Investigation of hydration of α-amino acids by means of absorption millimeter spectroscopy

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The hydration indexes for 19 protein α-amino acids are measured by means of absorption millimeter spectroscopy (AMS) at 31.42 GHz. The plot of the hydration indexes on the area of surface of aliphatic amino acid molecules accessible for water is a straight line located above the points corresponding to aromatic or polar amino acids. The contribution of nonpolar groups in the hydration index is greater than that of polar groups provided that their accessible surface areas are equal. The contribution to hydration of —OH and $-CONH_2$ groups in Ser, Gln, and Asn coincides in sign with that of pure hydrophobic hydration but the value of the contribution is significantly smaller. The change in mobility of water molecules, which is the basis of the AMS method, may serve as the physicochemical foundation for the construction of a new hydrophobicity scale for amino acids comparable with the already existing scales.

Key words: amino acids, hydration, hydrophobicity, absorption millimeter spectroscopy.

The side groups (R) of protein α-amino acids ($H_3N^+CHRCOO^-$) may be both of hydrophilic and hydrophobic nature. Nonvalence interactions of amino acid residues within one polypeptide chain and intermolecular interactions of these residues with water have a significant influence on the folding of globular proteins[1] that results in the formation of a space structure unique to each protein. The polarity of side groups R determines to a large extent the specificity of action for active sites of different enzymes,[2] as well as the nature of other physiologically important interactions, such as protein—protein and protein—ligand interactions.[3] The formation of the corresponding complexes should be preceded by destruction of the hydrate shell both of a ligand (substrate) and of side groups of amino acids which form binding centers.

Practically all the experimental and theoretical methods used in the physical chemistry of aqueous solutions are suitable for quantitative estimation of the hydration effect for side groups R. This makes it possible to obtain various indexes and, in some cases, hydration numbers, *i.e.*, the number of water molecules in a hydration shell. The values of hydration indexes as a function of physical nature of parameters are in one form or another associated not only with the reorganization of a solvent on a water—nonpolar fragment interphase (hydrophobic hydration) but also with the effect of polar (hydrophilic) hydration, *i.e.*, with the formation of H-bonds between water and a polar fragment. The interrelation of these effects are differently manifested in the parameters obtained by different experimental methods. This is probably one of the main reasons for the absence of a unified hydrophobicity scale.

Earlier, absorption millimeter spectroscopy (AMS) was used to study hydration effects. It was shown that the absorption coefficient α of electromagnetic radiation for aqueous solutions within the 1—10 cm^{-1} range depends on the state of an aqueous component. Hydration of aqueous solutions of alcohols,[4] amino acids, and a series of other amphiphilic compounds[5] was studied at 3—10 cm^{-1} (300—100 GHz), and it was shown that hydration numbers can be directly determined by this method. The absorption in this region is almost completely determined by the content of the fraction of mobile water molecules, which have not less than one rotation degree of freedom. The hydration numbers obtained allow us to estimate experimentally the number of water molecules, which are retained in hydration shell and lose their rotation mobility.

A new variant of the AMS method based on "wave guide dielectric resonance" effect, performed within the 0.75—1.3 cm^{-1} (23—40 GHz) range, was recently proposed.[6] This procedure was used to study the hydration of aliphatic amino acids[7,8] existing in the form of $H_3N^+CHRCOO^-$ zwitterions. At this frequency range, a

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noticeably higher absorption of solutions of hydrophobic compounds as compared with pure water is observed at room temperature. It was shown that the hydration indexes (N) determined are linearly dependent on the area of the surface of an amino acid molecule accessible to water, "accessible surface area" (ASA). For glycine, the ratio of the N index and ASA was not less than 4 times lower than that for hydrophobic amino acids such as valine, leucine, and isoleucine.

In the present work we used this procedure for determining the hydration indexes of 19 protein α-amino acids in diluted aqueous solutions, including acids sparingly soluble in water. These indexes were used to estimate the relation of the hydration effects of nonpolar groups (purely hydrophobic hydration) and hydration of polar groups.

Experimental

The amino acids used were purchased from Sigma (USA).

Measurements of the absorption of aqueous solutions of amino acids at a concentration 0.5—1 % (wt) were carried out at the frequency of 31.42 GHz at 30 °C with an accuracy not lower than ±4 % using the procedure of Ref.7. Absorption (α/dB) was estimated by the formula $\alpha = -\log I/I_0$, where I_0 is the intensity of electromagnetic radiation after passing through pure water and I is the intensity of the beam, which decreased after absorption of the radiation by the solution. The effect of amino acid hydration was expressed as the difference (δα) between the absorption of the solution (α_{exp}) and the theoretical contribution of the aqueous component according to the equation

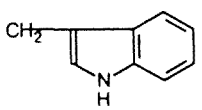
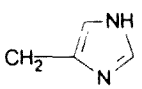
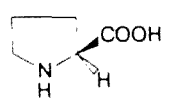
$$\delta\alpha = \alpha_{exp} - \kappa_l C_l, \qquad (1)$$

where $\kappa_l C_l$ is the calculated contribution of absorption by the aqueous component, C_l and κ_l are the molar concentration and extinction coefficient of pure water.

Results and Discussion

The δα values determined experimentally for 19 amino acids (all protein amino acids except cysteine) are rather different (Table 1), indicating the change in the state of water molecules in the hydration shells of the amino acids studied; otherwise, the δα values were equal to zero. Earlier it has been shown that the deviations of the α_{exp} values measured in the millimeter range, from the expected contributions of the aqueous phase to ab-

Table 1. Hydration indexes $N = \delta\alpha/\kappa_l C_2$ of α-amino acids at 31.42 GHz (30 °C)

Amino acid	R	C_2 / mol L^{-1}	δα / dB mm^{-1}	N	S_R[a] / Å^2	S_{np}[a] / Å^2
Gly	H	0.134	0.036	3.7	25	25
Ala	Me	0.112	0.123	15.1	67	67
Val	$CH(Me)_2$	0.0854	0.177	30.2	117	117
Leu	CH_2CHMe_2	0.076	0.177	33.7	137	137
Ile	CH(Me)Et	0.0762	0.201	37.4	140	140
Asp	CH_2COOH	0.0187	0.018	13.1	106	48
Ser	CH_2OH	0.0954	0.072	10.6	80	44
Trp		0.0122	0.027	32.7	222	195
Phe	CH_2Ph	0.0605	0.125	30.9	175	175
Asn	CH_2CONH_2	0.0759	0.060	10.8	113	44
Arg	$(CH_2)_3NHC(=NH)NH_2$	0.0574	0.078	19.6	196	89
His		0.0645	0.071	14.9	151	102
Gln	$CH_2CH_2CONH_2$	0.0685	0.072	15.0	144	53
Glu	CH_2CH_2COOH	0.0340	0.032	13.3	138	61
Lys	$(CH_2)_4NH_2$	0.0684	0.098	19.4	167	119
Met	CH_2CH_2SMe	0.0671	0.105	21.6	160	117
Pro[b]		0.0869	0.100	15.6	105	105
Thr	$CH(OH)CH_3$	0.0840	0.096	16.2	102	74
Tyr[c]	$CH_2C_6H_4OH$-p	—	—	27.0	187	144

[a] S_R and S_p are from Ref. 9. [b] Total amino acid formula. [c] Calculated from the data on hydration of Gly-Tyr since the solubility of Tyr is low.

sorption $\kappa_1 C_1$, *i.e.* from $\delta\alpha$ (equation (1)), are determined by the state of water in the hydration shells of dissolved molecules[6] (the absorption range of amino acids lies far from the millimeter wavelength range). The existence of the region for diluted solutions where $\delta\alpha$ is proportional to the concentration of the dissolved component C_2 (the region where the Lambert-Beer law can be applied)[5,7] allows one to regard the indexes

$$N = \delta\alpha/\kappa_1 C_2 \qquad (2)$$

as a concentration-independent measure of hydration.

The absorption deficit, $-\delta\alpha$, at 3—10 cm^{-1} (20 °C) was explained by the existence of a practically non-absorbing component, bound hydrate water. At the same time, there are particles in the bulk of water (rotators)[5] which strongly absorb millimeter radiation. Within the framework of the assumption that water loses its rotation mobility in a hydrate shell, the N_θ values introduced by equation (2) may be interpreted as hydration indexes.[5] Under our experimental conditions, *i.e.*, in the long-wave part of the millimeter region, the model of interaction of radiation with fractions of water molecules, which differ in the number of free and retarded degrees of freedom, is significantly more complex since the hydrate shell is heterogeneous and a contribution of the relaxation effects of bound water, which is not manifested in a shorter wavelength region, may be observed. Therefore, the N values introduced by equation (2) are treated by us not as numbers of water molecules but as hydration indexes.

As can be seen from Table 1, relatively large values of N are obtained for amino acids that are known as hydrophobic (Leu, Ile, Trp, Phe, *etc.*). This corresponds with the fact that hydrophobic hydration is accompanied by stabilization of a network of water H-bonds in the first hydration shell of hydrophobic molecules.[3]

It is known that hydrophobic hydration is substantially diminished with the rise of temperature that is manifested, for example, by a strong decrease in the heat capacity of hydration.[1] The AMS method at 1 cm^{-1} also appeared to be temperature-sensitive. For example, for some amino acids, the N indexes at 14 °C are even negative, −3.7 for Met, −3.6 for Pro, −0.6 for Thr, and −0.1 for Gln.

Figure 1 presents the dependence of the hydration indexes for 19 amino acids on the surface area of a side radical accessible for water molecules.[9] As we have mentioned before,[7] a good linear correlation $N_{alk} = a + bS_R$ is observed for aliphatic amino acids (Gly, Ala, Val, Leu, and Ile), that is typical for the majority of hydrophobicity scales. This is evidence of the heterogeneity of the hydration shell formed around aliphatic radicals and allows one to calculate[7] the specific increment of pure hydrophobic hydration per 1 $Å^2$ (b = 0.283±0.015). Multiplying b by the ASA of the alkyl group, one can estimate the hydrophobic hydration of this group. For example, if we assume the average

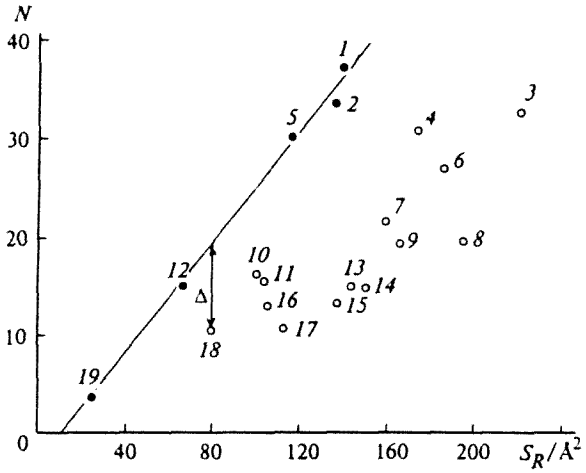


Fig. 1. Dependence of the hydration index of α-amino acids, N, on the surface area of side radicals accessible to water (ASA), S_R. *1* — Ile, *2* — Leu, *3* — Trp, *4* — Phe, *5* — Val, *6* — Tyr, *7* — Met, *8* — Arg, *9* — Lys, *10* — Thr, *11* — Pro, *12* — Ala, *13* — Gln, *14* — His, *15* — Glu, *16* — Asp, *17* — Asn, *18* — Ser, *19* — Gly. Dark circles correspond to aliphatic amino acids.

ASA for $-CH_2-$ group to be equal to 32 $Å^2$, the contribution of this group to the hydration $\Delta N_{-CH_2-} \approx 9$. The proportionality of hydrophobic hydration to ASA is, probably, a general property of hydrophobic hydration which is reliably measured not only by the AMS method but also by other methods (determination of heat capacity of hydration,[10] compressibility of solutions).[11]

As can be seen from Fig. 1, the experimental N values for all nonaliphatic amino acids lie under the N_{alk} straight line (the difference $\Delta = N_{alk} - N$ is also given in Fig. 1 while $\Delta > 0$). Thus, if we compare two compounds which contain nonpolar group in one case and polar group in the other with the same ASA, we can point that ΔN_{np} for nonpolar group is always higher than ΔN_p for polar group. The inequality $\Delta N_{np} > \Delta N_p$ is valid when $S_{np} = S_p$, where S_{np} is the ASA of the nonpolar group and S_p is the ASA of the polar group. On the plot of N *vs* S_R, nonaliphatic amino acids can be divided into two groups: 1) mainly hydrophobic cyclic amino acids, Phe, Trp, and Pro, which give the smallest deviation from the $N_{alk} = a + bS_R$ straight line, and 2) amino acids (Asp, Glu, Asn, Gln, Lys, Arg, His, Tyr, Ser, Met, and Thr) with heteroatoms (O, S, and N) in side chains which give the largest deviation.

The dependence of N on ASA of nonpolar groups of side radicals $S_{np} = S_R - S_p$ is given on Fig. 2. If there is no contribution of polar groups to hydration, the experimental points for all amino acids are located on the N_{alk} straight line. The deviation of the points corresponding to polar amino acids from the straight line in Fig. 2 characterizes the contribution of polar amino groups to hydration ΔN_p. For Ser, Asp, Asn, and Gln, the points

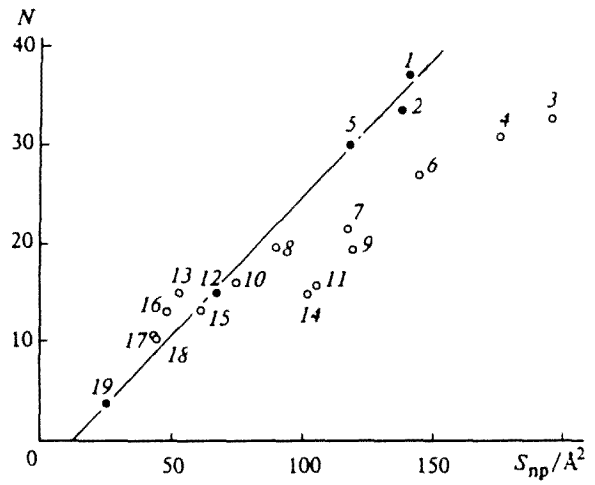


Fig. 2. Dependence of the hydration index of α-amino acids, N, on the surface area of nonpolar groups accessible to water (ASA) in side radicals $S_{np} = S_R - S_p$. Designation of amino acids is the same as in Fig. 1.

lie above the straight line; therefore, in this case, the contribution of polar group to hydration is positive. The contribution of His, Pro, Lys, and Met is of negative value.

One may quantitatively estimate the contribution of polar groups in R to hydration using the following reasoning. Let us consider hydration of the whole amino acid molecule as the sum of the hydration of its invariable part, $-CH(NH_3^+)COO^-$ (ΔN_0), the hydration of nonpolar groups of a side radical (ΔN_{np}), and the hydration of polar groups of a side radical (ΔN_p). Taking into account the linear dependence of hydrophobic hydration on the ASA, we obtain

$$N = \Delta N_0 + \Delta N_p + \Delta N_{np} = \Delta N_0 + \Delta N_p + bS_{np}. \quad (3)$$

Since the ASA for the whole side radical $S_R = S_p + S_{np}$ and $N_{alk} = \Delta N_0 + bS_R$ (according to the definition of ΔN_0), from equation (3) we obtain

$$N = \Delta N_0 + \Delta N_p + b(S_R - S_p) = N_{alk} + \Delta N_p - bS_p. \quad (4)$$

From equation (4), taking into account the expression $\Delta = N_{alk} - N$, we obtain the formula for calculating the contribution of the polar constituent to the hydration on the basis of the measured Δ values (Fig. 1):

$$\Delta N_p = bS_p - \Delta. \quad (5)$$

When obtaining the formula (5), we used the assumption that the coefficient b is constant for all non-polar groups, no matter to which groups they are bound. Equation (5) is valid for amino acids with uncharged side radicals since only in this case the total ΔN_0 contribution is strictly constant. Finally, this formula indicates that the Δ shown in Fig. 1 is a function of the polar part of the side radical only.

Table 2. Contributions of polar groups to the hydration index

Group	Amino acid	S_p / Å^2	Δ	ΔN_p	$\Delta N_p/S_p$ / Å^{-2}
—OH	Ser	36	8.6±0.2	1.6±0.6	0.04±0.02
—OH	Thr	28	9.2±0.3	−1.3±0.6	−0.05±0.02
$-CONH_2$	Gln	91	22.3±0.8	3.5±2.1	0.04±0.02
$-CONH_2$	Asn	69	17.7±0.8	1.8±1.8	0.03±0.03

The calculations based on the AMS method show (Table 2) that the contribution of the $-CONH_2$ group to hydration is of positive value, *i.e.*, has the same sign as that of nonpolar groups. The —OH group in Ser gives a positive contribution, but the contribution of the same group in Thr has the negative sign. The accuracy of ΔN_p determination is relatively low because this value is calculated as the difference of two values, but, in spite of this fact, one may conclude that the contribution of the —OH group to hydration is different for serine and threonine (1.6±0.6 and −1.3±0.6, respectively). For $-CONH_2$ groups in glutamine and asparagine, close values of ΔN_p are obtained. Thus, the principle of additivity of group contributions cannot be used in all cases for calculation of total hydration of amphiphilic compounds, and additional studies are needed. The hydration of polar groups calculated per 1 Å^2 (Table 2) is several times smaller than $b = 0.283$ corresponding to hydrophobic hydration. Thus, the dynamic mobility of water in a hydration shell is different for its interaction with polar and nonpolar fragments (hydration shell heterogeneity) at a time scale longer than 50 ps (1 cm^{-1} range). This is probably the reason for the formation of relatively weak H-bonds between water molecules in the case of hydrophobic hydration as compared with relatively strong H-bonds between water molecules and polar groups.

All the data given in this paper indicate that the AMS method depicts the difference in the state of water molecules interacting with polar and nonpolar groups. Therefore, one may consider that the N indexes may serve as a measure of hydrophobicity of amino acids (new hydrophobicity scale). Table 3 presents the ordinal numbers of amino acids enumerated according to the decrease in N for this and other scales, as well as the correlation coefficients of the ordinal numbers. A good correlation is obtained for the new scale which is based on determination of the mobility of water in the hydration shell of amino acids, with the scales based on the other physicochemical principles of determination of hydrophobicity. The largest differences are observed for glycine, which occupies the last position (Table 3), that is explained by the additional negative hydration of glycine.[5]

Thus, the hydration indexes of amino acids determined from the interaction of millimeter radiation with water molecules of a hydration shell, allow one to record

Table 3. Comparison of the hydrophobicity scale obtained using AMS method, with the scales published before

Amino acid	Ordinal number of amino acid according to the hydrophobicity scale corresponding to the work						
	Present work	Reference					
		10	12	13	14	15	16
Ile	1	3	3	2	5	4	4
Leu	2	4	1	6	4	5	3
Trp	3	1	5	1	1	1	1
Phe	4	2	2	3	2	2	2
Val	5	5	6	7	6	6	6
Tyr	6	10	4	5	3	3	7
Met	7	7	7	8	7	8	5
Arg	8	14	16	12	18	18	11
Lys	9	13	11	9	19	19	13
Thr	10	11	9	17	10	11	2
Pro	11	6	8	4	11	7	9
Ala	12	8	13	10	8	9	8
Gln	13	16	19	19	14	14	15
His	14	12	15	11	9	1	10
Glu	15	15	12	13	17	17	18
Asp	16	17	14	14	16	16	19
Asn	17	18	18	16	13	13	17
Ser	18	19	10	18	15	15	16
Gly	19	9	17	15	12	12	14
*r	1	0.89	0.90	0.90	0.83	0.84	0.94

* r is the correlation coefficient of the ordinal number of amino acid according to the hydrophobicity scale proposed in this paper with that of amino acid according to the already existing hydrophobicity scales.[10,12–16]

directly the effects of hydrophobic ordering of water and formation of H-bonds for polar groups. The rotation-vibrational mobility of water, which provides a basis for the absorption AMS method, may serve as a physico-chemical principle for construction of the hydrophobicity scale of amino acids comparable with the already existing hydrophobicity scales.

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