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Thermodynamic Parameters of Protolytic Equilibria of Selected Dipeptides in Aqueous Solutions

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Abstract—Heat effects of stepwise dissociation of D,L-valyl-D,L-leucine, L-leucyl-L-leucine, D,L-leucylglycine, and glycyl-D,L-leucine (298.15 K; 0.1, 0.5, or 1.0 mol/L KNO_3) have been determined by direct calorimetry. Standard thermodynamic parameters of the studied equilibria have been calculated.

Keywords: dipeptide, enthalpy, entropy, ionic strength, hydrophobicity, calorimetry

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The influence of the aqueous solution ionic strength on the heat effect of acid-base equilibrium involving dipeptides built of valine, alanine, glycine, serine, and asparagine have been analyzed earlier in [1–10], and standard thermodynamic parameters of the corresponding reactions (pK^0, $\Delta_r G^0$, $\Delta_r H^0$, and $\Delta_r S^0$) have been determined.

In this work we studied the dipeptides including leucine fragment: D,L-valyl-D,L-leucine, L-leucyl-L-leucine, D,L-leucylglycine, and glycyl-D,L-leucine. The constants of stepwise dissociation of their carboxylic and betaine groups were considerably different, thus allowing independent calorimetric determination of heat effects of reactions (1) and (2).

$$H_2L^+ = H^+ + HL, \quad (1)$$

$$HL = H^+ + L^-. \quad (2)$$

The enthalpy of dissociation of H_2L^+ and HL could be found as the difference between the mixing and the dilution enthalpies [Eq. (3)]:

$$\Delta_{dis}H = -(\Delta_{mix}H - \Delta_{dil}H)/\alpha, \quad (3)$$

where $\Delta_{mix}H$ being the enthalpy of mixing of HNO_3 solution with the dipeptide solution in the presence of the background electrolyte; $\Delta_{dil}H$ being the enthalpy of dilution of the HNO_3 solution in the background electrolyte solution; α being the degree of protonation of HL and L^-. Heat effects of dissociation of the studied dipeptides at 298.15 K in the presence of 0.1, 0.5, and 1.0 mol/L of KNO_3 are collected in Table 1.

The values of $\Delta_{dis}H$ found for the set of ionic strengths allowed calculation of the standard thermodynamic parameters of the studied equilibria. In order to extrapolate the heat effects to zero ionic strength we used a one-parametric Eq. (4) [11]:

$$\Delta_{dis}H - \Delta z^2\Psi(I) = \Delta_{dis}H^0 + bI, \quad (4)$$

where $\Delta_{dis}H$ and $\Delta_{dis}H^0$ being the reaction enthalpies at the finite and zero ionic strength, respectively; b being an empirical coefficient; Δz^2 being a difference of charges of the reaction products and the starting reactants; and $\Psi(I)$ being a theoretically derived function of ionic strength [11]. The corresponding thermodynamic parameters of the dipeptides dissociation constants were then calculated using the Vasilev equation (5) [11]:

$$pK^0 = pK + A\Delta z^2[I^{1/2}/(1+1.6I^{1/2}) - 0.05I] + 0.05I, \quad (5)$$

where K^0 and K being thermodynamic and concentration constants of dissociation, respectively, and A being a Debye–Huckel theory constant.

Concentration constants of dissociation of D,L-valyl-D,L-leucine, L-leucyl-L-leucine, D,L-leucylglycine, and glycyl-D,L-leucine have been earlier determined in [12–15]. Standard thermodynamic parameters of dissociation of the studied dipeptides are collected in Table 2 along with our results of similar calorimetry studies of a series of other dipeptides.

The results of this work are in good agreement with the earlier suggestions on the dependence of thermo-

Table 1. Heat effects (J/mol) of dissociation reactions at 298.15 K

Reaction	$I = 0.1$	$I = 0.5$	$I = 1.0$
D,L-Valyl-D,L-leucine			
$H_2L^+ = H^+ + HL$	–2171±145	–1809±144	–1167±137
$HL = H^+ + L^-$	46357±261	46271±182	46713±203
L-Leucyl-L-leucine			
$H_2L^+ = H^+ + HL$	–1865±218	–1549±164	–1054±222
$HL = H^+ + L^-$	45724±264	46839±270	48343±296
D,L-Leucylglycine			
$H_2L^+ = H^+ + HL$	687±120	931±117	1358±112
$HL = H^+ + L^-$	45788±209	46867±236	47398±189
Glycyl-D,L-leucine			
$H_2L^+ = H^+ + HL$	–2589±124	–2138±130	–1937±128
$HL = H^+ + L^-$	45732±204	46423±139	47201±175

dynamic parameters of protolytic equilibria in the dipeptide solutions on the nature of side groups of the corresponding N- and C-terminal units [2, 6]. The dissociation of the betaine groups resulted in similar heat effects. Somewhat increased $\Delta_{dis}H^0$ of the betaine group dissociation in the cases of alanine, valine, or leucine N-terminal unit (except for D,L-alanyl-D,L-asparagine) as compared with that in the case of glycine N-terminal unit could be due to the positive inductive effect of the corresponding aliphatic groups. However, the electron-donor effect of those groups was not likely the only factor, and the $\Delta_{dis}H^0$ value was

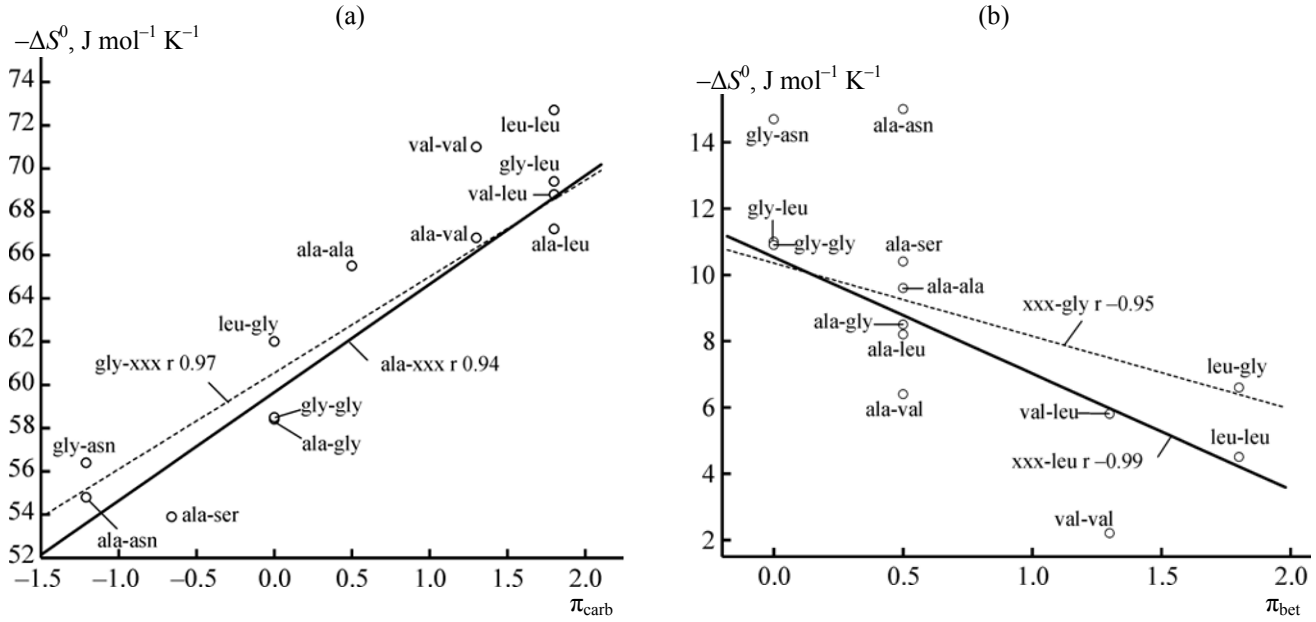

Fig. 1. Influence of hydrophobicity of side substituent in carboxylate (a) and betaine (b) amino acid residues on the ΔS^0 value for dissociation of the corresponding functional groups of the dipeptide (π is the Hansh index).

Table 2. Standard thermodynamic parameters of dissociation of selected dipeptides

Reaction	pK^0	$\Delta_{dis}G^0$, kJ/mol	$\Delta_{dis}H^0$, kJ/mol	$-\Delta_{dis}S^0$, J mol^{-1} K^{-1}
L-Leucyl-L-leucine (1.8, 1.8)[a]				
$H_2L^+ = H^+ + HL$	3.45±0.01	19.69±0.06	–1.97±0.22	72.7±0.8
$HL = H^+ + L^-$	8.12±0.01	46.35±0.06	45.01±0.30	4.5±1.0
D,L-Valyl-D,L-leucine (1.3, 1.8)[a]				
$H_2L^+ = H^+ + HL$	3.19±0.01	18.21±0.06	–2.31±0.15	68.8±0.5
$HL = H^+ + L^-$	8.33±0.01	47.55±0.06	45.82±0.28	5.8±0.9
D,L-Alanyl-D,L-leucine [1] (0.5, 1.8)[a]				
$H_2L^+ = H^+ + HL$	3.24±0.01	18.49±0.06	–1.54±0.17	67.2±0.6
$HL = H^+ + L^-$	8.48±0.03	48.40±0.17	45.97±0.23	8.2±1.0
Glycyl-D,L-leucine (0.0, 1.8)[a]				
$H_2L^+ = H^+ + HL$	3.17±0.02	18.09±0.11	–2.60±0.13	69.4±0.6
$HL = H^+ + L^-$	8.49±0.01	48.46±0.06	45.18±0.20	11.0±0.8
D,L-Leucylglycine (1.8, 0.0)[a]				
$H_2L^+ = H^+ + HL$	3.34±0.05	19.06±0.29	0.59±0.12	62.0±1.0
$HL = H^+ + L^-$	8.29±0.05	47.32±0.29	45.34±0.24	6.6±1.2
L-Valyl-L-valine [2] (1.3, 1.3)[a]				
$H_2L^+ = H^+ + HL$	3.38±0.01	19.29±0.06	–1.89±0.26	71.0±0.9
$HL = H^+ + L^-$	8.18±0.03	46.69±0.17	46.04±0.40	2.2±1.5
D,L-Alanyl-D,L-valine [3] (0.5, 1.3)[a]				
$H_2L^+ = H^+ + HL$	3.10±0.02	17.69±0.11	–2.23±0.24	66.8±1.0
$HL = H^+ + L^-$	8.43±0.02	48.12±0.11	46.22±0.40	6.4±1.4
D,L-Alanyl-D,L-alanine [4] (0.5, 0.5)[a]				
$H_2L^+ = H^+ + HL$	3.12±0.01	17.81±0.06	–1.72±0.11	65.5±0.5
$HL = H^+ + L^-$	8.54±0.01	48.75±0.06	45.89±0.56	9.6±1.9
D,L-Alanylglycine [5] (0.5, 0.0)[a]				
$H_2L^+ = H^+ + HL$	3.18±0.03	18.15±0.17	0.74±0.11	58.4±0.7
$HL = H^+ + L^-$	8.35±0.03	47.66±0.17	45.14±0.22	8.5±0.9
D,L-Alanyl-D,L-serine [6] (0.5, –0.66)[a]				
$H_2L^+ = H^+ + HL$	2.98±0.04	17.01±0.23	0.95±0.16	53.9±0.9
$HL = H^+ + L^-$	8.44±0.04	48.18±0.23	45.06±0.21	10.4±1.0
D,L-Alanyl-D,L-asparagine [4] (0.5, –1.21)[a]				
$H_2L^+ = H^+ + HL$	2.96±0.03	16.92±0.17	0.56±0.17	54.8±0.7
$HL = H^+ + L^-$	8.47±0.01	48.36±0.06	43.88±0.43	15.0±1.3

Table 2. (Contd.)

Reaction	pK^0	$\Delta_{dis}G^0$, kJ/mol	$\Delta_{dis}H^0$, kJ/mol	$-\Delta_{dis}S^0$, J mol^{-1} K^{-1}
Glycyl-L-asparagine [4] (0.0, –1.21)[a]				
$H_2L^+ = H^+ + HL$	3.06±0.02	17.48±0.11	0.65±0.21	56.4±0.8
$HL = H^+ + L^-$	8.69±0.03	49.60±0.17	45.21±0.52	14.7±1.2
Glycylglycine [7] (0.0, 0.0)[a]				
$H_2L^+ = H^+ + HL$	3.16±0.01	18.04±0.06	0.61±0.14	58.5±0.5
$HL = H^+ + L^-$	8.31±0.01	47.43±0.06	44.19±0.33	10.9±1.1

[a] The Hansh index values reflecting hydrophobicity of side groups in the corresponding betaine and carboxylate fragments of the dipeptides are given in parentheses [16].

governed by more complex intramolecular distribution of the electron density resulting from the mutual influence of the betaine and the carboxylic fragments of the dipeptide. The presence of an alkyl group in the N-terminal fragment of the dipeptide had no significant influence on the heat of dissociation of the carboxylic group.

The introduction of an aliphatic substituent at the C-terminal fragment of the dipeptide determined the exothermal effect of dissociation of carboxylic groups of D,L-valyl-D,L-leucine, L-leucyl-L-leucine, glycyl-D,L-leucine, D,L-alanyl-D,L-leucine, L-valyl-L-valine, D,L-alanyl-D,L-valine, and D,L-alanyl-D,L-alanine: hydrophobic alkyl groups of those dipeptides seemed to create a medium with a low dielectric constant [4] thus enhancing the interaction between the ammonium cation and the carboxylate anion. The enhancement of the interaction between the oppositely charged ions could also result in the changed distribution of the solvent molecules around the dipeptide molecule leading to a decrease in $\Delta_{dis}S^0$ of the carboxylic group and an increase in $\Delta_{dis}S^0$ of the betaine group due to the binding of more water

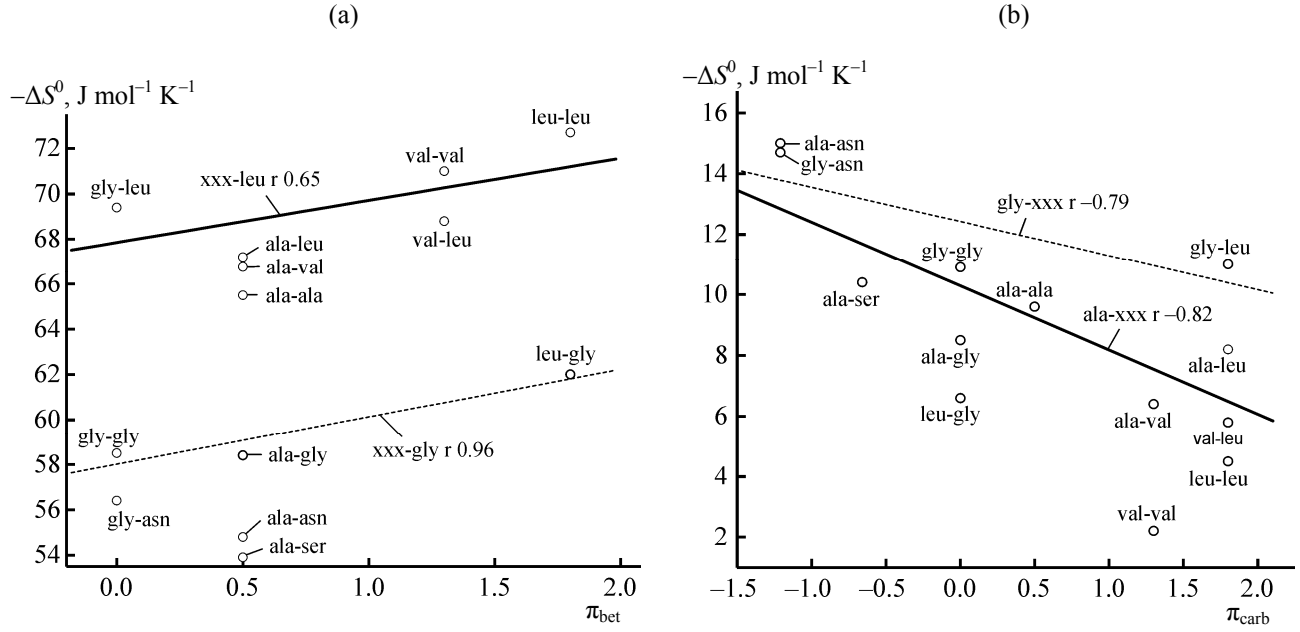

Fig. 2. Influence of hydrophobicity of side substituent in betaine (a) and carboxylate (b) amino acid residues on the ΔS^0 value for dissociation of the –COOH (a) and $-NH_3^+$ (b) groups (π is the Hansh index).

Table 3. Standard thermodynamic parameters of dissociation of selected dipeptide derivatives of β-alanine

Reaction	pK^0	$\Delta_{dis}G^0$, kJ/mol	$\Delta_{dis}H^0$, kJ/mol	$-\Delta_{dis}S^0$, J mol^{-1} K^{-1}
		β-Alanyl-β-alanine [8]		
$H_2L^+ = H^+ + HL$	4.02±0.02	22.93±0.11	2.42±0.28	68.8±1.0
$HL = H^+ + L^-$	9.59±0.03	54.74±0.15	46.70±0.28	27.0±1.3
		β-Alanylglycine [9]		
$H_2L^+ = H^+ + HL$	3.24±0.03	18.49±0.15	1.24±0.15	57.9±0.7
$HL = H^+ + L^-$	9.62±0.08	54.91±0.46	47.83±0.21	23.7±1.7
		Glycyl-β-alanine [10]		
$H_2L^+ = H^+ + HL$	4.04±0.05	23.06±0.29	1.36±0.18	72.8±1.1
$HL = H^+ + L^-$	8.37±0.06	47.78±0.34	42.97±0.28	16.1±1.5

Table 4. $\Delta_{dis}S^0$ (J mol^{-1} K^{-1}) values of overall dissociation reactions of selected dipeptides and of the functional groups of the corresponding amino acids[a]

Dipeptide	$-\Delta_{dis}S^0_\Sigma$	$-\Sigma\Delta_{dis}S^0$	$-\Delta_{dis}S^0_{carb}$	$-\Delta_{dis}S^0_{bet}$
	$H_2L^+ = 2H^+ + L^-$	$H_2L^+ = 2H^+ + L^-$	$H_2L^+ = H^+ + HL$	$HL = H^+ + L^-$
D,L-Alanyl-D,L-alanine	75.1±2.0 [4]	75.6±1.5	35.2±0.9 [17]	40.4±1.2 [17]
D,L-Alanylglycine	66.9±1.1 [5]	70.5±1.3	30.1±0.4 [18]	40.4±1.2 [17]
D,L-Alanyl-D,L-serine	64.3±1.3 [6]	68.3±1.7	27.9±1.2 [19]	40.4±1.2 [17]
D,L-Alanyl-D,L-asparagine	69.8±1.5 [4]	68.4±1.4	28.0±0.8 [20]	40.4±1.2 [17]
β-Alanyl-β-alanine	95.8±1.5 [8]	90.1±1.5	50.7±0.5 [17]	39.4±1.4 [17]
β-Alanylglycine	81.6±1.8 [9]	69.5±1.5	30.1±0.4 [18]	39.4±1.4 [17]
Glycylglycine	69.4±1.2 [7]	69.1±1.2	30.1±0.4 [18]	39.0±1.1 [18]
Glycyl-β-alanine	88.9±1.9 [10]	89.7±1.2	50.7±0.5 [17]	39.0±1.1 [18]
Glycyl-L-asparagine	71.1±1.4 [4]	67.0±1.4	28.0±0.8 [20]	39.0±1.1 [18]

[a] $\Delta_{dis}S_\Sigma^0$, the entropy change for the overall dissociation reaction of carboxylic and betaine groups of the dipeptide; $\Sigma\Delta_{dis}S^0 = \Delta_{dis}S^0_{carb} + \Delta_{dis}S^0_{bet}$; $\Delta_{dis}S^0_{carb}$ and $\Delta_{dis}S^0_{bet}$, entropy changes for stepwise dissociation reactions of carboxylic and betaine groups of the corresponding amino acids.

molecules with the corresponding zwitter ions. The said change of the reaction entropy is generally in line with the changes in the hydrophobicity of the side group (Figs. 1 and 2).

The introduction of further methylene groups into the glycine fragments of the dipeptides (along the glycylglycine–glycyl-β-alanine–β-alanylglycine, and β-alanyl-β-alanine series, Table 3) decreased the acidity of the corresponding carboxylic or betaine groups due to the enhanced inductive effect of the hydrocarbon moiety and led to the enhanced hydration of the dipeptide because of the increased distance between the positive and the negative charges.

The significant difference between the $\Delta_{dis}S^0$ values for dissociation of the functional groups of the dipeptides and the amino acids at the close $\Delta_{dis}S^0_\Sigma$ values for the overall reactions as well as the close sums $\Sigma\Delta_{dis}S^0$ for stepwise dissociation of carboxylic and the betaine groups of the corresponding amino acids (Table 4) was seemingly due to the changed hydration state of zwitter ions resulting from the varied distance between the ammonium and the carboxylate ions.

EXPERIMENTAL

Solutions of D,L-valyl-D,L-leucine, D,L-leucylglycine, and glycyl-D,L-leucine (all from Reanal, "analytically pure" grade) and L-leucyl-L-leucine (Sigma, "analytically pure" grade) were prepared by the dipeptides dissolution in the freshly bidistilled water. Solutions of KOH and KNO_3 were prepared from the "chemically pure" grade chemicals. The solutions concentration was determined by titration. Background ionic strength was supported by twice recrystallized KNO_3 "analytically pure" grade).

Calorimetry measurements were performed using an ampule calorimeter equipped with isothermal cover, thermistor temperature sensor, and an automated temperature recording device. Heat effects of mixing of 0.9484 mol/L HNO_3 solution with 0.0051–0.0104 mol/L solutions of D,L-valyl-D,L-leucine, L-leucyl-L-leucine, D,L-leucylglycine, and glycyl-D,L-leucine were measured (298.15 K; ionic strength of 0.1, 0.5, or 1.0 mol/L of KNO_3; pH 4.3 → 2.5 and 8.5 → 7.4). Heat of dilution of HNO_3 in the background electrolyte solution of the same ionic strengths was determined independently.

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