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Dissociation Constants of Protolytic Dissociation of Glutamyl-Glutamic and Glycyl-Glutamic Acids in Aqueous Solution at 298 K

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Abstract—Protolytic dissociation constants of stepwise dissociation of glutamyl-glutamic and glycyl-glutamic acids in aqueous solutions at 298.15 K at ionic strengths of 0.1, 0.5, and 1.0 M on the NaCl background were measured by potentiometric titration. The thermodynamic dissociation constants were calculated. Using the obtained and published data, we discuss the effect of additional carboxy groups in the side chains of the molecule on the protolytic dissociation constants of peptides.

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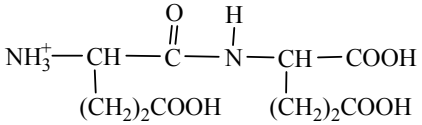
Acid–base interactions in peptide and protein solutions determine their ionic state and have a major influence on the course of many biochemical processes. The study of acid–base equilibria in solutions of peptides is complicated by the presence in their molecules of a sufficiently large number of active groups, and the individual stages of dissociation of these groups overlap. The peptides studied in this paper, glutamyl-glutamic acid (Glu-Glu, H_4L^+) and glycyl-glutamic acid (Gly-Glu, H_3A^+) in strongly acidic solutions exist as the protonated forms **I** and **II**, respectively. They occupy a central position in the metabolism of proteins. There is no data in the literature on the acid–base dissociation constants of glutamyl-glutamic acid, and for glycyl-glutamic acid they are extremely limited [1, 2]. The aim of our study is to determine the stepwise dissociation constants of these peptides in a wide range of ionic strength produced by sodium chloride at 298.15 K and to study the effect of acid–base groups in the side chains of peptides on these values.



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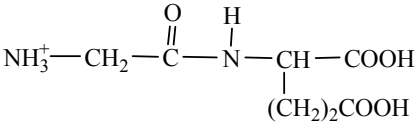

II

Experimental data obtained from potentiometric measurements were processed using the PHMETR software [3]. The calculations take into account equilibria (1)–(5) for the peptide Glu-Glu, which has four active groups, and (5)–(8) for the peptide Gly-Glu, which has three active groups:

$$H_4L^+ \rightleftarrows H_3L^{\pm} + H^+, \quad (1)$$

$$H_3L^{\pm} \rightleftarrows H_2L^- + H^+, \quad (2)$$

$$H_2L^- \rightleftarrows HL^{2-} + H^+ \quad (3)$$

$$HL^{2-} \rightleftarrows L^{3-} + H^+ \quad (4)$$

$$H_2O \rightleftarrows OH^- + H^+ \quad (5)$$

$$H_3A^+ \rightleftarrows H_2A^{\pm} + H^+ \quad (6)$$

$$H_2A^{\pm} \rightleftarrows HA^- + H^+ \quad (7)$$

$$HA^- \rightleftarrows A^{2-} + H^+ \quad (8)$$

The criterion of the adequacy of the chosen model with respect to the experiment was the difference between the calculated and experimental pH values,

Table 1. Dissociation constant pK = –log K of glutamyl-glutamic and glycyl-glutamic acids at 298K and at different values of ionic strength (NaCl)

Acid–base groups	pK			
	I 0.1	I 0.5	I 1.0	I 0
Glutamyl-glutamic acid				
α"-COOH ⇄ COO^- + H^+	3.43±0.04	3.30±0.02	3.40±0.03	3.40±0.1
γ'-COOH ⇄ COO^- + H^+	3.80±0.04	3.71±0.02	3.75±0.03	4.00±0.01
γ"-COOH ⇄ COO^- + H^+	4.60±0.04	4.43±0.02	4.58±0.03	4.97±0.05
α'-NH_3^+ ⇄ NH_2 + H^+	7.85±0.02	7.46±0.01	7.70±0.02	8.40±0.1
Glycyl-glutamic acid				
α"-COOH ⇄ COO^- + H^+	2.96±0.02	3.04±0.02	3.23±0.02	2.91±0.03
γ"-COOH ⇄ COO^- + H^+	4.32±0.01	4.20±0.01	4.26±0.02	4.50±0.03
α'-NH_3^+ ⇄ NH_2 + H^+	7.81±0.09	7.78±0.01	7.86±0.01	8.21±0.09

which did not exceed the experimental error, the latter had alternating sign in the entire range of pH from 2 to 11 units.

The obtained values of the constants of acid–base dissociation in the form pK = –log K for glycyl-glutamic and glutamyl-glutamic acid at 298.15 K and different ionic strengths are shown in Table 1. For glutamyl-glutamic acid the constants of acid–base dissociation we determined for the first time. The pK errors obtained characterize the mean square deviation of the pK values. Table 1 shows that with increasing ionic strength the pK values vary slightly. Thermodynamic dissociation constants were obtained by extrapolating the corresponding concentration values to zero ionic strength by the equation of one individual parameter [4]. The variation in the concentration pK_c values with increasing ionic strength of solution, ψ(I), is satisfactorily described by a linear dependence, as Figure 1 illustrates for glutamyl-glutamic acid. The thermodynamic dissociation constants at zero ionic strength, pK, calculated from these dependencies, are also listed in Table 1.

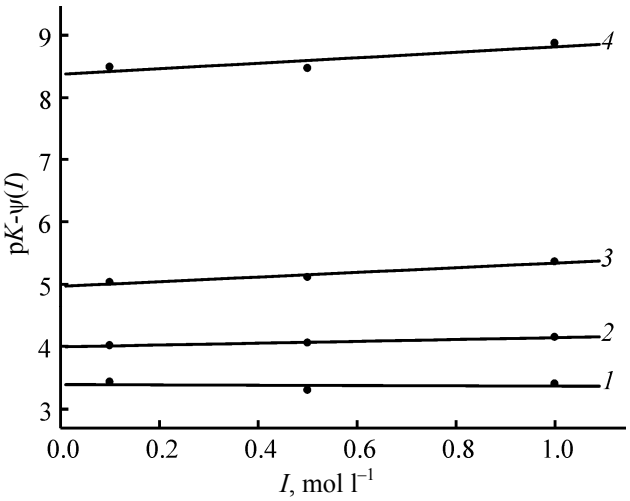

Fig. 1. The dependence of pK_c of Glu-Glu on the ionic strength of the solution at 298.15 K: (*1*) pK_1 (H_3L^+), (*2*) pK_2 (H_2L^-), (*3*) pK_3 (HL^{2-}), (*4*) pK_4 (L^{3-}).

Table 2 shows a comparison of the thermodynamic dissociation constant of the stepwise protolytic dissociation of the dipeptides of different composition with those of monomeric amino acids composing the peptide molecule. While going from the linear aliphatic amino acids to their peptide the distance between the active groups NH_3^+ and COOH increases. As is well known, the ability to protolytic dissociation of α-carboxy groups in this case falls (pK^0 increases) while of protonated α-amino groups rises (pK^0 decreases). Change in the pK^0 values in going from glycine to glycyl-glycine demonstrates this pattern. It was previously shown [5] that the increase in pK^0 of the dissociation of α-carboxy groups of peptides is associated with more unfavorable entropic effect of dissociation, and the decrease in pK^0 of the dissociation of protonated α-amino group is a result of reducing the unfavorable effect of the entropy due to the specific solvation of the amino group NH_2 appearing at the dissociation. Similar trends can be seen in the change of the dissociation constants in going from glycine (pK^0 = 9.60 for α-NH_3^+) and glutamic acid (pK^0 = 2.16 for α-COOH) to the dipeptide, the glycyl-glutamic acid (pK_1^0 = 2.91 for α"-COOH and pK_3^0 = 8.21 for α'-NH_3^+), as well as from glutamic acid (pK^0 =

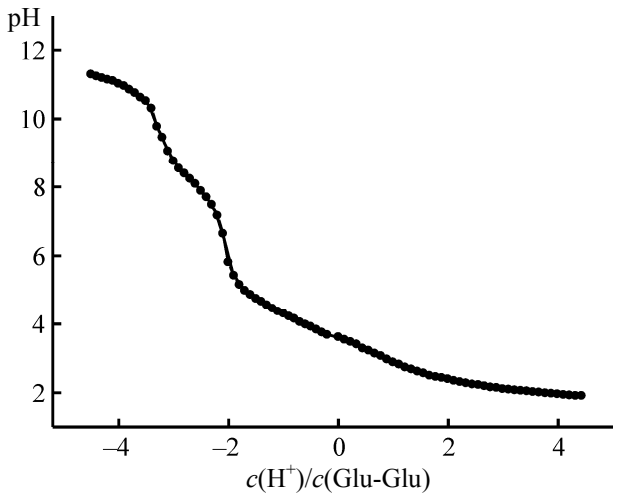

Fig. 2. Potentiometric titration curves of glutamyl-glutamic acid at the ionic strength 0.1 M, at 298.15 K.

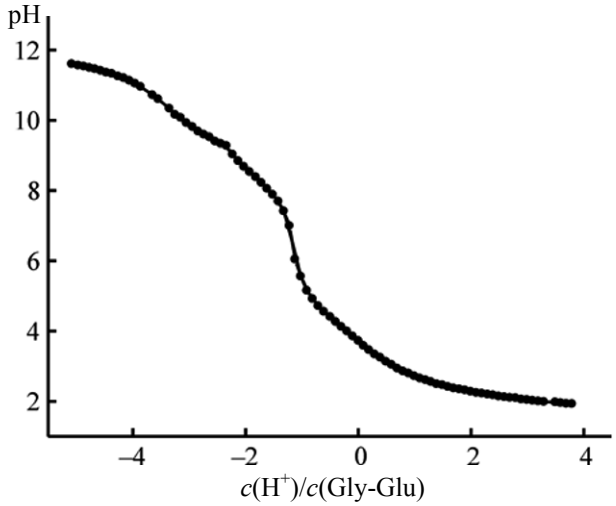

Fig. 3. Potentiometric titration curves of glycyl-glutamic acid at the ionic strength 0.1 M, at 298.15 K.

9.67 for α-NH_3^+ and pK^0 = 2.16 for α-COOH) to glutamyl-glutamic acid (pK_1^0 = 3.40 for α''-COOH and pK_4^0 = 8.40 for α'-NH_3^+).

Protolytic dissociation constants of the NH_3^+ group included in the composition of the dipeptide remain approximately constant. Among the peptides Gly-Gly, Gly-Glu, Gly-Asp, Glu-Glu, the pK^0 values range from 8.2 to 8.4 logarithmic units. Probably, the dissociation of a NH_3^+ group remote from the carboxy group in the molecules of all the above peptides weakly depends on the electronic effects induced by additional side chain carboxy groups in the Gly-Glu, Gly-Asp, Glu-Glu. The dominating factor determining the course of the dissociation of the group is the entropy factor, as shown previously for the peptide Gly-Gly [5].

The glutamic acid dipeptides are polybasic carboxylic acids. The mutual influence of the carboxy groups in the molecule leads to the appearance of some features in the protolytic dissociation of these peptides. The acidic properties of α''-COOH are more pronounced for dibasic acids Gly-Glu (pK^0 = 2.91) and Gly-Asp (pK^0 = 2.79) compared with the monobasic acid Gly-Gly (pK^0 = 3.16). The acidic properties of extra γ''-COOH group in the side chain becomes weaker in going from monomeric amino acids to the dipeptide: pK^0 = 4.32 for glutamic acid and pK^0 = 4.50 for Gly-Glu; pK^0 = 3.65 for aspartic acid and pK^0 = 4.31 for Gly-Asp. In the molecule of tribasic carboxylic acids Glu-Glu the ability to protolytic dissociation is redistributed by complex way among the three COOH groups. The acidic properties of α''-COOH groups are significantly weaker (pK^0 = 3.40). The ability to the protolytic dissociation of the carboxy groups in the side chains also changes: the dissociation of γ'-COOH group grows (pK^0 = 4.0) while that of γ''-COOH group falls (pK^0 = 4.97) compared to γ-COOH group of monomeric glutamic acid (pK^0 = 4.32). All three carboxy groups in the Glu-Glu molecule are rather distant from the amino group. Therefore, the

Table 2. Dissociation constants at 298 K of dipeptides compared with the amino acids

Active group	Gly [6]	Glu [6]	Asp [6]	Gly-Gly [7]	Gly-Asp [2]	Gly-Glu	Glu-Glu
α'-COOH	2.34	2.16	1.88				
α''-COOH				3.16	2.79	2.91	3.40
γ'-COOH		4.32	3.65				4.0
γ''-COOH					4.31	4.50	4.97
α'-NH_3^+	9.60	9.67	9.60	8.31	8.35	8.21	8.40

observed regularities of changes in the acidic properties can only be associated with the effects of intramolecular redistribution of the electron density due to interference of three carboxyl groups.

EXPERIMENTAL

Stepwise dissociation constants of glutamyl-glutamic and glycyl-glutamic acids in aqueous solutions were determined by potentiometric titration at 298.15±0.1 *K* at fixed ionic strength: 0.1; 0.5; 1.0 M, the supporting electrolyte was NaCl. Figures 2 and 3 show the titration curves of aqueous solutions of the peptides.

Peptide solution was prepared by dissolving weighed samples of the peptides from Sigma in freshly boiled distilled water just prior to the experiment. The product was dried to constant weight. The concentration of Glu-Glu and Gly-Glu in potentiometric studies was 5.0×10^{-3} mol l^{-1}. Sodium chloride of reagent grade was used for preparation of the supporting electrolyte. The titrants were the carbonate-free solution of 0.1 M NaOH and 0.1 M HCl solution.

The potentiometric titration procedure was described in detail previously [5]. To avoid absorption of carbon dioxide from the atmosphere, through the working solution during the experiment was passed inert gas nitrogen. The EMF of a circuit consisting of a glass electrode and a saturated silver chloride reference electrode was measured. The measurements were performed with a pH meter pH-mV-150 with digital display indicating EMF. Measurement accuracy was ±0.5 mV.

The acidity of the solution pH was determined by the equation:

$$pH = (E^0 - E)/\eta,$$

where E^0 and E are apparent standard potential of glass electrode and experimental EMF, respectively. Nernst coefficient was found to be $\eta = 0.05852$ V per a pH unit, by measuring EMF in buffer solutions at pH 1.68, 4.01, 6.86, and 9.01. The values of E^0 measured for standard solutions of HCl were 0.2060, 0.2103, and 0.2209 V at ionic strength of the solution 0.1, 0.5, and 1.0 M, respectively.

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