Ultrasonic Study on Aqueous Solutions of Amino Acids

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Ultrasonic experiments for aqueous solutions of amino acids (glycine, *l*-alanine, *l*-valine, and *l*-leucine) were carried out by means of pulse technique in order to study the nature of solute-solvent interactions. The sound velocity and absorption coefficient were determined for aqueous solutions in the frequency range 15–45 MHz, at temperatures 10–30°C. No relaxation frequency was observed in all the solutions. The absorption coefficient for glycine solutions at low concentrations is nearly equal to that for pure water. The result is consistent with the idea that glycine molecules behave as a structure breaker within water. Concerning the concentration dependency of sound velocity and absorption, no appreciable difference is observed in various solutions of amino acids in spite of difference in the size of hydrocarbon side chains in solute molecules. It is concluded that the behavior of *l*-alanine and its higher homologues in water dose not differ from that of glycine.

Recently, a number of studies have been made on the solute-solvent interactions in aqueous solutions.[1,2] Holtzer and Emerson[3] discussed the changes in the structure of water induced by nonelectrolytic solute molecules, based on a simple two state model.[4]

We pointed out from ultrasonic measurements that denaturing agents such as urea, guanidine hydrochloride *etc.* have a "cooperative breaking effect" on the "open-packed structure" or ice-like structure" in water.[5] The cooperative breaking effect of denaturing agents arises from the cooperative nature of structure formation and breaking in water.[6,7]

Since the proposition stated by Frank and Evans,[8] hydrocarbon molecules in water are believed to reinforce the structure (icebergs) around the molecules within water. We showed that 1,3-diethylurea molecules behave as a structure former due to the structure-forming ability of ethyl groups which is in sharp contrast to the behavior of urea and 1,3-dimethylurea molecules as a structure breaker.[9] Hydrophobic bonds which result from the structure-forming ability of hydrocarbon groups in water have been studied by many workers, since it is believed to be an important factor governing the conformation of protein molecules in water.[10,11]

There have been some ambiguities about the behavior of amino acids in water.[12] Tyrell and Zamann[13] reported that there is no appreciable difference between the behavior of glycine and that of *dl*-alanine in water.

On the contrary, Robinson,[14] Tanford[15], and Ellerton *et al.*[16] stated that in aqueous solutions of *l*-alanine and its higher homologues the behaviors of solutes definitely differ from that in glycine solutions.

We have investigated the influences of serveral neutral amino acids upon the structure of water by means of an ultrasonic pulse technique.

Experimental

An ultrasonic pulse method was used for the measurement of absorption coefficient and sound velocity. The apparatus and the procedures were described previously.[17] The sound velocity and absorption coefficient were measured at 5 MHz and in the frequency range 15–45 MHz at temperatures 10–30°C. The data of absorption coefficient were reproducible within ±2% and sound velocity data ±1 m/sec. Samples of glycine, *l*-alanine, *l*-valine, and *l*-leucine were obtained from Nakarai Chemicals Ltd. The shear viscosity in aqueous solutions of these amino acids were measured by means of a capillary viscometer of Ostwald type.

Results

Sound Velocity. The sound velocity data at 5 MHz measured for the aqueous solutions of glycine and *l*-alanine are plotted against temperature in Figs. 1 (a) and (b), respectively, together with the velocity data for pure water by Greenspan and Tschiegg.[18] The velocity *vs.* concentration curves obtained for aqueous solutions of glycine, *l*-alanine, *l*-valine, and *l*-leucine are plotted in Fig. 2 at various temperatures. Concentrations are given in volume %. The concentrations are calculated from the density and weight

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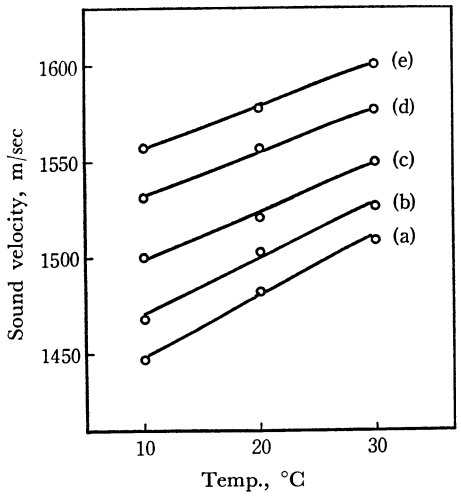

Fig. 1(a). Ultrasonic velocity of aqueous solutions of glycine.
Concn. (M: mol/*l*) (a) pure water, (b) 0.5 M, (c) 1.0 M, (d) 1.5 M, (e) 2.0 M

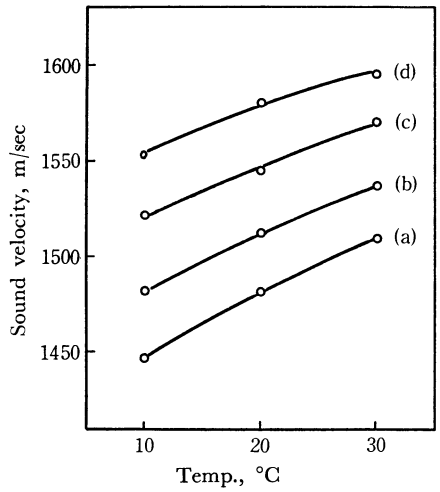

Fig. 1(b). Ultrasonic velocity of aqueous solutions of *l*-alanine.
Concn. (M: mol/*l*) (a) pure water, (b) 0.5 M (c) 1.0 M, (d) 1.5 M

of each amino acid sample in solid state. In Fig. 2, it is shown that the velocity *vs.* concentration relations for solutions of various amino acids form a single composite curve and that the sound velocity is not dependent on the size of hydrocarbon side chain of amino acids.

Ultrasonic Absorption. The absorption coefficient α was measured for the aqueous solutions of glycine in the concentration range 0.5–2.0 mol/*l*, of *l*-alanine in the range 0.5–1.5 mol/*l* of *l*-valine at 0.5 mol/*l* and of *l*-leucine at 0.1 mol/*l*. The frequency dependency of α/f^2 (f, frequency) is shown in Figs. 3 (a), (b), (c), and (d) for each solution. No relaxation frequency was observed for all the solutions tested. This coincides with the result for glycine solutions by Hammes and Pace.[19] In Figs. 4 (a) and (b) we see that at the same concentration for solutions of glycine and

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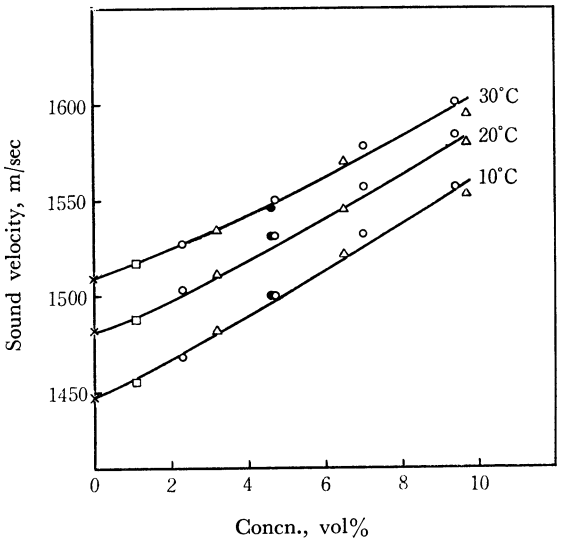

Fig. 2. Concentration dependence of ultrasonic velocity for amino acid solutions.
×, pure water, ○, glycine, △, *l*-alanine, ●, *l*-valine, □, *l*-leucine

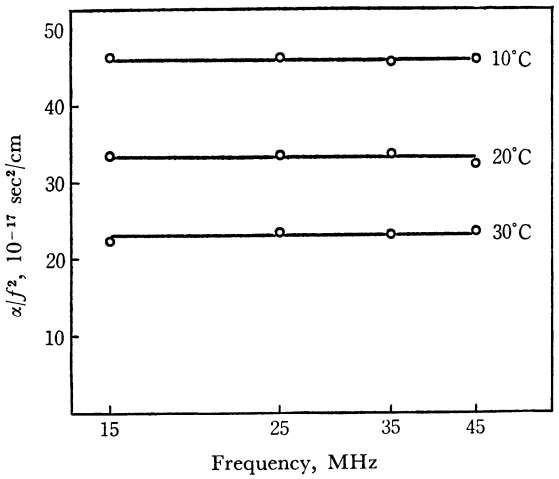

Fig. 3(a). Ultrasonic absorption of aqueous solutions of glycine. (2.0 M glycine)

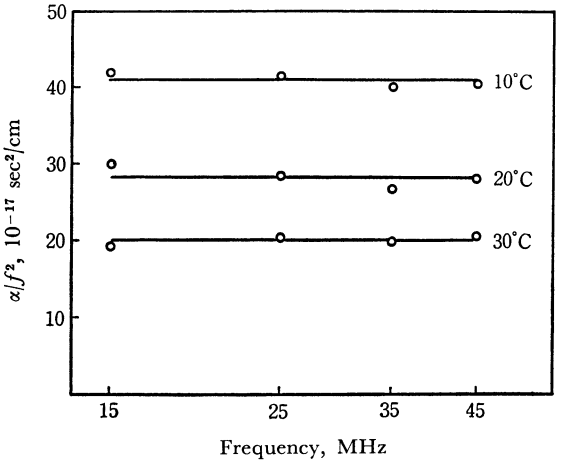

Fig. 3(b). Ultrasonic absorption of aqueous solutions of *l*-alanine. (1.0 M *l*-alanine)

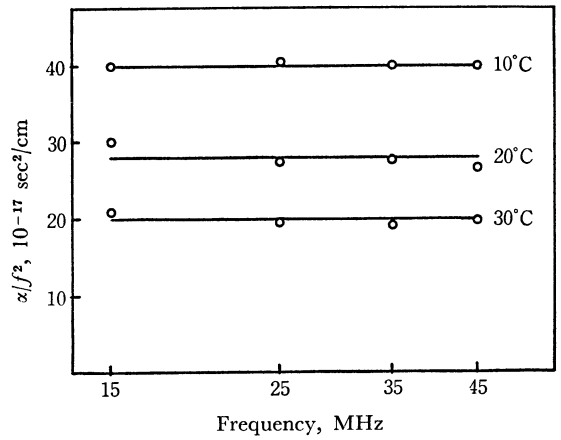

Fig. 3(c). Ultrasonic absorption of aqueous solutions of *l*-valine. (0.5 M *l*-valine)

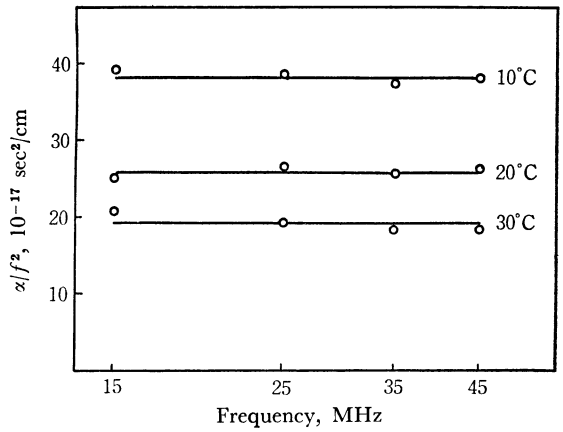

Fig. 3(d). Ultrasonic absorption of aqueous solutions of *l*-leucine. (0.1 M *l*-leucine)

l-alanine, the magnitudes of α/f^2 for both solutions are nearly equal. It is also seen that the magnitudes of of α/f^2 at low concentrations are essentially the same as that of pure water for both solutions. The concentration dependencies of α/f^2 for solutions of four amino acids are compared in Fig. 5. Concentrations are given in volume %. We see that the plot of α/f^2

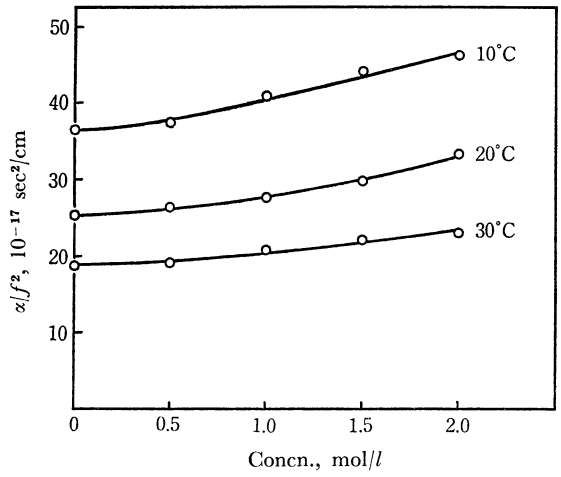

Fig. 4(a). Concentration dependence of ultrasonic absorption for glycine solutions.

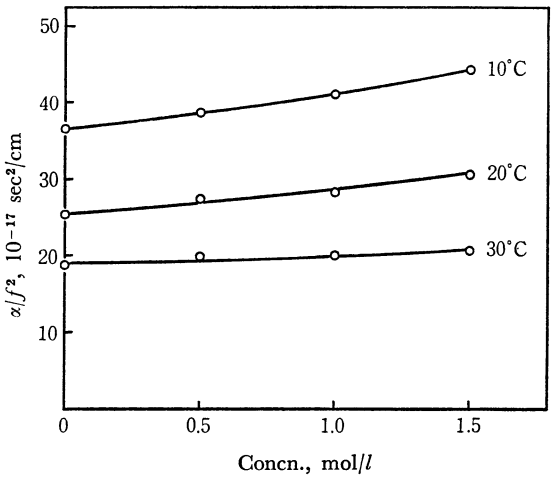

Fig. 4(b). Concentration dependence of ultrasonic absorption for *l*-alanine solutions.

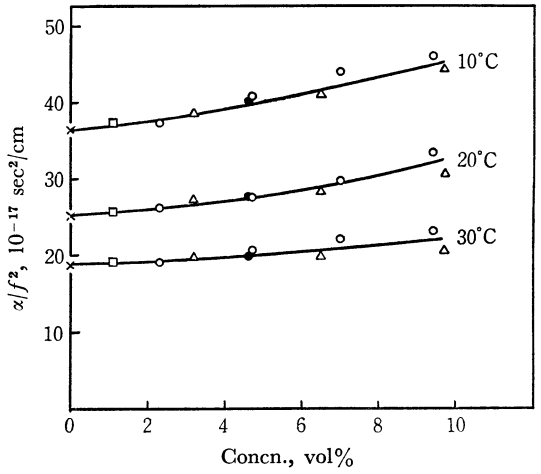

Fig. 5. Concentration dependence of ultrasonic absorption for amino acid solutions.
×, pure water, ○, glycine, △, *l*-alanine, ●, *l*-valine, □, *l*-leucine

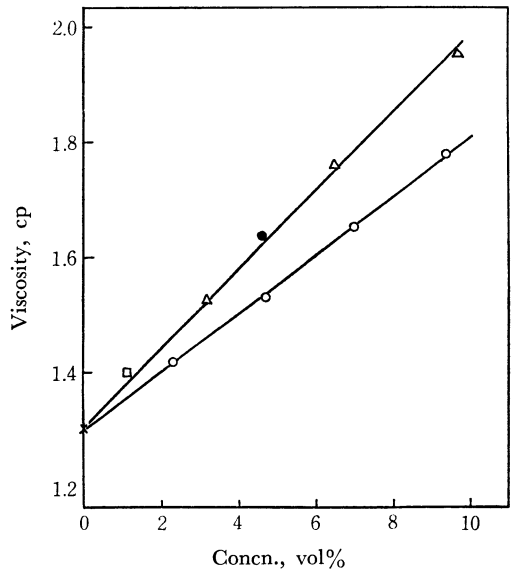

Fig. 6. Viscosity for aqueous solutions of amino acids at 10°C.
○, glycine, △, *l*-alanine, ●, *l*-valine, □, *l*-leucine, ×, pure water

vs. concentration relations for various amino acid solutions forms a single curve at various temperatures. The situation is quite similar to the case of sound velocity shown in Fig. 2. In order to obtain the magnitude of structural absorption $(\alpha/f^2)_{struct.}$ from the data given in Fig. 5, determination of the classical absorption is necessary. The classical absorption is given by

$$(\alpha/f^2)_{shear} = \frac{8\pi^2\eta}{3\rho V^3} \quad (1)$$

where ρ is density, V sound velocity, and η shear viscosity. The values of η were measured for all solutions and given in Fig. 6. Thus, the structural absorption is given in the equation

$$(\alpha/f^2)_{struct.} = (\alpha/f^2)_{obs.} - (\alpha/f^2)_{shear}. \quad (2)$$

The result obtained is shown in Fig. 7.

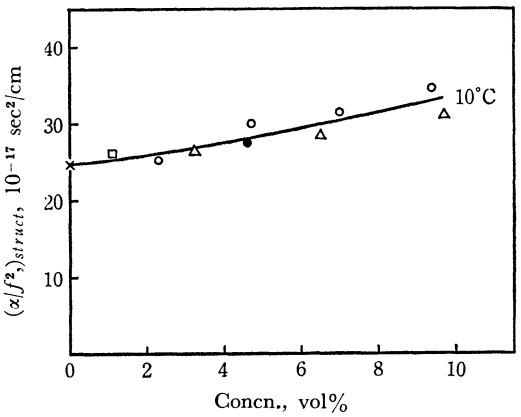

Fig. 7. Concentration dependence of structural absorption for amino acid solutions.
×, pure water, ○, glycine, △, *l*-alanine, ●, *l*-valine, □, *l*-leucine

Discussion

The Effect of Glycine upon the Structure of Water. Robinson *et al.*[20] pointed out from thermodynamical data that glycine behaves as a structure breaker in water while *l*-alanine and higher homologues behave as a structure former. Longsworth[21] supported this view. As for the behaviors of glycine in water, a number of workers have reached the same conclusion that glycine acts as a structure breaker.[13–16,20,21] As seen in Fig. 4 (a), the magnitude of absorption coefficient in an aqueous solution of glycine increases slowly with concentration. The tendency is similar to the case of 1,3-dimethylurea solution, in which the solute molecules behave as a structure breaker.[9] Thus, it might be said that the behavior of glycine in water is essentially similar to that of 1,3-dimethylurea. It seems that glycine molecules break the structure of water though the ability is supposed to be weaker than that of ordinary denaturing agents, such as urea.

The Effect of Side Chain of Amino Acids upon the Structure of Water. It is generally believed that the structure-forming ability of solute molecules in liquid water increases with the size of hydrocarbon side chain.[22,23] However, there has been some controversy concerning the behavior of *l*-alanine and higher homologues of amino acids in water.

Tanford and his collaborators[15,24] pointed out that *l*-alanine and higher homologues behave as a structure former while glycine behaves as a structure breaker. Recently, Spink and Auker[25] supported the view of Robinson, Tanford, and others, based on their thermodynamical data. Kay and Evans[26] also stated that the effect of hydrocarbon side chains of *l*-alanine and higher homologues on the structure in water differs from that of glycine.

On the contrary, Tyrell and Zamann[13] reported on the basis of thermodynamical data that there is no appreciable difference between the behavior of glycine and that of *dl*-alanine in water. This view is supported by the recent study of Kauzmann *et al.*[12]

The results in the present study clearly support the conclusion of Tyrell & Zamann and Kauzmann *et al.* As seen in Figs. 2,5, and 7, the ultrasonic data of sound velocity and absorption form a single curve at each temperature irrespective of the different size of side chains. Thus, it is concluded that the behavior of these four amino acids in water are similar essentially, and the structure-forming effect due to alkyl groups in amino acid molecules are cancelled out by the strong structure-breaking effect due to the dipolar field by zwitter ions ($NH_3^+CHRCOO^-$).

It can therefore be said that amino acids cannot be regarded as good models for studying hydrophobic bonding.[12]

Concluding Remarks

The following conclusion are obtained.

1) The effects of four amino acids on the ice-like structure in water are essentially similar to each other. 1-Alanine and its higher homologues do not always behave as a structure former in water.

2) Glycine molecules behave as a weak structure breaker for the ice-like structure in water.

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