

## Vibrational Spectroscopic Studies of the Interaction of Water with Serine

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The vibrational dynamics of water around serine was investigated by using Raman spectroscopy and inelastic incoherent neutron scattering. Experiments with serine in deuterium oxide were performed to assist the assignment. The study shows that for the serine, the exchange of protons–deuterons on the active  $-\text{NH}_3^+$  and  $-\text{OH}$  groups were relatively easy, whereas there were hardly any exchanged on the  $-\text{CH}$  or  $-\text{CH}_2-$  groups. The main features of the spectra for hydrated samples (versus the dry samples) were altered considerably; new sharp peaks in the measured spectra appeared, indicating that the hydrogen bonding between water and serine had disturbed the structure of the serine molecule.

### 1. Introduction

The thermodynamic behavior and the state of solvation of proteins and peptides in solution relies heavily on the interactions of water with various functional groups of the protein, which in turn contribute to the formation of the stable folded structure of the protein in solution. The direct study of these important protein–water interactions is difficult because of the complexity of the interactions in a protein macromolecule.<sup>1,2</sup> As amino acids are the basic building blocks of proteins, it is useful to study amino acid–water interactions that mimic some aspects of the protein structure.

Each amino acid consists of an amino functional group and a carboxyl acid group; they differ from each other by the composition of the side chain constituent ( $-\text{R}$  group). The interaction between amino acids and surrounding molecules through hydrogen bonding leads to considerable stability of the zwitterionic form in biological media. Understanding of the interaction between water and the individual amino acid is therefore fundamentally important for us to comprehend the role of water in biological systems. Vibrational spectroscopy techniques, such as IR/Raman and inelastic incoherent neutron scattering are powerful ways to investigate the interactions among the different constituents. In this study, we will concentrate on one of the important amino acids, serine.

The conformation of serine has been widely studied by theoretical and experimental methods.<sup>3–12</sup> Thomas et al. have reported the structure of anhydrous serine using X-ray diffraction.<sup>10</sup> The structure of the monohydrate was presented by Michel et al. using neutron diffraction studies.<sup>11</sup> However, there is little information in the literature relating to the vibrational dynamics of serine from the crystalline state to the hydrated form. In this contribution, we report results on the interaction of water with serine by Raman spectroscopy and inelastic incoherent neutron scattering (IINS). Bands appearing in the

neutron scattering spectrum (predominated by H motion) in the low energy transfer region are mainly due to hydrogen bonding between water and amino acids, or between water molecules. Raman spectroscopy can provide information in a much wider energy transfer range than inelastic neutron scattering and with a better resolution at the high-energy transfer ( $\text{C}-\text{H}$ ,  $\text{O}-\text{H}$ ,  $\text{N}-\text{H}$  bending and stretching) region. By combining Raman and IINS data, we can facilitate the interpretation of the vibrational spectra to obtain a better assignment of the vibrational peaks. Experiments with samples where exchangeable protons were replaced with deuterons were also performed. Some assignments are tentative, but the peak shifts are obvious and reproducible.

### 2. Experimental Details

The amino acid samples were purchased from Sigma. Samples referred to in this paper as “dry” were obtained by drying them under vacuum at 80 °C for over a day. The water contents added were determined by weight difference and expressed throughout this paper as mole proportion versus the dry sample. The dry samples were kept under vacuum to avoid the adsorption of atmospheric water and then saturated with the designated amount of water for 12 h before the experiment. The deuterated serine was made by diluting a sample in  $\text{D}_2\text{O}$  and then recrystallized by slow evaporation using a vacuum drier.

The Raman scattering measurements were performed with a multichannel modular triple Raman system (JY-T64000) with confocal microscopy. A long focus 50X microscope objective lens was used to focus the laser beam on the sample surface and to collect the scattered light. The excitation wavelength at 488 nm of an  $\text{Ar}^+$  ion laser was used in this experiments. The laser power on the samples was kept at 3 mW and the typical spectrum acquisition time was 200 s. All Raman measurements were taken at room temperature.

The neutron scattering experiments were performed on the TOSCA instrument at the ISIS pulsed spallation neutron source of the Rutherford-Appleton Laboratory at 15 K ( $\pm 5$  K) to reduce multiphonon scattering contribution, the spectra are hence predominated by the one-phonon modes. TOSCA is an indirect

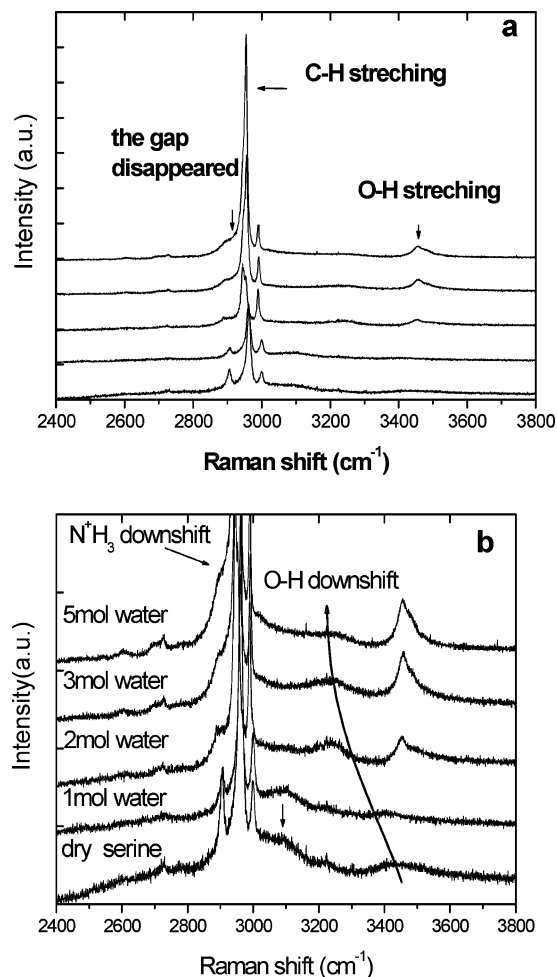
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**Figure 1.** OH, NH and CH stretching region in the Raman spectra of serine with different concentrations of H<sub>2</sub>O. (a) is a panorama of the Raman shift region from 2400 to 3800 cm<sup>-1</sup> and (b) shows the peak shifts of OH and NH in detail.

geometry time-of-flight spectrometer with an optimal energy transfer range of 20–4000 cm<sup>-1</sup> (or 2–500 meV). The best results are normally obtained below 2000 cm<sup>-1</sup> (250 meV). The resolution of the spectrometer is determined by a number of factors but, for practical purposes, can be taken to be 2% of the energy transfer.<sup>13,14</sup>

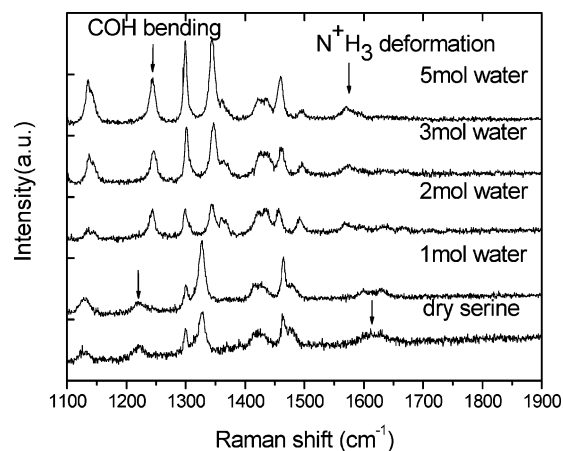
### 3. Results and Discussion

The Raman spectra of OH, NH, and CH stretching regions of serine with different amounts of water are presented in Figure 1. The assignment of some high-frequencies of X–H stretching modes (X = C, N, O) is tentative; others are difficult to assign because they overlap with other modes. In the case of serine, the OH stretching overlapped with CH and NH stretching modes.<sup>3</sup> The ab-initio calculations made by Chakraborty et al.<sup>15</sup> have predicted that the OH stretching frequency is at 3392 cm<sup>-1</sup> in the zwitterionic form with an average error of 10.1 cm<sup>-1</sup>, but there were no corresponding experimental data available to compare until now. In Figure 1, we can see the broad “polymeric” band in the range 3400–3500 cm<sup>-1</sup> for dry serine. Although overlapping with CH vibrations, the three NH<sub>3</sub><sup>+</sup> stretching vibrations at 3162, 3118 and 3022 cm<sup>-1</sup> can still be distinguished respectively, agreeing well with prediction.<sup>15</sup> As the concentration of water increases, the OH group and NH<sub>3</sub><sup>+</sup> group on the serine form hydrogen bonds with water; thus the broad band shifts to ~3230 cm<sup>-1</sup> from 3407 cm<sup>-1</sup>, whereas

**TABLE 1: Details of Hydrogen Bonds: D–H...A in Serine and Serine·H<sub>2</sub>O<sup>a</sup>**

serine <sup>10</sup>			serine·H <sub>2</sub> O <sup>11</sup>		
D--A	H--A	D–H...A	D--A	H--A	D–H...A
2.887	1.96	167	<b>2.769</b>	<b>1.804</b>	153.4
2.871	1.90	159	<b>2.877</b>	<b>1.822</b>	177.4
2.840	1.91	161	<b>2.785</b>	<b>1.843</b>	166.8
2.918	2.10	153	<b>2.809</b>	<b>1.868</b>	173.2
			2.913	1.901	168.1
			2.919	2.130	132.4

<sup>a</sup> The relatively strong hydrogen bonds are signified in bold and italic type. Bonds in angstroms and angles in degrees.



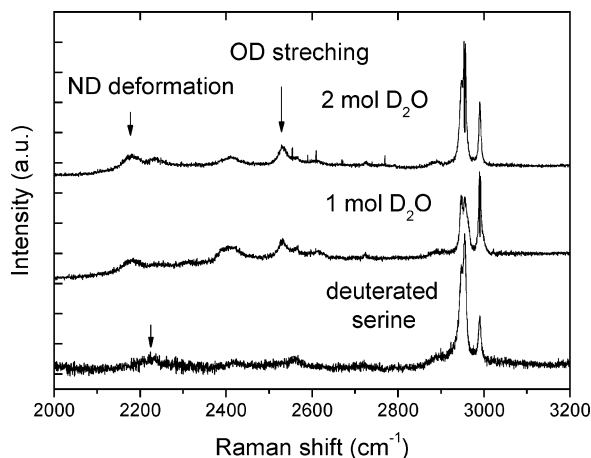
**Figure 2.** Raman spectra of serine with different concentrations of H<sub>2</sub>O measured at room temperature in the region from 1100 to 1900 cm<sup>-1</sup>.

NH<sub>3</sub><sup>+</sup> stretching vibrations downshift and overlap with the CH vibrations. A sharp peak at 3454 cm<sup>-1</sup> appears, representing free OH stretching vibration.<sup>16</sup>

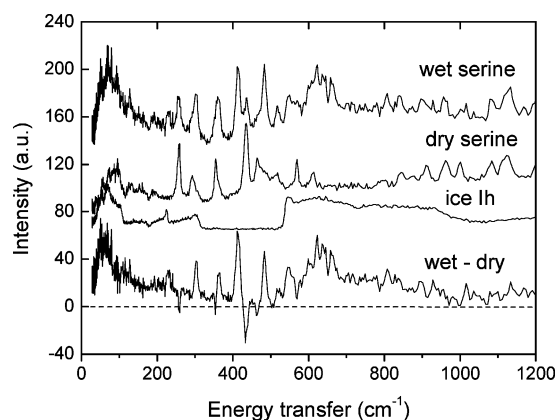
When the relative intensities for the different hydrations are compared, the OH group vibration at 3230 cm<sup>-1</sup> of the serine decreases but the free OH vibration of the serine increases. Meanwhile, the intensity associated with hydrogen bonding between water and NH<sub>3</sub><sup>+</sup> increases so much that the gap between 2906 and 2962 cm<sup>-1</sup> disappears. This interesting phenomenon can be explained by assuming that the water molecule first penetrates into serine multimers to form hydrogen bonds with the OH group. Along with the deformation of serine molecule, it is likely to transfer to NH<sub>3</sub><sup>+</sup>.

In Table 1, the hydrogen bond lengths and angles in serine and serine·H<sub>2</sub>O are listed. In anhydrous crystalline serine, the O–H groups partake in an infinite series of weak hydrogen bonds (2.918 Å for O–H...O and 2.10 Å for H...O), i.e., forming intermolecular hydrogen bonds between O–H groups. However, the N–H groups form stronger hydrogen bonds to the more electronegative carboxylate oxygen atoms.<sup>10</sup> From Table 1, we can see that the hydrogen bonds formed were stronger with the addition of 1 mol of water than in anhydrous serine.<sup>11</sup>

In Figure 2, the sharp peak at 1244 cm<sup>-1</sup> for the wet sample is COH bending, which shifted from 1220 cm<sup>-1</sup> for the dry sample. The NH<sub>3</sub><sup>+</sup> asymmetry bending modes at 1628 and 1637 cm<sup>-1</sup> shift to lower energy transfer at 1567 and 1575 cm<sup>-1</sup>, respectively.<sup>15</sup> There are some peaks that are difficult to assign. At 1462 and 1475 cm<sup>-1</sup>, two peaks separated when water was added. The peak at 1329 cm<sup>-1</sup> for the dry sample disappeared and two peaks at 1345 and 1360 cm<sup>-1</sup> appeared in wet samples. The region from 1300 to 1500 cm<sup>-1</sup> is mainly CH<sub>2</sub>, CH and



**Figure 3.** Raman spectra of deuterated serine isotopomers with different concentrations of  $D_2O$  in the region from 2000 to 3200  $cm^{-1}$ . The shift of ND deformation can be seen clearly compared with the NH downshift.



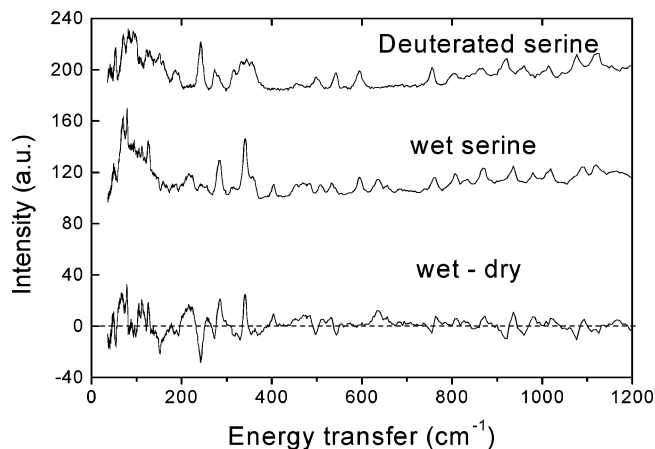
**Figure 4.** Inelastic incoherent neutron scattering spectra of serine recorded on TOSCA at  $\sim 20$  K. Spectra of wet, dry, ice Ih and difference spectrum (wet-dry) are ranked from top to bottom.

CC vibrations.<sup>5,15</sup> These shifts mentioned above show the skeleton deformation of serine by water molecules.

The Raman spectra of deuterated serine are presented in Figure 3. The assignments below 1800  $cm^{-1}$  are complicated. In normal circumstances the vibrational frequencies should shift after isotope substitution,  $\nu_2:\nu_1 = (m_1:m_2)^{1/2}$ , where  $\nu_1$  and  $\nu_2$  are the vibrational frequencies and  $m_1$  and  $m_2$  are the different masses of the isotopes. From Figure 3, we can see that the  $NH_3^+$  stretching vibrations at  $\sim 2224$   $cm^{-1}$  shift to lower energy transfer at 2180  $cm^{-1}$  and the intensity of OD stretching at 2530  $cm^{-1}$  increases considerably. However, the C–H stretching modes can be seen in the spectra of the deuterated serine, indicating that the exchange of protons–deuterons on the active  $NH_3^+$  and OH groups was relatively easy compared to the H on the CH or  $CH_2$  groups.

The spectroscopy of inelastic incoherent neutron scattering offers significant advantages in the study of molecular motions in crystals. Because hydrogen nuclei exhibit a particularly large cross section for the incoherent scattering of thermal neutrons, internal modes in which hydrogen atoms participating produce IINS spectra of much higher intensity than other modes.

Inelastic neutron scattering spectra of serine samples measured on TOSCA are presented in Figure 4. Contrast experiments were performed with a dry sample and a 1:1 mol hydration (also called wet sample). The difference spectrum  $I_{diff}(\omega)$  obtained by subtracting the spectrum of the dry  $I_{dry}(\omega)$  sample from that of the wet sample  $I_{wet}(\omega)$  is shown in Figure 4. Because the



**Figure 5.** Inelastic incoherent neutron scattering spectra of deuterated serine measured on TOSCA at  $\sim 20$  K. The peak shifts can be seen at lower energy region.

**TABLE 2: Experimental Frequencies ( $cm^{-1}$ ) Change of Vibrational Modes of Hydrated Serine Compared with Anhydrous Serine**

anhydrous serine	hydrated serine	approximate assignment
	3454	free OH stretching
3407	3230	OH stretching
around 3100	around 2900	$NH_3^+$ stretching
1637	1575	$NH_3^+$ asymmetry bending
1628	1567	$NH_3^+$ asymmetry bending
1475	1495	$CH_2$ , CH and CC vibrations
1462	1460	$CH_2$ , CH and CC vibrations
1329	1360	$CH_2$ , CH and CC vibrations
	1345	$CH_2$ , CH and CC vibrations
1220	1244	COH bending
494	487	$\gamma(N-H\cdots O)$
468		$\gamma(N-H\cdots O)$
439	415	OH torsion + $\gamma(N-H\cdots O)$
358	364	NCC bending
296	overlapped	CCC bending
261	258	CCN bending
190	190	CCO bending

volume of samples in the beam line was imprecise, to subtract the features from the dry sample as a routine procedure of data treatment, we introduced a variable  $f$ , i.e.,  $I_{diff}(\omega) = I_{wet}(\omega) - f \cdot I_{dry}(\omega)$ . The adjustment  $f$  varies from 0.8 to 1.5, aiming to remove all the contribution of signals from the dry sample as we did for other amino acids using inelastic neutron scattering.<sup>18</sup>

As shown in Figure 4, we observed the peaks at 190, 261, 296 and 358  $cm^{-1}$  of the dry serine corresponding to the deformation vibrations of the C–C–N, C–C–C, N–C–C and C–C–O moieties mixed with the torsional vibrations of the carboxylic and amino groups.<sup>12</sup> These peaks shift toward smaller wavenumbers of 186, 242, 273 and 355  $cm^{-1}$  when hydrogen has been replaced by deuterium, indicating a large contribution of amino group vibrations (see Figure 5). The relative intensities of these modes changed in the wet serine due to overlapping with the hydrogen bonding of water. A slight peak shift can be observed from the subtracted spectra in both hydrated and deuterated serine, indicating the ambient change of amino group (Table 2).

The peak at 439 and two peaks at 468 and 494  $cm^{-1}$  of the dry serine may be assigned to the vibrational modes of the  $NH_3^+$  and OH groups because these peaks are not observed in the spectrum of the deuterated sample. In the region of the hydrogen bonding vibrations, the three hydrogen atoms on the  $NH_3^+$  group produce a triplet structure, which shifts to lower frequency after the deuteration. They can be interpreted as the out-of-plane  $\gamma$ -

(N–H···O) vibrations related to hydrogen bonds on the nitrogen atom of the amino group and the oxygen atoms belonging to the carboxylate group of the adjacent molecule. The frequency shift from 439 to 316  $\text{cm}^{-1}$  corresponds to the  $1/\sqrt{2}$  factor anticipated for these modes, as shown in Figure 5. This peak was also assigned to the OH torsion, its intensity is also decreased after hydration whereas the N–H···O vibration at 439  $\text{cm}^{-1}$  shifts to lower energy transfer at 415  $\text{cm}^{-1}$  (see Figure 4). The corresponding deuterated spectra agree well with the above. The two weak peaks at 468 and 494  $\text{cm}^{-1}$  combine to a strong peak at 487  $\text{cm}^{-1}$ , which implies a new hydrogen bonding formed. This may explain the interlaced peaks in the subtracted spectrum in this region.

Other vibrational modes from 536 to 968  $\text{cm}^{-1}$  may be difficult to analyze due to the influence of the librational band of water. The subtraction spectra in this region resemble neither serine nor water. The two sharp peaks relative to the translational motion of the hydrogen bonds in water at 226 and 302  $\text{cm}^{-1}$  are characterized by ice Ih structure surrounding the amino acid, and the librational band from 536 to 968  $\text{cm}^{-1}$  is perturbed by interaction with serine.<sup>13,17,18</sup> Michalarias et al. have found that the ice around DNA resembles high-density amorphous ice, but here we do not observe a similar phenomenon.<sup>1</sup>

#### 4. Conclusions

In this paper, we have reported our recent studies of vibrational dynamics of water around one particular amino acid–serine. This study enables us to build a better picture for the interaction between water and serine and allows us to conclude that the hydrogen bonding between water and serine is very strong; as a result, most hydrogen vibrations in the serine molecule are shifted. The two components, serine and water, are completely integrated into one molecular group, forming a new structure, indicating its hydrophilic nature. Water molecules around serine seem likely to attach to the  $\text{NH}_3^+$  group than to

the OH group. Stronger hydrogen bonds are formed with water molecules than in anhydrous serine.

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