Development of Time-Resolved Vacuum-Ultraviolet Circular Dichroism Spectroscopy and its Application to the Interaction Analysis betweenβ-Lactoglobulin and Lipid Membrane

Satoshi Hashimoto[a], Koichi Matsuo[b]

[a]*Graduate School of Advanced Science and Engineering, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8526, Japan*
[b]*Hiroshima Synchrotron Radiation Center, Hiroshima University, 2-313 Kagamiyama, Higashi-Hiroshima 739-0046, Japan*

Keywords: Circular dichroism, β-Lactoglobulin, Membrane interaction, Secondary structures, Time-resolved measurement

Structure of protein changes depending on the solvent environments including the presence or absence of membrane, leading various physicochemical phenomena. In this study, vacuum-ultraviolet circular dichroism (VUVCD) spectroscopy combined with time-resolved measurement was used for the analysis of conformational changes of protein induced by membrane interactions. In the time-resolved measurement, we have made a microfluidic cell that can efficiently mix protein solution and lipid membrane solution at a low flow velocity [1-2], and installed it in the VUVCD system equipped with a Schwarzschild focusing system that can miniaturize the synchrotron radiation light below 100 micrometers.

β-Lactoglobulin (βLG) was used as a model protein being capable of interacting with membrane such as lysophosphatidylglycerol lipids (LysoDMPG). Time-resolved VUVCD spectra were measured from 260-180 nm in the range of time resolution from 1 to 15sec at the lipid/protein concentration ratio = 50. After a liner dichroism has no effect on the CD spectrum at the low flow situation, the time-resolved spectra of βLG were analyzed. The result shows that the secondary structure of βLG changes from β-strand to α-helix structures as the time after the mixing passes, indicating that βLG forms a membrane-bound structure within a few seconds. The time-dependent VUVCD spectra were analyzed by singular value decomposition method, and it was found that the structural change was composed of two states.

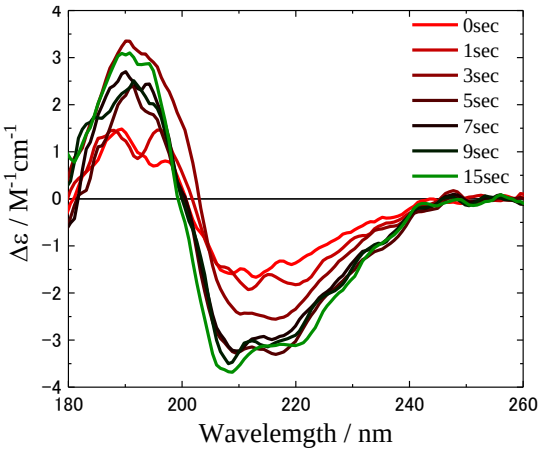

FIGURE 1. Time-resolved VUVCD spectra of βLG for interaction with LysoDMPG in the range of 260-180 nm at the lipid/protein concentration ratio = 50

References

1. A. S. Stroock, S. K. W. Dertinger, A. Ajdari, I. Mezic, H. A. Stone, G. M. whitesides, Chaotic Mixer for Microchannels, *Science*, **295** 647-651 (2002).
2. T. J. Kwak, Y. G. Nam, M. A. Najera, A. W. Lee, J. R. Strickler, W. J. Chang, Convex Grooves in Staggered Herringbone Mixer Improve Mixing Efficiency of Laminar Flow in Microchannel, *PLOS ONE*, e0166068 (2016)