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ULTRASONIC STUDIES ON HYDRATION OF CARBOXYLIC ACIDS, AMINO ACIDS AND PEPTIDES IN AQUEOUS ETHANOLIC SOLUTIONS*

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Hydration numbers of the simplest carboxylic acids, amino acids and di- and tripeptides were determined by measurements of ultrasonic velocity in the ethanolic-aqueous solutions. Hydration numbers of the functional groups present in the amino acids and peptides were also determined.

1. Introduction

Understanding of the nature of interactions between biologically active substances, such as amino acids, peptides, proteins or nucleic acids and water is, according to many investigators, the key to solution of numerous problems connected with the role and interaction of these substances in the living organisms. Hence, the ample literature comprises both theoretical and experimental works with the application of various techniques [1–10].

The investigations of the problem of hydration of amino acids and peptides are presented in this paper. The experiments consisted of measurements of the hydration numbers of carboxylic acids, amino acids and di- and tripeptides at different temperatures by using the ultrasonic method proposed by YASUNAGA *et al.* [11, 12]. The purpose of these investigations was, besides the determination of the hydration numbers of various types of compounds, to calculate the contribution of individual functional groups present in the tested compounds in the overall hydration and, also, the determination of hydration of alkyl groups.

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2. Experiment

In all experiments the following pure or chemically pure substances were used: glycine, α- and β-alanine, α-aminobutyric acid, diglycine, glycylalanine and glycylvaline produced by Reanal (Hungary), glycylproline produced by Koch Light Laboratories (England), alanylglycine made by Fluka (Germany) and alanylalanine made by Janssen (Belgium). Other substances, such as acetic acid, propionic acid, *n*-butyric acid, triglycine and glycylalanylglycine were produced in Poland. Triple-distilled water was used throughout. The second distillation of water was carried out from diluted KOH and $KMnO_4$ solution. Ethyl alcohol (99.8 %), pure for analysis, added to aqueous solutions of substances under investigations, was from POCh, Gliwice, Poland. Water content in the alcohol was checked by means of the Karl Fischer method. The water content determined was in agreement with that given by the manufacturer within the limits of ±0.1 % error.

The measurement method was described in detail previously [13, 14]. Hydration was determined from the following relation:

$$W_x = W_0 - \frac{A_1 W_1}{A_0}, \tag{1}$$

where W_x is the amount of water bound by the solute, A_0 and W_0 are the amounts of alcohol and water at the maximum of ultrasound velocity for the ethanol-water system, and A_1 and W_1 are the amounts of alcohol and water at the maximum velocity for ethanolic-aqueous solutions, containing a known amount of the solute.

The amount of water moles per 1 mole of the solute was calculated from the formula:

$$n_t = W_x M d_0 / M_0 m, \tag{2}$$

where d_0 is the density of water, m is the mass concentration of the solute, M_0 is the molecular weight of water and M is the molecular weight of the solute. The concentrations of the solutes were within the range 0.1–1 mole per kG H_2O, depending on their solubility.

The results of experiments are shown in Tables 1–4 and Figs 1–3. The results given in Tables are mean from 4–6 measurements. The hydration numbers quoted in the literature and obtained by other methods were also given for comparison.

3. Results and discussion

The temperature dependence of the n_t values given in the tables can be expressed by the relation of the same type as the one obtained previously for

Table 1

	n	n_t							error	literature data	
		°C	10	15	20	25	30	35	± δ	[21]	[22]
CH_3COOH	3,0		3.6	4.0	4.4	4.8	5.4	6.0	0.2	3.0	2.0
C_2H_5COOH	6.0		6.7	7.1	7.5	8.0	8.5	9.0	0.2	3.0	3.0
C_3H_7COOH	8.0		8.6	9.0	9.4	9.8	10.4	10.9	0.4	4.0	4.0

Table 2

	n	n_t							error	literature
		°C	10	15	20	25	30	35	± δ	data
glycine	4.0		4.5	4.9	5.3	5.7	6.4	7.0	0.2	6.0 [21]; 3.3 [24]; 8.2 [30];
α-alanine	6.0		6.6	7.0	7.4	7.8	8.3	8.9	0.2	7.6 [21]; 3.3 [24]; 2.6 [30];
β-alanine	5.0					6.8	7.2	7.7	0.4	
dl-α-amino-butyric acid	8.0		8.5	8.9	9.4	9.9	10.5	11.0	0.4	$4.1^{(a)}$[25]
dl-valine	12.0					13.6	14.0	14.5	0.6	$4.3^{(a)}$[29]
dl-lysine	11.0		11.7		12.4	12.8		13.9	0.6	
dl-proline	6.0			7.0		7.9		8.9	0.4	

(*a*) – the mean number of moles of water per mole of different amino acids.

Table 3

	n	n_t					error	literature data
		°C	20	25	30	35	± δ	[24]
diglycine	7.0		8.5	8.9	9.3	9.8	0.5	4.5 25°C; $6.0^{(a)}$
triglycine	11.0		12.4	12.8	13.2	13.7	1.0	5.5 25°C; $7.5^{(a)}$
glycyl-l-α-alanine	9.0		10.6	11.0		12.0	0.5	
glycyl-dl-α-alanylglycine	12.0			13.5	14.0	14.5	1.0	
dl-alanyl-dl-alanine	11.0		12.5	12.8		13.9	0.5	
l-alanylglycine	9.0			10.8		11.9	0.5	
glycyl-l-proline	9.0			10.7		11.8	0.5	
glycyl-l-valine	16.0			17.6		18.8	1.0	

(*a*) – obtained by extrapolation to 0°C.

other compounds [13–20]:

$$n_t = n + At + Bt^2, \tag{3}$$

where n is the number of water moles per 1 mole of the solute at 0°C, A and B are experimental coefficients equal, respectively, to 3.83×10^{-2} and 1.3×10^{-3}, and t is temperature in °C.

Assuming that at 0 °C water molecules are in the ice-like structure, one can suggest that the number n ilustrates the interaction between the solutes and the water lattice.

The further terms of the equation, i.e. At and Bt^2, are related to the properties of water itself, independent of the type of the solute, and they determine the population of "free" water at different temperatures; at 25°C, $At + Bt^2 = 1.8$.

Similar relationships between n_t and temperature, obtained for the different types of compounds, suggest a similar mechanism of the hydration of these substances: a certain ice-like structure of considerable stability is formed around solute molecules. At the concentration of alcohol corresponding to the maximum of ultrasonic velocity, a structure, composed of quasi crystalline hydrates of the examined substances, an aqueous alcoholic structure, similar to those above and to that of "free" water, whose molecules do not build the ice-like skeleton, is formed.

Comparison of the results obtained for the individual groups of chemical compounds allows formulation of some relationships between hydration numbers and structure of these compounds:

1) the hydration numbers increase with the growth of the hydrocarbon chain, the mean number of water molecules per $-CH_2-$ group is 2.

2) the hydration numbers of di- and tripeptides are, respectively, smaller by 1 or 2 than the sum of the hydration numbers of amino acids composing the given peptide. Glycylvaline with the hydration number equal to the sum of the hydration numbers of glycine and valine is an exception to this rule.

3) the shift of the amine group into the β-position of the alkyl chain (β-alanine) or the introduction of an additional amine group into the ε-position (lysine) causes a decrease in the hydration number by 1 as compared with the respective α-amino acids.

The results obtained allow some conclusions to be drawn regarding the mode of binding water molecules by the different groups present in the substances tested.

Taking into account the low degree of dissociation of carboxylic acids in aqueous solutions, the presence of ions may be neglected and such solutes may be treated as polar molecules. The binding of water by these molecules will take place mainly by free electron pairs of oxygen atoms and with protons in carboxylic groups. The alkyl groups of acids will probably be hydrated in the same way as alkyl groups of non-electrolyte molecules.

The situation is slightly different in the case of amino acids and peptides.

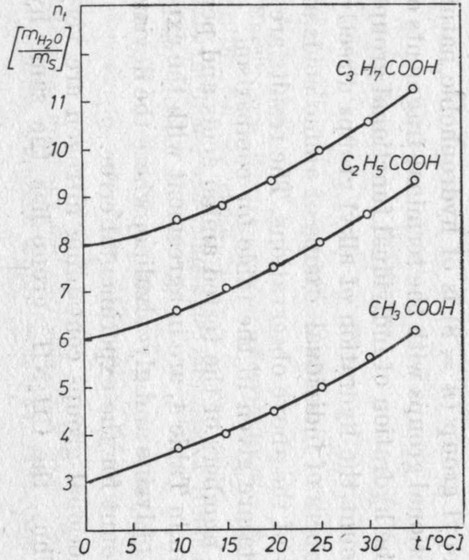

Fig. 1. Dependence of the hydration numbers n_t of carboxylic acids on temperature

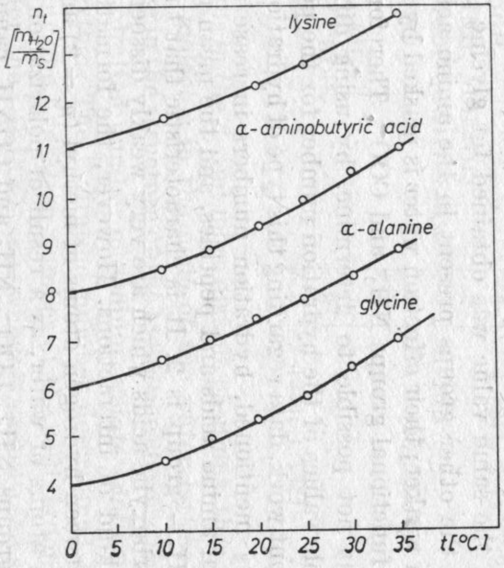

Fig. 2. Dependence of the hydration numbers n_t of amino acids on temperature

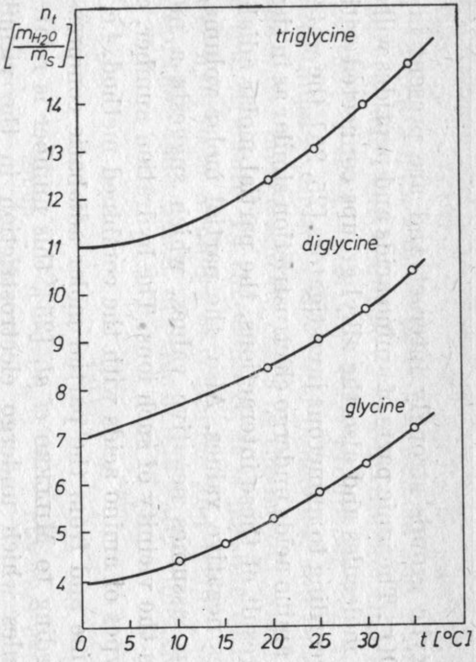

Fig. 3. Dependence of the hydration numbers n_t of glycine oligopeptides on temperature

Amine and carboxylic groups strongly interact and are present in the ionic forms NH_3^+ and COO^-. The ionic parts of amino acids and peptides will affect the surrounding water molecules and also the alkyl groups connected with them in a different way. According to numerous investigators [25, 26], the water molecules which surround amino acids undergo electrostriction similar as in the presence of ions, and, as a result of these interactions, the partial molar adiabatic compressibility assumes negative values. Also, the partial molar volume, affected and electrostriction, assumes negative values, which suggests a more dense packing of water in the vicinity of such ions. The hydration number, calculated for the different types of amino acids with the combined method, i.e. from the partial molar volume and from the partial molar adiabatic compressibility, is about 4 and, according to MILLERO *et al.* [25], this number is related only to such water molecules which undergo electrostriction in the vicinity of the $NH_3^+CHCOO^-$ group.

In this paper, the same value was obtained for glycine ($NH_3^+CH_2COO^-$). The interaction of the other groups present in the amino acids and peptides with water is much weaker; their effect on water is masked by the strong interactions of charged functional groups NH_3^+ and COO^-. Therefore, in the former experiments, it was not possible to determine, by using different detecting methods, the reliable values of the hydration number for the peptide and alkyl groups. In the present work, data regarding this type of hydration were obtained.

As was already mentioned, hydration numbers increase with the length of the alkyl chain of amino acids and peptides, and the mean number of water molecules per $-CH_2-$ group is 2. It is characteristic that the same result is obtained for the carboxylic acids which are very weakly dissociated. This could suggest the same kind of interactions. However, the formation of the weak hydrogen bonds between hydrogen atoms of methyl ($n = 2$) and ethyl ($n = 4$) groups and oxygen atoms of water, as a result of polarization of these groups by the functional groups NH_3^+, COO^-, NH_2 and CONH, cannot be excluded. This hydration would be of hydrophilic nature. On the other hand, it seems that hydration of isopropyl group ($n = 8$) is of hydrophobic nature due to weaker interactions of functional groups with the terminal fragments of the alkyl chain. Determination of the hydration of individual functional groups should be made by taking into account the hydration of alkyl groups directly bound to them. The hydration numbers of individual groups of amino acids and peptides were determined in view of the above observations. The results are given in Table 4. Some literature data are given in the table for comparison.

The hydration numbers of the tested amino acids and peptides, calculated from the data shown in Table 4, are in agreement with the experimental values, with the exception of lysine and glycylvaline, where the deviations of one water molecule could account for the experimental error.

Generally, functional groups containing nitrogen are very weakly, or not at all, hydrated. Thus, the $CH_2NH_3^+$ group has the same hydration number as the $CH_2(CH_3)$ group, α-alanine as propionic acid and α-aminobutyric acid as that of *n*-butyric acid. This could be caused by the fact that the dimensions and geometry of the $-NH-$, $-NH_2$ and NH_3^+ groups, as well as those of the NH_4^+ ion [16], are similar to those of the water molecules, and these can replace both nodes of the water lattice and its cages in the ice-like water structure without disturbing this structure. In this context, it is possible to clarify the problem of the lower hydration numbers of β-alanine and lysine, as in the structures of hydrates of these substances, in place of one water molecule found in the vicinity of alkyl groups in the β- and more distant positions, there occurs a fragment of the hydration molecule in the node of the water lattice and this does not lead to any change in the structure of the hydration sheath. The results of measurements of the hydration number of sodium acetate confirm the above assumption, to some extent. The hydration number determined for this compound is $n = 8$. Assuming the hydration number of the sodium ion as 5 [16], a value of 3 is obtained for the CH_3COO^- ion, which is exactly the same as the hydration number of the CH_2COO^- group.

Table 4

group	n	literature data
$CHCOO^-$, CH_2COO^-	3	
COOH	3	3–4 [21]; 2 [22]; 2 [28]
$CHNH_3^+$, $CH_2NH_3^+$	2	
$NH_3^+CHCOO^-$, $NH_3^+CH_2COO^-$	4	4 [25]
CONH	2	1–1.5 [28]; 3 [27]
CH_3	2	
CH_3CH_2	4	
CH_3CHCH_3	8	
CH_2	2	2 [23]; 1 [22]

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