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Hydration water dynamics in bovine serum albumin at low temperatures as studied by deuterium solid-state NMR

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ABSTRACT

Solid state ^{2}H NMR was used to investigate changes in the structure and dynamics of hydration waters of bovine serum albumin (BSA) due to glass transitions. The ^{2}H NMR spectra were separated into fast and slow components based on differences in spin-lattice relaxation time T_1. The fast components corresponded to water molecules interacting with protein while the slow components were the water molecules similar to bulk water and deuterons of the protein backbone. Simulation analysis of the ^{2}H NMR spectra of the fast components was used to assess the mode and rate of motions of hydration waters around the protein. At low temperatures, the water molecules underwent a 180° flip and slow reorientation in the tetrahedral sites. The distribution of the rate of the 180° flip and the D-O-D angle of water molecules were clarified. The distribution of the D-O-D angle of water molecules spread with decreasing temperature. The marked slowing down in the reorientation of water molecules was observed at a glass transition of around 200 K, which is linked to the disordered region of the protein. In contrast, the 180° flip of water molecules occurred frequently, even below 200 K. A freeze of the 180° flip of water molecules was observed around the glass transition temperature of 110 K, where primary hydrate water formed a direct hydrogen bond with the protein, making it perfectly immobile.

1. Introduction

The changes in the structure and dynamics of proteins are caused by glass transition and these changes are directly related to the biological activity of proteins [1–4].

The glass transition of bovine serum albumin (BSA) has been investigated by thermal analysis [5,6]. A sample quenched from 300 K down to 80 K showed a jump in heat capacity indicating a glass transition temperature T_g of 170 K. When the sample was annealed at 200–240 K, the relaxation effects resulted in three glass transition temperatures: $T_g = 110$, 135 and >180 K. The glass transition above 180 K is considered to originate from a rearrangement in the motion of the disordered region of the protein. Based on dielectric measurements, the corresponding glass transition was observed at 200 K [7,8]. The transition accompanying the change in the dynamics of protein and hydration water, also known as the dynamic transition, was found at about 200 K [1–4]. The glass transitions at $T_g = 110$ and 135 K are considered to be caused by a rearrangement in the motions of primary hydrate water forming a direct hydrogen bond with the protein and part of the internal water localized in the opening of a protein's structure, respectively. Thus, for hydrated proteins, the dynamics of hydration waters plays very important roles in the glass transition. Therefore, detailed analysis of the dynamics of hydration waters around the glass transition is important to investigate the physical properties of proteins. Previously, the relations between the dynamics of hydration waters and the glass or dynamical transition were investigated by various methods [7–16].

NMR is an effective method to study molecular dynamics and local structures in hydrated proteins [17–29]. There have been several investigations of the structure and dynamics of BSA using NMR [26,29]. To investigate the dynamics of water molecules, solid-state ^{2}H NMR is especially efficient, since the rate and mode of reorientational motions of molecules can be analyzed strictly from the line shape of a broadline spectrum and T_1 [30–40]. Thus far, the dynamic properties of hydration waters in elastin, collagen, and myoglobin have been analyzed using ^{2}H NMR (T_1) [41–43]. Although the deuteration causes a slight change in its bonding properties, the glass transition behavior and dynamics of water were little affected by deuteration [16]. In the present work, we

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tried to apply simulation analysis of solid-state ^{2}H NMR spectra to BSA to obtain more detailed information of the dynamics and structure of hydration waters and to clarify the relations between these two properties and glass transitions. Simulation of ^{2}H NMR broadline spectra, including distribution of the motions of water molecules, was performed. Changes in the motional mode, rate and D-O-D angles of hydration waters in BSA with decreasing temperature were investigated using the simulation analysis of ^{2}H NMR broadline spectra and T_1.

2. Materials and methods

BSA was obtained from Wako Pure Chemical Industries. BSA powder hydrated with deuterium oxide was prepared by repeated recrystallization three times from heavy water. The hydration level of samples was determined from the observed mass change of dried samples. Although the contents of small particles such as salt ions potentially present may give uncertainness, the hydration level of samples was estimated as h = 0.26 (h: gram of water per gram of protein).

The ^{2}H NMR spectra and T_1 were measured by using a Chemagnetics CMX-300 spectrometer at 45.825 MHz. The sample temperature was controlled with a nitrogen-gas-flow temperature controller (JEOL VT1A). The sample was sealed in a glass tube 6 mm in diameter and about 20 mm in length. The ^{2}H NMR spectra were observed using a quadrupole echo sequence $(90^\circ)_x - t - (90^\circ)_y - t - t_{acq}$, where t and t_{acq} are the interval of echo and acquisition time, respectively. The 90° pulse width and t were 2.7 and 20 μs, respectively. The partially relaxed spectra for saturation recovery were observed using a sequence $(90^\circ - t_s)_n$–$90^\circ - t_r - (90^\circ)_x - t - (90^\circ)_y - t - t_{acq}$. n was 5. t_s, t and t_{acq} were 100, 20 and 2048 μs, respectively. ^{2}H NMR T_1 was measured by the inversion recovery method. The simulation of ^{2}H NMR partially relaxed spectra was performed by homemade Fortran programs using double precision [39].

3. Results and discussion

3.1. ^{2}H NMR spectra and T_1

Fig. 1 shows the temperature dependence of the ^{2}H NMR broadline spectrum. Above 273 K, a sharp spectrum due to the rapid isotropic rotation of water molecules was mainly observed. Below 273 K, a broad component with peaks at about ±60 kHz due to the freezing of the motions of hydration waters appeared in the spectrum and its intensity increased with decreasing temperature. The line width of the central component increased with decreasing temperature. The broad central component below 213 K indicates the existence of the 180° flip of water molecules representing the distribution of the D-O-D angle. The central component of the spectrum disappeared and the ^{2}H NMR spectrum became an almost rigid powder pattern below T_g = 110 K accompanied by the freezing of primary hydrate water.

Fig. 2 shows partially relaxed ^{2}H NMR spectra for the inversion recovery method at 203 K. As shown in the spectrum at a recovery time of t_r = 10 ms, recovery of the central sharp component was faster than that of the broad component. Fig. 3 shows the recovery of magnetization for the inversion recovery method. The recovery of magnetization was analyzed by two-component fitting using

$$M(t_r)/M_0 = A_f\left[1 - 2\alpha\exp\left(\left(-t_r/T_{1f}\right)^{\beta_f}\right)\right] + \left(1 - A_f\right)\left[1 - 2\alpha\exp\left(-(t_r/T_{1s})^{\beta_s}\right)\right]. \quad (1)$$

Here, $M(t_r)$ is the magnetization at recovery time t_r and M_0 is the thermal-equilibrium value of magnetization. A_f is the ratio of the fast component. T_{1f}, β_f and T_{1s}, β_s are the spin-lattice relaxation times and the stretching parameters for fast and slow components, respectively. α shows imperfection in the 180° pulse. The deuterated parts in the protein backbone as well as water molecules undergoing slow motion are considered to be included in the slow component. Only one component was observed below 160 K. The mean T_{1f} and T_{1s} values were obtained by Ref. [41–43].

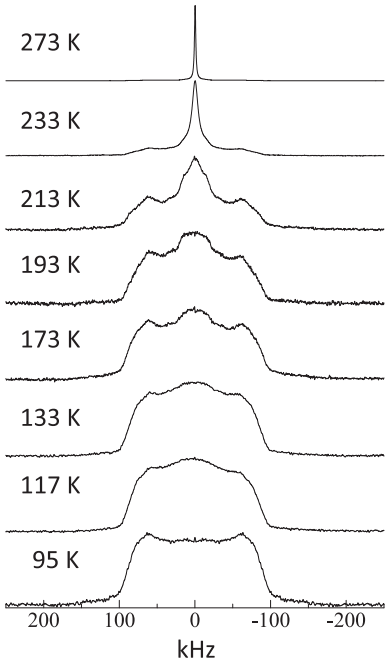

Fig. 1. Temperature dependence of ^{2}H NMR spectrum for BSA.

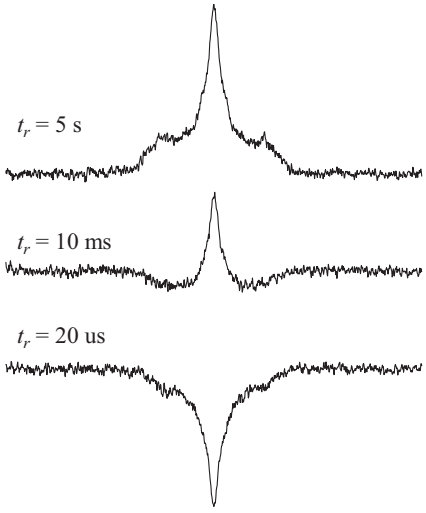

Fig. 2. Partially relaxed ^{2}H NMR spectrum of BSA for inversion recovery method at 233 K τ_r shows the recovery time after a 180° pulse.

$$\langle T_{1i}\rangle = \frac{T_{1i}}{\beta_i}\Gamma\left(\frac{1}{\beta_i}\right), \quad (i = f,\ s) \quad (2)$$

where $\Gamma(x)$ is the Gamma function.

Fig. 4 shows the temperature dependence of $\langle T_1\rangle$ for fast and slow components. $\langle T_{1s}\rangle$ increased with decreasing temperature.