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Natural-Abundance Oxygen-17 Magnetic Relaxation in Aqueous Solutions of Apolar Amino Acids and Glycine Peptides

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The spin-lattice relaxation times, T_1, of $H_2{}^{17}O$ have been measured for the aqueous solutions of 11 apolar amino acids and 5 glycine peptides as a function of the concentration at 25 °C. The coordination numbers, n_h, and the rotational correlation times, τ_c^h, of water molecules around the amino acids and peptides were estimated and compared with that of pure water, τ_c^0. The value of $\tau_c^h/\tau_c^0=1.87$ for norleucine is the largest, while those of τ_c^h/τ_c^0 for glycine peptides are almost the same 1.2. The value of $n_h(\tau_c^h/\tau_c^0-1)$ was defined as the dynamic hydration number (DHN). The DHN showed a good correlation with several physicochemical properties, such as the molecular weights, partial molar volumes, adiabatic compressibilities, heat capacities, *B*-coefficients of the activity coefficent, and limiting diffusion coefficients of amino acids and glycine peptides in aqueous solutions.

The physicochemical properties of amino acids and oligo peptides in aqueous solutions have drawn much attention for several decades. These properties, closely related to the solute–water interaction, have been chiefly studied by thermodynamic methods. The importance of the hydration of amino acids is generally recognized in connection with the structure and function of proteins. It has been pointed out that there is a dynamic correspondence between the enzyme function and the dynamic structure of water.[1] The dynamic characteristics of the hydration of amino acid as a model compound of protein, therefore, is very important. Not enough such studies, however, have yet been carried out; moreover, there have been few attempts to relate the thermodynamic properties to the dynamic properties.

In the investigation of such thermodynamic properties as the volumes,[2,3] heat capacities,[4,5] and compressibilities[6] of aqueous solutions of amino acids or peptides, frequent attempts have been made to examine the linear correlation between such properties and the number of carbon atoms or peptide groups in the backbone.[2,4] It is very difficult to compare the properties of amino acids with those of peptides, since these properties are plotted as a function of a variable with a different base. It is, therefore, necessary to use the same variable for the purpose of investigating consistently the hydration of amino acids and peptides.

Recently, it was shown that the hydration properties of sugars in water can be systematically explained by the dynamic hydration number (DHN), obtained by measuring the ^{17}O relaxation rates of water in aqueous sugar solutions.[7] In this paper we report the spin-lattice relaxation times, T_1, of natural-abundance ^{17}O nuclei of water in the aqueous solutions of 11 apolar amino acids and 5 glycine peptides as a function of the concentration at 25 °C, since amino acids possess exchangeable protons. The concentration dependence of water-^{17}O relaxation rates is interpreted by means of the characteristics of the hydration of amino acids. Then we discuss the relation between the physicochemical properties (molecular weight, volume, compressibility, *B*-coefficient of activity coefficient, heat capacity, and diffusion coefficient) and the DHN.

Experimental

The tri-, tetra-, penta-, hexaglycine, leucine, and isoleucine were purchased from Sigma, while the glycine, diglycine, alanine, β-alanine, α-, β-, γ-aminobutyric acids, valine, norvaline, and norleucine were purchased from Tokyo Kasei. All of the amino acids were of a.G.R. grade and were used without further purification. Distilled and deionized water was used.

All natural-abundance oxygen-17 NMR experiments were performed using a JEOL GX-500 spectrometer operating at 67.8 MHz. The oxygen-17 T_1 was measured by using the inversion recovery sequence (180°–τ–90°). For the ^{17}O relaxation in neutral water, $T_2<T_1$ is observed in consequence of ^{17}O–^1H spin–spin coupling.[8] The water and all the solutions were maintained at pH<4 by adding a small amount of a dilute HCl solution, since, in this pH region, the proton exchange becomes sufficiently fast, making $T_2=T_1$ for ^{17}O in water.

The temperature was maintained at 25±0.3 °C by means of a gas thermostat.

Results and Discussion

Dynamic State of Aqueous Solutions of Amino Acids and Glycine Peptides. In Fig. 1, the selected values T_1^0/T_1 for $H_2{}^{17}O$ in aqueous solutions of amino acids and peptides are plotted against their molalities, where T_1^0 and T_1 are the spin-lattice relaxation times of the ^{17}O of water in pure water and in solutions respectively. All of the experimental values of T_1^0/T_1 in this work are adequately represented by an empirical equation of this form:

$$T_1^0/T_1 = 1 + B\,m \qquad (1)$$

where m is the molality of the solute. The solid lines in Fig. 1 are those calculated by the least-squares method. The correlation coefficient, γ, were in the

range of 0.8—1.0 except for hexaglycine and norleucine.

Under extreme motional narrowing conditions, we obtain the following relation according to the two-state model:[7]

$$55.5\,B = n_h\left(K\frac{\tau_c^h}{\tau_c^0} - 1\right) \quad (2)$$

where the superscripts h and 0 refer to the water of the hydration sphere (cosphere) and bulk water respectively, and where n_h is the coordination number. τ_c is the rotational correlation time of ^{17}O, and K is the ratio of the quadrupole coupling constant of ^{17}O in the cosphere and the bulk water. The value of K is 1 or 0.75.[7] The values of DHN, n_{DHN}, and their correlation coefficients, γ, for apolar amino acids and peptides are given the 4th column of Table 1.

If the value of n_h is known, we can obtain the value of τ_c^h/τ_c^0. We can estimate n_h by first calculating the water-accesible surface area using the data of the van der Waals volume of constituent atoms reported by Edward[9] and dividing these by the effective surface area of the water molecule. The calculated values of the van der Waals volumes and the n_h for amino acids and peptides are given in the 3rd and 5th columns of Table 1.

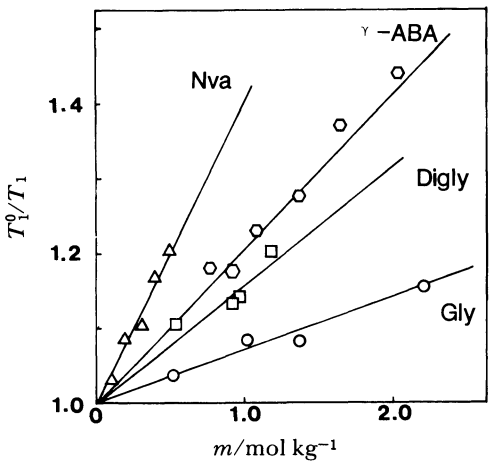

Fig. 1. T_1^0/T_1 of $H_2{}^{17}O$ in aqueous solutions of norvaline, γ-ABA, diglycine, and glycine as a function of molality.

As is seen in Table 1, all the n_{DHN} values are positive. This result means that all of the apolar amino acids and glycine peptides studied are structure makers as entire molecules. However, each constituent group in the molecule is not always a structure maker, because the observed hydration property is the sum of the contribution of the hydrophobic and hydrophilic hydrations.[10,11] Although diglycine and leucine have the same molecular weight, the n_{DHN} value of diglycine is about one third that of leucine. The n_{DHN} values for valine and tetraglycine are nearly equal, but the value of τ_c^h/τ_c^0 of valine is larger than that of tetraglycine. These results show that the thermal motion of water molecules around an apolar group is more restricted than that around the peptide groups.

The alkyl group is a typical hydrophobic structure maker, and the carboxyl group is an electrostrictive structure maker, while the amino group is a structure breaker.[13] Kresheck and Benjamin[13] and Uedaira[14] considered the peptide group as structure-breaking based on their thermodynamic studies.

The n_h value increases with the increase in the molecular size. The τ_c^h value of the water molecule around a structure-making group is larger than that of τ_c^h and increases with the increase in the size of the group, but τ_c^h value of the water molecule around a structure-breaking group is smaller than that of τ_c^0.[15] Thus, the thermal motion of water molecules in the cosphere of glycine peptides differs with that in local

Table 1. Van der Waals Volumes and Hydration Characteristics of Apolar Amino Acids and Glycine Peptides in Aqueous Solutions at 25°C

No.	Compound	M_w	$V_w\times10^3$ / nm³	DHN (γ)	n_h	τ_c^h/τ_c^0 K=1;	0.76
1	Glycine	75.07	67.4	3.9 (0.98)	24.6	1.16	1.53
2	α-Alanine	89.10	84.4	10.7 (0.99)	27.0	1.40	1.84
3	β-Alanine	89.10	84.4	9.3 (0.76)	27.0	1.34	1.77
4	α-ABA[a]	103.12	101.4	15.2 (0.94)	29.1	1.56	2.06
5	β-ABA	103.12	101.4	10.9 (1.00)	29.1	1.38	1.81
6	γ-ABA	103.12	101.4	11.4 (0.98)	29.1	1.39	1.83
7	Valine	117.15	118.4	20.1 (0.97)	31.2	1.64	2.16
8	Norvaline	117.15	118.4	22.1 (0.88)	31.2	1.71	2.25
9	Leucine	131.18	135.4	26.6 (0.93)	33.1	1.80	2.37
10	Isoleucine	131.18	135.4	19.0 (0.98)	33.1	1.58	2.07
11	Norleucine	131.18	135.4	28.8 (0.42)	33.1	1.87	2.46
12	Diglycine	132.12	116.2	8.9 (0.98)	42.3	1.21	1.59
12	Triglycine	189.17	165.0	11.4 (0.80)	60.1	1.19	1.57
14	Tetraglycine	246.22	213.8	18.2 (0.99)	77.8	1.23	1.62
15	Pentaglycine	303.27	262.6	23.4 (0.69)	95.6	1.25	1.64
16	Hexaglycine	360.32	311.4	16.2 (0.50)	113.3	1.14	1.50

a) Amino butyric acid.

regions peripheral to the different groups. That is, the thermal motion of the hydrated water is more restricted around the structure-making groups, while it is more rapid around the peptide and amino groups, than in bulk water. The value of τ_c^h/τ_c^0 calculated by Eq. 2 is determined by these opposing influences on the thermal motion; it represents an average dynamic state over all the water molecules in the cosphere. Consequently, τ_c^h/τ_c^0 values of peptides are smaller than those of apolar amino acids and are close to unity. Recently, Uedaira and Uedaira[15] found that the *m*-benzenedisulfonate ion has a larger n_h value, but its n_{DHN} and τ_c^h/τ_c^0 values are smaller than those of the benzenesulfonate ion, because the sulfonate group is the structure-breaker.

According to the molecular dynamic study of alanine peptide, Rossky and Karplus[16] showed that the polar groups (C=O and NH) of dipeptide have little influence on the mobility of the water molecule around them, but water molecules near the apolar groups are substantially hindered in both translational and rotational motion. Thus, their molecular dynamics calculations support our experimental results.

The τ_c^h/τ_c^0 values for the glycine peptides are almost the same as that of glycine. For sugars and the alkylsulfonate ions, it was found that the τ_c^h/τ_c^0 value for an oligomer is almost the same as that of a monomer.[7,15]

Relation between Physicochemical Properties and DHN. Many physicochemical properties of amino acids or petides have been plotted as functions of the number of carbon atoms or peptide groups by many authors.[3-6] For the purpose of comparing the properties of amino acids and peptides, however, such plots are not adequate, as has been mentioned above.

Gill et al.[17] showed that the coordination number of a water molecule around a apolar solute generally correlates with all the thermodynamic properties in water. This empirical rule presupposes that all the water molecules in the cosphere of the solute molecule exist in the same dynamic state. This rule cannot apply in the cases of amino acids and peptides, since the water molecules in the vicinity of the different groups of the solute molecule exist in different dynamic states. The DHN, which is a product of the statical and the dynamical quantities, therefore, is considered to be a more reasonable indication of the hydration characteristics of amino acids and peptides than is the coordination number. Table 2 lists the physicochemical properties of amino acids and peptides, which are explained below on the basis of the DHN.

In Table 2, the values of ΔV_h were calculated from this relation;[22] $\Delta V_h = \overline{V}_0 - N_A V_A$, where N_A is Avogadro's number. We calculated the values of D_0 for the tetra-, pepnta-, and hexaglycine by means of the shell model.[20] In the calculation, we assumed that the microviscosity around an oligomer is the same as that around the monomer.[20] This assumption is valid judging from the results shown in Table 1, since the microviscosity around a solute molecule is proportional to the τ_c^h of the water molecule in the cosphere.

All of the properties in Table 2 can be expressed by the following linear equation of n_{DHN}:

$$Y = a + b\,n_{DHN} \tag{3}$$

The values of *a* and *b* are given in Table 3. The value of *a* indicates that of *Y* of a hypothetical solute which has the same interaction as a water–water one.

In general, the value of *b* in Eq. 3 depends on the size of the solute molecule and on the dynamic state of the

Table 2. Physicochemical Properties of Apolar Amino Acids and Glycine Peptides in Aqueous Solutions at 25 °C

No.	Compound	$\overline{V}_0$[a]	$-\overline{K}_s^0\times10^4$[b]	B[c]	$\overline{C}_p^0$[a]	ΔV_h	$D\times10^6$[e]
		$cm^3\ mol^{-1}$	$cm^3\ mol^{-1}\ bar^{-1}$		$J\ K^{-1}\ mol^{-1}$	$cm^2\ mol^{-1}$	$cm^2\ s^{-1}$
1	Glycine	43.26	27.0	5.0	39	2.66	10.554
2	α-Alanine	60.54	25.0	−1.2	141.4	9.71	9.097
3	β-Alanine	58.28	26.36	−0.2	91	7.45	
4	α-ABA	75.6	21.8	−2.5	224	14.53	8.305
5	β-ABA	76.3	18.7	−2.0	182	15.23	
6	γ-ABA	73.2	27.0	1.6	154	12.13	
7	Valine	90.75	24.0		307	19.44	7.722
8	Norvaline	91.80			335	20.49	
9	Leucine	107.77	31.8		382	26.22	
10	Isoleucine	105.80				24.25	
11	Norleucine	107.73			400	26.18	
12	Diglycine	76.27	35.91	12.3	105.0	6.28	7.909
13	Triglycine	111.81	44.36	16	185.9	12.43	6.652
14	Tetraglycine	149.7	53.14		283	20.93	5.497
15	Pentaglycine	187.1			373	28.94	4.820
16	Hexaglycine						4.310

a) Refs. 3 and 18. b) Refs. 6 and 18. c) Mean values calculated from the data of Ref. 19. d) Ref. 4. e) Refs. 20 and 21.

Table 3. Coefficients of Empirical Equations between n_{DHN} and Physicochemical Properties of Aqueous Solutions of Apolar Amino Acids and Glycine Peptides

Y	Homologue	a	b	γ
M_w	Amino acid	71.01	2.193	0.929
	Peptide	36.09	11.641	0.992
$\overline{V}_0$	Amino acid	38.59	2.621	0.938
	Peptide	16.64	7.365	0.994
$\overline{K}_s^0 \times 10^4$	Amino acid	−28.26	0.261	−0.700
	Peptide	−20.42	−1.855	0.986
B	Amino acid	6.79	−0.652	−0.855
	Peptide	−0.754	1.469	1.000
$\overline{C}_p^0$	Amino acid and peptide	−16.65	15.56	0.985
ΔV_h	Amino acid and peptide	−28.76	1.047	0.940
$1/D_0 \times 10^{-5}$	Amino acid	0.866	0.0217	0.994
	Peptide	0.767	0.0573	0.999

water molecules in the cosphere. As may be seen from Table 3, the absolute values of b for apolar amino acids are smaller than those of b for peptides. It is clear from Eq. 2 that the $(K\tau_c^h/\tau_c^0-1)$ term enlarges the effect of n_h (intrinsic property) if the value is larger than unity, and diminishes the effect if it is smaller. In other words, the water structure in the cosphere becomes bulky or compact according whether the values of $(K\tau_c^h/\tau_c^0-1)$ are large or small respectively. Therefore, the physicochemical properties of apolar amino acids have small values of b.

In this connection, it is of interest to compare the values b for the $\overline{K}_s^0$ of amino acid and peptide. As has been clarified above, the peptide groups is the structure-breaker, and the thermal motion of water molecules is more vigorous than that of bulk water. As a result, the hydration sphere becomes more compact, and so the $\overline{K}_s^0$ value of peptide becomes more negative.

It seems that $\overline{C}_p^0$ and ΔV_h depend only on the state of water in the cosphere, regardless of the intrinsic properties of the apolar amino acids and peptides. This is true of ΔV_h by definition. In Fig. 2, the values of the partial molar heat capacity for amino acids and peptides are plotted against the n_{DHN}. Figure 2 is important in connection with the heat capacity change of the denturaion of a globular protein. Privalov[23] elucidated that the interaction of apolar amino acid residues with water is indeed the most important contributor to this change on the unfolding of the proteins.

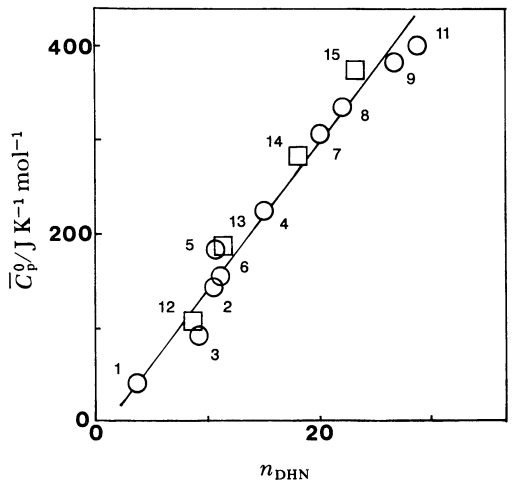

Fig. 2. Relation between the partial molar heat capacity, $\overline{C}_p^0$, and n_{DHN}. The numbers in figure denote the same compound as in Table 1.

Conclusion

In summary we obtained the following results:

(1) The values of τ_c^h/τ_c^0 for apolar amino acids increase with an increase in the size of the hydrophobic group.

(2) The peptide group is the structure-breaker, and the values of τ_c^h/τ_c^0 for peptides are almost the same, 1.2.

(3) The physicochemical properties of apolar amino acids and peptides in aqueous solutions are expressed by the linear equation of n_{DHN}.

These conclusions suggest that the details of the motion and structure of the hydration water can play a deterministic role both in dynamic and in static properties of aqueous solutions.

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