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Protonation Constants of Glycylisoleucine at Different Ionic Strengths and Various Ionic Media

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ABSTRACT

The protonation of different species of glycylisoleucine were studied in the pH range of 1.5 - 10 at 25 °C using potentiometric technique. Investigations were performed in sodium perchlorate and tetraethylammonium iodide as background electrolytes at I (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6) mol dm⁻³. The parameters based on the protonation constants were calculated, and their dependences on ionic strength are described by a Debye-Huckel type equation. The dependence on ionic strength of the protonation constants was also analyzed by the specific ion interaction theory (SIT) model. The constants at infinite dilution, obtained using this model, are $\log K_1^\circ = 2.72$ and $\log K_2^\circ = 8.24$. The specific interaction coefficients are also reported.

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INTRODUCTION

Determination of protonation constants of some organic and inorganic compounds have a long history back to the end of the 19th century, when the first pK_a was measured. Since then a vast body of data on acidities in various media has been collected.¹⁻⁵ The acidity or basicity of a compound in a given medium is influenced by both the electronic effects of the substituents and the solvent as well the ionic strength effects of the medium.

Amino acids and peptides are of importance in several fields: in medicine, in biochemistry, in food conservation, and in industry.⁵ So, a great deal of work has been done in determining the protonation constants of these biologically ligands in different

media so far.⁶⁻⁸ The supporting background electrolytes have been often employed in studies of the protonation constants of amino acids and peptides because they are not thought to react with the compounds. However, there are many evidences of interaction of these background salts on the ionic species formed in solution. The interactions between the formed ions in solution are usually separated in the literature into specific and nonspecific. As a result, a linear combination with few parameters for description of the effects is proposed.⁹⁻¹⁶ One of the most interesting is that proposed by specific ion interaction theory (SIT) model that was used to extrapolate the protonation constants of all species found at infinite dilution and to calculate specific interaction parameters.

The present work deals with the study of protonation of glycyl-L-isoleucine in aqueous solution at 25 °C and different ionic strengths of sodium perchlorate and tetraethylammonium iodide (0.1 to 0.6 mol dm⁻³) using a potentiometric technique. The parameters which define this dependency were analyzed with the aim of obtaining further information with regard to their variation as a function of charges involved in the species formed reactions. Moreover, a Debye-Huckel type equation was established for the dependence of protonation constant on ionic strength. This equation gives the possibility of

estimating a formation constant at a fixed ionic strength when its value is known at another ionic strength in the range $0.1 < I < 0.6$ mol dm⁻³, and therefore may give a significant contribution of solving many analytical and speciation problems. In this work, the specific ion interaction theory (SIT) model was also used to extrapolate the protonation constants species found at infinite dilution to calculate the thermodynamic values and also the specific interaction coefficient.

Experimental

Chemicals

Glycyl-L-isoleucine, $NH_2-CH_2-CO(NH)-CH(CH_3-CH_2-CH-CH_3)-COOH$, (Fluka, analytical reagent grade) has been used as received. The NaOH solution was prepared from titrisol solution (Merck) and its concentration was determined by several titrations with standard HCl. Perchloric acid, sodium perchlorate, and tetraethylammonium iodide were supplied from Merck and Fluka, respectively, as analytical reagent grade materials and were used without further purification. Dilute perchloric acid solution was standardized against standard NaOH solution. All dilute solutions were prepared using double-distilled water with conductivity equal to $1.5 \pm 0.1 \mu\Omega^{-1} \text{ cm}^{-1}$.

Apparatus

An Eyela pH-meter, PHM 2000, was used for $-\log[H^+]$ measurements. The hydrogen ion concentration was measured with an Ingold UO 3234 glass electrode and an Ingold UO 3236 calomel electrode. To exclude carbon dioxide and oxygen from the system, a stream of purified nitrogen was passed through a sodium chloride and then bubbled slowly through the reaction solution.

Measurements

All measurements were carried out at 25 ± 0.1 °C. The ionic strength was maintained to 0.1-0.6 mol dm⁻³ with sodium perchlorate or tetraethylammonium iodide. The pH-meter was calibrated for the relevant H^+

concentration with a solution of 0.01 mol dm⁻³ perchloric acid solution containing 0.09 mol dm⁻³ sodium perchlorate (for adjusting the ionic strength to 0.1 mol dm⁻³). The same procedure was performed for the other ionic strengths. For these standard solutions, we set $-\log[\text{H}^+] = 2.00$.¹⁷ Junction potential corrections have been calculated from eq 1

$$-\log[\text{H}^+]_{\text{real}} = -\log[\text{H}^+]_{\text{measured}} + a + b[\text{H}^+]_{\text{measured}} \quad (1)$$

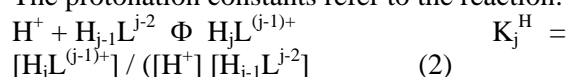
Here a and b were determined by measuring of hydrogen ion concentration for two different solutions of HClO₄ with sufficient NaClO₄ to adjust the ionic media.

Procedure

A 50 cm³ of glycyloisoleucine was titrated with an alkali solution (0.1 mol dm⁻³ NaOH), both of the same ionic strength. The $-\log[\text{H}^+]$ was measured after addition of a few drops of titrant, and this procedure was extended up to the required $-\log[\text{H}^+]$. In all cases, the procedure was repeated at least three times and the resulting average values and corresponding deviations from the average are shown in the text and tables.

Results and Discussion

The protonation constants refer to the reaction:



where j is equal to 1 for the protonation of amine and is 2 in the case of protonation of carboxylate groups, respectively. The protonation constants were determined in different ionic strengths of sodium perchlorate and tetraethylammonium iodide using potentiometric technique and calculated using a computer program which employs a nonlinear least-squares method.¹⁸ These values are listed in Table 1 together with the values reported in literature, which are in good agreement with those reported earlier.¹⁹ The protonation constants of glycyloisoleucine in molality scale are reported in Table 2. The values of protonation constants in molality scale have been determined with the method described by Baes and Mesmer.²⁰⁻²¹

Ionic strength dependence

The dependence of the protonation constants on ionic strength can be described by a semi-empirical equation.²²⁻²⁶

$$\log K(I) = \log K(I^*) - f(I) + CI \quad (3)$$

where $f(I) = Z^*AI^{1/2}/(1 + BI^{1/2})$, $K(I)$ and $K(I^*)$ are the stability constants of the actual and the reference ionic media, respectively. A is the parameter of Debye-Hückel equation, ($A = 0.509 \text{ mol}^{-1/2} \text{ dm}^{3/2}$ at 25 °C), $Z^* = \sum(\text{charges})_{\text{reactants}}^2 - \sum(\text{charges})_{\text{products}}^2$, C is an empirical parameter that its value is considered, and B is set equal to 1.5 mol^{-1/2} dm^{3/2} (a small error in fixing B is absorbed in the linear term C ²⁴). Results of a series of investigations done by Daniele et al.,²⁷⁻²⁸ De Stefano et al.,²⁹⁻³⁰ and Gharib et al.²²⁻²⁶ showed that, when all the interactions occurring in the solution are considered, in the range $0 \leq I \leq 1 \text{ mol dm}^{-3}$, the empirical parameters are dependent on the stoichiometry of the formation reaction. If an approximate value of C is known, the stability constant can be determined for the variation of ionic strength from I^* to I by the equation

$$\log K(I) = \log K(I^*) - f(I, I^*) + C(I - I^*) \quad (4)$$

where

$$f(I, I^*) = Z^*A[I^{1/2}/(1 + 1.5I^{1/2}) - I^{*1/2}/(1 + 1.5I^{*1/2})] \quad (5)$$

I and I^* are the ionic strength of the solution by appropriate electrolyte. A preliminary analysis of the data showed that if a fixed value is assigned to C , the fit with eq 5 is not always good over the whole range of ionic strength from 0.1 to 0.6 mol dm⁻³. This equation may be useful for small changes of ionic strength, but a better fit is obtained by adding a further terms of the form $DI^{3/2}$ (D is another adjustable parameter). Therefore the data were fitted to the eq 6.

$$\log K(I) = \log K(I^*) - f(I, I^*) + C(I - I^*) + D(I^{3/2} - I^{*3/2}) \quad (6)$$

It is noticeable that the introduction of the term $D(I^{3/2} - I^{*3/2})$ very often improves the goodness of the fit. For example, for the $\log K_1$ in tetraethylammonium iodide media, from eq 6, we obtained two sets of values depending on whether or not we take into account the term in D :

$$C = 0.48, S = 1.93 \times 10^{-2}$$

$$C = -3.41, D = 3.67, S = 5.03 \times 10^{-3}$$

The squares sum, S , shows that there is a significant improvement in the fit when D term is introduced. The parameters for the dependence on ionic strength (C and D) were calculated by the fitting method and reported in Table 3.

The dependence of $\log K$ on ionic strength determined in NaClO_4 and tetraethylammonium iodide as background electrolytes, Fig. 1, show a regular trend and is in good agreement with other complex species.²²⁻³⁰ Our previous results on ionic strength dependence of complex formation constants²²⁻²⁶ and this work reveal the $\log K$ values are nearly always at their minimum at an ionic strength range (0.3 - 0.7) mol dm^{-3} , that is a characteristic of the curve $\log K = f(I)$. According to the theory of electrolytic solutions³¹ the $AI^{1/2}$ term in equation 6 accounts for Coulomb interactions between ions screened by the ion atmosphere, while the BI term accounts for disturbances in ion-solvent interaction. At low ionic strength (less than about 0.1 mol dm^{-3}) these interactions are of primary importance. However, as the ionic strength increases, the ionic atmosphere becomes more compressed and screens the ionic charges more effectively, so that intermolecular interactions (dipole-dipole or multipole-multipole) become more important. These forces at higher ionic strength possibly have primary role between the ions and contribute to the C and D terms in eq 6.

Determination of the thermodynamic protonation constants

To obtain the thermodynamic protonation constants, we applied the specific ion interaction theory model, SIT.⁹⁻¹⁶

According to SIT model, formation constants may be expressed as

$$\log K_m = \log K^\circ - Z^*d + f(\varepsilon, I_m) \quad (7)$$

where K_m is the molal formation constant, K° is the thermodynamic formation constant, d is the Debye-Huckel term $[0.509I_m^{1/2}/(1+1.5I_m^{1/2})]$, I_m is the ionic strength in molality, and $f(\varepsilon, I_m)$ is a linear term dependent on ionic strength and

interaction coefficients, usually called SIT parameter. For the species HL the SIT parameters in the supporting electrolyte, NaClO_4 , assumes the form⁹⁻¹⁶

$$f(\varepsilon, I_m) = I_m[\varepsilon(L^-, \text{Na}^+) + \varepsilon(\text{H}^+, \text{ClO}_4^-)] \quad (8)$$

for the second species H_2L^+ ,

$$f(\varepsilon, I_m) = I_m[\varepsilon(L^-, \text{Na}^+) + 2\varepsilon(\text{H}^+, \text{ClO}_4^-) - \varepsilon(\text{H}_2\text{L}^+, \text{ClO}_4^-)] \quad (9)$$

The concentrations of the ions of the ionic medium are usually very much larger than those of the reacting species. Hence, the ionic medium ions will make the main contribution to the value of ε for the reacting ions. The ion interaction coefficients assume to be zero for ions of the same charge sign and for uncharged species with the supporting electrolyte, but the activity coefficient can be obtained from the well-known relationship,⁹⁻¹⁶ $\log \gamma = \lambda I$.

In our study, the apparent stability constants are converted to molality units according to the conversion factors determined by Baes and Mesmer,²⁰⁻²¹ and are listed in Table 2. Also, we needed to know several interaction coefficients, ε , for the different species mentioned in eqs 8 and 9. $\varepsilon(\text{H}^+, \text{ClO}_4^-)$ obtained from the literature.⁹⁻¹⁶ However, $\varepsilon(L^-, \text{Na}^+)$ and $\varepsilon(\text{H}_2\text{L}^+, \text{ClO}_4^-)$ were calculated from the dependence of protonation constants on ionic strength and are listed in Table 4, assuming $\lambda_{\text{HL}} = 0.1$ on the basis of several values reported for similar system.⁹⁻¹⁶ Usually, when some interaction coefficients are not known, eq 7 can be rearranged to the following form, eq 10

$$\log K_m = \log K^\circ - Z^*d + I_m \Delta \varepsilon \quad (10)$$

where $\Delta \varepsilon$ is the summation of the specific interaction terms. Using these coefficients, we determined ε values for the species of glycyloisoleucine by fitting the formation constants at different ionic strengths from eq 10, and are listed in Table 4. This fitting also allows us to obtain the thermodynamic formation constants that are: $\log K^\circ_1 = 2.72$ and $\log K^\circ_2 = 8.24$.

It is clear from cursory examination of the results in Table 1, the first protonation constant, K_1 , shows the trend in sodium perchlorate > tetraethyl ammonium iodide.

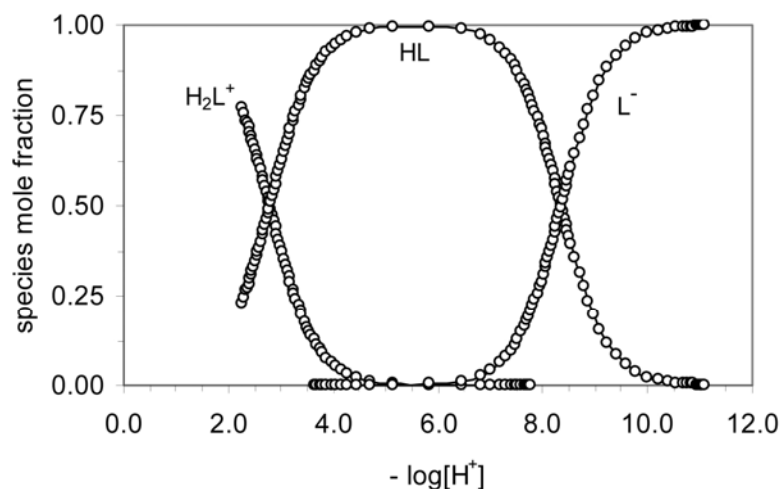


Fig. 1. The equilibrium distribution of the different species of glycyloleucine system as a function of $-\log[\text{H}^+]$ at 25 °C and ionic strength $0.1 \text{ mol dm}^{-3} \text{ NaClO}_4$.

But, this rule is not exactly governed in the case of K_2 and a fluctuation can be observed. To clarify this point, we calculated the percentage contribution of both parameters, C and D , on the effect of different media on the protonation constant values. The percentage contributions are almost the same for K_1 and with a little difference in the case of K_2 . So, this result is not able to clarify the point. However, a reasonable difference in the SIT interaction coefficients can be observed between L^- , HL , and H_2L^+ , Table 4.

We believe this has the most probable responsibility for the differences. Unfortunately, the SIT parameters have not computed for $(\text{C}_2\text{H}_5)_4\text{NI}$ yet, and so we are not able to proceed the discussion further and clarify the point exactly.

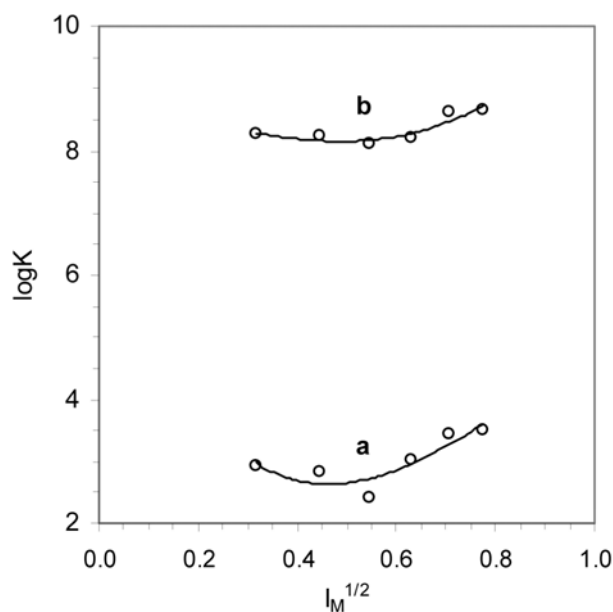


Fig. 2. Plots of $\log K$ versus square root of ionic strength in the case of sodium perchlorate (in molar scale) for glycyloisoleucine at 25 °C, (a) $\log K_1$, (b) $\log K_2$

Table 1. Protonation constants of glycyloisoleucine at 25 °C and different ionic strengths of sodium perchlorate and tetraethylammonium iodide in molar scale

$I_M / \text{mol dm}^{-3}$	NaClO_4		$(\text{C}_2\text{H}_5)_4\text{NI}$		ref.
	$\log K_1$	$\log K_2$	$\log K_1$	$\log K_2$	
0.1	2.94 ± 0.07	8.27 ± 0.08	2.79 ± 0.08	8.36 ± 0.11	this work
0.2	2.85 ± 0.06	8.24 ± 0.09	2.71 ± 0.05	8.08 ± 0.09	this work
0.3	2.43 ± 0.07	8.10 ± 0.10	2.81 ± 0.05	8.29 ± 0.12	this work
0.4	3.04 ± 0.06	8.21 ± 0.09	2.89 ± 0.06	8.38 ± 0.08	this work
0.5	3.43 ± 0.08	8.62 ± 0.11	2.94 ± 0.04	8.58 ± 0.09	this work
0.6	3.51 ± 0.07	8.67 ± 0.12	2.98 ± 0.05	8.62 ± 0.11	this work
	3.18	8.21	-	-	19

Table 2. Protonation constants of glycyloleucine at 25 °C and different ionic strengths of sodium perchlorate in molal scale

$I_m / mol\ Kg^{-1}$ ($NaClO_4$)	$NaClO_4$	
	$\log K_1$	$\log K_2$
0.101	2.93	8.27
0.202	2.84	8.23
0.305	2.41	8.10
0.409	3.02	8.21
0.513	3.41	8.61
0.619	3.48	8.65

Table 3. Parameters for the dependence on ionic strength of the different species of glycyloleucine at 25 °C

species	$NaClO_4$		$(C_2H_5)_4NI$	
	C	D	C	D
$\log K_1$	- 20.26	19.93	- 3.41	3.67
$\log K_2$	- 11.38	11.36	- 10.54	10.41

Table 4. SIT interaction coefficients

species	SIT coefficients
$\varepsilon(H^+, ClO_4^-)$	0.14 ^a
$\varepsilon(L^-, Na^+)$	1.01
$\varepsilon(H_2L^+, ClO_4^-)$	-0.39
$\Delta\varepsilon_{111}$	1.15
$\Delta\varepsilon_{122}$	1.68

^a Were obtained from reference [20].

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