The secondary structure of aggregates and aggregational mechanism of equine serum albumin studied by FT-IR spectorscopy

Akira Okuno

The secondary structure and aggregational mechanism of equine serum albumin (ESA) are investigated on the viewpoint of physical chemistry by using FT-IR spectroscopy. This doctoral thesis is constructed from the four chapters.

In CHPTER 1, the historical background of the physicochemical study of proteins and pressure-induced protein denaturation are described. Moreover, the meaning for using ESA as a sample and the importance of pressure effect on the protein aggregation are described. Understanding of aggregational mechanism of ESA on the base of the thermodynamic parameters is the main purpose of this study.

In CHAPTER 2, the feature of high pressure cell and the methods of measurements of both high pressure FT-IR and high pressure light scattering are described. The determination of the thermodynamic parameters from FT-IR spectral data and the kinetic analysis of heat-induced aggregation of ESA are explained.

In CHAPTER 3, the comparison of the pressure-induced aggregation of ESA with the heat-induced one is shown on the secondary structural viewpoint by pressure- and temperature-tuning FT-IR spectroscopy. There are secondary structural differences between the pressure- and heat-induced aggregation. The structure of the heat-induced aggregates is the intermolecular β-sheet, but that of the pressure-induced aggregates is an amorphous form without intermolecular β-sheet. Moreover, it is shown that the structural flexibility relates to the reversibility of the pressure-induced aggregation of ESA. On the other hand, the aggregation-preventing and aggregates-dissociating pressure effects of ESA are studied on the secondary structural and volumetric viewpoints in order to explain the secondary structural and mechanical differences between heat- and pressure-induced aggregates. The aggregation mechanism is clarified based on the kinetic and thermodynamic parameters. It is shown that the volume differences and energy barrier between native and aggregated states are important for secondary structural property.

In CHAPTER 4, the results and discussions of this thesis are summarized and the future outlook of the study of protein aggregation is referred on the viewpoint of physical chemistry.