Hydration of Amino Acids from Ultrasonic Measurements

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In this paper the results of compressibility of aqueous solutions of amino acids in water and in aqueous HCl and NaOH solutions at 25 °C are presented. The effect of the charged protonated amino groups and deprotonated carboxylic groups on the hydration number was tested. The idea of additivity of the hydration number with the constituents of the solute molecule was successfully applied and discussed.

1. Introduction

Compressibility is known as an independent and effective tool for investigating biologically active compounds and finds increasing use as a means for characterizing protein systems.[1,2]

Remembering that biological macromolecules are physiologically active in aqueous solutions, the knowledge of interaction of proteins and their groups with water is necessary to understand the role of this solvent when solvated to soluble organics in living cells. Some difficulty arises from the fact that amino acids exist mainly in zwitterionic forms in aqueous solutions. This suggests that their volume and compressibility properties are intermediate between those of electrolytes and organic nonelectrolytes. The importance of hydrophobic hydration of these solutes is another problem, as this mechanism could be competitive with the electrostatic one.[3] Such competition was also proved by Ide et al., who found that the structure of water in solutions of amino acids with nonpolar side chains is enhanced, while around amino acids with polar or charged side chains it is destroyed.[4]

The volume properties of proteins and amino acids are characterized by a decrease in volume when these substances are dissolved in water, similar to what occurs for electrolytes.[5,6] This phenomenon is usually attributed to electrostriction due to water−solute interactions, suggesting that amino acids or proteins behave as electrolytes. However, it is also possible that the so-called "bound water" behaves more like water solvated to organic solutes than bulk water.[7,8] This interpretation is evidently too simplified if one keeps in mind that amino acids are traditionally considered as hydrophobic substances and that their hydrophobicity is well correlated with the length of the chain. Hecht et al. compared different rankings of the amino acids' hydrophobicity and found that the size of the water clathrate around the hydrophobic side chain increases in the order Gly < Ala < Val, Ile, Leu.[9] Note that some hydrophobic character can be attributed even to the shortest amino acid, glycine.

In this work we apply a different approach. It was recently found that the hydration numbers determined ultrasonically, i.e., from the density and speed of sound data, in two-component aqueous solutions of simple organic nonelectrolytes are additive with both the length and the character of the constituents of the solute molecule.[10–12] It was also proved that for different solute molecules the functional groups bring the same increment to the total hydration number observed. Thus, the idea of determining such contributions for amino acids seems possible and attractive, because of the possibility of estimation of the impact of water solvent on the geometrical aspects of the amino acids' interactions and reactivity, as was shown by Pratt and Pohorille in their review devoted to the models and structure simulations of organic molecules, in particular amphiphilic, at an interface.[3]

We also applied two different solvents—0.2 M HCl and 0.2 M NaOH—to check how the pH of the system affects the calculated hydration numbers. It should be stressed that for reacting systems, i.e., amino acid + HCl or NaOH, the Pasynski method yields values of hydration numbers which result from compressibility of substances engaged in occurring reactions.

Performing studies in aqueous HCl and NaOH solutions essentially means that the pH values will change. Thus, the conditions which may exist in biological systems, including the human body, can be practically simulated in the laboratory.

2. Experimental Section

Glycine (POCH, Poland, p.a.), L-alanine (ROTH, Germany, for biochemistry ≥99%), L-valine (AppliChem, Germany, >99%), L-leucine (Reanal, Hungary, 98%), L-isoleucine (Reanal, Hungary, 98%), L-phenylalanine (AppliChem, Germany, pure Ph. Eur., USP grade, >98.5%), L-serine (MP Biomedicals, LLC, Germany, USP grade), and L-lysine monohydrate (Loba-Feinchemie, Austria, pure) were used without additional purification.

Glycine, L-alanine, L-valine, L-leucine, L-isoleucine, L-phenylalanine and L-serine hydrochlorides, L-lysine dihydrochloride, and glycine, L-alanine, L-valine, L-leucine, L-isoleucine, L-phenylalanine, L-serine, and L-lysine sodium salts were prepared by mixing equimolar amounts of the respective amino acid and hydrochloric acid or sodium hydroxide. The latter were prepared from Fixanal chemicals.

Solutions were prepared before measurements by weighing using doubly distilled, freshly prepared water.

The speed of sound was determined using a computer-steered OPKUD 01/100 apparatus (Optel, Wrocław, Poland), with an absolute accuracy better then ±0.2 $m \cdot s^{-1}$ and a precision of similar order. Measurements are based on the determination of the time an acoustic signal needs to pass through a sample of known length. All samples were degassed before measurements in an ultrasonic cleaner.

The density was measured using a vibrating tube Ecolab MG-2 (Kraków, Poland) apparatus with an accuracy of ca. ±0.1 $kg \cdot m^{-3}$.

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The temperature of the measurements was 25 ± 0.01 °C, stabilized by a precision Julabo F25-ME (Germany) thermostat. Its stability was controlled by a digital thermometer built into the density apparatus and the absolute value by a precision mercury thermometer.

From the speed of sound and density data the adiabatic compressibility coefficients, κ_S, were calculated, using the Laplace equation:

$$\kappa_S = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_S = \frac{1}{du^2} \quad (1)$$

where V is the volume, P is the pressure, d is the density, u is the speed of sound, and the index S denotes the adiabatic condition.

The relative change of compressibility was assumed to be caused by some fraction of water molecules being engaged in hydration spheres, where they become incompressible. This leads to the well-known formula for the hydration number, n_h, known as the Pasynski equation[13,14] (see also a review by Stuehr and Yeager[15]):

$$n_h = \frac{n_1}{n_2}\left(1 - \frac{\kappa_S}{\kappa_S^0}\right) \quad (2)$$

where n_1 and n_2 are the numbers of moles of water and solute in solution, respectively, κ_S is the adiabatic compressibility coefficient of the solution, and κ_S^0 is that of pure solvent. The applicability of the Pasynski equation in nonelectrolytic aqueous solutions was recently examined by us.[10]

There were three series of experiments performed, differing by the type of solvent: pure water, 0.2 M HCl, and 0.2 M NaOH. The two latter were prepared using Fixanal chemicals.

3. Results and Discussion

In this work only very dilute systems were investigated. The mole fraction of the solutes never exceeded 0.01; i.e., there were always more than 100 water molecules per solute molecule. For such dilutions one can neglect interactions between the solute molecules, as well as assume that individual solvation shells are effectively separated by bulk water molecules.

It is both surprising and meaningful that, in the investigated range of concentrations, all the solutes are characterized by a perfectly linear dependence of the adiabatic compressibility coefficients κ_S on the concentration, as can be seen in Figure 1 (the R^2 coefficient was always 0.999 or better). This means that the hydration numbers of nonelectrolytes obtained by the Pasynski formula are undoubtedly constant for such low concentrations.

3.1. Calculations of the Contributions of Functional Groups to the Observed Total Hydration Numbers of Amino Acids. The values of the hydration numbers are now assumed by us to be a sum of the contributions from individual constituents of the solute molecule, as was proposed recently.[11] This idea arises from the well-known fact that in a homological series of simple organic solutes the changes of many physical parameters are regular. The linear or almost linear variability with extension of the solute hydrocarbon chain is very well-known for partial molar volumes and adiabatic compressibilities.[33–35] However, the linearity of compressibility changes with elongation of the solute molecule suggests a similar linearity of hydration numbers also. Such ideas were presented by Hedwig et al.,[36] Cheng et al.,[37] and others.[38,39] For a series of poly(vinyl alcohol)s, Inzelt and Grof found the hydration number additive with the number of monomers (i.e., with the chain length) and equal to 3.7−4.0 per −OH group.[40] This value is in perfect agreement with the results of Sasahara and Uedaira, who estimated the contribution of a monomer to the total hydration number of poly(ethylene glycol) as 3.9.[41]

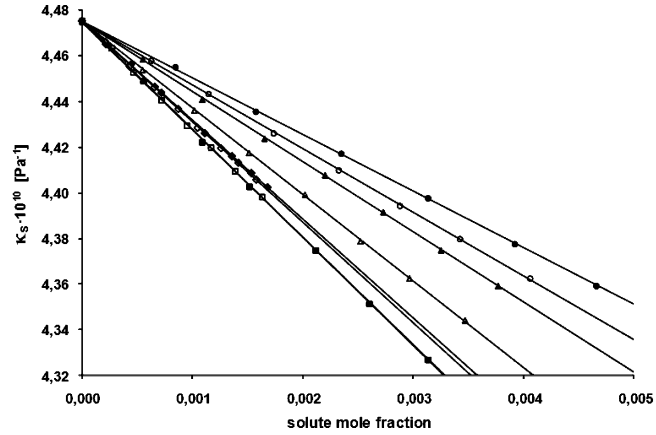

Figure 1. Example of experimental results: κ_S vs solute mole fraction for amino acids in water at 25 °C. Key: ●, glycine; ○, L-alanine; ▲, L-serine; △, L-valine; ◆, L-leucine; ◇, L-isoleucine; ■, L-lysine; □, L-phenylalanine.

Assuming additivity of hydration numbers with the geometry of the solute molecules and collecting respective experimental data for a series of compounds, a number of linear equations with the number of unknowns equal to the number of constituents were obtained which were easy to solve. A similar procedure was applied in the study reported in ref 35 and is described in detail there.

With this assumption, we determined the hydration numbers of amino acids and respective amino acid hydrochlorides and amino acid sodium salts in water at 25 °C. The obtained data are collected and presented in Table 1 together with the data available in the literature.

In the fitting procedure for amino acid hydrochlorides and sodium salts we also used the ionic n_h values of the Na^+ cation (4.0) and Cl^- anion (3.1) given in ref 42, which were also determined from the acoustic Pasynski method at 25 °C on the basis of data collected by Allam and Lee.[43,44]

When taking the above into consideration, the set of data obtained in this way allows the hydration numbers of different groups (fragments) of a solute molecule to be fitted in the manner presented in ref 35. These contributions are listed in Table 2, and the n_h values calculated from them for all the solutes applied for the fitting are included in Table 1. The differences between experimental and calculated hydration numbers are surprisingly low, and thus, the assumption expressed in the Introduction seems to be very well confirmed. It is worth noting that the calculated sum of squares of deviations (SQ), the parameter minimalized in fitting and being the probe of the goodness of the fitting, was as low as 2.66.

It could seem strange to some that constituents such as $-CH_3$ or $-CH_2-$ also have contributed their values to the overall hydration number. This was discussed in the Introduction (the effect of the size of the molecule), but one should also remember that dielectrically water + amino acid systems rather resemble emulsions, containing two kinds of spherical solutes.[31] Therefore, this picture suggests the importance of hydrophobic interactions in the overall hydration.

TABLE 1: Hydration Numbers (Experimental and Calculated) of Amino Acids and Their Respective Hydrochlorides and Sodium Salts in Water at 25 °C Together with Data Available in the Literature[a]

	hydration number (n_h)			
	this work			
solute	$n_{h,exptl}$	$n_{h,calcd}$	Δn_h	from the literature[b]
glycine	5.5	5.4	0.1	2.63, 3.34, 3.67, 3.97,[8] 5.5,[14] 4.4,[16] 5.7,[17–19] 3.40,[20] 12.8,[21] 3.26,[22] 3.56, 3.23, 2.63, 3.52,[23] 2.63, 3.52,[24] 8.2,[25] 2.72,[26] 2.9,[27] 7.0,[28] 2.2,[29] 11.4[30]
L-alanine	6.2	6.4	−0.2	3.16 (D), 3.41 (D), 3.57 (D), 3.94 (D), 3.16 (L), 3.41 (L), 3.57 (L), 3.94 (L), 3.09 (DL), 3.57 (DL), 3.94 (DL),[8] 6.5,[14] 7.8,[17–19] 3.35,[20] 19.6,[21] 2.89 (DL),[22] 3.06 (L), 3.43 (L), 3.48 (L), 4.66 (L),[23] 3.41 (DL), 4.65 (DL),[24] 2.6 (DL), 3.3 (L),[25] 3.49 (DL),[26] 3.8,[27] 1.75 (L)[31]
L-valine	8.5	8.5	0.0	3.43 (L), 3.94 (L), 3.78 (L), 4.16 (L),[8] 13.6 (DL),[17–19] 4.22,[20] 30.6 (L),[21] 3.40 (L), 3.92 (L), 3.54 (L), 5.15 (L),[23] 3.43 (L), 5.18 (L),[24] 4.9 (DL),[25] 3.21 (DL),[26] 3.9,[27] 7.37 (L)[31]
L-leucine	9.7	9.6	0.1	3.93 (L), 3.94 (L), 4.30 (L), 4.96 (L),[8] 4.53,[20] 41.1 (L),[21] 4.96 (L), 7.09 (L),[24] 13.9 (DL), 8.3 (L),[25] 5.5,[27] 8.53 (L)[31]
L-isoleucine	9.8	9.6	0.2	38.5 (L),[21] 24.0 (L),[25] 8.60 (l)[31]
L-phenylalanine	10.6	10.7	−0.1	4.15, 4.28, 4.64, 5.22,[8] 14.1 (DL),[19] 4.48,[20] 46.9 (L),[21]4.94 (DL),[26] 7.51 (L)[31]
L-serine	6.9	6.9	0.0	6.9 (DL),[19] 4.04,[20] 4.2 (DL),[25] 3.91 (L),[26] 3.1[c,32]
L-lysine	10.5	10.5	0.0	12.8 (DL),[17–19] 20.1 (L),[25] 2.8[c,32]
glycine hydrochloride	6.7	6.0	0.7	
L-alanine hydrochloride	7.0	7.0	0.0	
L-valine hydrochloride	8.7	9.1	−0.4	
L-leucine hydrochloride	10.1	10.2	−0.1	17.2 (L)[19]
L-isoleucine hydrochloride	9.5	10.2	−0.7	15.4 (L)[19]
L-phenylalanine hydrochloride	11.4	11.3	0.1	15.5 (DL)[19]
L-serine hydrochloride	7.9	7.5	0.4	
L-lysine dihydrochloride	13.1	13.7	−0.6	
glycine sodium salt	9.4	9.9	−0.5	
L-alanine sodium salt	10.6	10.9	−0.3	
L-valine sodium salt	13.7	13.0	0.7	
L-leucine sodium salt	14.2	14.1	0.1	
L-isoleucine sodium salt	14.5	14.1	0.4	
L-phenylalanine sodium salt	15.2	15.2	0.0	
L-serine sodium salt	10.9	11.4	−0.5	
L-lysine sodium salt	13.6	15.0	−1.4	

[a] The theoretical (calculated) hydration numbers, $n_{h,calcd}$, were obtained using values from Table 2. Note that the literature hydration numbers were obtained using different methods, including nonacoustic ones. [b] D stands for the D-enantiomer, L for the L-enantiomer, and DL for a mixture of D- and L-stereoisomers. [c] Hydration number of the amino acid side chain.

TABLE 2: Calculated Hydration Numbers of Functional Groups, That Is, Their Contributions to the Observed Total Hydration Numbers, Together with Their Values Available in the Literature

group	this work	contribution to n_h from the literature
$-NH_2$	0.92	−1 to 0[17]
−COOH	1.32	2−3[17]
$-NH_3^+$	0.45	for the $-CH<^{NH_3^+}_{COO^-}$ group: 4.1 ± 0.4,[8] 4[17]
$-COO^-$	3.83	
−OH	0.80	2.6−4.0[30]
$-CH_3$	1.47	1−2[17]
$>CH_2$	1.17	1−3,[17] 1.0,[30] 3,[31] 2.1[45]
>CH−	0.63	
$-C_6H_5$	4.64	

TABLE 3: Comparison of Experimental and Calculated Values of Hydration Numbers of Amino Acids in Water, 0.2 M HCl, and 0.2 M NaOH[a]

	in water	in 0.2 M HCl		in 0.2 M NaOH	
solute	$n_{h,exptl}$	$n_{h,exptl}$	$n_{h,calcd}$	$n_{h,exptl}$	$n_{h,calcd}$
glycine	5.5	4.9	3.9	0.2	0.0
L-alanine	6.2	5.3	4.9	1.6	1.0
L-valine	8.5	6.7	7.0	3.9	3.1
L-leucine	9.7	7.9	8.1	4.7	4.2
L-isoleucine	9.8	7.7	8.1	5.0	4.2
L-phenylalanine	10.6	9.4	9.2	5.3	5.3
L-serine	6.9	6.0	5.4	1.2	1.5
L-lysine	10.5	9.5	9.5	4.4	5.1

[a] The calculated values of n_h in 0.2 M HCl and in 0.2 M NaOH were obtained using eqs 4 and 6, respectively.

The contribution for a nonprotonated amino group is a little higher than for its protonated form, a rather strange behavior when electrostriction is supposed to cause a more pronounced effect. By analogy, the relatively high value for $-COO^-$ compared to the much lower one for −COOH is caused by additional electrostriction. This effect is undoubtedly worth further investigation.

3.2. Hydration Numbers of Amino Acids in Acidic and Alkaline Solutions. We also determined the hydration numbers of amino acids in aqueous 0.2 M HCl and 0.2 M NaOH at 25 °C to check how they are influenced by the pH of the system. Table 3 collects these results together with respective values obtained in pure water.

Amino acids exist in aqueous solution in zwitterionic form, and their oppositely charged groups undergo protolytic reactions with acids or bases. As can be seen from Table 3 the values of the hydration numbers of amino acids in 0.2 M HCl are on average lower by about 1.5 than those in pure water. The hydration numbers of amino acids in 0.2 M NaOH are much

lower than those in aqueous and acidic solutions. The difference between those in pure water and alkaline solutions is on average about 5.0.

The answer comes from the analysis of hydration numbers of simple electrolytic solutes, presented by us recently[42] and calculated on the basis of data collected by Allam and Lee.[43,44] It was found that n_h of the OH^- anion is equal to 5.9, while that of the H^+ cation is equal to -1.0. Independent of the negative n_h of the latter, which is unique and unexpected and undoubtedly needs deeper investigations and explanation, these values suggest that applying alkaline or acid solvents influences the obtained hydration numbers if the solute reacts with OH^- or H^+ ions. For amino acids in HCl one should consider the following reaction (R denotes a hydrocarbon chain):

$$\mathrm{RCH}<^{\mathrm{NH_3^+}}_{\mathrm{COO^-}} + \mathrm{H^+} + \mathrm{Cl^-} \rightarrow \mathrm{RCH}<^{\mathrm{NH_3^+}}_{\mathrm{COOH}} + \mathrm{Cl^-} \quad (3)$$

This means that when an amino acid is added to a HCl solution, a reaction of the $-COO^-$ group with H^+ ions takes place. As a result of this reaction, the respective cations of the amino acid appear in solution, and simultaneously an equivalent number of H^+ ions disappear from the system, while the number of Cl^- anions remains unchanged. In the case of L-lysine protonation of bare $-NH_2$ group also occurs.

Therefore, taking the above into consideration, i.e., making a balance of water molecules engaged in the process of formation of ions and their solvates, the hydration numbers of the amino acid in hydrochloride acid solution can be calculated from the following equation:

$$n^{0.2\mathrm{M\ HCl}}_{h\,\mathrm{RCH}<^{\mathrm{NH_3^+}}_{\mathrm{COO^-}}} = n_{h\,\mathrm{RCH}<^{\mathrm{NH_3^+}}_{\mathrm{COOH}}} - n_{h\,\mathrm{H^+}} \quad (4)$$

where

$$n^{0.2\ \mathrm{M\ HCl}}_{\mathrm{h,RCH}<^{\mathrm{NH_3^+}}_{\mathrm{COO^-}}}$$

is the hydration number of the amino acid in 0.2 M HCl

$$n_{\mathrm{h,RCH}<^{\mathrm{NH_3^+}}_{\mathrm{COOH}}}$$

is the hydration number of the respective amino acid cation in pure water, and n_{h,H^+} is the hydration number of the H^+ cation ($n_{h,H^+} = -1.0$; see ref 42).

In a similar way we can explain why the values of the hydration numbers of amino acids in 0.2 M NaOH solutions are much more lower than their respective values in pure water. Let us consider the following reaction:

$$\mathrm{RCH}<^{\mathrm{NH_3^+}}_{\mathrm{COO^-}} + \mathrm{OH^-} + \mathrm{Na^+} \rightarrow \mathrm{RCH}<^{\mathrm{NH_2}}_{\mathrm{COO^-}} + \mathrm{H_2O} + \mathrm{Na^+} \quad (5)$$

As a result of the above reaction 1 H_2O molecule appears in the system as a product of deprotonation of the $-NH_3^+$ group, and also 5.9 molecules of water appear which formed before the hydration shell of the OH^- anion ($n_{h,OH^-} = 5.9$; see ref 42). The number of Na^+ cations remains unchanged.

When making a balance of water molecules engaged in the process of formation of ions and their solvates, the hydration numbers of amino acids in sodium hydroxide solution can be calculated as follows:

$$n^{0.2\mathrm{M\ NaOH}}_{h\,\mathrm{RCH}<^{\mathrm{NH_3^+}}_{\mathrm{COO^-}}} = n_{h\,\mathrm{RCH}<^{\mathrm{NH_2}}_{\mathrm{COO^-}}} - n_{h\,\mathrm{OH^-}} \quad (6)$$

where

$$n^{0.2\ \mathrm{M\ NaOH}}_{\mathrm{h,RCH}<^{\mathrm{NH_3^+}}_{\mathrm{COO^-}}}$$

is the hydration number of the amino acid in 0.2 M NaOH

$$n_{\mathrm{h,RCH}<^{\mathrm{NH_2}}_{\mathrm{COO^-}}}$$

is the hydration number of the respective amino acid anion in pure water, and n_{h,OH^-} is the hydration number of the OH^- anion.

Therefore, the values of the hydration numbers of cations

$$n_{\mathrm{h,RCH}<^{\mathrm{NH_3^+}}_{\mathrm{COOH}}}$$

and anions

$$n_{\mathrm{h,RCH}<^{\mathrm{NH_2}}_{\mathrm{COO^-}}}$$

can be easily calculated from the contributions of the functional groups presented in Table 2. The results of these theoretical calculations of the hydration numbers of amino acids in acidic and alkaline solutions are shown in Table 3.

Such a simple arithmetic analysis explains very well the observed values of the hydration numbers and leads to calculated n_h values very close to the experimental ones. This was also recently proved by us for reacting systems such as amines in acidic and carboxylic acids in alkaline solutions.[46]

The hydration numbers presented in this work cannot be understood as absolute values. The acoustic method observes an average number of water molecules which are assumed to become incompressible as an effect of electrostriction, formation of hydrogen bonds, increased rigidity of the water network close to hydrophobic groups of molecules, etc. The latter phenomenon meaningfully affects neither intermolecular water−water distances nor angles between them. For example, it was found from neutron diffraction that there are ca. 3.0 water molecules coordinated to the amino group of glycine. The intermolecular distances suggest that a hydrogen bond is formed between the amino group and the nearest neighbor water molecules in aqueous solution.[47] The value listed in Table 1 ($n_h = 5.5$) is higher, most probably because of the hydrophobic hydration effect mentioned above, hardly detectable by neutron scattering.

In general, inspection of Table 1 shows that the reproduction of hydration numbers when applying the assumed additivity of them with solute molecule constituents and applying the contributions determined and shown in Table 2 is surprisingly good. This is a good test of the correctness of our initial assumptions and the applied procedure. However, the reason the protonation of the amino group has little effect on its

hydration while deprotonation of the carboxyl group increases its hydration number ca. 3-fold is unknown and needs further studies; the same concerns the negative value of the hydration number of the H^+ ion, mentioned already.

4. Conclusions

In this study, the speeds of sound and densities of diluted aqueous solutions of chosen simple amino acids and their hydrochlorides and sodium salts were measured at 25 °C. On the basis of these data, the hydration numbers of the solutes were calculated using the concept of Pasynski. These values are very close to the literature n_h values obtained by the same technique and on the same order as those obtained by other methods.

It was shown for diluted aqueous solutions of amino acids that the effect of solutes on the hydration numbers is strongly and directly dependent on the length of the solute molecules and their constituents. It seems possible now to predict the hydration numbers of amino acids and their salts only from their structural formulas.

It was also tested how the pH of the system affects the calculated hydration numbers. It should be stressed that for reacting systems, i.e., amino acid + HCl or NaOH, the Pasynski method yields values of hydration numbers which are combinations of the compressibilities of substances engaged in the occurring reactions.

The results of our experiments on aqueous solutions of simple amino acids and their salts are important for the general knowledge about their hydration and could be useful in understanding the behavior of biologically active macromolecules in an aqueous environment.

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