–	–

[8] 0.2 *M* KCl, 20 °C, used in this work. [10] 0.2 *M* KCl, 25 °C. [11] 0.1 *M* KCl, 25 °C. [12] 0.16 *M* KNO_3, 25 °C. [13] 0.1 *M* $NaClO_4$, 25 °C. [+] determined and used in this work.

pH-titrations

All pH-measurements were performed using a Schott pH-meter CG 803 equipped with a standard glass electrode. Titrations were carried out at metal/ligand ratios between 1:1.5 and 1:4. The concentration of cadmium chloride was 2.000 m*M*, the concentration of mercury chloride was 1.987 m*M* in all titrations. The systems were titrated with 0.1 or 0.05 *M* NaOH. All investigations were carried out under nitrogen atmosphere at 20 °C and ionic strength of 0.2 *M* KCl. The stability constants were calculated using a FORTRAN program on the CDC computer of the University of Innsbruck.

PMR Measurements

PMR spectra were obtained by a Varian EM-360-L 60 MHz spectrometer at an ionic strength of 0.2 *M* KCL in H_2O. The spectra were recorded at a sweep rate of 0.4 Hz/sec and at a probe temperature of 30 °C, the sweep width being 120 Hz. The concentration of the dipeptide gly-gly was 0.1 *M*. Cadmium chloride was added in equimolar amounts. 0.3 *M* KCL was added to solutions containing only gly-gly to give an ionic strength comparable to that of solutions containing cadmium chloride. HCL or NaOH was added to bring the solutions to the desired pH. TMA (tetramethylammonium chloride) was used as an internal standard. Chemical shifts are reported relative to the central resonance signal of the TMA triplet.

Method of Calculation

For the general case of a metal M^{n+} reacting with a proton containing ligand LH_2:

$$\lambda \cdot M^{n+} + \mu LH_2 \rightleftharpoons Complex^{n-\nu} + \nu \cdot H^+$$

the following equations can be formulated:

$$(1)\quad [M]_{total} = [X] + \sum_i \lambda_i c_i$$

$$(2)\quad [L]_{total} = [Y] + \sum_i \mu_i c_i$$

$$(3)\quad [NaOH] = [OH^-] - [H^+] + \sum_i \nu_i c_i$$

$$(4)\quad K_i = \frac{c_i [H^+]^{\nu}}{x^{\lambda} \cdot y^{\mu}}$$

where [X] is the concentration of free metal ions, [Y] the concentration of the free ligand and c_i the concentration of the various complex species at equilibrium. K_i are the equilibrium constants. $[M]_{total}$, $[L]_{total}$, [NaOH] and $[H^+]$ are known or measured. With an initial guess for the K_i, the [X] and [Y] are numerically determined, leading to a calculated value for [NaOH], which is compared with the measured quantity [NaOH]. The K_i values are optimized by minimizing the difference $[NaOH]_{calc} - [NaOH]_{measured}$. In our case, this procedure was performed using the data of titration curves obtained for various metal/ligand ratios over the whole pH range *simultaneously*. As a measure of the statistical significance of the resulting pK values, the quantity dpK has been defined. A change of pK by ± dpK leads to an increase of $\sum_i ([NaOH]_i^{calc} - [NaOH]_i^{measured})^2$ by a factor of 2. If dpK is larger than ±0.5, the corresponding species is regarded to be not significant. As a further criterion for the decision, whether a species might be existing, its percent contribution to the system (≥3%) and its influence on the graphic simulation of the titration curve are considered.

Results and Discussion

pH-titrations

Cd(II) forms 1:1 complexes of the type $CdLH^+$ and 1:2 complexes of the type CdL_2H_2. Hg(II) shows similar properties, but the tendency for the formation of the 1:2 complexes is small. If LH_2 denotes the zwitterionic form of the dipeptide, the following reactions can be defined:

TABLE II. Dissociation Constants for Complexes between Cadmium(II) and Dipeptides. pK3 and pK4 notation see text; superfixes correspond to references; for criteria of ± deviations of pK values see text (dpK).

Dipeptide	pK3		pK3 Lit.	pK4		pK4 Lit.
gly-gly	2.72	±0.09	2.87[1] 3.08[3] 2.76[5] 2.70[4] 2.86[6] 3.33[15]	1.78	±0.28	2.53[1] 2.57[3] – 2.45[4] 2.49[6] 2.54[15]
gly-d,l-ala	2.87	±0.07	–	2.38	±0.09	–
gly-d,l-leu	2.85	±0.08	–	2.21	±0.12	–
gly-l-pro	3.25	±0.08	–	2.47	±0.11	–
d,l-ala-gly	2.39	±0.07	–	1.69	±0.29	–
d,l-leu-gly	2.24	±0.14	2.49[14]	1.97	±0.28	2.35[14]
l-pro-gly	3.06	±0.05	–	2.20	±0.13	–
d,l-ala-d,l-ala	2.56	±0.13	–	2.08	±0.24	–
d,l-ala-d,l-leu	2.57	±0.07	3.16[2]	1.57	±0.36	–
l-pro-l-ala	3.15	±0.08	–	2.44	±0.17	–
l-pro-l-leu	3.02	±0.06	–	2.88	±0.05	–

[1] I = 0, 15 and 25 °C. [2] 0.5 *M* KNO_3, 20 °C, l-ala-l-leu. [3] 25 °C. [4] 0.1 *M* KNO_3, 25 °C. [5] I = 0, 25 °C, PMR. [6] 0.1 *M* KNO_3, 25 °C. [14] I = 0, 25 °C, l-leu-gly. [15] I = 0, 25 °C.

K1: $LH_2 + H^+ \rightarrow LH_3^+$

K2: $LH_2 \rightarrow LH^- + H^+$

K3: $MLH^+ \rightarrow M^{2+} + LH^-$

K4: $ML_2H_2 \rightarrow MLH^+ + LH^-$

The simulation of the titration curves including the formation of further complex species, *e.g.* where the ligand loses a further proton, did not improve the quality of the simulation. The constants for the protolysis of the pure peptides were mostly taken from literature [8]. Table I lists the dipeptides and their constants of protolysis. Table II and III present the stabilities of the Cd(II) and the Hg(II) complexes. Some of these dipeptides were used in the d,l-form; the calculated dissociation constants have to be regarded therefore as mean values for all stereoisomers present [9].

TABLE III. Dissociation Constants for Complexes between Mercury(II) and Dipeptides. pK3 and pK4 notation see text; for criteria of ± deviation of pK values see text (dpK).

Dipeptide	pK3		pK4	
gly-gly	2.58	±0.07	–	–
gly-d,l-ala	2.80	±0.10	2.20	±0.20
gly-d,l-leu	2.77	±0.09	1.84	±0.27
gly-l-pro	3.13	±0.05	2.15	±0.22
d,l-ala-gly	2.42	±0.10	–	–
d,l-leu-gly	2.31	±0.13	–	–
l-pro-gly	3.28	±0.04	–	–
d,l-ala-d,l-ala	2.83	±0.10	–	–
d,l-ala-d,l-leu	2.79	±0.07	–	–
l-pro-l-ala	3.19	±0.08	2.92	±0.12
l-pro-l-leu	3.13	±0.10	2.89	±0.16

Influence of the Side Chains on the Stability of the Cadmium Dipeptide Complexes

Cadmium has a tendency to form 1:1 complexes rather than 1:2 complexes. The stability of the 1:1 complexes follows approximately that of the 1:2 complexes. This suggests a similar structure of the two types of complexes. The C-terminal amino acid has no steric influence, but it has an effect on the stability of the complexes due to its influence on pK2 (Fig. 1). The plot of pK3 against pK2 leads to a linear relationship. Other systems also show this linear correlation, which is generally believed to prove complexation mainly at the amino group [2, 14]. Datta *et al.* [14] suggest the formation of a chelate complex via amino group and peptide oxygen (Figs. 9 and 10). On the one hand the N-terminal amino acid influences the basicity and thus the stability of the complex, whereas it may function as a steric hindrance in the case of bulky side chains R. This explains why gly-pro forms a more stable complex

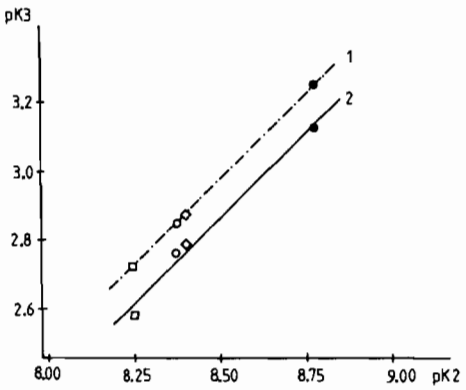

Fig. 1. Linear relationship between pK3 and pK2 for a series of glycyl-amino acids: gly-gly □; gly-d,l-leu ○; gly-d,l-ala ◇; gly-l-pro ●; 1) Cd(II). 2) Hg(II).

with Cd(II) than pro-gly, for example, though the latter has a more basic amino group; the higher basicity is overcompensated by the steric hindrance caused by proline in the N-terminal position.

Influence of the Side Chains on the Stability of the Mercury(II) Dipeptide Complexes

The influence of the basicity of the amino group is quite similar as in the case of Cd(II) (Fig. 1), but some differences between Cd(II) and Hg(II) can be detected. Formation of 1:2 complexes is only found if there is a strong tendency to form the 1:1 complex. Though Hg(II) generally shows a stronger complex formation tendency than does Cd(II), the stabilities of the Cd(II) dipeptide complexes are often higher, especially in the case of 1:2 complexes. The steric hindrance of complex formation caused by the bulky side chain R is not so remarkable as in the case of Cd(II), possibly due to the larger binding distance of Hg(II). The other differences between the metals can be explained mainly by their behaviour in solutions containing chloride. While $CdCl_2$ is dissociated to a great extent in solutions containing chloride, $HgCl_2$ forms stable chloro-complexes [16], as illustrated in Fig. 2. This figure also helps to explain the relatively small tendency of Hg(II) to form 1:2 complexes with dipeptides; the complexation of dipeptides can occur only if chloride is displaced by the dipeptide. Figures 3–6 show some examples of the relative distribution of the species depending on pH. The lower pK2 and the higher pK3 and pK4, the greater the amount of complex formed at a given pH. The pH scale represents the range covered by the experiment.

PMR Measurements

Gly-gly has two non-equivalent methylene groups.

$H_3\overset{+}{N}-CH_2-CO-NH-CH_2-COO^-$

β α

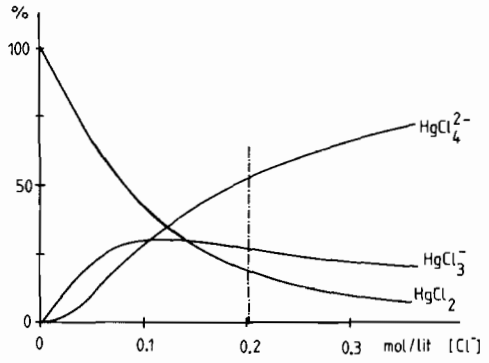

Fig. 2. Distribution of $HgCl_2$, $HgCl_3^-$ and $HgCl_4^{2-}$ as a function of the total concentration of chloride. The values were computed with the constants published by Godfrey *et al.* [16]. Total concentration of Hg(II): 0.002 *M*. —·—·—·— conditions as in the pH-titrations, neglecting the complex formation of Hg(II) with dipeptides.

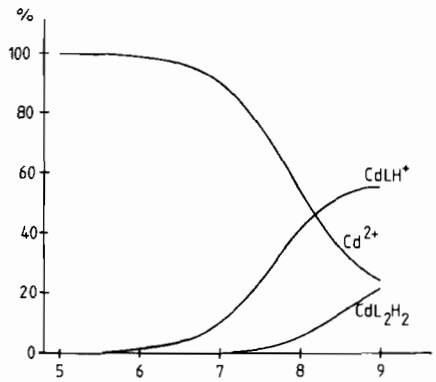

Fig. 3. Distribution of species as a function of pH. Concentration is given in percent of the total metal concentration. Total concentration of cadmium(II): 0.002 *M*. Total concentration of gly-l-pro: 0.004 *M*.

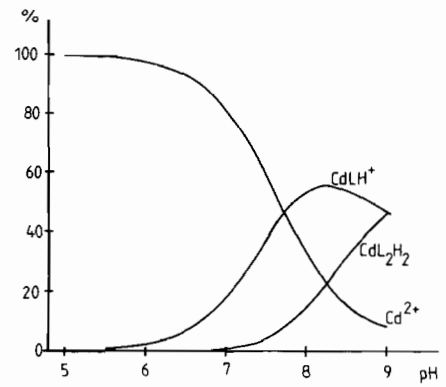

Fig. 4. Distribution of species as a function of pH. Concentration is given in percent of the total metal concentration. Total concentration of cadmium(II): 0.002 *M*. Total concentration of gly-l-pro: 0.008 *M*.

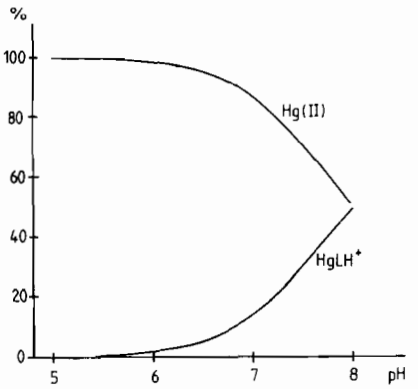

Fig. 5. Distribution of species as a function of pH. Concentration is given in percent of the total metal concentration. Total concentration of mercury(II): 0.002 *M*. Total concentration of gly-gly: 0.008 *M*.

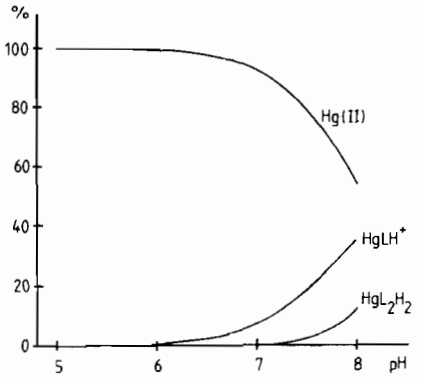

Fig. 6. Distribution of species as a function of pH. Concentration is given in percent of the total metal concentration. Total concentration of mercury(II): 0.002 *M*. Total concentration of l-pro-l-ala: 0.008 *M*.

Both signals appear as broad singlets in H_2O because of exchange effects. The influence on the chemical shift by addition of cadmium chloride to gly-gly as well as the influence of pH on both cadmium-free and complexed gly-gly should serve as a powerful tool in the discussion of possible complex structures. The assignment of the signals is facilitated by previous works [5, 7] on gly-gly and its complexes with transition metals. Figures 7a, b illustrate the distribution of the species depending on pH (under conditions as described in the experimental section of PMR measurements). Figure 8 illustrates the chemical shift of the α and β methylene protons of gly-gly relative to TMA. All signals appear at lower field than does the TMA standard signal.

Pure gly-gly

The significant downfield shift of the α methylene proton signal at pH = pK1 is caused by the protonation of the carboxylate group. In this pH range the

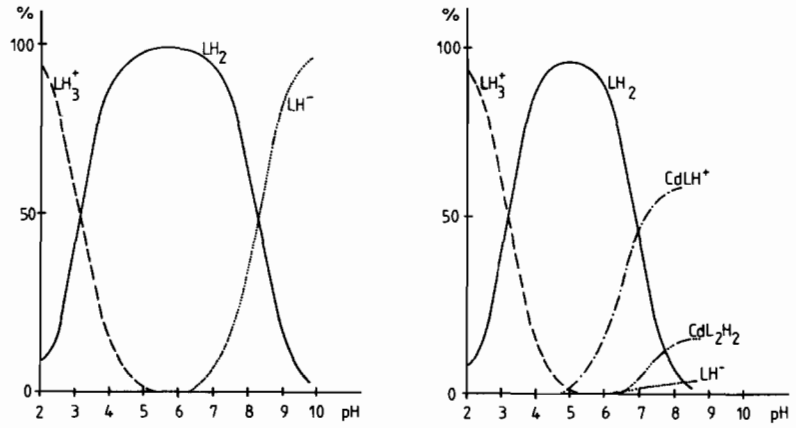

Fig. 7. a) Distribution of species as a function of pH. The concentration is given in percent of the total gly-gly concentration. Total concentration of gly-gly: 0.1 *M*. b) Distribution of species as a function of pH after addition of 0.1 *M* cadmium chloride.

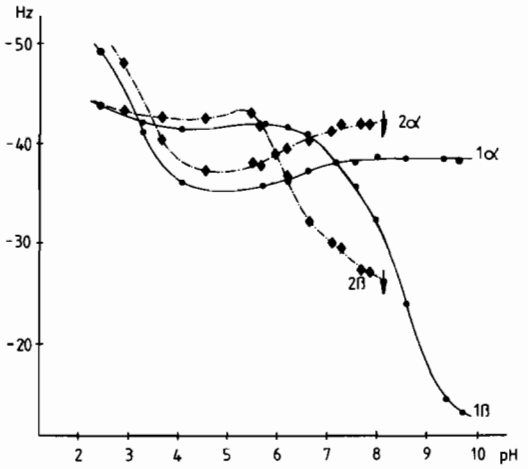

Fig. 8. Chemical shift of the α and β methylene protons relative to TMA as a function of pH. 1) –●———●– pure gly-gly. 2) –◆–·–·–◆– gly-gly + cadmium chloride.

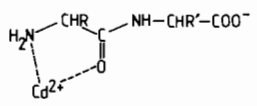

Fig. 9. Postulated structure of the complexes $CdLH^+$.

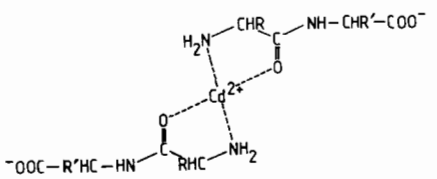

Fig. 10. Postulated structure of the complexes CdL_2H_2.

shift of 1β is relatively small. The sigmoid shape of the 1β curve at pH = pK2 is due to the loss of a proton of the amino group. In this pH range 1α slightly shifts downfield.

Gly-gly + Cadmium Chloride

The formation of the complex influences the shift of the β methylene protons to a great extent. The results presented in this work are in accordance with those published by Tewari *et al.* [7], who examined the complex formation of gly-gly with zinc(II) and $Hg(NO_3)_2$, applying the same method. When the formation of the complex can be detected at first, which occurs at pH ~ 5, a proton of the amino group is replaced by Cd(II), this leads to a strong upfield shift of 2β. The total loss of a proton has still a greater shielding effect on the β methylene protons; therefore, the 1β- and 2β-curves cross over the alkaline pH range. At pH values greater than 8, $Cd(OH)_2$ is precipitated (marked by arrows in Fig. 8). The smooth curve 2β is distorted at the beginning of formation of the 1:2 complex (pH 6.5). The interpretation of curve 2α is more difficult. 2α is shifted downfield by complex formation. This effect is probably caused by the formation of a chelate complex via amino group and peptide oxygen. The assumption of a chelate complex formation coordinated at amino group and peptide oxygen [14] is thus confirmed by the PMR results. It should be mentioned that, compared to the 1α curve, 2α is shifted throughout the whole pH region. Rabenstein and Libich [5] assumed the formation of the weak complex $CdLH_2^{2+}$ ($H_3\overset{+}{N}–CH_2–CO–NH–CH_2COO^-\cdots Cd^{2+}$), which cannot be detected by the method of pH-titration. Nag and Banerjee [4] determined its dissociation constant by polarography and published a value of 1.00.

Acknowledgements

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