

## ULTRASONIC STUDIES OF THE HYDRATION OF AMINO ACIDS AND OLIGOPEPTIDES

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Hydration number of selected amino acids, their hydrochlorides and their oligopeptides with glycine were determined by the ultrasonic method. Assuming additivity of contributions of ionic and non-ionic groups to the total hydration of the studied substances, the hydration numbers of the free amino acids were calculated. The results obtained are interpreted on the basis of the pseudocrystalline model of water structure.

### 1. Introduction

In the model of interactions of biologically active substances such as amino acids, peptides and proteins, hydration numbers of these substances are of fundamental importance. In the previous paper [1] the hydration numbers of selected water-soluble amino acids and peptides were presented. This paper is an extension of this study into amino acids slightly soluble in water. In order to evaluate the hydration numbers of these amino acids the hydration numbers of their hydrochlorides and their dipeptides with glycine were determined first. Both the hydrochlorides and dipeptides are soluble in water. Assuming additivity of contributions of ionic and non-ionic groups to the total hydration of a given compound, the hydration number of the free amino acid can be calculated. The ultrasonic method of hydration number determination consists in determination of the maximum of the parabola-like dependency of ultrasonic velocity on ethanol concentration in both water-ethanol and water-ethanol-cosolvent systems [2, 3]. Introduction of a third component to a water-ethanol solution leads to a shift of this maximum. The value,  $n_t$ , of this shift (expressed in moles of  $H_2O$  per mole of solute) is a measure of hydration and is described by the following formula:

$$n_t = \frac{1000(m_0 - m_1)}{m_0 m_s m_w} \quad (1)$$

where:  $m_0$  and  $m_1$  are the molal concentrations (mole  $\text{kg}^{-1}\text{H}_2\text{O}$ ) of ethanol at the maxima of ultrasonic velocity in binary and ternary systems, respectively;  $m_s$  is the molal concentration of the solute;  $M_w$  is the molecular weight of water. With use of the above method the hydration numbers of the following substances were determined: carboxylic acids, amino acids and peptides [1], 1-1 and 2-1 electrolytes [4-6], non-electrolytes [7-9], sugars and their derivatives [10, 11], nucleosides [12], nucleotides and nucleic acids [13, 14], and macromolecules [15, 16].

For some of the above listed substances the measurements were performed not only in aqueous ethanolic solutions but also in solutions of other cosolvents such as propanol, acetone, dioxane, tetrahydrofuran, dimethylformamide and dimethylsulphoxide [6-8]. In the case of substances slightly soluble in water, the above method gives results with a high error, because the shifts of maximum velocities are usually small, often lying within the experimental error. The proposed procedure of determination of hydration numbers of amino acids slightly soluble in water through measurements in solutions of their derivatives is an attempt to eliminate this limitation.

## 2. Experimental

The examined amino acids and oligopeptides were from Sigma (USA). Hydrochlorides of the amino acids were obtained through the reaction of these amino acids with 1 M HCl. Triply distilled water and 99.8% ethanol, analytically pure (POCH, Gliwice) were used. The measurements of ultrasonic velocity were carried out at  $25 \pm 0.01^\circ\text{C}$  with use of "sing around" gauge. The details of the experimental procedure were described previously [10, 17]. The obtained results are listed in Tables 1-3. These results are mean of 4-6 measurements. For comparison the hydration numbers of amino acids and peptides obtained previously [1] are also included in the Tables.

**Table 1.** Hydration numbers of amino acids determined in aqueous-ethanolic solutions at  $20^\circ\text{C}$

Compound	$n_{25^\circ\text{C}}$	$n^0$
Glycine <sup>(a)</sup>	$5.7 \pm 0.2$	3.9
$\alpha$ -alanine <sup>(a)</sup>	$7.8 \pm 0.2$	6.0
DL- $\alpha$ -amino butyric acid <sup>(a)</sup>	$9.9 \pm 0.4$	8.1
DL-valine <sup>(a)</sup>	$13.6 \pm 0.6$	11.8
DL-lysine <sup>(a)</sup>	$12.8 \pm 0.6$	11.0
DL-proline <sup>(a)</sup>	$7.9 \pm 0.4$	6.0
DL- $\beta$ -phenylalanine	$14.1 \pm 1.0$	12.3
DL-serine	$6.9 \pm 1.0$	5.1
D- $\beta$ -asparagine	$4.3 \pm 1.0$	2.5
DL-ornithine	$11.0 \pm 1.0$	9.2
L-arginine	$6.9 \pm 1.0$	5.1

<sup>(a)</sup> - Ref. [1]

**Table 2.** Hydration numbers of amino acids monohydrochlorides in aqueous-ethanolic solutions at 25°C

Compound	$n_{25^{\circ}\text{C}}$	$n^0$
L-arginine monohydrochloride	$8.6 \pm 1$	6.8
DL-ornithine monohydrochloride	$12.2 \pm 1$	10.4
L-lysine monohydrochloride	$13.6 \pm 1$	11.8
DL- $\beta$ -phenylalanine monohydrochloride	$15.5 \pm 1$	13.7
L-leucine monohydrochloride	$17.2 \pm 1$	15.4
D- $\beta$ -asparagine monohydrochloride	$5.4 \pm 1$	3.6
DL-tryptophan monohydrochloride	$11.2 \pm 1$	9.4
DL-methionine monohydrochloride	$11.7 \pm 1$	9.9
L-histidine monohydrochloride	$6.5 \pm 1$	4.7
L-iso-leucine monohydrochloride	$15.4 \pm 1$	13.6

**Table 3.** Hydration numbers of oligopeptides determined in aqueous-ethanolic solutions at 25°C

Compound	$n_{25^{\circ}\text{C}}$	$n^0$
Diglycine <sup>(a)</sup>	$8.9 \pm 0.5$	7.1
triglycine <sup>(a)</sup>	$12.8 \pm 1.0$	11.0
glycyl-L- $\alpha$ alanine <sup>(a)</sup>	$11.0 \pm 0.5$	9.2
glycyl-DL- $\alpha$ -alanyl glycine <sup>(a)</sup>	$13.5 \pm 1.0$	11.7
L-alanylglycine <sup>(a)</sup>	$10.8 \pm 0.5$	9.0
DL-alanyl-DL-alanine <sup>(a)</sup>	$12.8 \pm 0.5$	11.0
glycyl-L-proline <sup>(a)</sup>	$10.7 \pm 0.5$	8.9
glycyl-L-valine <sup>(a)</sup>	$17.6 \pm 1.0$	15.8
glycyl-L-leucine	$20.0 \pm 1.5$	18.2
DL-leucylglycine	$20.0 \pm 1.5$	18.2
D-leucylglycylglycine	$25.0 \pm 1.0$	23.2
glycyl-L-asparagine	$6.8 \pm 1.0$	5.0
glycyl-L-methionine	$14.0 \pm 1.5$	12.2
glycyl-L-tyrosine	$11.0 \pm 1.0$	9.2

<sup>(a)</sup> — Ref. [1]

### 3. Results and discussion

The values of  $n^0$  corresponding to hydration numbers at 0°C are listed in Tables 1–3. These values were obtained on the assumption that the temperature dependence of the hydration numbers  $n_i$  of the studied amino acids, their hydrochlorides and their oligopeptides with glycine is of the same type as that obtained previously for simple amino acids and peptides [1], 1–1 electrolytes [4, 5], sugars [10, 11],

non-electrolytes [7, 8] and macromolecules [15, 16]:

$$n_t = n^0 + At + Bt^2, \quad (2)$$

where:  $A$  and  $B$  are coefficients equal to  $0,0383 \text{ mole } ^\circ\text{C}^{-1}$  and  $0,0013 \text{ mole } ^\circ\text{C}^{-2}$ , respectively;  $t$  is temperature in  $^\circ\text{C}$ . Assuming that at  $0^\circ\text{C}$  all water molecules are fixed in the pseudocrystalline, ice-like water structure, the value of  $n^0$  can be interpreted as a number of water molecules of the ice-like net which constitute the closest vicinity of a solute molecule. The remaining fragment of eq. (2)  $At + Bt^2$  is dependent on the properties of water irrespective of the type of solute and determines the population of "free" water at different temperatures. At  $25^\circ\text{C}$   $At + Bt^2 = 1.8$ . By subtracting this value from experimental results  $n_t$  at  $25^\circ\text{C}$  the values of  $n^0$  were obtained. The values of this parameter were calculated for the free amino acids which in the study were used in the form of hydrochlorides and oligopeptides with glycine. For this calculation it was assumed that  $n^0$  is an additive function of contributions of ionic and non-ionic groups of these substances. In the case of the hydrochlorides the hydration number of  $\text{Cl}^-$ ,  $n_{\text{Cl}^-}^0$  - obtained previously from the study of hydration of 1-1 electrolytes [5, 4] was used, and it was assumed that hydration within the pairs

**Table 4.** Hydration numbers  $n^0$  of amino acids obtained from direct measurements and calculated from measurements of hydration numbers of hydrochlorides of amino acids and oligopeptides of amino acids with glycine

	Values obtained from direct measurements	Values calculated	
		from measurements in hydrochlorides of amino acids under assumption $n_{\text{Cl}^-}^0 = 2$	from measurements in oligopeptides of amino acids with glycine under assumption $n_{\text{glyc}}^0 = 3$
	$n^0$	$n_c^0$	$n_p^0$
glycine	3.9		4.0
$\alpha$ -alanine	6.0		6.0
DL-proline	6.0		5.9
DL-valine	11.8		12.8
L-arginine	5.1	4.8	
D- $\beta$ -asparagine	2.5	1.6	2.0
DL-ornithine	9.2	8.4	
DL-lysine	11.0	10.0	
DL-phenylalanine	12.3	11.7	
L-leucine		13.4	15-16
L-isoleucine		11.6	
DL-serine	5.1		
DL-methionine		7.9	9.2
DL-tryptophan		7.4	
L-histidine		2.7	
L-tyrosine			6.2

of groups  $-\text{NH}_3^+$ ,  $-\text{NH}_2$  and  $-\text{COO}^-$ ,  $-\text{COOH}$  is of the same order. In the case of the dipeptides of the amino acids with glycine it was assumed that hydration of glycine group in all dipeptides is the same as that of glycine group in diglycine. For diglycine  $n^0 = 7$  and for glycine  $n^0 = 4$ . Therefore it can be assumed that 3 water molecules correspond to glycine group in a dipeptide i.e.  $n_{\text{glyc.}}^0 = 3$ . Correctness of this assumption was confirmed by the results obtained for glycyllalanine, alanyl-glycine and glycyproline for which the values of  $n^0$  obtained this way for alanine and proline are in agreement with the values of  $n^0$  obtained directly for these amino acids in free state. The results of the performed measurements are listed in Table 4. It follows from these results that the values of hydration numbers  $n^0$  calculated by the proposed method for the other amino acids under study are in agreement with each other within the experimental error. Therefore it seems that the proposed method is a useful way of determination of hydration numbers of amino acids slightly soluble in water, for which other methods fail.

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