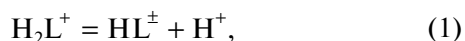


Fig. 1. Equilibrium diagram in an aqueous solution of glycyl-D-phenylalanine at $T = 298.15$ K.

Protolytic equilibria in an aqueous solution of glycyl-D-phenylalanine can be described by the equations



In a neutral environment, the dipeptides are in the zwitterionic form HL^\pm . The fractional distribution of H_2L^+ , HL^\pm , and L^- species at different pH values (Fig. 1) indicates the possibility of independent determination of the thermal effects of the dissociation of the cationic acid and zwitterion of the dipeptide (processes (1) and (2)). It follows from the diagram that only HL^\pm and H_2L^+ are present in solution at pH of 1–4; only L^- and HL^\pm are present at pH of 7–11; and all the three species, at pH of 4–7. Based on the equilibrium diagram in an aqueous solution of the peptide, the concentration conditions for the calorimetric experiment were chosen.

The equilibrium constants of these processes K_1 and K_2 , respectively, were found quite reliably [9–14]; the literature data are presented in Table 1. These studies were performed at various ionic strengths against the background of different supporting elec-

Table 1. Literature data on the constants of stepwise dissociation of glycyl-phenylalanine (determined by potentiometric titration)

Reference	Medium	T , K	$\text{p}K_1$	$\text{p}K_2$
[9]	0.1 M (NaNO_3)	298	3.24	7.92
[10]	0.15 M (NaClO_4)	310	2.87	8.03
[11]	0.2 M (KNO_3)	298	2.99	8.08
[12]	0.1 M (KNO_3)	298	3.07	8.96
[13]	0.2 M (KCl)	298	2.99	8.09
[14]	0.1 M (KNO_3)	298	2.983	8.157

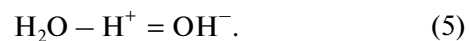
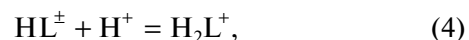
trolytes. Therefore, in order to compare and analyze the literature data, it is necessary to find the most probable values of dissociation constants for each value of ionic strength. The constants thus obtained were extrapolated to zero ionic strength by the least squares method using an equation with one individual parameter [15].

The aim of this work was direct calorimetric determination of the thermal effects of dissociation and neutralization of glycyl-D-phenylalanine in the presence of various electrolytes at several values of ionic strength and the calculation of the corresponding standard values.

EXPERIMENTAL

Glycyl-D-phenylalanine (ReaKhim, 98.8% main component) was used without additional purification. The calorimetric measurements were performed in the ampule of a calorimeter with an isothermal shell and a KMT-14 thermistor temperature sensor, while automatically recording the temperature changes over time [16, 17]. The operation of the calorimeter was checked according to the thermal effect of the potassium chloride dissolution in water. The agreement of the experimentally measured values with the most reliable literature data [18] indicated the absence of noticeable systematic errors in the operation of the calorimeter. To maintain the given value of the ionic strength of the solution, potassium and sodium nitrates and sodium chloride were used, which were prepared from recrystallized reagents of reagent grade. The samples of solutions were weighed on a VLR-200 balance with an accuracy of 2×10^{-4} g.

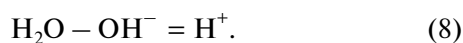
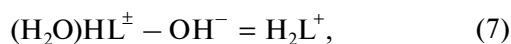
When determining the thermal effect of dissociation of H_2L^+ , it was taken into account that the following processes occur in the system:



The following procedure was used to determine the thermal effect of proton addition to the carboxyl group of glycyl-D-phenylalanine. A 0.01 M solution of the peptide ($\text{pH}_{\text{init}} 3.6$) served as a calorimetric liquid. The pH was created by introducing an exact amount of the supporting electrolyte. The ampule contained accurate samples of solutions of HNO_3 (with a concentration of 0.5903 mol/kg, 0.6131 M) or HCl (with a concentration of 0.9902 mol/kg, 1.0355 M). After mixing, the pH of the solutions was close to 2.0. In this way the enthalpies of interaction ($\Delta_{\text{mix}}H_1$) of a mineral acid solution with a peptide solution were determined (Table 2). In addition, the enthalpies of dilution ($\Delta_{\text{dil}}H_1$) were determined in a separate series of experiments. The ampule contained solutions of mineral

acids (of the same concentration as in the first series of the experiment when determining ($\Delta_{\text{mix}}H_1$), and supporting electrolyte solutions served as a calorimetric liquid. The measurements were performed at ionic strengths of solution of 0.5, 0.75, and 1.0 M. The experimental data are given in Table 2.

When determining the thermal effects of peptide neutralization in the system, the following processes were considered:



The thermal effect of glycyl-D-phenylalanine dissociation at the second step was studied at pH in the range from 10.2 to 8.1. For this, we measured the thermal effects ($\Delta_{\text{mix}}H_2$) of the interaction of a 0.02 M glycyl-D-phenylalanine solution with solutions of carbonate-free hydroxides NaOH, LiOH, and KOH, prepared by the standard procedure from reagents of reagent grade. When determining the enthalpies of dilution ($\Delta_{\text{dil}}H_2$), pH of the supporting electrolyte solution was set equal to the initial pH of the peptide solution; thus, dilution occurred without changing the equilibrium composition of the system. To maintain the given ionic strength, exact amounts of NaCl, NaClO₄, NaNO₃, KNO₃, and LiNO₃ were introduced in the solution. In the initial solution, pH 8.1 was created by adding the required amount of the corresponding alkali. The experimental data in the form of arithmetic means over three or four experiments are given in Table 3.

RESULTS AND DISCUSSION

The change in enthalpy during the acid dissociation of the carboxyl group of glycyl-D-phenylalanine $\Delta_{\text{dis}}H(\text{H}_2\text{L}^+)$ was found by the equation

$$\Delta_{\text{dis}}H(\text{H}_2\text{L}^+) = -(\Delta_{\text{mix}}H_1 - \Delta_{\text{dil}}H_1)/\alpha_1, \quad (9)$$

where $\Delta_{\text{mix}}H_1$ is the thermal effect of the interaction of a mineral acid solution with a peptide solution; $\Delta_{\text{dil}}H_1$ is the change in the enthalpy of dilution of a mineral acid solution in a supporting electrolyte solution; and α_1 is the completeness of protonation of HL^\pm .

The equilibrium composition of solutions before and after the calorimetric experiment was calculated using the universal KEV program [19]. The calculation showed that nitric and hydrochloric acids react with the zwitterion according to a scheme that is the reverse of process (1) by 70–80%. The thermal effects of dissociation of glycyl-D-phenylalanine at the first step at different values of ionic strength are given in Table 2.

Table 2. Thermal effects (J/mol) of the interaction of a 0.01 M glycyl-D-phenylalanine solution with solutions of HNO₃ (0.6131 M) and HCl (1.0355 M), thermal effects of dilution of mineral acids in supporting electrolyte solutions, and $\Delta_{\text{dis}}H(\text{H}_2\text{L}^+)$ values of the peptide calculated from them

Electrolyte	<i>I</i> , M	$\Delta_{\text{mix}}H_1$	$\Delta_{\text{dil}}H_1$	$\Delta_{\text{dis}}H(\text{H}_2\text{L}^+)$
NaCl	0.5	303 ± 120	1200 ± 115	2214 ± 120
	0.75	3150 ± 110	1034 ± 120	2550 ± 130
	1.0	3004 ± 100	627 ± 110	2864 ± 110
NaNO ₃	0.5	2494 ± 100	711 ± 110	2148 ± 120
	0.75	3104 ± 120	1080 ± 100	2439 ± 130
	1.0	3591 ± 100	1339 ± 110	2714 ± 120
KNO ₃	0.5	2268 ± 120	562 ± 120	2048 ± 120
	0.75	2551 ± 100	638 ± 100	2320 ± 100
	1.0	2825 ± 100	706 ± 120	2573 ± 120

Table 3. Thermal effects (J/mol) of the interaction of a glycyl-D-phenylalanine solution with KOH, NaOH, and LiOH solutions and thermal effects of dilution of the peptide solution in solutions of the corresponding supporting electrolytes $T = 298.15$ K

Electrolyte	<i>I</i> , mol/L	$-\Delta_{\text{mix}}H_2$	$-\Delta_{\text{dil}}H_2$	$(\Delta_{\text{mix}}H_2 - \Delta_{\text{dil}}H_2)/\alpha_2$
NaCl (NaOH)	0.5	9373 ± 210	460 ± 110	9833 ± 230
	0.75	8417 ± 230	376 ± 130	8793 ± 250
	1.0	6571 ± 200	1046 ± 130	7617 ± 270
NaClO ₄ (NaOH)	0.5	9149 ± 190	502 ± 100	9651 ± 260
	0.75	7850 ± 200	627 ± 130	8477 ± 260
	1.0	6374 ± 210	836 ± 110	7210 ± 230
NaNO ₃ (NaOH)	0.5	8759 ± 200	711 ± 110	9470 ± 270
	0.75	7406 ± 200	836 ± 100	8242 ± 280
	1.0	4995 ± 210	1673 ± 120	6668 ± 260
LiNO ₃ (LiOH)	0.5	8073 ± 190	2120 ± 120	10193 ± 260
	0.75	7480 ± 210	1673 ± 130	9153 ± 230
	1.0	6957 ± 180	1339 ± 120	8296 ± 250
KNO ₃ (KOH)	0.5	9698 ± 260	815 ± 120	10513 ± 260
	0.75	8600 ± 210	1027 ± 100	9627 ± 220
	1.0	6693 ± 200	2121 ± 120	8814 ± 220

The thermal effect of dissociation of the betaine proton of the peptide ($\Delta_{\text{dis}}H(\text{HL}^\pm)$) was calculated by the equation

$$\Delta_{\text{dis}}H(\text{HL}^\pm) = \left(\frac{\Delta_{\text{mix}}H_2/\Delta_{\text{dil}}H_2}{m_{\text{HL}^\pm}} \right) = \left(\frac{\Delta[\text{L}^-]}{C_{\text{HL}^\pm}} \right) \Delta H_6 + \left(\frac{\Delta[\text{H}_2\text{L}^+]}{C_{\text{HL}^\pm}} \right) \Delta H_7 + \left(\frac{\Delta[\text{H}^+]}{C_{\text{HL}^\pm}} \right) \Delta H_8 + \Delta H_w, \quad (10)$$

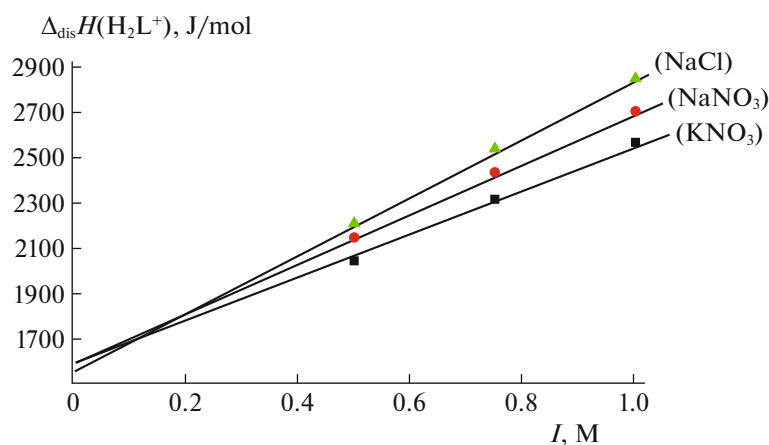


Fig. 2. Determination of the standard thermal effects of dissociation of glycyl-D-phenylalanine at the first stage at 298.15 K.

where $\Delta[L^-]$, $\Delta[H_2L^+]$, and $\Delta[H^+]$ is the difference between the final and initial equilibrium concentrations of the corresponding species; m_{HL^\pm} is the mass of the peptide; and C_{HL^\pm} is the peptide concentration (M).

Since the completeness of the peptide neutralization process was 99.9%, and, consequently, the contributions of other terms can be neglected, Eq. (10) can be written in a simplified form:

$$\Delta_{\text{dis}}H(HL^\pm) = (\Delta_{\text{mix}}H_2 - \Delta_{\text{dil}}H_2)/\alpha_2 + \Delta H_w, \quad (11)$$

where $\Delta_{\text{mix}}H_2$ is the thermal effect of the interaction of the peptide solution with the alkali solution; $\Delta_{\text{dil}}H_2$ is the thermal effect of dilution of the peptide solution in the supporting electrolyte solution; α_2 is the completeness of peptide neutralization; and ΔH_w is the thermal effect of water dissociation in the supporting electrolyte solution.

The value of ΔH_w was taken from [20] for the supporting electrolytes used in our work. The error was determined as the standard deviation of the average value from three or four parallel experiments.

The enthalpies of stepwise dissociation of glycyl-D-phenylalanine at zero ionic strength were found using the equation with one individual parameter [15]:

$$\Delta H - \Delta Z^2\Psi(I) = \Delta H^\circ + bI, \quad (12)$$

where ΔH and ΔH° is the change in enthalpy at the final value of the ionic strength and at $I = 0$, respectively; $\Psi(I)$ is the theoretically calculated function of the ionic strength; ΔZ^2 is the difference in the squares of the charges of the reaction products and the initial components; and b is the empirical coefficient.

For processes with $\Delta Z^2 = 0$, Eq. (12) takes the form

$$\Delta H = \Delta H^\circ + bI. \quad (13)$$

The results of graphical processing of the obtained experimental data on the thermal effects of dissocia-

tion and neutralization of glycyl-D-phenylalanine are presented in Figs. 2 and 3. It can be seen that the points quite satisfactorily fit the straight lines for each of the supporting electrolytes NaCl, NaClO₄, NaNO₃, KNO₃, and LiNO₃, and that the segments cut off by these straight lines on the y axis give the values of the standard thermal effect. The most probable values of standard thermal effects are found as the arithmetic mean of the ΔH° values obtained for each of the supporting electrolytes.

According to Fig. 2, an increase in the concentration of NaCl, NaNO₃, and KNO₃ causes some increase in the endothermicity of acid dissociation of the dipeptide. However, even at $I = 1.0$ mol/L, the difference in the enthalpies of dissociation of glycyl-D-phenylalanine at the first step, found against the “background” of NaCl and KNO₃, is only 300 J/mol. Thus, it can be noted that for both aliphatic amino acids and dipeptides, there is no significantly different effect of the nature of supporting electrolytes on the thermal effect of acid dissociation.

The standard thermodynamic characteristics of the stepwise dissociation of glycyl-D-phenylalanine are presented in Table 4. It was noted [21] that the equilibrium constants related to the final value of ionic strength are currently often used to calculate the

Table 4. Standard thermodynamic characteristics of dissociation and neutralization of glycyl-D-phenylalanine at a temperature of 298.15 K

Characteristic	Process	
	$H_2L^+ = HL^\pm + H^+$	$HL^\pm = L^- + H^+$
$\Delta_r H^\circ$, kJ/mol	1.5 ± 0.26	44.32 ± 0.42
$\Delta_r G^\circ$, kJ/mol	17.00 ± 0.10	46.40 ± 0.10
$\Delta_r S^\circ$, kJ/(mol K)	-51.9 ± 1.0	-6.9 ± 1.4

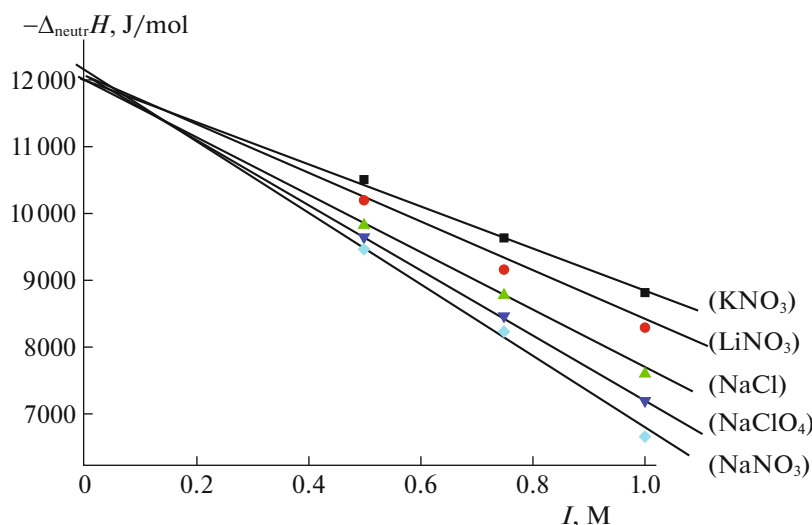


Fig. 3. Determination of the standard thermal effects of neutralization of glycyl-D-phenylalanine at 298.15 K (second stage).

change in the standard Gibbs energy and, further, to calculate the change in entropy. Numerous literature data also show that the standard thermodynamic functions are often calculated from the concentration constants found at ionic strengths of 0.1, 0.5, 3.0, etc. created by different supporting electrolytes.

We recalculated the constants for zero ionic strength of solution using the Davies equation for 298.15 K. These are exactly the parameters ($I = 0.0$ M and $T = 298.15$ K) that characterize the standard state, to which the entropies calculated by us refer.

The stepwise dissociation of peptides, as well as aliphatic amino acids, is accompanied by a negative and small change in entropy [22–24]. This significantly distinguishes the dissociation of amino acids in an aqueous solution from the ionization of weak organic acids [25, 26].

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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