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Thermodynamic Characteristics of Acid–Base Equilibria of DL-α-Alanyl–DL-Norleucine in Aqueous Solutions at 298 K

A. I. Lytkin, V. V. Chernikov, O. N. Krutova, S. A. Bychkova, and I. A. Skvortsov
Ivanovo State University of Chemistry and Technology, Ivanovo, 153460 Russia
e-mail: kdvkonkpd@dsn.ru

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Abstract—Protolytic equilibria in aqueous solutions of DL-α-alanyl–DL-norleucine are studied via potentiometry and calorimetry. Measurements are made at 298.15 K and ionic strengths of 0.5, 1.0, and 1.5 (against a background of potassium nitrate). The thermodynamic characteristics (pK, ΔG, ΔH, ΔS) of the stepwise dissociation of the dipeptide both in aqueous–salt solutions and in standard solution are obtained for the first time.

Keywords: thermodynamics, solutions, chemistry, calorimeter, peptides.

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INTRODUCTION

Data on the thermodynamic characteristics of dipeptide solutions are needed in the many fields where these compounds are used (pharmacology, medicine, the food and cosmetic industries) for the development, validation, and optimization of technological processes involving such compounds and their complexes with metals. This information can be used to study the thermodynamic properties of more complex protein systems, and for the needs of biotechnology.

The dissociation of DL-α-alanyl–DL-norleucine ($NH_3^+CH(CH_3)CONHCH{-}CH_2CH(CH_3)_2COO^-$) in aqueous solutions can be represented by the following scheme:

$$H_2L^+ \leftrightarrow HL^{\pm} + H^+, \quad (1)$$

$$HL^{\pm} \leftrightarrow L^- + H^+. \quad (2)$$

There is no information on the thermodynamics of acid-base equilibria involving DL-α-alanyl–DL-norleucine ($C_9H_{18}N_2O_3$) in the literature. The aim of this work was to determine the dissociation and protonation constants of the dipeptide via potentiometry and to measure the heat effects of the reactions at 298.15 K and $I = 0.5$, 1.0, 1.5 (KNO_3) by direct calorimetry, and to calculate the corresponding thermodynamic characteristics under standard conditions.

EXPERIMENTAL

Chromatographically homogeneous DL-α-alanyl–DL-norleucine (Reanal, Hungary) was used without further purification. The dipeptide solutions were prepared by dissolving precisely weighed samples of the reagent in freshly double-distilled water immediately prior to each experiment. Potassium nitrate (pure grade) was recrystallized twice from double-distilled water. Solutions of KOH and HNO_3 were prepared from reagent grade chemicals. The concentration of working solutions was determined using conventional titrimetric methods [1].

Potentiometric titration of DL-α-alanyl–DL-norleucine was performed at 298.15 K and ionic strength of 0.5 (KNO_3). Accurately measured volumes of the peptide solution with the required ionic strength were placed into a thermostatic cell. The peptide concentration was 0.003 mol/L. Titration was conducted with standard solutions of KOH (to determine the dissociation constant of betaine protons) and HNO_3 (to determine the protonation constant of carboxyl groups) containing a background electrolyte to avoid changes in ionic strength during titration, due to dilution; the amount of the added titrant was determined gravimetrically.

The equilibrium concentration of hydrogen ions was determined by measuring the emf of a circuit consisting of an ESL-43-07 glass electrode and an EVL-1MZ saturated silver chloride electrode. The glass electrode potential was controlled using a P-363/3 potentiometer. A pH-340 pH-meter-millivoltmeter was used as a null-instrument. The accuracy of measuring the potential was ±0.1 mV. The accuracy of maintaining the temperature of the potentiometric cell and electrodes was ±0.05 K. Before plotting a titration curve, the potentiometric cell was calibrated against standard solutions of HNO_3 and KOH containing

Table 1. Heat effects (J/mol) of interaction between a 0.02 M solution of DL-α-alanyl—DL-norleucine and HNO_3 solution (pH_{init} 3.5, pH_{fin} 2.5), $T = 298.15$ K

I	$-\Delta_{mix}H$	$-\Delta_{dil}H$	$\Delta_{dis}H(H_2L^+)$
0.5	2.28 ± 0.29	0.89 ± 0.25	1.39 ± 0.25
1.0	2.60 ± 0.20	0.63 ± 0.28	1.94 ± 0.28
1.5	2.90 ± 0.29	0.29 ± 0.28	2.52 ± 0.28

Table 2. Heat effects (kJ/mol) of interaction between HNO_3 solution (1.1673 mol/kg) and a 0.02 M solution of DL-α-alanyl—DL-norleucine (pH_{init} 9.4; pH_{fin} 7.9), $T = 298.15$ K

I	$-\Delta_{mix}H$	$-\Delta_{dil}H$	$\Delta_{dis}H(HL^{\pm})$
0.5	45.18 ± 0.29	1.08 ± 0.25	44.10 ± 0.35
1.0	46.31 ± 0.20	1.40 ± 0.28	44.91 ± 0.32
1.5	47.50 ± 0.29	1.75 ± 0.28	45.75 ± 0.37

Table 3. Standard thermodynamic characteristics (kJ/mol) of acid—base interaction between DL-α-alanyl—DL-norleucine and their temperature-dependent and -independent components ($T = 298.15$ K)

Process	pK^0	Δ_rH^0	Δ_rG^0	$-A$	$-\Delta_rH^0_{dep}$	$-\Delta_rH^0_{ind}$	Δ_rS^0, J/(mol K)
(1)	3.32 ± 0.04	0.86 ± 0.28	−18.95 ± 0.15	5196	1.07	1.93	−58.2 ± 1.0
(2)	7.82 ± 0.19	43.22 ± 0.45	−44.64 ± 0.35	2167	4.15	40.49	−4.8 ± 1.4

potassium nitrate to attain the required ionic strength.

Calorimetric measurements were performed on an ampoule calorimeter equipped with an isothermal shell and a KMT-14 thermistor temperature sensor with automatic recording of the temperature [2]. The operation of the calorimeter setup was verified using a conventional calorimetric standard: the heat effect of the dissolution of crystalline potassium chloride in water. Potassium chloride of reagent grade was recrystallized twice from double-distilled water and dried in an oven at 393.15 K up to a constant weight. The experimentally obtained heat effect from the dissolution of crystalline KCl in water ($\Delta_{sol}H(\infty H_2O) = -17.25 \pm 0.06$ kJ/mol) was consistent with the most reliable data in the literature [3], indicating that there was no detectable systematic error in the operation of the calorimeter setup.

The heat effects from mixing DL-α-alanyl—DL-norleucine solutions with HNO_3 solutions were measured in different pH ranges on a calorimeter equipped with an isothermal shell and automatic recording of the temperature—time curve [3]. The samples of the solutions were weighed on a VLR-200 balance with an accuracy of 2×10^{-4} g.

To determine the heat effect from the protonation of the carboxyl group of DL-α-alanyl—DL-norleucine, a 0.02 M solution of the peptide (pH_{init} 3.5) was used as the calorimeter liquid, and a precisely weighed sample of HNO_3 solution (concentration, 0.8904 mol/kg) was placed into an amploule. After mixing the solutions, pH was close to 2.5. The experimental data on the heat effects of mixing and dilution are given in Table 1.

The following procedure was used to determine the heat effect from the dissociation of $HL^{\pm}$: the heat effects of the interaction between HNO_3 solution (1.1638 mol/kg) and a 0.02 M solution of the peptide were measured in the pH range of 9.4—7.9, along with the corresponding heat effects from dilution. The data on the heat effects from mixing the solutions and diluting mineral acid in the solution of background electrolyte are presented in Table 2. The experiments were performed at 298.15 K and ionic strengths of 0.5, 0.1, and 1.5 (KNO_3).

The aim of this work was to study the effect the concentration of background electrolyte had on the heat effects from the dissociation of DL-α-alanyl—DL-norleucine via direct calorimetry, and to calculate the standard thermodynamic characteristics of acid—base interactions in solutions of the dipeptide.

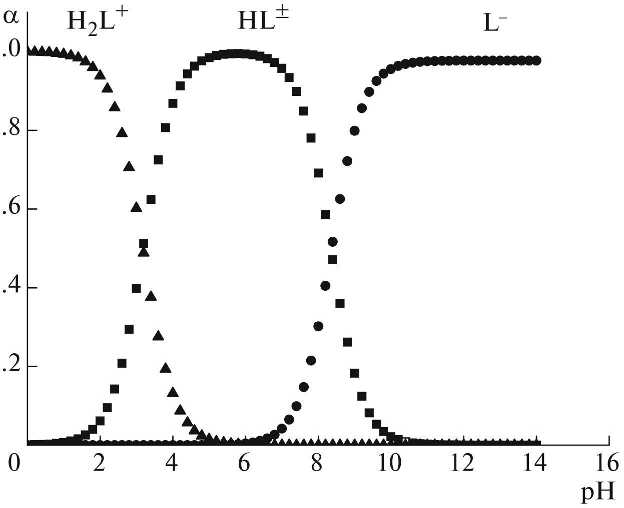
RESULTS AND DISCUSSION

Calculations for the dissociation and protonation constants of DL-α-alanyl—DL-norleucine were done using the PHMETR program [4], which is based on the principle of finding the minimum of criterial function F from the variation in pK values, determined at each iteration using a modified Hooke and Jeeves algorithm. The criterial function has the form

$$F = \Sigma(\log[H^+]_{j,exp} - \log[H^+]_{j,calc})^2 \rightarrow \min, \qquad (3)$$

where $\log[H^+]_{j,exp}$, $\log[H^+]_{j,calc}$ are the logarithms of the equilibrium concentrations of H^+, measured experimentally and calculated at the actual pK values. The calculation of the equilibrium concentrations was carried out according to the Brinkley method [5]. Dissociation constants were recalculated to zero ionic strength using the Davies equation. To interpret the results from calorimetric measurements, we used the concentration dissociation constants of the peptide, recalculated to fixed values of ionic strength using the equation proposed in [6]. The calculated pK^0 values are shown in Table 3.

The figure shows the diagram of equilibria in aqueous solution of DL-α-alanine-DL-norleucine, calculated using our pK values.



Equilibrium diagram for an aqueous solution of DL-α-alanyl–DL-norleucine at 298.15 K.

The heat effect from the dissociation of $HL^\pm$ was determined using the data on the heat effects from the interaction between HNO_3 solution and DL-α-alanyl–DL-norleucine solution in the pH range of 9.4–7.9. Since the degree of completeness of the protonation reaction under the conditions of our calorimetric experiments was more than 99.9%, the contribution from the heat effect from the formation of water from H^+ и OH^- was negligible, and the change in enthalpy was calculated using the relation

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

where $\Delta_{mix}H$ is the heat effect from the interaction between HNO_3 solution (1.1638 mol/kg) and a 0.02 M solution of the peptide in the pH range of 9.4–7.9; $\Delta_{dil}H$ is the enthalpy change during the dilution of HNO_3 solution in the background electrolyte solution. The heat effects that we obtained from the dissociation of betaine proton of DL-α-alanyl–DL-norleucine are shown in Table 2.

The heat effect from the dissociation of H_2L^+ particles ($\Delta_{dis}H(H_2L^+)$) was calculated using the equation:

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

where $\Delta_{mix}H$ is the heat effect from the interaction between 0.1M solution of HNO_3 and DL-α-alanyl–DL-norleucine solution, $\Delta_{dil}H$ is the heat effect of the dilution of a solution of mineral acid in a solution of the background electrolyte, and α_1 is the degree of completeness of the protonation reaction of $HL^\pm$ particle (α = 83%). The compositions of the solutions before and after each calorimetric experiment were calculated using the versatile RRSU program [6]. The results from our calculations are presented in Table 1.

The heat effects from the dissociation of DL-α-alanyl–DL-norleucine in standard solution were found by extrapolating the heats of stepwise dissociation at fixed values of ionic strength to zero ionic strength according to the equation proposed in [7]:

$$\Delta_r H_i - \Delta z^2 \psi(I) = \Delta_r H_i^0 + bI , \tag{6}$$

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

Points in the coordinates $(\Delta H - \Delta z^2\Psi(I))-I$ satisfactorily fit a straight line. The enthalpies of dissociation of D,L-α-alanyl–DL-norleucine at zero ionic strength were obtained using the least-squares method: $\Delta_{dis}H(H_2L^+)$ = 863 ± 250 J/mol and $\Delta_{dis}H(HL^+)$ = 43 220 ± 450 J/mol. The standard thermodynamic characteristics of the stepwise dissociation of DL-α-alanyl–DL-norleucine were obtained for the first time and are presented in Table 3.

An approach based on Herny's concepts proved to be useful for analyzing thermodynamic data on acid–base interactions and reactions of complex formation with bioligands, as was described in detail in [8]. The change in enthalpy can be presented as the sum of temperature-dependent ($\Delta_r H_{dep}$) and temperature-independent ($\Delta_r H_{ind}$) components:

$$\Delta_r H = \Delta_r H_{dep} + \Delta_r H_{ind}. \tag{7}$$

It was proposed in [8] that proton-accepting particles should be divided into two groups, depending on the contribution from $|\Delta_r H_{ind}| = |\Delta_r G_{ind}|$. The first group is characterized by a high value of this contribution and group comprises all the particles containing an amino group. Numerical values of the contribution from $|\Delta_r H_{ind}| = |\Delta_r G_{ind}|$ upon the protonation of particles of this type are, as a rule, 30–40 kJ/mol or more. This group is referred to as nonelectrostatic. The value of the temperature-dependent contribution upon the protonation of particles of this type is quite low. For particles from the second group, protonated with an oxygen atom, the magnitude of the temperature-independent contribution is far less and rarely exceeds 12 kJ/mol. The temperature-dependent contribution $\Delta_r H_{dep}$ is approximately the same for the particles of this group.

In our case, the dipeptide molecule contains both of these functional groups. The protonation of the $-NR_2$ group obviously occurs in the same manner as for particles belonging to the first group (for DL-α-alanyl–DL-norleucine $\Delta_r H_{ind}$ = 40.49 kJ/mol), while the protonation of the oxygen-containing moiety proceeds in the same manner as that of the particles from the second group (for DL-α-alanyl–DL-norleucine $\Delta_r H_{\text{ind}} = -1.93$ kJ/mol) (Table 3).

The inductive effect is weakened upon moving from amino acids to peptides, since the amino groups are separated from the carboxyl groups by the greater number of carbon atoms in the peptides than in the amino acids. The reduction in the inductive effect upon moving from amino acids to peptides weakens the nitrogen–hydrogen bonds in the latter, so the charged amino groups of the peptides can bind more water than the NH_3^+ groups in amino acid. This is particularly evident in that $\left|\Delta S_1^0\right|$ upon the dissociation of peptides is greater in magnitude than $\left|\Delta S_1^0\right|$ upon the dissociation of amino acids [9]. For α-alanine, $\Delta S_1^0 = -35.2 \pm 0.5$ J/(mol K); for D,L-α-alanyl–DL-norleucine, $\Delta S_1^0 = -58.2 \pm 1.0$ J/(mol K).

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