Water Structure in Proteins in Solid State Studied by Near Infrared Spectroscopy

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**Abstract.** Water adsorption in proteins is the crucial process of protein folding and structure stabilizing. Adsorption of water on proteins can be evaluated by near-infrared spectroscopy, a useful technique for observing combination frequency of a water molecule. In this work, albumin, lysozyme, and silk, were used as models for α-helix and β-pleated sheet proteins. Their NIR spectra during water adsorption process were measured by using an NIR spectrometer equipped with a transflectance accessory. Moreover, the quantitative adsorption of water was determined by gravimetric technique. The results indicate that, there are five different NIR absorptions arise from the OH combination frequencies of water adsorbed by albumin in the 5300-5100 cm-1 region. But there are only four absorptions for lysozyme and silk. The OH combination frequencies arising from water molecules in albumin indicate that it acquires free water molecules (5280 cm-1) and adsorbed water molecules through carbonyl-water interactions (5248 and 5160 cm-1) and amino-water interactions (5200 and 5120 cm-1). Interestingly, there is no indication for the presence of free water molecules in lysozyme and silk. Furthermore, the gravimetric results indicate that the rate of water adsorbed follows the order RW.Alb<RW.Lys<RW.Sil  and total mass of water adsorbed per gram solid follows the order WAlb<WLys=WSil.

Introduction

Water is the most natural abundant on the earth, which plays the vital role in several biological processes, including protein folding and structure stabilizing [1]. Hydration of water onto protein molecules is very important not only for their conformations, but also for the activities such as improving the catalytic activity of the enzyme, lubricating and so on [2,3]. Generally, there are three categories of water that associated with protein structure. Firstly, bulk water, free water surrounding the protein and can assist in protein diffusion mechanism. Secondly, hydration water, water network interacts to protein surface. The last type is individually bound water molecules, which form hydrogen bonds with polar atoms on protein [4]. Three-dimensional structure of proteins allows water penetration into inside cavities as a buried water to stabilize functional groups of amino acids [5]. There are some research work that can be found in the literature on the function and interaction of water molecules in protein, which suggested that water can locate on the protein surface and trapped inside the space in molecules. Several different techniques were used in these studies [5,6,7,8]. However, the feature of water molecule bound with protein has not been described clearly in these articles. Recently, several research workers employed near infrared spectroscopic technique to study water behavior in food, opal, and so on [9,10,11]. In 2010, Iwamoto studied the interaction between water and carbonyl group of amide by using near infrared spectroscopy, revealing that water can perform hydrogen bond with carbonyl amide in two manners; one-bound water and two-bound water [12]. Moreover, according to Christy’s work [13,14], there are evidences supported that macromolecule such as polysaccharide, adsorbed water molecules which can be determined by NIR spectroscopy [13,14]. Therefore, to get a clearer understanding, molecular adsorption of water into three different proteins; albumin, lysozyme and silk, were studied by using near infrared spectroscopy in this work. Second and fourth derivative spectra acquired during the water adsorption process were analyzed. OH combination frequencies were characterized and classified to various types of hydrogen bonding interactions between water and protein samples. Furthermore, the amount of water adsorbed in samples was determined by using gravimetric measurement.

**Experimental**

 **Materials and sample preparation**. Three different protein samples, which are Bovine serum albumin, Lysozyme from hen egg and French silk, have been used in this work. These samples were purchased from Sigma Aldrich, Fluka BioChemika and Lagartun, respectively. Small amount of them were ground into fine powder, then dried by using the ceramic heater (BA electric Bunsen from electrothermal, UK) controlled by an external power supply and evacuated by using a powerful vacuum pump (Edwards, UK) to remove water at 100-120 ˚C for 1 hour. The drying temperature was measured by digital thermometer with a K type thermocouple (Clasohlson, UK). Experiment was carried out at two humidity, 40% and 60% RH, which were measured by a small thermometer-hygrometer (Thermometerfabriken Viking AS, Sweden). The dried samples were kept in glass vial for water adsorption determination.

 **Near infrared (NIR) measurement**.The near infrared spectra of all samples were measured by PerkinElmer Spectrum One NTS FT-NIR spectrometer (PerkinElmer Ltd., UK) equipped with PerkinElmer transflectance accessory and deuterated triglycine sulphate detector. Samples vials were placed directly on the crystal, then the spectra of dried samples and samples during water adsorption process were collected in the region 10000-4000 cm-1. Total of 10 scans were made each time. The obtained spectra were converted to log(1/R), then the second and forth derivative spectra were obtained and saved by using PerkinElmer spectrum software.

 **Gravimetric measurement**. The water adsorption process on each dried samples was evaluated by gravimetric technique. Glass vials of samples were placed quickly on a Mettler electronic balance connected to computer through an RS-232 port, then the increasing mass was recorded twice every second by locally written software. The obtained data were plotted in Excel workbook and presented in comparison graph for result discussion.

**Results and Discussion**

Near infrared spectra during water adsorption process of three protein samples at relative humidity of 40% shown in Fig.1 reveal the increasing of OH combination and overtone peak around 5200 and 7000 cm-1, respectively. The band assignment of NIR peaks of proteins with water molecules adsorbed is shown in Table.1.



Fig. 1. NIR spectra during water adsorption of a) Albumin, b) Lysozyme and c) Silk

Table 1 Near Infrared bands assignment for albumin, lysozyme and silk

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| --- | --- |
| **Peak [cm-1]** | **Band assignment** |
| 9000-8000 | Second overtone of CH stretching  |
| 7100-6800 | Overtone OH of water |
| 6600-5600 | First overtone of protein functional groups |
| 5300-5100 | Combination band of OH stretching and bending of water molecules bonded to functional groups of proteins |
| 4860 | Combination of Amide A and Amide II |
| Approx. 4600 | Combination of Amide B and Amide II |
| 4418 | β-pleated sheet (silk) |
| 4700-4000 | Combination of CH stretching in proteins |

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Fig. 2. Fourth derivative profiles of NIR spectra of protein samples with adsorbed water molecule in the range of 5350-5030 cm-1

 To observe the hydrogen bonding interaction between water and protein samples, second and fourth derivative profiles were carried out. Fig. 2 displays the fourth derivative spectra of albumin, lysozyme and silk in the region of 5350-5030 cm-1, which relate to water adsorbed by polar functional groups within their molecules. Four peaks arising from hydrogen bonding can be characterized into two major types of interaction; carbonyl-water interaction and amino-water interaction. OH combination frequency of water molecules bonded to carbonyl oxygen atoms of protein backbone with only one hydrogen bond appear around 5248 cm-1[12], while forming of two hydrogen bonds of water toward carbonyl oxygen provide peak at lower wavenumber around 5160 cm-1 [12] due to stronger hydrogen bonding. On the other hand, absorption band around 5200 cm-1 arises from hydrogen bonding between nitrogen of amino groups and hydrogen atom of water, resulting quaternary ammonium structure. The last adsorption band, which appear around 5120 cm-1, refer to bonding between hydrogen of amino groups and water. Interestingly, derivative profiles indicate that albumin, which has the biggest molecular structure among our samples, acquire free water molecules resulting adsorption peak at 5280 cm-1 [13], but there is no presence of free water molecules in lysozyme and silk.

 The adsorption intensities of bands due to OH combination frequencies of water molecule adsorbed on oxygen atom of carbonyl groups (5248 cm-1) and free water (5280 cm-1) in albumin are shown in Fig. 3a). The increasing rate of intensities reflect to the rate of water adsorption on albumin molecule. It is clear that water adsorption at carbonyl group has the higher rate than acquisition of free water and the trends are similar, suggesting that water can be adsorbed through hydrogen bonding interaction more than acquiring as free water molecules.

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Fig. 3. a) Time correlation of the intensities of fourth derivative bands at 5280 and 5248 cm-1 which refer to free water molecule and carbonyl-water interaction, respectively. b), c) and d) Plot showing the masses of water adsorbed by albumin, lysozyme and silk, respectively comparison between 40% and 60% RH.

 Plot of adsorption of water respect to time in albumin, lysozyme and silk are shown in Fig. 3b), c) and d), respectively. Silk, β-pleated sheet protein, show the highest rate of water adsorption, while lysozyme adsorbs water at a slower rate than silk. In the case of albumin, it has the slowest rate of water adsorption. The saturation of water adsorption of albumin took place over 30 hours, resulting the order of water adsorption rate follows RW.Alb<RW.Lys<RW.Sil . The amount of water adsorbed in lysozyme and silk are 0.1 gram per gram sample at 40%RH and 0.12 grams per gram sample at 60%RH. However, even albumin took the longest time for adsorption, it can adsorb water only 0.06 and 0.1 gram per gram albumin at 40% and 60%RH, respectively. This result indicates that silk is the highly water adsorbing material. It can adsorb water molecule greater than silica gel, which has water adsorbed on the molecule only 0.04 grams per gram of silica gel in 60 minutes [15].

**Conclusion**

 Near Infrared spectroscopy has been used to study molecular adsorption of water onto proteins. Second and fourth derivative profiles were used effectively for confirmation and assignment of five different adsorption bands to each type of hydrogen bond interaction between water and proteins. There are two main types of hydrogen bonding were observed, which are carbonyl-water interaction and amino-water interaction. Remarkably, there is no evidence of free water molecules that can be adsorbed by lysozyme and silk. Moreover, the gravimetric result suggested that both silk and lysozyme can adsorb water molecule more than albumin. Interestingly, β-pleated sheet protein shows the significantly faster adsorption rate than protein that contain α-helix structure.

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