

Effect of Temperature on the Thermodynamic Characteristics of Acid–Basic Reactions in Aqueous Solutions of DL-Valyl-DL-leucine

A. I. Lytkin^{a,*}, V. V. Chernikov^a, O. N. Krutova^a, and D. K. Smirnova^a

^aIvanovo State University of Chemistry and Technology, Ivanovo, 153000 Russia

*e-mail: kdvkonkpd@yandex.ru

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Abstract—Temperature effects of the reaction between a DL-valyl-DL-leucine solution and solutions of HNO₃ and KOH are measured via calorimetry in different ranges of pH, at temperatures of 288.15 and 308.15 K and ionic strengths of 0.5, 1.0, and 1.5 (using KNO₃ and LiNO₃ as background electrolytes). The thermal effects of the stepwise dissociation of the dipeptide are determined. Standard thermodynamic characteristics ($\Delta_r H^\circ$, $\Delta_r G^\circ$, $\Delta_r S^\circ$, ΔC_p°) are calculated for the acid–base reactions in aqueous solutions of DL-valyl-DL-leucine. The connection between the thermodynamic characteristics of dipeptide dissociation and the structure of this compound is considered.

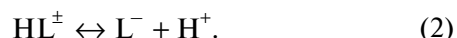
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INTRODUCTION

Data on the thermodynamic characteristics of dipeptide solutions are needed in different areas where these compounds are used (e.g., pharmacology, medicine, the food and cosmetic industries) and in the development, validation, and optimization of technological processes with these compounds and complexes of them with metals. This information can be used in studying the thermodynamic properties of more complex protein systems and for the needs of biotechnology.

The dissociation of DL-valyl-DL-leucine in an aqueous solution can be presented as



Data on the ionization constants of DL-valyl-DL-leucine are given in the literature [1–3]. The dissociation constants of zero ionic strength were converted using the Davies equation in [4] for $I < 0/5$:

$$\text{p}K^\circ = \text{p}K^c + A\Delta Z^2 \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.2I \right), \quad (3)$$

where $\text{p}K^c$ and $\text{p}K^\circ$ are negative logarithms of the concentration and thermodynamic constants, respectively; ΔZ^2 is the difference between the squares of the charges of the reaction products and initial reagents; A is the constant of the Debye limit law (0.5107 at 25°C); and I is the ionic strength of the solution.

Figure presents a diagram of equilibria in a DL-valyl-DL-leucine aqueous solution, constructed by calculating the equilibrium composition of the peptide solution at different pH values using the RRSU software [5].

There is little data on the heat of the acid–base reaction with DL-valyl-DL-leucine. The heat of the stepwise dissociation of DL-valyl-DL-leucine at a temperature of 298.15 K (zero ionic strength) was determined via direct calorimetry in [6]: $\Delta_{\text{dis}} H_{\text{HL}^\pm} = 45.82 \pm 0.28$ and $\Delta_{\text{dis}} H_{\text{H}_2\text{L}^+} = -2.31 \pm 0.15$ kJ/mol. The effect of the temperature of the thermodynamic characteristics of the stepwise dissociation of DL-valyl-DL-leucine was not studied. In [7], the heat of the stepwise dissociation of the peptide at a temperature of 298.15 K was determined via calorimetry: $\Delta_{\text{dis}} H_{\text{HL}^\pm} = 45.90$ and $\Delta_{\text{dis}} H_{\text{H}_2\text{L}^+} = -1.6$ kJ/mol. The accuracy of the measurements was not given.

This aim of this work was to study the effect the temperature and concentration of background electrolyte had on the heat effects of the stepwise dissociation of dipeptide, based on experimental determination of the enthalpy of DL-valyl-DL-leucine ionization in an aqueous solution at 288.15 and 308.15 K and ionic strengths of 0.5, 1.0, and 1.5 against a background of potassium nitrate, and calculations of the standard thermodynamic characteristics of stepwise dipeptide dissociation.

Table 1. Heat effects (kJ/mol) of the reaction between a 0.02 M solution of DL-valyl-DL-leucine and a solution of HNO₃ (pH_{in} 3.7; pH_{fin} 2.1)

<i>I</i> (KNO ₃)	$-\Delta_{\text{mix}}H$	$-\Delta_{\text{dil}}H$	$-\Delta_{\text{dis}}H_{\text{H}_2\text{L}^+}$
288.15 K			
0.5	541 ± 150	1320 ± 180	1025 ± 230
1.0	898 ± 200	1565 ± 180	890 ± 270
1.5	1473 ± 180	1981 ± 150	669 ± 230
308.15 K			
0.5	-475 ± 150	1599 ± 180	2730 ± 230
1.0	172 ± 200	1790 ± 180	2130 ± 230
1.5	-877 ± 180	2003 ± 150	1482 ± 220

Table 2. Heat effects (kJ/mol) of the reaction between a solution of HNO₃ (1.1638 mol/kg solution) and a 0.02 M DL-valyl-DL-leucine solution (pH_{in} 9.2; pH_{fin} 7.5)

<i>I</i> (KNO ₃)	$-\Delta_{\text{mix}}H$	$\Delta_{\text{dil}}H$	$\Delta_{\text{dis}}H_{\text{HL}^\pm}$
288.15 K			
0.5	44.60 ± 0.25	2.38 ± 0.28	46.98 ± 0.38
1.0	44.18 ± 0.24	3.19 ± 0.28	47.37 ± 0.37
1.5	43.61 ± 0.25	3.99 ± 0.25	47.59 ± 0.35
308.15 K			
0.5	43.58 ± 0.27	2.76 ± 0.28	46.34 ± 0.33
1.0	43.73 ± 0.29	2.91 ± 0.28	46.64 ± 0.36
1.5	43.01 ± 0.27	3.85 ± 0.25	46.86 ± 0.35

EXPERIMENTAL

DL-Valyl-DL-leucine drug (C₁₁H₂₂N₂O₃, chromatographically homogeneous) produced by Reanal (Hungary) was used without additional purification. The dipeptide solutions were prepared by dissolving precisely weighed amounts of the reagent in freshly prepared bidistilled water immediately prior to each experiment. Potassium nitrate ("ch." (pure) grade) was twice recrystallized from bidistilled water. Solutions of KOH and HNO₃ were prepared using chemicals of "kh. ch." (chemically pure) grade. The concentration of the working solutions was determined via ordinary titrimetry [8].

Calorimetric measurements were made in an ampoule calorimeter equipped with an isothermal shell, a KMT-14 thermistor temperature sensor, and automatic recording of temperature changes over time [9]. The accuracy of the calorimetry setup was checked against a universal calorimetric standard: the heat of the dissolution of crystalline potassium chloride in water. KCl was purified via double recrystallization of the salt of "kh. ch." (chemically pure) grade from bidistilled water. The potassium chloride was preliminarily dried to a constant weight in an oven at 393.15 K. Comparison of the experimentally obtained heats of dissolution of KCl(cr.) in water ($\Delta_{\text{sol}}H(\infty\text{H}_2\text{O}) =$

-17.25 ± 0.06 kJ/mol) and the most reliable literature data [10] showed there was no notable systematic error in the operation of the calorimetric setup. The solutions were weighed on a VLR-200 scale with an accuracy of 2×10^{-4} g.

A peptide solution (0.02 M, pH_{in} 3.7) was used as the calorimetric liquid in determining the heat effect of adding protons to the carboxyl group of DL-valyl-DL-leucine. A precisely weighed portion of the HNO₃ solution (concentration, 0.9004 mol/kg solution) was placed in the ampoule. After the solutions were mixed, the pH value was close to 2.1. Our experimental data on the heats of mixing and dilution are given in Table 1.

To determine the heat effect of HL[±] dissociation, we determined the heat of the reaction between a HNO₃ solution (1.1638 mol/kg) and a 0.02 M peptide solution in the pH range of 9.2–7.5, and the corresponding heat of dilution. Table 2 presents the data on the solutions' heat of mixing and the dilution of the mineral acid in the solution of the background electrolyte. The experiments were performed at temperatures of 288.15 and 308.15 K and ionic strength values of 0.5, 1.0, and 1.5 (KNO₃).

RESULTS AND DISCUSSION

The distribution of H₂L⁺, HL[±], and L⁻ particles at different values of the medium's pH (Fig. 1) showed we could determine the heat of the dissociation of cations and zwitterions of DL-valyl-DL-leucine independently. The equilibrium compositions of the solutions before and after each calorimetric experiment were calculated using the RRSU universal software [5]. The choice of concentrations in the calorimetric experiments was made on the basis of diagrams of equilibria in an aqueous solution of DL-valyl-DL-leucine (Fig. 1).

The heat of the HL[±] dissociation was determined from data on the heat of the reaction between HNO₃ solutions and DL-valyl-DL-leucine solutions in the pH range of 9.2–7.5. Since the completeness of the protonation reaction under the conditions of the calorimetric experiment exceeded 99.9%, the contribution from the heat effect of water forming from H⁺ and OH⁻ ions was negligible and the change in enthalpy was calculated as

$$\Delta_{\text{dis}}H_{\text{HL}^\pm} = -(\Delta_{\text{mix}}H - \Delta_{\text{dil}}H), \quad (4)$$

where $\Delta_{\text{mix}}H$ is the heat effect of the reaction between a HNO₃ solution (1.1638 mol/kg) and a 0.02 M peptide solution in the pH range of 9.2–7.5; $\Delta_{\text{dil}}H$ is the change in enthalpy upon dilution of the HNO₃ solution in the background electrolyte solution. The obtained heats of the dissociation of the betaine protons of DL-valyl-DL-leucine are given in Table 2.

The heat effect of the dissociation of the H_2L^+ ($\Delta_{\text{dis}}H_{H_2L^+}$) particle was calculated with the equation

$$\Delta_{\text{dis}}H_{H_2L^+} = -(\Delta_{\text{mix}}H - \Delta_{\text{dil}}H)/\alpha_1, \quad (5)$$

where $\Delta_{\text{mix}}H$ is the heat of the reaction between a HNO_3 solution and a DL-valyl-DL-leucine solution; $\Delta_{\text{dil}}H$ is the heat of dilution of the mineral acid solution in the solution of the background electrolyte; and α_1 is the completeness of the protonation reaction of HL^\pm particles ($\alpha_1 \approx 0.76$).

The composition of the solutions before and after each calorimetric experiment was calculated using the RRSU universal software [5]. The calculation results are given in Table 1.

The heat effects of DL-valyl-DL-leucine dissociation in the standard solution were found by extrapolating the heats of stepwise dissociation at fixed values of the ionic strength to zero ionic strength, using the equation proposed in [11]:

$$\Delta_r H_i - \Delta z^2 \Psi(I) = \Delta_r H_i^0 + bI, \quad (6)$$

where $\Delta_r H_i$, $\Delta_r H_i^0$ are the changes in enthalpy at the final value of the ionic strength and at $I = 0$, respectively; $\Psi(I)$ is a function of the ionic strength, calculated theoretically; Δz^2 is the difference between the squares of the charges of the reaction products and the initial components; and b is an empirical coefficient.

The points in the $(\Delta H - \Delta z^2 \Psi(I))$ from I) coordinates fit the line satisfactorily. The enthalpy of DL-valyl-DL-leucine dissociation at zero ionic strength was obtained using the least squares approach.

The standard thermodynamic characteristics of the stepwise dissociation of DL-valyl-DL-leucine, which we obtained for the first time, are given in Table 3.

The temperature dependence of the heat effects of the dissociation of H_2L^+ and HL^\pm particles was linear. The changes in heat capacity (ΔC_p) during the dissociation of the dipeptide were negative and fell in absolute

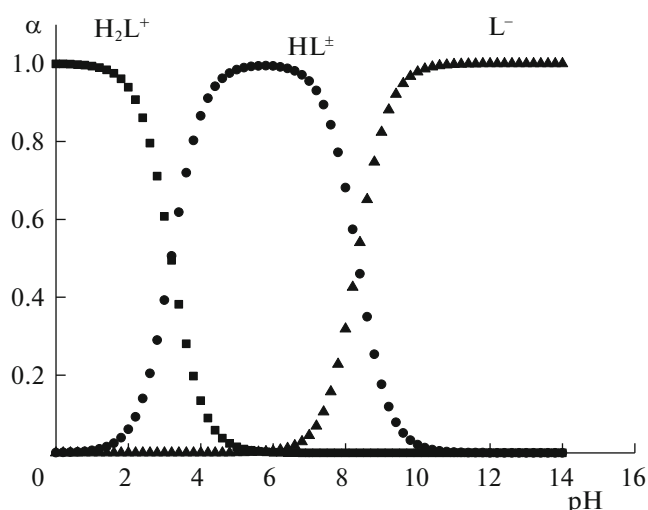


Fig. 1. Equilibrium diagram in DL-valyl-DL-leucine aqueous solution at 298.15 K.

value as the concentration of the background electrolyte rose.

As with similar structures [12, 13], the heat effect of HL^\pm dissociation changed negligibly in the investigated range of temperatures. Meanwhile, the rise in temperature from 288.15 to 308.18 K resulted in a substantial reduction of $\Delta_{\text{dis}}H(H_2L^+)$.

Temperature Θ at which the heat effect of dissociation of the carboxyl group changes sign can be estimated with the equation

$$\Theta = 298.15 - \Delta_r H_{298.15} / \Delta_r C_p. \quad (7)$$

The applicability of Eq. (7) in determining Θ values was demonstrated in [14] while studying the acid–base interaction in solutions of iminodiacetic and ethylenediaminetetraacetic acids.

It can be seen from the plots that the line crosses the x axis at a temperature of 277 K; i.e., the heat effect of process (1) is zero when $\Theta = 277$ K. This value of the temperature coincides with the one calculated with

Table 3. Standard thermodynamic characteristics (kJ/mol) of the acid–base reaction of DL-valyl-DL-leucine in an aqueous solution

T , K	pK°	ΔG° , kJ/mol	ΔH° , kJ/mol	$-\Delta S^\circ$, J/(mol K)	$-\Delta C_p^\circ$, J/(mol K)
$H_2L^+ = HL^\pm + H^+$					
288.15	3.24 ± 0.05	17.87 ± 0.11	-1.21 ± 0.25	66.2 ± 1.0	0.1 ± 15
298.15*	3.19 ± 0.01	18.21 ± 0.06	-2.31 ± 0.15	68.8 ± 0.5	
308.15	3.15 ± 0.05	18.58 ± 0.11	-3.36 ± 0.30	71.2 ± 1.0	
$HL^\pm = L^- + H^+$					
288.15	8.41 ± 0.03	46.39 ± 0.12	46.11 ± 0.25	0.9 ± 1.4	36 ± 15
298.15*	8.33 ± 0.01	47.55 ± 0.06	45.82 ± 0.28	5.8 ± 0.9	
308.15	8.25 ± 0.03	48.67 ± 0.17	45.38 ± 0.30	10.6 ± 1.3	

* Literature data.

Eq. (7). Since the endothermicity of process (1) rises along with the ionic strength, we would expect Θ to shift to the area of high temperatures as the concentration of background electrolyte grows.

This pattern was observed in studying dicarboxylic acids and hydroxy acids. The temperature coefficient of enthalpy of dissociation ΔC_p also changed considerably as the ionic strength rose. This was likely due to there being a deficit of water molecules in the solution at higher electrolyte concentrations, which reduced the contribution from freezing in the hydration shells of ions and lowered the absolute value of ΔC_p .

Raising the temperature intensified the chaotic thermal movement of water molecules, leading to destruction of its structure. The orientation of the molecules around the ions was therefore made easier, and a rise in temperature resulted in more negative values of $\Delta_{\text{dis}}S$ at a constant ionic strength, as was confirmed by the data in Table 3.

The change in entropy during the first stage of dipeptide dissociation was considerable in absolute value, and the growth of the anionic particle charge had a dramatic effects on the $\Delta_{\text{dis}}S$ value. When the temperature rose, the effect of water structuring by anionic particles was more pronounced, reducing the entropy of ionization. The increase in particle charge produced a more dramatic change in $\Delta_{\text{dis}}S$ as the temperature rose.

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