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Thermal properties and hydration structure of poly-L-lysine, polyglycine, and lysozyme

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ABSTRACT

Differential scanning calorimetry (DSC) and X-ray diffraction (XRD) measurements of hydrated poly-L-lysine, polyglycine and lysozyme at hydration levels (mass of water/mass of peptide and protein) = 0.30–0.82, 0.27–0.49, and 0.30–0.81, respectively, were performed in the temperature range of 180–298 K. The DSC data showed that the interfacial hydration water of the polypeptides and the protein is not frozen even at 180 K. The X-ray radial distribution functions revealed that with decreasing temperature the interactions of the polypeptides and the protein with hydration water molecules gradually become remarkable, resulting in the development of the structure of their hydration water. The structure change of the hydration water with lowering temperature is monotonous and could not be the origin of dynamic transition (glass transition and fragile-to-strong crossover) for hydrated proteins as previously observed by neutron scattering.

1. Introduction

Water plays an important role in various biological processes. To clarify the relationship between the biological processes and water on a molecular level, many studies using X-ray and neutrons have so far been performed for various biomolecules [1–5]. Inelastic X-ray scattering measurements enabled us to obtain information on collective dynamics of vibration motions of hydrated proteins [6–8], whereas elastic and quasi-elastic neutron scattering methods provided us root mean square displacements, structure relaxation, and collective dynamics of hydrated proteins [2,3,9–13]. From these measurements of protein dynamics, dynamic transitions, such as glass transition (a transition from harmonic to anharmonic atomic motions) [9,10,12,13] and dynamic crossover (fragile-to-strong crossover [11]) were found to take place at 220–230 K. It should be noted that these dynamic transitions and dynamic crossover occurred at a hydration level above 0.37 [13]. Interestingly, the onset of biological activity of proteins has been found at a similar hydration level. Hence, clarification of the origin of the dynamic transitions will be a key to reveal the role of water in biological processes. To our knowledge, however, it is not clear whether or not these dynamic transition and crossover arise from the structure change of hydration water.

In the present study, to reveal the origin of the dynamic transition and the dynamic crossover of protein hydration water, thermal properties and structure of hydrated polypeptides (poly-L-lysine and polyglycine) and lysozyme are investigated using DSC and XRD. DSC clarifies melting and freezing temperatures and the amount of freezing water in hydrated proteins. XRD gives the microscopic structure of hydration water of proteins in terms of radial distribution function. Since proteins consist of both hydrophilic and hydrophobic residues, the interaction between proteins and hydration water is complex. Thus, the same techniques are applied to hydrated polypeptides which consist of single amino acid residues to look into the low temperature behavior of hydration water around single amino acid residues. Finally, a comparison of hydration water in the polypeptides and lysozyme is made with water confined in mesoporous silica.

2. Experimental

2.1. Samples

Powdery lysozyme, poly-L-lysine, and polyglycine were purchased from Sigma-Aldrich and used without further purification. The powder was dried with a turbo molecular pump for one day. The water absorbed protein sample was prepared as follows: the dry powder was kept in a dessicator which contained saturated KCl, KNO_3 aqueous solutions, or pure water to reach a desired hydration level, h. The saturated vapor pressure of water of the saturated KCl and KNO_3 aqueous solutions is 86.0% and 93.0% of that of pure water, respectively [14,15]. For the preparation of hydrated lysozyme at a hydration level above 0.5, protein powder and water were mixed together in a cell used for subsequent DSC and XRD measurements at a desired hydration level. Then, the

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mixture was sealed and kept in an oven at 333 K which is below the temperature (~343 K) of thermal denaturation of lysozyme.

2.2. DSC measurements

DSC measurements were made on an EXSTAR DSC6100 (Seiko Instruments) equipped with a cooling system using liquid nitrogen. The samples ($h = 0.3$–0.81) of typically ~10 mg were sealed in aluminum cells of 70 μL. The cooling and heating rates were 2 K min^{-1}, respectively, over a temperature range between 180 K and 300 K. The temperature was controlled to within ±0.05 K. The empty cell was used as a reference.

2.3. X-ray diffraction measurements

Both hydrated ($h = 0.27$–0.82) and dried lysozyme and polypeptide powders were sealed in each quartz capillary (W. Müller) of 2 mm diameter and 0.01 mm wall thickness. X-ray scattering intensities from the hydrated and dry samples were measured over a temperature range of 298–180 K with an X-ray diffractometer (R-AXIS RAPID-II, Rigaku) using a curved imaging plate detector. An empty capillary was also measured at room temperature. The X-rays were generated at a Mo anode tube operated at 50 kV and 40 mA and then monochromatized with a flat graphite crystal to obtain Mo*K*α radiation (the wavelength λ = 0.7107 Å). A double-hole type collimator, whose hole-diameters are 0.5 mm each, was used to obtain the parallel X-ray beam. The exposure time was 1 h for each measurement at each temperature. Cooling of the sample was carried out by blowing cold N_2 gas generated from a refrigerating equipment (Rigaku) on to the capillary. The temperature of the sample was measured with a copper–constantan thermocouple and controlled within ±1 K with a temperature control unit (Rigaku).

The two dimensional diffraction pattern of samples recorded on an imaging plate was integrated to one dimensional intensities with a 2DP program (Rigaku). After absorption correction of sample and capillary, the intensity of an empty quartz capillary was subtracted from those of the hydrated and dry samples. The subtracted intensities were corrected for Compton scattering as described elsewhere [16], and normalized to absolute units in a usual manner [17–19]. The structure functions, $i(s)$, were normalized to the stoichiometric volume of an oxygen atom in proteins.

The radial distribution function, RDF, $D(r)$, was calculated by means of Fourier transform as

$$D(r) = 4\pi r^2\rho_0 + 2r\pi^{-1}\int_{s_{min}}^{s_{max}} si(s)M(s)\sin(rs)ds. \quad (1)$$

Here, $\rho_0 (=[\sum x_i f_i(0)]^2/V)$ stands for the average scattering density of a sample powder in the stoichiometric volume V, and s_{min} and s_{max} are the minimum and the maximum s values attained in the measurements, respectively (s_{min} = ~0.3 $Å^{-1}$ and s_{max} = ~12 $Å^{-1}$). A modification function $M(s)$ of the form $[[\sum x_i f_i^2(0)]/[\sum x_i f_i^2(s)]]\exp(-0.01s^2)$ was used. Spurious peaks below 1 Å in RDF arises from systematic errors, such as the truncation of the structure function at a definite s value, imperfection of the X-ray diffraction geometry and uncertainty of absorption coefficient and the scattering factor. The structure function of the spurious peaks is calculated by the following equation:

$$i_{spur}(s) = M(s)^{-}\int_0^{r_{max}}\left\{\sum P_{ij}(r) - D(r)\right\}\frac{\sin(sr)}{sr}dr, \quad (2)$$

where $P_{ij}(r)$ is the function which reproduces theoretical peaks in the range of 0 ~ r_{max}. The $P_{ij}(r)$ is calculated for the short intramolecular interaction ($r < 1.5$ Å) within water and amino acid molecules. The $i_{spur}(s)$ is added to the experimental $i(s)$ values, and the Fourier transform is performed to give the RDF without spurious peaks [20]. All treatments of the X-ray diffraction data were carried out with the program KURVLR [21].

3. Results and discussion

3.1. Thermal properties of hydration water of polypeptides and protein

Fig. 1 shows the DSC curves for water absorbed polylysine at $h = 0.3$ and 0.57, polyglycine at $h = 0.3$ and 0.57, and lysozyme powders at $h =$ 0.3 and 0.8. All DSC data at $h = 0.3$ show no peaks due to the forming of ice, suggesting no crystallization of water in the temperature range of 298 to 180 K. The cooling curve for hydrated lysozyme at $h = 0.8$ indicates that the hydration water freezes at 252 K. In the heating process, a shoulder and a broader peak are observed at 261 and 265 K, respectively, implying that the frozen water of lysozyme would have some different components as in the case of hydrated dextran gel [22].

The cooling and heating curves for hydrated polyglycine at $h = 0.57$ show that the freezing and melting temperatures of the hydration water are 258 K and 269 K, respectively. These values are similar to those of pure water (256 and 273 K) [23]. Moreover, the peak in the heating curve is sharper than that for lysozyme. These features imply that polyglycine perturbs water structure less effectively than lysozyme.

The cooling curve for hydrated polylysine at $h = 0.57$ shows that the freezing temperature of hydration water is 220 K. In the heating process, two broad peaks are found at 230 and 242 K. This indicates that two kinds of hydration water exist around polylysine. The freezing and melting temperatures of polylysine are lower than those of lysozyme. These differences in freezing and melting temperature between polypeptides and lysozyme could be due to the different hydration of the side chains of the polylysine and lysozyme exposed to water since polylysine has a random structure and hence more side chains of polylysine are exposed to water than those of lysozyme having folded structure. The freezing temperature of hydrated ubiquitin ($h = 0.4$) measured by DSC and NMR measurements was reported to be ~220 K [24,25]. This value is similar to that of polylysine found in the present study. Further

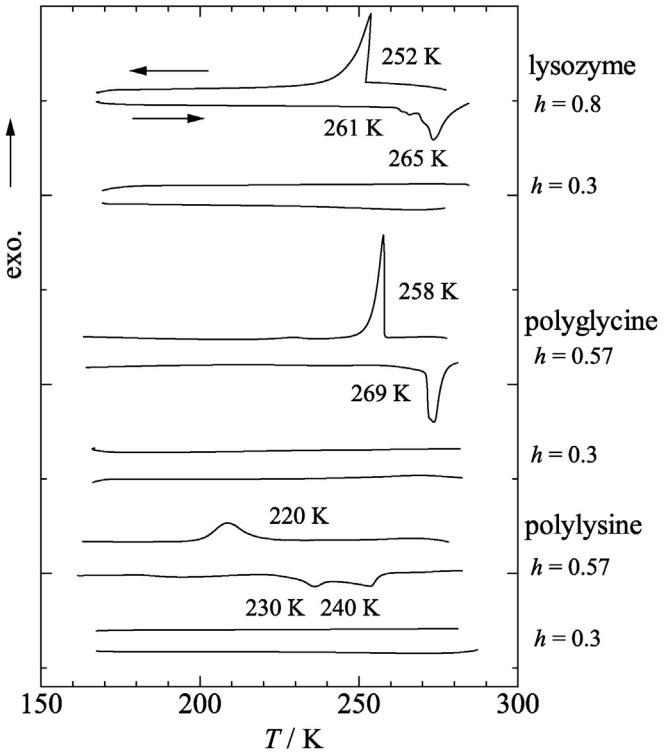

Fig. 1. DSC curves of hydrated poly-L-lysine at $h = 0.3$ and 0.57, polyglycine at $h = 0.3$ and 0.57, and lysozyme at $h = 0.3$ and 0.81.

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