Temperature effects on denaturation of a protein in alcohol-water binary solvents

T. Takamuku[a], M. Katsuyama[a], C. Higuma[a], K. Sasaki[a]

[a]*Saga University*

Denaturation of protein is the key to the serious diseases such as Alzheimer's and Perkinson's diseases. However, the mechanism for denaturation of proteins has not yet been completely clarified on the molecular level.

We have investigated the effects of alcohols on the denaturation of protein, hen egg white lysozyme (HEWL). Circular dichroism (CD) spectroscopy was applied to observe the change in the secondary structure of HEWL with increasing alcohol mole fraction x_A. Here, alcohols included ethanol, 2-propanol, trifluoroethanol (TFE), and hexafluoroisopropanol (HFIP). As previously reported [1], the effects of fluorinated alcohols, especially, HFIP, on the denaturation of HEWL was more significant compared to aliphatic alcohols. Additionally, we succeeded in evaluating the change in the geometry of HEWL with increasing alcohol content using small-angle neutron scattering (SANS) technique [2].

In the present study, we have tried to elucidate the synergy effect of alcohol and temperature on the denaturation of HEWL. Both CD and SANS measurements were made on HEWL/alcohol-water solution at x_A = 0.1 as a function of temperature.

In the aqueous solution, the ratios of α-helices and β-sheets are kept at 21 and 26 %, respectively, until 60 °C (Fig. 1). The native structure does not change below the temperature. The former decreases above the temperature, while the latter increases. For the HFIP-water solution at 25 °C, the ratio of α-helices increases to 43% from that for native, while the ratio of β-sheets decreases to 4.3%. These ratios do not drastically change with rising temperature. When the temperature again decreases from the maximum to 25.0 °C, the change in the ratios follows the same route.

The SANS profiles for all the solutions were fitted through the Schulz spheres model by assuming the spherical geometry of HEWL. Fig. 2 displays the mean radius of HEWL estimated for the D_2O and the four alcohol-D_2O systems as a function of temperature. As expected from the CD results, the value for the HFIP system at 25 °C is much larger than that for the native (16 Å). With increasing temperature, the size of HEWL increases in all the systems, especially, the HFIP system. When the temperature decreases again to 25.0 °C, the mean radius for the D_2O, TFE, and HFIP systems recovered to the starting value. In the next beamtime, we will examine the recoverability for the other systems.

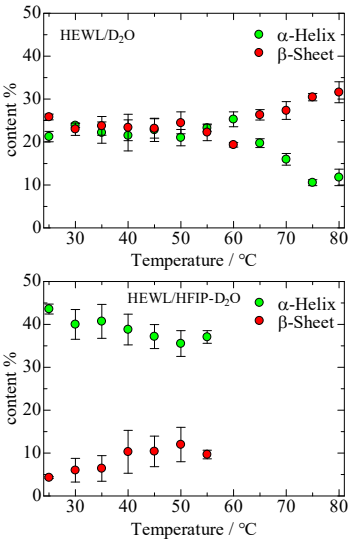

Fig.1 Ratios of α-helices and β-sheets.

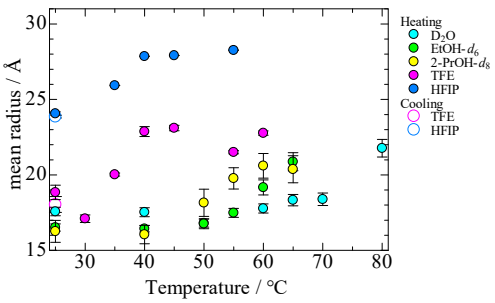

Fig. 2 Mean radius of HEWL.

[1] D.-P. Hong et al., J. Am. Chem. Soc., **121**, 8427 (1999). [2] T. Takamuku et al., J. Phys. Chem. B, **128**, 4076 (2024).