Structure and dynamics of β-lactogloblin in alcohol-water mixture

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The secondary and the higher-order structures of protein are affected by the hydrophilic and lipophilic balance (HLB) of solvent. In alcohol-water mixture, we can control the HLB of solvent by changing the composition of alcohol. In fact, alcohol-induced α-helix formation of peptides and proteins is well known and has been widely used in biophysics and biochemistry. So far, in order to investigate the alcohol effect on the native structure of monomer protein, we have measured the circular dichroism (CD) spectra of chymotrypsin inhibitor 2 (CI2) as a function of alcohol mole fraction in aqueous mixtures of methanol, ethanol, trifluoroethanol (TFE), and hexafluoro-isopropanol (HFIP)[1]. The CD spectra have shown that the secondary structure of CI2 changes from β-strand to α-helical structure at alcohol mole fractions characteristic of the individual alcohols in an order of HFIP $>$ TFE $>$ ethanol $>$ methanol. In the present study, small-angle neutron scattering (SANS) and neutron spin echo (NSE) measurements were performed on alcohol aqueous solutions of β-lactogloblin (β-Lg) in order to investigate the alcohol effect on the higher-order structure and dynamics of the protein.

The β-Lg powder ($>$90 %) was lyophilized in D_2O to exchange labile hydrogens with deuterons. The β-Lg powder was resolved in D_2O with deuterated hydrochloric acid and stirred with a vortex mixer for 30 s. The concentration of DCl was adjusted to 0.1 M in the final solution. And then alcohols were added to the solution up to a desired alcohol concentration and stirred with a vortex mixer for 30 s. Deuterated alcohols used were methanol-d_4, ehtanol-d_6, 2-propanol-d_8, and 2,2,2-trifuluoroethanol-d_3. In this study, the unit of alcohol concentration is volume percent.

The wavelength used for small-angle neutron scattering was 6 Å. The neutron beam size at the sample position was 7 mmϕ. A sample solution was kept in a quartz cell of 2 mm path length. The cell was inserted into a temperature-controlled chamber. The temperature in the chamber was 298K and controlled within $\pm$0.1K. The distances between the sample and detector were 1 and 4 m, corresponding to the momentum transfer (Q) of 0.007 - 0.14 Å^{-1}. The measuring time was 10 min to 1 h depending on scattering intensity. Measurements were also made for background, an empty cell, and lupolen used for intensity normalization. The SANS data of the sample solutions and the empty cell were corrected for absorption by using transmission data measured, and then the empty cell data were subtracted as background from the data of the sample solutions. Correction for the detector efficiency and normalization to absolute units were made by using the intensity of lupolen. The SANS profiles of β-Lg in alcohol-water mixtures showed that β-Lg exists as a monomer at low alcohol concentrations ($<$30% methanol, $<$20% ethanol, $<$20% 2-propanol, and $<$10% TFE). The radius of gyration (R_G) of β-Lg was calculated with the Gunier equation. The values of R_G of β-Lg were almost independent of alcohol in these alcohol concentrations. The CD spectra indicated that the degree of α-helical structure enhanced with the alcohol addition was less than 20% in these alcohol concentrations [2]. Hence, β-Lg is likely to still keep the native structure in these alcohol concentrations. With increasing alcohol concentration, the large scattering intensity in a low Q region was observed. The SANS profile could be fitted with a power function whose exponent was 1.7 independent

of alcohol. This implies that a homogenous network of β-Lg was grown. The CD spectra exhibited that the α-helical structure of β-Lg was dominant in these alcohol concentrations [2].

For the NSE measurements, the wavelength used was 7.3 Å. The scattering vector Q covered was 0.01 - 0.1 $Å^{-1}$. The Fourier time was varied from 0.15 to 15 ns. The measuring time was 4 to 12 h for each Q range depending on scattering intensity. A plate of Grafoil was measured for resolution correction. The sample temperature was 292K and was controlled within $\pm$0.3K. The intermediate scattering functions (ISF) of β-Lg at various Q values were measured in pure D_2O, 20% ethanol-, and 10% TFE- D_2O mixtures. The ISFs were fitted with a single exponential function. The Q^2 dependence of the relaxation rate in pure D_2O, 20% ethanol-, and 10% TFE-D_2O mixtures are shown in Figure 1. The diffusion coefficients (D) were obtained by using a least-square fitting procedure over $0 < Q^2 < 0.01$ $Å^{-2}$. Hydrodynamic radius (Stokes radius) R_H was determined by the Stokes-Einstein equation. The R_H value in pure water (40.2Å) is smaller than that in alcohol-water mixtures (43.4 and 43.1Å in 20% ethanol- and 10% TFE-D_2O mixtures, respectively). Because the radii of gyration of β-Lg in these solvents are almost the same as mentioned above, the increase in R_H in alcohol-water mixture would not arise from an increase of the Stokes friction. On the other hand, the R_H values obtained from dynamical light scattering (DLS) measurements were almost the same irrespectively of solvents [3]. This difference would be explained in terms that NSE would detect internal motion of β-Lg due to a smaller wave length of neutrons. That is, even small addition of alcohol which seldom affects the ternary structure of β-Lg would retard internal motion of the protein. This is consistent with the results of molecular dynamics simulation of a small peptide in TFE-water mixture [4].

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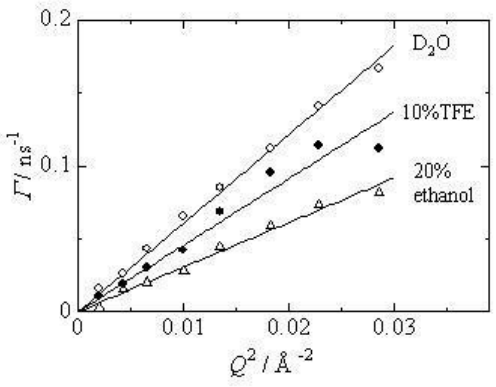

Fig. 1. Q^2-dependence of the relaxation rates of β-lactoglobulin obtained from ISF of NSE in aqueous solutions of 10% TFE and 20% ethanol.