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PHYSICAL CHEMISTRY OF SOLUTIONS

Standard Enthalpies of Formation of Glycyl-Phenylalanine and Products of Its Dissociation in an Aqueous Solution

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Abstract—Direct calorimetry is used to study the heats of dissolution of crystalline glycyl-phenylalanine in water and solutions of potassium hydroxide at 298.15 K. The standard enthalpy of formation of glutathione in the crystalline state is calculated according to additive groups, based on group systematics with fragments classified in a manner similar to Benson's approach, which considers the influence of the primary environment for atoms. The standard enthalpies of formation are calculated for glycyl-phenylalanine and products of its dissociation in an aqueous solution.

Keywords: glycyl—phenylalanine, peptides, calorimetry, enthalpy, solutions

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INTRODUCTION

It is important to obtain new data on the enthalpies of dissolution of amino acids and dipeptides in water, since features of the solvation (hydration) of simple structural elements (amino acids and oligopeptides) largely determine the behavior of complex biosystems. It is not surprising that these compounds are of great interest to numerous researchers who consider them from various points of view [1–4].

There are reliable data in the literature on the constants of ionization of glycyl-phenylalanine (Table 1) [5–10]. These works were performed at different ionic strengths of the solution, against backgrounds of supporting electrolytes that differ in nature. We recalculated the values of pK_1 and pK_2 to zero ionic strength in order to compare constants obtained by different authors for the stepwise dissociation of the peptide.

Constants of the dissociation of glycyl-phenylalanine to zero ionic strength were recalculated using the Davis equation [11] (for $I < 0.5$)

$$\mathrm{p}K^o = \mathrm{p}K^c + A\Delta Z^2\left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.2I\right) \qquad (1)$$

and Eq. (2) (for $I > 0.5$):

$$\mathrm{p}K^o = \mathrm{p}K^c + A\Delta Z^2\left(\frac{\sqrt{I}}{1+1.6\sqrt{I}} - 0.05I\right) - \delta I, \qquad (2)$$

where pK^c and pK^o are the negative logarithms of the constants of concentration and thermodynamic dissociation; ΔZ^2 is the difference between the squares of the charges of the reaction products and initial materials; A is the constant of the limiting Debye law, equal to 0.5107 at 25°C; δ is an empirical coefficient equal to 0.05; and I is the ionic strength of the solution (mol/L). The thermodynamic constants of stepwise dissociation were also determined graphically [12]:

$$\mathrm{p}K^c = \mathrm{p}K^o - \Delta Z^2 A\frac{\sqrt{I}}{1+1.6\sqrt{I}} + \delta I. \qquad (3)$$

After processing the literature data, we may assume the constants of stepwise dissociation are the most probable values of the constants of thermodynamic dissociation at 298.15 K: $\mathrm{p}K_1^\circ = 2.98 \pm 0.03$, $\mathrm{p}K_2^\circ = 8.13 \pm 0.03$.

The aim of this work was to determine the standard enthalpies of formation of glycyl-phenylalanine and products of its dissociation in an aqueous solution according to the thermal effects of dissolving the peptide in water and in aqueous solutions of KOH at 298.15 K.

Table 1. Literature data on the constants of the stepwise dissociation glycyl-phenylalanine

Source	Medium	T, K	pK_1	pK_2
[5]	0.1 M ($NaNO_3$)	298	3.24	7.92
[6]	0.15 M ($NaClO_4$)	310	2.87	8.03
[7]	0.2 M (KNO_3)	298	2.99	8.08
[8]	0.1 M (KNO_3)	298	3.07	8.96
[9]	0.2 M (KCl)	298	2.99	8.09
[10]	0.1 M (KNO_3)	298	2.983	8.157

Table 2. Enthalpies of dissolution glycyl-phenylalanine in water at 298.15 K

$m_{\rm p}$, г	$M \times 10^3$, mol $HL^{\pm}$/1000 kg H_2O	C, mol H_2O/mol $HL^{\pm}$	$\Delta_{\rm sol}H$, kJ/mol	$-\Delta_{\rm f}H^\circ(HL^{\pm}$, sol., $n H_2O$, hyp., undiss., 298.15 K), kJ/mol
0.0058	0.619	89608	13.51 ± 0.25	698.1
0.0074	0.790	70233	13.55	698.1
0.0085	0.907	61144	13.62	698.2
0.0096	1.025	54138	13.68	698.2
0.0125	1.335	41578	13.69	698.2
0.0236	2.520	22022	13.85	698.4
0.0244	2.606	21300	13.89	698.4
0.0314	3.353	16552	13.92	698.5
0.0421	4.496	12345	14.06	698.6
0.0568	6.066	9150	14.35	698.9
0.0672	7.177	7734	14.32	698.9
0.0895	9.558	5807	14.43	699.0
0.0912	9.740	5699	14.51	699.1
0.1256	13.414	4138	14.59	699.1

$m_{\rm P}$ is the weighed peptide; C is breeding.

EXPERIMENTAL

ReaChem crystalline glycyl-phenylalanine was used in this work. The content of the main component was 98.8% with no additional purification. Before use, the crystalline peptide was dried to constant weight at 353 K. The operation of the calorimetric setup [13, 14] was tested against a generally accepted calorimetric standard (the heat of dissolution of crystalline potassium chloride in water). The KCl preparation was purified via double recrystallization of the chemically pure reagent from bidistillate. Before sampling, the potassium chloride was dried to constant weight in an oven at 393.15 K. The match between experimentally obtained heats of dissolution KCl(cr.) in water $\Delta_{\rm sol}H(\infty H_2O) = 17.25 \pm 0.06$ kJ/mol and the most reliable published data [15] showed there was no notable systemic error in the operation of the calorimetric setup. Samples of the solutions were weighed on a VLR-200 balance with an accuracy of 2×10^{-4} g.

The confidence interval of the mean ΔH was calculated with a probability of 0.95. The equilibrium composition of the solutions was calculated using the RRSU program, allowing for the simultaneous occurrence of several processes of acid–base interaction and the dissociation of water [16].

RESULTS AND DISCUSSION

The dissolution of glycyl-phenylalanine in water can be described by the scheme

$$HL^{\pm}(\text{cr.}) + nH_2O = HL^{\pm}(\text{sol.}, nH_2O). \qquad (4)$$

Standard enthalpies of formation of glycyl-phenylalanine in solution at different dilutions were calculated using the equation

$$\begin{aligned} &\Delta_{\rm f}H^\circ(HL^{\pm}, \text{sol.}, nH_2O, 298.15\ \text{K}) \\ &= \Delta_{\rm f}H^\circ(HL^{\pm}, \text{cr.}, 298.15\ \text{K}) \\ &+ \Delta_{\rm sol}H(HL^{\pm}, \text{cr.}, 298.15\ \text{K}), \end{aligned} \qquad (5)$$

where $\Delta_{\rm f}H^\circ(HL^{\pm}$, cr., 298.15 K) is the standard enthalpy of formation of crystalline glycyl-phenylalanine, and $\Delta_{\rm sol}H(HL^{\pm}$, 298.15 K) is the heat of dissolution of the peptide (Table 2).

Standard enthalpies of the combustion and formation of glycyl-phenylalanine were calculated using the additive group approach [17–19], based on group systemics with a classification of fragments similar to Benson's, which considers the influence of the initial environment for atoms. The enthalpies of combustion and formation of the test compound were calculated using the formula

$${}_{\rm c(f)}H^\circ_{\rm (s)} = \sum A_i \Delta_{\rm c(f)} H_i^\circ, \quad i = 1, 2, 3, \ldots, n, \qquad (6)$$

where $\Delta_{\rm c(f)}H_i^\circ$ is the energy contribution to the heat of combustion and formation of a certain atomic group, H_i° is the number of such atomic groups in a molecule, and n is the number of types of atomic groups in a molecule.

The initial data for calculating $\Delta_{\rm f}H^\circ_{\rm (s)}(C_{11}H_{14}N_2O_3) = -690.4 \pm 1.9$ kJ/mol and $\Delta_{\rm c}H^\circ_{\rm (s)}(C_{11}H_{14}N_2O_3) = -5678.8 \pm 1.9$ kJ/mol are presented in Table 3.

Heat of combustion $\Delta_c H^\circ_{(s)}(C_{11}H_{14}N_2O_3)$ = −5645.1 kJ/mol of the given dipeptide was determined experimentally in [20]. The energies of the combustion of the compound were associated with the combustion reaction, described by the general scheme

$$C_{11}H_{14}N_2O_3 + 13O_2 \rightarrow 11CO_2 + 7H_2O + N_2. \quad (7)$$

The enthalpy of formation of crystalline glycyl-phenylalanine was calculated using the formula

$$\begin{aligned}&\Delta_f H^\circ(C_{11}H_{14}N_2O_3,\ \text{cr., } 298.15\ \text{K})\\ &= 11\Delta_f H^\circ(CO_2,\ \text{g, } 298.15\ \text{K})\\ &+ 7\Delta_f H^\circ(H_2O,\ \text{l, } 298{,}15\ \text{K})\\ &- \Delta_c H^\circ(C_{11}H_{14}N_2O_3,\ \text{cr., } 298.15\ \text{K}).\end{aligned} \quad (8)$$

Standard enthalpies of formation of CO_2 and H_2O were taken from [21]: $-\Delta_f H^\circ(CO_2,\ \text{g, } 298.15\ \text{K})$ = 393.51 ± 0.05 kJ/mol and $-\Delta_f H^\circ(H_2O,\ \text{l, } 298.15\ \text{K})$ = 285.83 ± 0.04 kJ/mol. As a result, $\Delta_f H^\circ_{(s)}(C_{11}H_{14}N_2O_3) = -684.3$ kJ/mol.

Table 2 shows that the heat of formation of glycyl-phenylalanine in an aqueous solution in the considered range of concentrations is virtually independent of the degree of dilution, which is not surprising for such high values.

The standard enthalpy of formation of a glycyl-phenylalanine zwitterion in a hypothetically undissociated state upon final dilution in an aqueous solution was found using the equation

$$\begin{aligned}&\Delta_f H^\circ(HL^{\pm},\ \text{sol., } nH_2O,\ \text{std.s., undiss., } 298.15\ \text{K})\\ &= \Delta_f H^\circ(HL^{\pm},\ \text{sol., } nH_2O,\ 298.15\ \text{K})\\ &+ \alpha(H_2L^+)\Delta_{dis}H^\circ(H_2L^+) - \alpha(L^-)\Delta_{dis}H^\circ(HL^{\pm}),\end{aligned} \quad (9)$$

where $\alpha(H_2L^+)$, $\alpha(L^-)$ are fractions of particles H_2L^+ and L^-, respectively, calculated using the RRSU universal program [16]. The value of $\alpha(H_2L^+)$ varied from 0.00144 to 0.00234; that of $\alpha(L^-)$, from 0.000534 to 0.000323 at the given concentrations. The thermal effects of the stepwise dissociation of particle H_2L^+ were $\Delta_{dis}H(H_2L^+)$ = 1.7 ± 0.25 kJ/mol and $\Delta_{dis}H(HL^{\pm})$ = 43.9 ± 0.32 kJ/mol.

Values of $\Delta_{dis}H^\circ(H_2L^+)$ and $\Delta_{dis}H^\circ(HL^{\pm})$ were determined in [21]. The total contribution from the second and third terms on the right side of Eq. (9) did not exceed 0.25 kJ/mol or change in the considered range of concentrations.

The standard enthalpy of formation of glycyl-phenylalanine in a hypothetical non-dissociated state at infinite dilution was found by extrapolating values obtained with Eq. (9) to the zero value of molality m of the solution (Fig. 1).

Least squares were used to find

$$\Delta_f H^\circ(HL^{\pm},\ \text{sol., } H_2O,\ \text{std. s., hyp. undiss., } 298.15\ \text{K}) = -698.1 \pm 1.9\ \text{kJ/mol}.$$

Table 3. Individual contributions of energy to the enthalpies of formation of glycyl-phenylalanine, kJ/mol

Group	$-\Delta_f H_i^\circ$	$-\Delta_c H_i^\circ$
(C)–NH_2	50.8 ± 21.2	232.1 ± 20.4
(N)$(C)_2$–CH	21.6 ± 20.4	516.7*
(C)(N)–C=O	182.3 ± 20.6	211.7*
$(C)_2$–NH	−28.9 ± 38.1	168.1 ± 36.7
(C)–COOH	435.3 ± 4.3	100.7 ± 2.2
$(C)_2$–CH_2	27.8 ± 1.8	651.7 ± 22.3
(C)–C_6H_5	−41.1*	3127.5*
(C)(N)–CH_2	42.6 ± 21.6	640.3 ± 19.9

Asterisks mark the values given by the authors without error. Number n of the types of atomic groups in a molecule is 1.

The standard enthalpy of formation for particle L^- in an aqueous solution was determined using data on the heat of dissolution of the peptide in alkali solutions at an equivalent ratio of at least 1 : 2 (Table 4). The dissolution of the peptide in a solution of KOH is described by the scheme

$$\begin{aligned}&HL^{\pm}(\text{cr.}) + OH^-(\text{sol., } nH_2O)\\ &= L^-(\text{sol., } nH_2O) + H_2O(\text{l}).\end{aligned} \quad (10)$$

Calculations showed that reaction (10) was at least 99.9% complete.

Since $\Delta z^2 = 0$ in reaction (10), the thermal effects of peptide dissolution at zero ionic strength were calculated using the equation [12]

$$\Delta_r H_{(10)} = \Delta_r H^\circ_{(10)} + iI, \quad (11)$$

where $\Delta_r H_{(10)}$ and $\Delta_r H^\circ_{(10)}$ are thermal effects of process (10) at finite and zero values of the ionic strength.

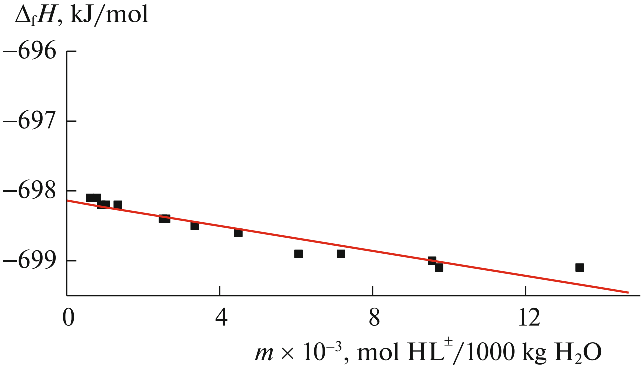

Fig. 1. Graphic definition of the standard enthalpy of formation glycyl-phenylalanine in a hypothetical undissociated state at infinite dilution.

Table 4. Enthalpies of dissolution of glycyl-phenylalanine in solution KOH at different concentrations and $T = 298.15$ K (kJ/mol)

m, g	c°_{KOH}, mol/L	$\Delta_{\mathrm{sol}}H$ kJ/mol
0.0202	0.004273	26.73 ± 0.27
0.0203		26.55 ± 0.26
0.0201		26.58 ± 0.28
0.0406	0.008548	27.35 ± 0.25
0.0407		27.42 ± 0.28
0.0407		27.34 ± 0.28
0.0609	0.01282	28.19 ± 0.25
0.0609		28.21 ± 0.27
0.0610		28.18 ± 0.26

m is the weighing weight.

Table 5. Standard enthalpies of formation of glycyl-phenylalanine and its dissociation products in aqueous solution

Particle	State	$\Delta_f H^{\circ}$(298.15 K), kJ/mol
$HL^{\pm}$	Cryst.	−684.3 ± 1.9
	sol., H_2O std. s., hyp. undis.	−698.1 ± 1.9
H_2L^{+}	sol., H_2O std. s., hyp. undis.	−699.6 ± 1.9
L^{-}	sol., H_2O std. s.	−654.3 ± 1.9

We calculated the standard enthalpy of anion formation using the obtained values of $\Delta_r H^{\circ}_{(10)}$ and definition $\Delta_f H^{\circ}(OH^-$, sol., H_2O std. s., 298.15 K), $\Delta_f H^{\circ}(H_2O$, l, 298.15 K) recommended in [22]:

$$\begin{aligned}\Delta_f H^{\circ}(L^-,\ \text{sol., } H_2O,\ \text{std. s., } 298.15\ \text{K}) \\ = \Delta_f H^{\circ}(HL^{\pm},\ \text{cr., } 298.15\ \text{K}) \\ + \Delta_f H^{\circ}(OH^-,\ \text{sol., } H_2O,\ \text{std. s., } 298.15\ \text{K}) \\ + \Delta_r H^{\circ}_{(10)} - \Delta_r H^{\circ}(H_2O,\ \text{l, } 298.15\ \text{K}).\end{aligned} \tag{12}$$

The standard enthalpy of formation of particle $HL^{\pm}$ in std. s., hip. non-diss. was also calculated using the equation

$$\begin{aligned}\Delta_f H^{\circ}(HL^{\pm},\ \text{sol., } H_2O,\ \text{std. s., hyp. undiss.,} \\ 298.15\ \text{K}) = \Delta_f H^{\circ}(L^-,\ \text{sol., } H_2O,\ \text{std. s.,} \\ 298.15\ \text{K}) - \Delta_{\text{dis}} H^{\circ}(HL^{\pm},\ 298.15\ \text{K}).\end{aligned} \tag{13}$$

The standard enthalpy of formation of the peptide's zwitterion agrees satisfactorily with the value obtained earlier. The weighted average value based on results from two independent determinations was assumed to be the one most likely: $\Delta_f H^{\circ}(HL^{\pm}$, sol., H_2O std. s., hyp. undiss., 298.15 K) = −698.1 ± 1.9 kJ/mol.

The standard enthalpy of formation of particle H_2L^+ was calculated using the equation

$$\begin{aligned}\Delta_f H^{\circ}(H_2L^+,\ \text{sol., } H_2O,\ \text{std. s., hyp. undiss.,} \\ 298.15\ \text{K}) = \Delta_f H^{\circ}(HL^{\pm},\ \text{sol., } H_2O,\ \text{std. s.,} \\ \text{hyp. undiss., } 298.15\ \text{K}) \\ - \Delta_{\text{dis}} H^{\circ}(H_2L^+,\ 298.15\ \text{K}).\end{aligned} \tag{14}$$

CONCLUSIONS

Standard enthalpies of formation of glycyl-phenylalanine and products of its dissociation in an aqueous solution (Table 5) were obtained for the first time. They are the key quantities in the thermochemistry of the peptide and allow us to perform rigorous thermodynamic calculations for systems with glycyl-phenylalanine.

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