Solvent effect on structure and dynamics of beta-lactoglobulin in alcohol-water mixtures

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The effect of alcohol on denaturation of protein is known in biophysics and biochemistry fields. Alcohols have a tendency to increase the content of α-helix structure in proteins. So far, the secondary structure of α-helix forming peptide and protein, Melittin and β-lactoglobulin (BLG), was investigated in aliphatic and fluorinated alcohols-water mixtures [1]. The denaturation effect of these alcohols changes according to the size and nature of the hydrophobic groups of the alcohols. However, an underlying mechanism in alcohol denaturation of proteins is still unclear. The investigation of configurations (size, shape and aggregation of proteins) and diffusive dynamics of proteins in alcohol-water mixture will help us to understand the mechanism of alcohol denaturation of the protein.

In the present study, small-angle neutron scattering (SANS) of BLG in ethanol- and trifluoroethanol(TFE)-water mixtures was measured in order to reveal the solvent effect on three-dimensional structure and aggregation state of BLG. Neutron spin echo (NSE) of BLG in water was also measured to reveal the diffusive dynamics of BLG. To minimize the contribution of exchangeable hydrogen atoms of the protein, BLG powder was dissolved in D_2O and then was lyophilized. The dried BLG powder obtained was dissolved in ethanol-D_2O and TFE-D_2O mixtures. The SANS of BLG in both mixtures was measured at 298 K. Concentrations of BLG solutions were 5, 10, and 20 mg ml^{-1} for each solvent. A sample was kept in a quartz cell of 2-mm path length. The cell was inserted into a temperature-controlled chamber. The distances between the sample and detector were 1 and 4 m, corresponding to Q of 0.007 - 0.3 Å^{-1}. Measurements were also made for background, solvent, and lupolen used for intensity normalization. The NSE signal for an D_2O solution of BLG of 40 mg ml^{-1} was measured at 298 K. A sample was kept in a quartz cell of 2-mm path length. The scattering vector Q covered was 0.055 - 0.12 Å^{-1}. The Fourier time was varied from 0.15 to 15 ns. The temperature of sample was controlled within $\pm$ 0.3 °C with circulated water.

Figure 1 shows SANS profile of BLG in ethanol-D_2O mixture at various ethanol volume fractions. The peak corresponding to the correlation between proteins was observed at 0.08 Å^{-1} in ethanol-D_2O mixture of 10 vol% ethanol. With increasing of ethanol fraction, the peak position does not change up to 30 volFor BLG in TFE-water mixtures, the position of the correlation peak between proteins drastically shifted to a small Q region at $\sim$20 vol% of TFE. In analogy with the case of ethanol-water mixture, the specific TFE composition of the structural transition of BLG is consistent with that for solvent structure transition from the tetrahedral-like water clusters to the chain-like alcohol ones for TFE-water mixture. The NSE signal of BLG in D_2O could be simulated by the single exponential function. The relaxation time obtained at Q = 0.102 Å^{-1} was 13.1 ns. We will discuss the correlation between diffusion constant and three-dimensional structure of BLG obtained from SANS. The further analysis is in progress. [1] D. Hong, M. Hoshino, R. Kuboi, and Y. Goto, J. Am. Chem. Soc. 121, 8427 (1999). [2] M. Matsumoto, N. Nishi, T. Furusawa, M. Saita, T. Takamuku, M. Yamagami, and T. Yamaguchi, Bull. Chem. Soc. Jpn. 68, 1775 (1995).

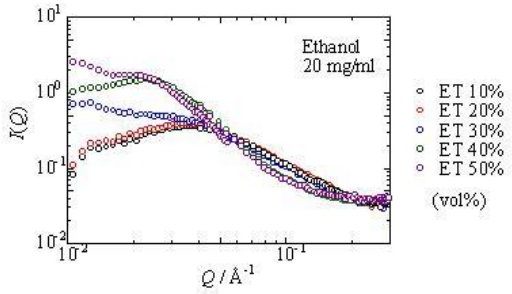

Fig. 1. SANS profiles of BLG in ethanol-water mixtures at various ethanol volume fractions.