Journal of Thermal Analysis and Calorimetry, Vol. 87 (2007) 2, 561–565

THERMOCHEMICAL INVESTIGATION OF ACID-BASE INTERACTIONS IN PEPTIDE SOLUTIONS

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Heat effects of interaction of *D*,*L*-α-alanyl-*D*,*L*-α-alanine, glycil-γ-aminobutyric acid, glycil-*L*-asparagine and *D*,*L*-α-alanyl-*D*,*L*-asparagine with KOH, LiOH and HNO_3 solutions were measured by the direct calorimetry method at 288.15, 298.15, 308.15, 318.15 K and at several values of the ionic strength created by adding KNO_3 and $LiNO_3$.

The standard dissociation enthalpies of the investigated ligands were obtained by the extrapolation to zero ionic strength. The standard thermodynamic characteristics (ΔG^0, ΔH^0, ΔS^0, ΔC_p^0) of the processes of acid-base interaction in dipeptide solutions were calculated. Several peculiarities of acid-base interaction reactions in the solutions of biologically important ligands were found. The correlations between the thermodynamic characteristics of the protolytic equilibria in the dipeptide and aminoacids solutions and the structure of these compounds were determined.

Keywords: *aminoacid, calorimetry, dipeptide, dissociation, temperature, thermodynamic characteristics*

Introduction

The study of low-molecular-mass peptides is very important nowadays. These compounds play an active part in a great number of vital processes. A variety and importance of peptide regulator functions are well known. The peptides can be used as the model compounds for more complicated biosystems. As all biochemical processes proceed in aqueous solutions it is of great importance to study the peptide properties in the solutions.

At present the literature data concerning the thermodynamic characteristics (ΔG^0, ΔH^0, ΔS^0, ΔC_p^0) of protolytic equilibria in the peptide solutions are not sufficient. The temperature influence as well as nature and concentration of a background electrolyte on the thermodynamic characteristics of processes of acid-base interaction with a participation of the compounds under study have not practically been investigated.

At present work the thermodynamic study of the processes of geometrical isomers step-wise dissociation: glycil-γ-aminobutyric acid which structure does not contain side chains, and *D*,*L*-α-alanyl-*D*,*L*-α-alanine possessing a branched structure, has been done. To research methyl group influence on the thermodynamics of acid-base interaction in the peptide solutions glycil-*L*-asparagine and *D*,*L*-α-alanyl-*D*,*L*-asparagine have been chosen.

Acid-base equilibria in the solutions of the studied dipeptides can be presented by the schemes:

$$H_2L^+ \Leftrightarrow HL^{\pm} + H^+ \quad K_1 = [HL^{\pm}][H^+]/[H_2L^+] \qquad (1)$$

$$HL^{\pm} \Leftrightarrow L^- + H^+ \quad K_2 = [L^-][H^+]/[HL^{\pm}] \qquad (2)$$

There are a lot of works at [1, 2] devoted to the potentiometric research of protolytic equilibria in the studied ligand solutions. These works had been conducted on sufficiently high experimental level. Stepwise dissociation constants have been determined at different ionic strength values made by different background electrolytes. For the comparison of the results on the dissociation constants obtained in different concentration conditions constant values were recalculated on zero ionic strength by means of Davies equation [3]. For the interpretation of the results of calorimetric measurements the most probable values of the thermodynamic constants of peptide dissociation were recalculated on the fixed values of ionic strength by means of equation given in [4].

Experimental

The heat effects were measured with the isothermic shell calorimeter and the calorimetric experiment curves were automatically recorded [5]. The calorimeter was calibrated against electric current. At present work dipeptides from 'Reanal' (Hungary) of chromatographically pure grade were used without additional purification. Solutions of the dipeptides were obtained by dissolving weighed amounts of the substances in freshly prepared doubly distilled water directly prior to use in calorimetric experiments. Before weighing, the substances were dried at 343 K un-

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1388–6150/$20.00
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til its mass ceased to vary. The content of H_2O in the samples did not exceed 0.2–0.3%. The required ionic strength was maintained by adding KNO_3 and $LiNO_3$ recrystallized from reagent of ch (pure) grade. Carbonate-free KOH, LiOH and HNO_3 solutions were prepared from reagents of 'chemically pure' grade as recommended in [6]. Measurements were taken at 288.15, 298.15, 308.15 and 318.15 K and ionic strength values of 0.5, 0.75, 1.0, 1.5. Two independent procedures were used to determine the stepwise dissociation heats of the peptides. Equilibrium solution compositions under all experimental conditions were calculated by the RRSU program [7].

Results and discussion

A great difference between the step-wise dissociation constants of the dipeptides allowed to carry out independent determination of ionization heat effects in the solutions of these compounds on each step.

According to the diagram of equilibria acidifying of peptide aqueous solutions causes the protonation of the carboxyl group of these compounds.

Two independent procedures were used to determine the heat effect of the protonation of $HL^{\pm}$ particle. According to the first procedure an ampoule with an accurately weighed amount of the peptide solution was broken in a solution of nitric acid (pH was changed approximately from 5.5 to 1.0). The enthalpy change in the peptide cation dissociation was determined by the equation:

$$\Delta_{dis}H(H_2L^+) = -(\Delta_{mix}H_1 - \Delta_{dil}H_1)/\alpha_1 \qquad (3)$$

where $\Delta_{mix}H_1$ is the heat of interaction between solutions of peptide and HNO_3; $\Delta_{dil}H_1$ is the heat of dilution of the peptide solution by a solution of the background electrolyte; α_1 is the completeness of the protonation of $HL^{\pm}$ particle which equals, for this procedure, no less than 97%.

When the second procedure was used the heat of dissociation of the peptide protonated particle (H_2L^+) was determined from the heats of interaction between solutions of nitric acid and the peptide in the pH range 3.5–2.5 by the equation:

$$\Delta_{dis}H(H_2L^+) = -(\Delta_{mix}H_2 - \Delta_{dil}H_2)/\alpha_2 \qquad (4)$$

where $\Delta_{mix}H_2$ is heat effect of interaction between solutions of HNO_3 and the peptide; $\Delta_{dil}H_2$ is the heat of dilution of HNO_3 by a solution of the background electrolyte; α_2 is the completeness of the protonation of $HL^{\pm}$ particle (according to the second procedure α_2 equals 70–80%).

The dissociation of NH_3^+-group proton proceeds on pH increasing in the peptide aqueous solutions. The heat effect of $HL^{\pm}$ neutralization was defined in pH range 5.5–12.0. KOH solution was used as a calorimetric liquid, a precise sample of the peptide solution was in an ampoule. The heat effect of dissociation of the betaine proton from peptide was calculated by the equation:

$$\Delta_{dis}H(HL^{\pm}) = (\Delta_{mix}H - \Delta_{dil}H)/\alpha + \Delta H_w \qquad (5)$$

where $\Delta_{mix}H$ is the heat of interaction between solutions of peptide and KOH; $\Delta_{dil}H$ is heat of dilution of the peptide solution by solution of the background electrolyte; α is the completeness of the neutralization of peptide which is no less than 99.5%; ΔH_w is the heat of water dissociation in a solution of KNO_3 [8].

The values of the heat effects of the stepwise peptide dissociation in the standard solution were found by extrapolation to zero ionic strength the obtained values according to the equation with one individual parameter [9].

$$\Delta H - \Delta Z^2\Psi(I) = \Delta H^0 + bI \qquad (6)$$

where ΔH and ΔH^0 are the enthalpies at finite and zero ionic strengths, respectively; $\Psi(I)$ is the theoretical ionic strength function; ΔZ^2 is the difference of the squares of charges of the reaction products and initial components; b is an empirical coefficient.

The dissociation heats of the carboxyl group of glycil-*L*-asparagine were graphically extrapolated to a zero ionic strength by the Eq. (6).

The heat effects of dissociation of H_2L^+ determined by two independent procedures were in close agreement with each other. Weighed mean values based on the results of the above-mentioned procedures were chosen as the most probable values of standard heats of the stepwise dissociation of the substances under study.

The standard thermodynamic characteristics of the processes of the dipeptides dissociation studied are listed in Table 1. An analysis of the results given in Table 1 reveals certain trends characterizing acid-base interactions in solutions of dipeptide. The dissociation of the amino group in these compounds is accompanied by substantial positive heat effects. It appears that the endothermic contribution of the dissociation of the nitrogen-hydrogen bond far exceeds the exothermic contribution of solvation of amino group and proton.

The numeral values of the temperature-dependent and temperature-independent enthalpy components were calculated using the Gerney scheme described in details [10]. The results of the calculation are given in Table 1. In case proton dissociation of amino group the temperature-dependent enthalpy components are much smaller than the temperature-independent contributions and ΔC_p^0 value is close to zero. For the dissociation of the proton from carboxyl group the absolute

Table 1 Standard thermodynamic characteristics of the stepwise ionization of some amino acids and dipeptides and their temperature-dependent and temperature-independent constituents

Process	T/K	pK°	ΔH^0/ J mol^{-1}	$-\Delta S^0$/ J mol^{-1} K^{-1}	ΔC_p^0/ J mol^{-1} K^{-1}	ΔH_d^0/ J mol^{-1}	ΔH_{ind}^0/ J mol^{-1}
1	2	3	4	5	6	7	8
$AlaH_2^+=AlaH^{\pm}+H^+$	298.15	2.43±0.01	3390±210	35.2±0.9	130±10	5400	8800
	308.15	2.41±0.01	2070±110	39.4±0.9		6500	8600
	318.15	2.39±0.01	870±120	43.0±0.9		7700	8600
$AlaH^{\pm}=Ala^-+H^+$	298.15	10.09±0.01	45540±720	40.4±1.2	55±20	6000	51500
	308.15	9.83±0.01	45060±560	42.0±1.4		6400	51500
	318.15	9.59±0.01	44430±700	44.0±1.4		7100	51500
$GlyH_2^+=GlyH^{\pm}+H^+$	298.15	2.35±0.02	4430±50	30.1±0.4	110±2	5030	9450
	308.15	2.32±0.02	3290±50	33.7±0.4		5990	9260
	318.15	2.30±0.02	2300±50	36.8±0.4		6970	9250
$GlyH^{\pm}=Gly^-+H^+$	298.15	9.78±0.01	44190±320	39.6±1.1	83.5±20	5790	49780
	308.15	9.53±0.01	43470±320	41.3±1.1		6670	50140
	318.15	9.29±0.01	42520±320	44.9±1.1		7690	50220
L-$AsnH_2^+=$*L*-$AsnH^{\pm}+H^+$	298.15	2.16±0.04	3880±90	28.5±0.9	95±10		
	308.15		2890±120	31.6±0.9			
	318.15		1970±120	34.4±0.9			
L-$AsnH^{\pm}=$*L*-Asn^-+H^+	298.15	8.97±0.08	40076±450	35.0±2.2	70±30		
	308.15		40000±460	37.5±2.1			
	318.15		39400±460	39.5±2.2			
D,*L*-α-Ala-*D*,*L*-α-$AlaH_2^+=$ *D*,*L*-α-Ala-*D*,*L*-α-$AlaH^{\pm}+H^+$	288.15	3.12±0.01	–510±210	61.4±1.1	100±10	6460	5950
	298.15	3.12±0.01	–1720±110	65.5±0.7		7730	6010
	308.15	3.13±0.01	–2440±120	67.8±0.7		8920	6480
D,*L*-α-Ala-*D*,*L*-α-$AlaH^{\pm}=$ *D*,*L*-α-Ala-*D*,*L*-α-Ala^-+H^+	288.15	8.82±0.01	45690±720	10.3±2.1	0	2970	48660
	298.15	8.54±0.01	45890±560	9.6±1.5		3360	49250
	308.15	8.28±0.01	45690±700	10.2±1.9		3850	49540
Gly-γ-$AbuH_2^+=$ Gly-γ-$AbuH^{\pm}+H^+$	298.15	4.22±0.02	1640±200	76.0±0.3	130±20	8490	10130
	308.15	4.22±0.02	430±100	79.3±0.1		9940	10370
	318.15	4.22±0.02	–1330±420	84.9±1.0		11620	10290
Gly-γ-$AbuH^{\pm}=$ Gly-γ-Abu^-+H^+	298.15	8.34±0.01	42620±490	16.7±1.5	0	3920	46540
	308.15	8.10±0.01	42430±710	17.3±2.1		4470	46900
	318.15	7.87±0.01	42250±570	17.9±1.6		5030	47280
Gly-*L*-$AsnH_2^+=$ Gly-*L*-$AsnH^{\pm}+H^+$	288.15	3.07±0.02	1580±200	53.2±0.5	100±10	5900	7480
	298.15	3.06±0.02	650±140	56.4±0.5		7020	7670
	308.15	3.06±0.02	–340±150	59.7±0.3		8200	7860
Gly-*L*-$AsnH^{\pm}=$ Gly-*L*-Asn^-+H^+	288.15	8.96±0.03	45620±480	13.2±1.1	40±30	3180	48800
	298.15	8.69±0.03	45210±520	14.7±1.2		3750	48960
	308.15	8.43±0.03	44850±430	15.8±0.8		4340	49190
D,*L*-α-Ala-*D*,*L*-$AsnH_2^+=$ *D*,*L*-α-Ala-*D*,*L*-$AsnH^{\pm}+H^+$	288.15	2.97±0.03	1680±300	51.1±0.4	120±20	5760	7440
	298.15	2.96±0.03	560±170	54.8±0.7		6900	7460
	308.15	2.95±0.03	–670±280	58.8±0.3		8120	7450
D,*L*-α-Ala-*D*,*L*-$AsnH^{\pm}=$ *D*,*L*-α-Ala-*D*,*L*-Asn^-+H^+	288.15	8.74±0.01	43710±400	15.6±1.2	0	3340	47050
	298.15	8.47±0.01	43880±430	15.0±1.3		3780	47660
	308.15	8.22±0.01	44020±470	14.5±1.3		4220	48240

values of temperature-dependent and temperature-independent enthalpy contributions are close to each other and ΔC_p^0 is no less than –100 J mol^{-1} K^{-1}. It may be a criterion of the process in which a change of the reaction conditions causes the change of heat effect sign. The temperature at which the heat effect changes its sign can be estimated by the equation:

$$\Theta=298.15-\Delta H^0_{298.15}/\Delta C^0_p \quad (7)$$

It was found that with increase of an ionic strength magnitude Θ moves to higher temperatures. The standard heat effect of dissociation of *D*,*L*-α-alanyl-*D*,*L*-α-alanine carboxyl group is exothermic even at the temperature of 288.15 K (Table 1).

For the purpose of the comparative analysis of dipeptides and their structural elements – amino acids – the data on standard thermodynamic characteristics of dissociation of some amino acids and their temper-

ature-dependent and temperature-independent constituents obtained before [11, 12] in our laboratory are given in Table 1. Much attention is paid to the difference in entropy values characterizing the processes of stepwise dissociation of amino acids and peptides. The absolute magnitude of the entropy change for α-amino acids during COOH-group ionization is a bit less (for absolute value) than the same characteristics for the dissociation process of the betaine proton. ΔS^0 value is more negative for carboxyl group dissociation than ΔS^0 value for amino group dissociation for the peptides. It is connected with zwitter-ion formation as a result of acid dissociation of the peptides. Zwitter-ion has the carriers of negative and positive charge, which are situated in peptides on a greater distance than in the amino acids. Thus, the dipeptide zwitter-ions are highly hydrated particles. Such as proton dissociation from the charged amino group does not greatly change the hydration equilibria.

ΔS_1^0 and ΔS_2^0 sum for amino acids as well as peptides has a close value. The summarizing ΔS for alanine and *D*,*L*-α-alanyl-*D*,*L*-α-alanine is near 75 J mol^{-1} K^{-1} at the temperature of 298.15 K. Probably, the hydration processes for amino acids and dipeptides dissociation are of the same nature. One can assume that in the stepwise ionization processes the solvation shells of the functional groups of these compounds are changed in general.

An analysis of the thermodynamic parameters of acid-base equilibria in solutions of peptides [13] lends support to the suggestion that dipeptides have β-conformations in their acid, neutral and base forms. Calculation results and the NMR data [14] show that dipeptides in solutions have a propensity to form intermolecular ion pairs, which arise as a result of interaction between their terminal amino- and carboxyl groups. Dipeptide molecules exhibit a higher conformational mobility compared with amino acids. Therefore the effect of the field increases in peptide solutions as COO^- and NH_3^+-groups can be spatially closer to each other.

It has been determined previously [15, 16] that the ammonium ion is highly solvated and causes the greater solvent compression than the carboxylate-ion. It was noted by King [2] that water has abnormally low entropy in the field of carboxylate-ion. It is characteristic for quasi-clathrate structures formation [17]. Chipens *et al.* [18] when answering the question why pair interactions of amino acid radicals of the opposite polarity are more preferable than pair interactions of the same polarity radicals concluded that the united cooperative system of linked water is formed in case of overlapping ordered hydrated shells of different nature. As a result a peculiar strengthening effect is observed which leads to the stability increase of ordered structures.

Probably, not only the electrostatic interaction exists between the charged terminal groups but also the interaction of their hydrate shells takes place in dipeptide solutions. The more hydrophobic area around the carboxylate-ion, the more hydrated is amino group and the closer are two functional groups.

The inductive effect action for the transition from amino acids to peptides decreases, as amino group in peptides is separated from the carboxyl group by the greater number of carbon atoms than in amino acids. The decreasing of an inductive effect action for the transition from amino acids to peptides leads to the decreasing of nitrogen-hydrogen bond in the latter and, as a consequence, the peptide charged amino group could bind the greater amount of water molecules than amino acids NH_3^+-group. Owing to this fact $\left|\Delta S_1^0\right|$ for the peptide dissociation is greater than $\left|\Delta S_1^0\right|$ for the amino acid dissociation. The hydrophilic hydration of an ammonium group and the hydrophobic hydration of a carboxyl group depend on the nature of the side chains located near.

D,*L*-α-alanyl-*D*,*L*-α-alanine, glycil-*L*-asparagine and *D*,*L*-α-alanyl-*D*,*L*-asparagine, consisting of α-amino acids, can be used for the analysis of the side chain influence on the thermodynamics of acid-base interaction in the peptide solutions. Conformational analysis of the aliphatic side chains [19] showed that the values of rotation angle around C^α–C^β links can be 60, 180 and –60°, while –60° is more preferable, as C^γ atom in this case is located between small N and H atoms. The aliphatic side chain has a stretched conformation.

Table 1 shows that the changing of hydrogen atom of glycil-*L*-asparagine into methyl group causes the decreasing of heat effect of *D*,*L*-α-alanyl-*D*,*L*-asparagine dissociation in comparison with glycil-*L*-asparagine. According to Rodante and Fantauzzi [20] all that takes place, obviously, due to the influence of methyl group steric factor which prevents NH_3^+ and COO^- groups convergence in *D*,*L*-α-alanyl-*D*,*L*-asparagine. The hydrophilic CH_2CONH_2-group and the hydrophobic CH_3-group interacting by their solvate shells form the united cooperative system of bonded water in *D*,*L*-α-alanyl-*D*,*L*-asparagine which also complicates the interaction of the charged functional groups of the peptide.

Table 1 shows that the dissociation enthalpies of *D*,*L*-α-alanyl-*D*,*L*-asparagine and glycil-*L*-asparagine carboxyl groups are close in spite of the influence of methyl group electron donor effect. Evidently, the high electron acceptor properties of carbonilic oxygen of the side chain of asparagine play the main role

in the dissociation processes of glycil-*L*-asparagine and *D*,*L*-α-alanyl-*D*,*L*-asparagine.

Conclusions

In the series of the investigated dipeptides the most endothermic process of the ammonium group dissociation and the most exothermic process of the dissociation of the carboxyl group of *D*,*L*-α-alanyl-*D*,*L*-alanine are caused by the substantial mutual influence of the charged functional groups in *D*,*L*-α-alanyl-*D*,*L*-alanine. The methyl groups of this dipeptide form the medium with a low permittivity, which ensures strong interaction between the ammonium-cation and carboxylate-anion. A similar decrease in ΔH the carboxyl group dissociation was observed in [2] the series of α-methyl, ethyl and isobutylsubstituents of glycil-glycine.

In the set of the dipeptides the most endothermic process of carboxyl group dissociation and the less endothermic process of amino group dissociation is observed for glycil-γ-aminobutyric acid. It is due to the inductive effect action decreasing and weak electrostatic interaction between the charged groups when the distance between NH_3^+ and COO^- dipeptide groups is increasing, since COO^- group in γ-aminobutyric residue is located in γ-position in relation to carbon α-atom. The decrease of the inductive effect action in the dipeptide which includes γ-aminobutyric acid increases the ability of NH_3^+-group to be solvated, providing the great value (absolute magnitude) of ΔS_1^0 (Gly-γ-Abu) at 298.15 K if hydrocarbon radical is located near COO^--group. Thus, the solvation possibilities of NH_2-group of glycil-γ-aminobutyric acid are greater in comparison with the other investigated dipeptides, i.e. /ΔS_2^0 (Gly-γ-Abu)/ at 298.15 K in zwitter-ion dissociation is greater than ΔS_2^0 for the peptides consisting of α-aminoacids. Glycil-γ-aminobutyric acid β-conformation, which is asymmetric *vs*. the amide bond, complicates COO^- and NH_3^+-groups interaction in this peptide solution. This fact explains the more negative value of $\Delta C_{p_1}^0$ (Gly-γ-Abu) in the series of the studied dipeptides.

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Received: May 5, 2006
Accepted: September 5, 2006
OnlineFirst: December 18, 2006

DOI: 10.1007/s10973-006-7679-y