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Note

COMPLEXATION REACTION OF METAL IONS WITH PEPTIDE SYSTEMS. VII. EQUILIBRIUM STUDIES OF *N*-BENZOYL-L-VALINE, *N*-BENZOYL-GLYCYL-L-VALINE AND ISOVALERIC ACID WITH METAL(II) ION SYSTEMS

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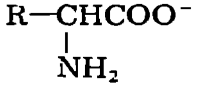
The interactions between metals and amino acids and peptides have become of considerable interest as coordination phenomena, as models for metal—protein reactions, as well as for biological systems in which the properties of proteins are modified by the fact that the metal atoms are attached to them [1]. Simple amino acids exist as zwitterions (I) but almost invariably coordinate as the anionic species of type (II) [2]

$$\mathrm{R{-}\underset{\displaystyle NH_3^+}{\underset{|}{C}}HCOO^-}$$

(I)



$$\mathrm{R{-}\underset{\displaystyle NH_2}{\underset{|}{C}}HCOO^-}$$

(II)

In this work we have studied the interaction of metal(II) ions (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+}) with *N*-benzoyl-L-valine (*N*-bz-L-val), *N*-benzoyl-glycyl-L-valine (*N*-bz-gly-L-val) and isovaleric acid to evaluate the effects of the substituent on the amino acid and the peptide. The pH titration technique of Bjerrum [3] and Calvin and Wilson [4], as modified by Irving and Rossotti [5,6], has been employed to evaluate the log K values at 25, 35 and 45°C at 0.15 M (KNO_3) ionic strength in 50 vol. % water—dioxane medium.

EXPERIMENTAL

L-Valine was benzoylated with benzoylchloride in strongly alkaline medium similar to that used for benzoylation of leucine [7]. The benzoyl derivative was repeatedly crystallized to a constant m.p. of 131°C.

The benzoylation of glycyl-L-valine was carried out in the same manner as the benzoylation of glycine. Benzoyl-glycyl-L-valine was repeatedly crystallized to a constant m.p. of 122°C.

Isovaleric acid was used as obtained from E. Merck. Dioxane was purified by the method of Vogel [8].

The ligand solutions were prepared in a 50 vol. % dioxane—water mixture just before use. Solutions of divalent metal ions were made in double distil-

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led carbon dioxide free water from AnalaR grade metal nitrates. These solutions were standardized by titration with EDTA using the usual procedures [9]. The following solutions were prepared in a 50 vol. % dioxane—water mixture, the total volume in each case being 50 ml. An appropriate quantity of potassium nitrate (1 M) was added to bring the ionic strength to the required level (0.15 M). All pH measurements were carried out at 25 ± 0.1, 35 ± 0.1 and 45 ± 0.1°C in a cell immersed in a thermostated bath. An inert atmosphere was maintained in the cell by passing nitrogen gas through the solution. A Toshniwal pH meter CL 46 with glass and calomel electrodes of 0.01 unit sensitivity was used to measure the pH. It was calibrated with suitable buffers before use: (A) 5 ml 0.005 M HNO_3; (B) A + 5 ml 0.025 M ligand; (C) B + 5 ml 0.005 M metal nitrate.

The mole ratio of metal ions to ligand was kept at 1 : 5 in order to fulfil the maximum coordination number of the metal ions. These solutions were titrated potentiometrically with a standard carbonate free potassium hydroxide solution (0.05 M) prepared in a 50 vol. % dioxane—water mixture. The concentrations were corrected for the changes in volume produced following the addition of alkali during titrations. The pH meter readings were corrected by applying the procedure of Van Uitert and Haas [10] for dioxane—water medium to obtain the pH values. Plots of pH vs. volume of alkali added were of usual shape.

RESULTS AND DISCUSSION

The formation curve was obtained by plotting the degree of formation ($\bar{n}_A$) of the proton—ligand complex vs. the pH value. Values of $\bar{n}_A$ were calculated using the relationships of Irving and Rossotti [5,6]. The proton—ligand stability constant was obtained with the help of the Bjerrum [3] half integral and graphical methods [5,6].

The results suggested that *N*-bz-L-val and *N*-bz-gly-L-val are monobasic, the carboxyl group being the acid centre. The high acidity of amino acids and peptides in general [e.g. L-valine pK_1 = 3.75 and glycyl-L-valine pK_1 = 4.55 as compared to isovaleric acid pK_1 = 6.35 (Table 1) in 50 vol. % dioxane—water medium at 0.15 M (KNO_3) ionic strength at 35°C] is due to the inductive effect of the positively charged nitrogen. Benzoylation of the amino group neutralizes this positive charge and introduces the less electronegative amide substituent, thus resulting in an increase in the pK values, e.g. pK_1 values of *N*-bz-L-val and *N*-bz-gly-L-val are 5.42 and 5.30, respectively. The benzene ring is too far removed from the carboxylate group to exert any influence. The pK values of *N*-bz-L-val and *N*-bz-gly-L-val are still less than that for isovaleric acid due to the inductive effect of the amide group. The pK_1 values for *N*-bz-L-val and *N*-bz-gly-L-val decrease with rise in temperature.

The values of formation function $\bar{n}$ (the average number of ligands attached per metal ion) and free ligand exponent pL, were calculated by applying the standard expressions [3—6]. The values of $\bar{n}$ were calculated up to pH 6 which is well below the pH range of hydrolysis of metal ions. All the

TABLE 1

pK_1 values

	Temperature (°C)		
	25	35	45
Benzoyl-L-valine	5.49	5.42	5.36
Benzoyl-glycyl-L-valine	5.35	5.30	5.23
Isovaleric acid		6.35	
Glycyl-L-valine		4.55	
L-valine		3.75	

systems were investigated with different total concentrations of the metal ions and ligands gave results within the error limits, indicating that the separation of the metal—ligand titration curves from the ligand titration curve was not due to hydrolysis of metal ions. It also indicates that only mononuclear complexes were present. The value of $\bar{n}$ does not exceed 1 in any of the metal ions, which shows the formation of only 1 : 1 complexes. Bjerrum's half integral [3], pointwise calculation and graphical methods [5,6] were used to calculate log K values. The mean values of log K_1 for complexes of various metal ions with N-bz-L-val and N-bz-gly-L-val at 25, 35 and 45°C are summarized in Table 2, while those of isovaleric acid at 35°C are given in Table 3. For each ligand the stabilities of the metal complexes follow the order Pb(II) > Cu(II) > Co(II) > Ni(II) > Zn(II) > Cd(II). This order is not in agreement with that of Irving and Williams [11]. The stability of Co(II), which is greater than that of Ni(II), may be attributed to the additional stabiliziation due to Jahn Teller distortion present in the case of Co(II). The values of log K_1 decrease with increase in temperature for all the metal ions.

The data in Table 3 indicate that the stabilities of the complexes of these metal ions with N-bz-L-val, N-bz-gly-L-val and isovaleric acid are of the same order. This indicates that association of the metal ions with N-bz-L-val and N-bz-gly-L-val probably takes place in the same way as that with isovaleric acid, i.e. only with the carboxylate group. These complexes do not involve appreciable bidentate chelation such as would result from the association of the metal ions with both the carboxylate group and amide group through oxygen or nitrogen atoms. Similar results have been reported for benzoylglycine, acetyl glycine and acetyl-glycyl-glycine with different metal ions [12—16]. However, the small amounts of chelated species may also be present in equilibrium with major monodentate complexes. Some metal ions do coordinate with peptide ligands via a deprotonated amide nitrogen atom in a complexed peptide in the pH ranges 4—6, 7—8 and 10—11 for Cu(II), Ni(II) and Co(II), respectively [17].

An attempt was made to investigate the presence of species in which metal ions coordinate via deprotonated nitrogen, but the insolubilities of the metal hydroxides and the low stabilities of the monodentate complexes prevented the study through the titration process.

TABLE 2

Values of metal—ligand stability constants and thermodynamics functions at three temperatures

Cation	Constant	Temperature (°C)			$-\Delta G$(kcal mole^{-1}) 35°C	ΔH(kcal mole^{-1}) 35°C	ΔS(cal mole^{-1} deg^{-1}) 35°C
		25	35	45			
Benzoyl-L-valine							
Pb(II)	log K_1	3.40	3.30	3.24	4.64	−3.44	3.90
Cu(II)	log K_1	3.26	3.19	3.11	4.49	−3.26	4.00
Co(II)	log K_1	3.04	3.01	2.98	4.23	−1.30	9.53
Ni(II)	log K_1	3.01	2.98	2.95	4.19	−1.30	9.39
Zn(II)	log K_1	2.94	2.90	2.86	4.08	−1.73	7.61
Cd(II)	log K_1	2.87	2.81	2.77	3.95	−2.15	5.83
Benzoyl-glycyl-L-valine							
Pb(II)	log K_1	3.38	3.26	3.20	4.59	−3.85	2.39
Cu(II)	log K_1	3.24	3.17	3.08	4.48	−3.48	3.18
Co(II)	log K_1	3.06	3.01	2.94	4.23	−2.61	5.26
Ni(II)	log K_1	3.01	2.94	2.87	4.13	−3.03	3.57
Zn(II)	log K_1	2.88	2.84	2.79	3.99	−2.01	6.42
Cd(II)	log K_1	2.85	2.80	2.75	3.94	−2.16	5.77

TABLE 3

Metal—ligand stability constants (log K) at 35°C

	Pb(II)	Cu(II)	Co(II)	Ni(II)
Benzoyl-L-valine	3.30	3.19	3.01	2.98
Benzoyl-glycyl-L-valine	3.26	3.17	2.99	2.96
Isovaleric acid	3.20	3.14	2.95	2.93

The values of overall changes in free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) accompanying the complexation have been determined using the temperature coefficient and the Gibbs—Helmholtz equation. These values are summarized in Table 2. The ΔH values are negative for all the metal ions, indicating the exothermic nature of the reactions. The entropy changes are positive for all the metals.

The log K, ΔG, ΔH and ΔS values are given to the required number of significant figures [18]. The error limits are ±0.05 for log K_1, ±0.08 kcal mole^{-1} for ΔG, ±0.1 kcal mole^{-1} for ΔH and ±0.2 cal mole^{-1} deg^{-1} for ΔS.

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