Structure and dynamics of β-lactoglobulin aggregates by small-angle neutron scattering and neutron spin echo methods

K. Yoshida[1], T. Yamaguchi[1], H. Endo[2], M. Shibayama[2]

[1] *Department of Chemistry, Faculty of Science, Fukuoka University,*[2] *Institute for Solid State Physics, The University of Tokyo*

Many proteins aggregate and gelate when they take a misfolded state. The gelation of β-lactoglobulin(BLG) is triggered by heat, pressure, and addition of alcohol. The pH dependence of the aggregation state of a heat-induced gel has been studied[1], however, the mechanism of alcohol-induced gelation is not clear. Understanding the mechanism of aggregation of denatured BLG would provide a hint to reveal how misfolded protein forms an aggregate such as amyloid fibril. Small-angle neutron scattering and neutron spin echo measurements provide useful information of the structure and dynamics of the BLG aggregates.

BLG was lyophilized from D_2O solution to substitute exchangeable hydrogen of BLG to deuterium. The BLG powder was dissolved into ethanol- and trifluoroethanol(TFE)-water mixtures at various volume fractions of alcohol and followed by addition of 100 mM of DCl and the BLG concentrations were 10 and 20 mg ml^{-1}. A sample was kept in a quartz cell of 2-mm path length for SANS measurement. The cell was inserted into a temperature-controlled chamber at 298 K. 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 and lupolen used for intensity normalization. A quartz cell of 4-mm path length for NSE measurement. 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 at 298 $\pm$ 0.3 K with circulated water.

Figure 1 shows the SANS intensity of BLG in ethanol-water mixture. This indicates that BLG exists as a monomer up to 20 vol% of ethanol and the aggregation occurs above 30 vol% where the secondary structure BLG changes. A similar trend was also found in TFE-water mixture. The alcohol-induced BLG aggregation could be well related to the secondary structure change. The relaxation of BLG obtained from NSE intermediate scattering functions retards drastically by addition of ethanol and TFE. The relaxation was hardly found at an energy resolution of the NSE spectrometer at 30 vol% of ethanol and 20 vol% of TFE where the solutions gelate, indicating the formation of a rigid BLG network structure.

The present results will be compared with those of heat- and pressure-induced aggregation of BLG. We will discuss the role of solvent on gelation of BLG at the molecular level.

References

[1] S. Takata, T. Norisuye, N. Tanaka, M. Shibayama, Macromolecules **33**, 5470-5475 (2000).

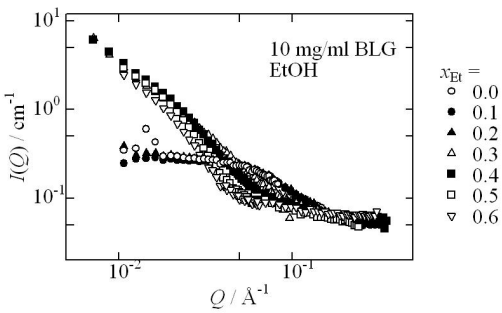

Fig. 1. SANS intensity curves of BLG at various volume fraction of ethanol in ethanol-water mixture with 100 mM DCl.