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Inelastic Neutron Scattering Studies of the Interaction between Water and Some Amino Acids

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The interaction between water and some of amino acids (glycine, L-glutamine, L-threonine, L-cysteine and L-serine) was studied by inelastic incoherent neutron scattering (IINS). The vibrational spectra of dry amino acids and amino acids with a water content (e.g., 1 mol water/1 mol amino acid) were recorded. Comparing the difference spectra obtained by subtracting the spectrum of dry sample from those of wet sample with the spectra of ice Ih, we obtained that the difference spectrum for serine changed greatly from normal ice spectrum; but on the other hand, the difference spectra for the other amino acids such as glycine, glutamine, threonine, and cysteine changed slightly. The results demonstrate that serine has stronger hydrophilic character than glycine, glutamine, threonine, and cysteine. This is the first time the hydrophilic or hydrophobic character of amino acids was studied by using inelastic neutron scattering techniques, which provides important information for theoretical modeling and force field refinement for the interaction between water and the amino acids studied here.

Introduction

Water plays a vital role in organisms. Understanding the interaction between water and biomolecules is fundamentally important toward our understanding of the bioprocess in living organisms. By X-ray crystallography,[1] NMR,[2] and neutron scattering,[3,4] many investigators have studied the interaction of water with DNA and proteins to clarify the mechanism of formation of higher-order structure of proteins. The behavior of amino acids, the molecular units that make up proteins, in water also has been studied in many laboratories using a variety of experimental techniques.[5−10] To understand the interaction of water with biomolecules, proteins for example, an understanding of the interaction between water and amino acids is essential.

Each amino acid molecule consists of an amino functional group and a carboxyl acid group and differs from other amino acids by the composition of side constituent −R. In the gas phase, most amino acids occur in their neutral form, while in solution or in the solid state they exist as zwitterions NH_3^+−CH (−R) -COO^-. The interaction with surrounding molecules through hydrogen bonding makes the zwitterionic form significantly more stable compared to the neutral form.

Much research has been done on the interaction between water and amino acids by theoretical[11−15] and experimental methods[16,17] using different approaches. Ide et al. investigated the interaction between various amino acids and water molecules through analysis of the O−H stretching of water in aqueous solution by Raman spectroscopy and ^{1}H-NMR.[16] Fischer et al. analyzed the structural changes of water in aqueous solutions of two amino acids (alycine and alanine), focusing on the region above 2800 cm^{-1} using FTIR and Raman difference spectroscopy.[17] These studies concentrated on the high-frequency vibrations in aqueous solutions[16,17] or the gas state.[13] Less is known about the low-frequency vibrations. A study of 1:1 1 mol water/1 mol amino acid by incoherent inelastic neutron scattering techniques[18] is important, as inelastic incoherent neutron scattering can be employed as a vibrational spectroscopy method that provides more detailed information in the low energy ranges than do infrared and Raman spectroscopy methods. Also, the neutron spectrum (predominated by H motion) in the low frequency region is mainly due to hydrogen bonding between water and amino acids, or between water molecules.

The aim of the present study is to investigate the interaction between amino acids (e.g., serine, glycine, glutamine, threonine, and cysteine) zwitterions and the water molecules through analyses of the inelastic neutron scattering spectra in the 10−130 meV (80−1040 cm^{-1}) range, and may reveal why the hydrophilic abilities of given amino acids are different.

Experimental Details

All of the amino acid samples were purchased from Sigma Company. "Dry" samples were obtained by drying them for about 12 h under vacuum. The dried samples were kept under vacuum to avoid the adsorption of atmospheric water. "Wet" samples with 1:1 by mol water content were obtained by adding distilled water to the dry samples in a desiccator, which

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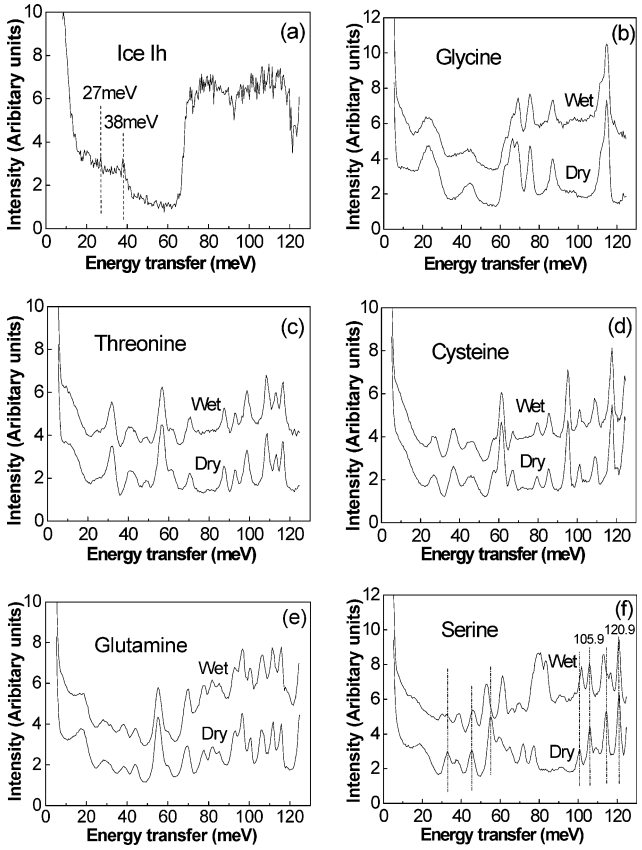

Figure 1. Inelastic neutron scattering spectra of wet and dry samples of (b) glycine, (c) threonine, (d) cysteine, (e) glutamine, (f) serine measured on the HET spectrometer at ISIS. Spectrum of ice Ih (a) is also shown here for comparison purposes.

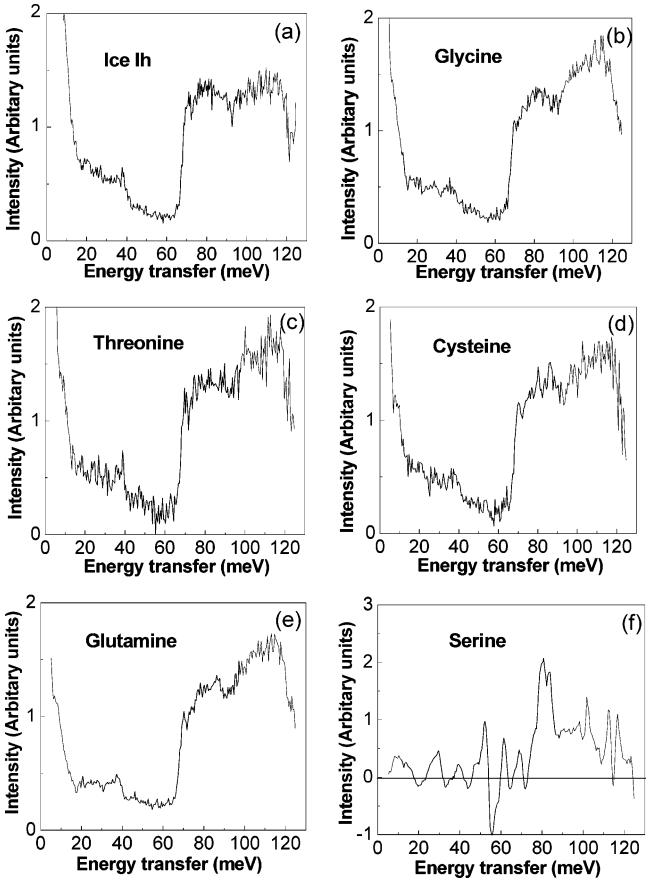

Figure 2. Difference spectra obtained by subtracting the spectra of the dry samples from those of the wet samples: (b) glycine, (c) threonine, (d) cysteine, (e) glutamine, (f) serine. The spectrum of ice Ih (a) is also shown for comparison.

contained saturated K_2SO_4 solution, providing for constant partial pressure of water vapor, and then allowing them to equilibrate for more than 12 h. For the convenience of IINS spectra analysis, we prepared 0.025 mol dry amino acids (e.g., 2.627 g for serine) and the wet sample of amino acids with 0.025 mol water (0.45 g).

IINS measurements were performed on the HET spectrometers at the ISIS pulsed spallation neutron source of the Rutherford Appleton Laboratory at temperatures about 15 K ($\pm$ 2 K) in order to reduce multiphonon scattering. The spectra hence are predominated by the one-phonon contribution. HET is a direct geometry spectrometer, in which single incident energy of the neutrons is selected by a monochromating chopper, and the final energy and momentum transfer is analyzed by time-of-flight and detector angles. The energy resolution (i.e., dE/E) is about 2−6%, depending on choice of the chopper. The background from the empty can was also measured under similar conditions and was subtracted from the original data. Finally, the measured data were transferred to the one-phonon spectrum by subtracting the multiphonon contributions calculated using the iteration technique.[19]

Results and Discussion

Inelastic neutron scattering spectra of all the dry and wet samples measured at ~15 K on HET using a standard CCR system are presented in Figure 1. The spectrum of ice Ih is also shown for comparison. Amino acid molecules have a zwitter-ionic structure when they are in the solid phase. Indeed, this case allows hydrogen bonds to be formed, stabilizing the ionic conformation $NH_3^+-CH(-R)-COO^-$. From Figure 1 we can see that there are one or two peaks below 40 meV in all the neutron scattering spectra of dry amino acids. We consider these low-frequency stretching vibrations as due to hydrogen bonds, resembling two main peaks at 27 meV and 38 meV in ice Ih[20] (see Figure 1a). Except for serine, almost all of the IINS spectra of wet samples are similar to their dry samples as shown in Figures 1b−e (at least in terms of the peak positions), which implies that the structures of glutamine, glycine, threonine, and cysteine are hardly changed. However, the IINS spectrum of wet serine is extremely different from that of dry serine in Figure 1f. Many peaks shift, except for two peaks at 106 meV and 121 meV, which implies that there is a strong interaction between water and the serine molecules.

The difference spectra $I_{diff}(\omega)$ obtained by the subtracting the spectra of the dry $I_{dry}(\omega)$ samples from that of the wet samples $I_{wet}(\omega)$ are shown in Figure 2. Since the exact amounts of dry and wet samples in the beam were hard to control, we therefore subtracted the dry spectra from the corresponding wet spectra with a fraction f:

$$I_{diff}(\omega) = I_{wet}(\omega) - f\,I_{dry}(\omega)$$

where f varies from 0.8 to1.2, aiming to remove features of the dry materials. If we over subtracted, negative peaks would appear in the difference spectrum. However, if a peak for the wet sample is shifted from dry sample, a negative peak is inevitable, see Figure 2f. If there are no interactions between water and amino acids, we expect that the difference spectra should match the spectrum of ice Ih.

After the procedure of careful subtraction, as one can see, the difference spectra for glycine, threonine, glutamine, and

TABLE 1: Details of Hydrogen Bonds, D(onator)−H···A(cceptor) in Serine, Glycine, Glutamine, Threonine, Cysteine, and Serine·H_2O (refs 21−26)[a]

serine[21]			glycine[22]			glutamine[23]		
D···A	H···A	D-H···A	D···A	H···A	D-H···A	D···A	H···A	D-H···A
2.887 Å	1.96 Å	167°	***2.770*** Å	***1.728*** Å	169.3°	***2.772*** Å	***1.752*** Å	164.2°
2.871	1.90	159	***2.855***	***1.832***	168.5	***2.866***	***1.854***	163.3
2.840	1.91	161	3.075	2.121	154.0	2.911	1.919	167.3
2.918	2.10	153	3.277	2.390	137.4	2.948	1.941	167.3
			2.955	2.365	115.5	2.937	2.088	141.3
			3.362	2.453	140.0			

threonine[24]			cysteine[25]			serine·H_2O26		
D···A	H···A	D-H···A	D···A	H···A	D-H···A	D···A	H···A	D-H···A
2.660 Å	***1.716*** Å	159.6°	***2.762*** Å	***1.710*** Å	174.1°	***2.769*** Å	***1.804*** Å	153.4°
2.794	***1.791***	162.6	***2.784***	***1.770***	164.5	***2.877***	***1.822***	177.4
2.917	1.900	166.1	3.025	2.066	156.3	***2.785***	***1.843***	166.8
3.124	2.202	149.1	3.391	2.400	134.0	***2.809***	***1.868***	173.2
			3.840	2.750	140.0	2.913	1.901	168.1
						2.919	2.130	132.4

[a] The relatively strong hydrogen bonds are emphasized by bold italic characters.

cysteine are all similar to the spectrum of ice Ih in Figure 2b−e, only that of serine is different from that of other amino acids or the spectrum of ice Ih (see Figure 2f).

A possible explanation for serine having this anomalous behavior may be that there is a different type of hydrogen bond in the crystal structure of serine, compared to the crystal structures of the other amino acids (glycine, glutamine, threonine, and cysteine). Details of the hydrogen bond lengths and angles in every anhydrous amino acid crystal (serine, glycine, glutamine, cysteine, threonine) are given in Table 1, as determined by X-ray diffraction and neutron diffraction techniques.[21−25] The hydrogen bond strength depends on its length and angle. For single donor acceptor systems, the strongest hydrogen bonds are collinear; and small deviations of linearity in the bond angles (up to 20°) have a relatively minor effect. The dependency on bond length is more important and has been shown to exponentially decay with distance. Table 1 shows that there are relatively strong hydrogen bonds with short H···A bond length (between 1.7Å and 1.9Å) in glycine, glutamine, cysteine, threonine. However, the length of the strongest hydrogen bond in serine is 1.90 Å. When 1:1 mol ratio of water is added to such a system, the original weak hydrogen bonds are broken more easily and new stronger hydrogen bonds begin to form with water in this system. Neutron diffraction studies of the crystalline serine monohydrate[26] (serine·H_2O) agree with our hypothesis. The last column of Table 1 gives data for hydrogen bonds in single crystals of serine·H_2O. In the serine monohydrate there exist six hydrogen bonds per asymmetric unit in the crystal structure, including four relatively stronger hydrogen bonds (H···A = 1.804, 1.822, 1.843, and 1.868 Å; for the details see Table 1).

Conclusions

We have investigated the hydrophilic character of serine, glycine, glutamine, threonine, and cysteine by inelastic incoherent neutron scattering at low frequencies. By comparing the spectra obtained with dry samples and wet samples with the spectrum of ice Ih, we concluded that serine has a more unstable crystal structure compared the other amino acids due to weaker hydrogen bonds. When water is added to the dry serine, it forms new stronger hydrogen bonds with the serine molecules.

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