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Dynamic Observation of Interaction Process between β-Lactoglobulin and Membrane by Time-Resolved Vacuum-Ultraviolet Circular Dichroism

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Membrane-bound proteins closely relate to various biological functions such as drag delivery into the membrane, stabilization of myelin in central nervous system, and antibacterial properties. Although it is difficult to observe the conformation change of protein due to the membrane interaction, a vacuum-ultraviolet circular dichroism (VUVCD) spectroscopy has a great advantage to monitor the protein structure in the presence of membrane and has been applied to characterize the conformation of membrane-bound protein such as myelin basic protein (MBP) [1] and α_1-acid glycoprotein (AGP) [2]. However, this synchrotron radiation CD technique can only obtain the structural information before and after membrane interaction, requiring the necessity of the dynamic parameters in the processes of membrane interaction. Recently, we constructed a microchannel cell [3, 4] with sequential flow method and installed into VUVCD system to measure the time-resolved (TR) CD spectra. In this study, this system was applied to observe the structural dynamics of β-lactoglobulin (bLG) interacting with two types of membrane (LysoDMPG and sodium dodecyl sulfate: SDS). TR-CD spectra of bLG were measured between 1 and 60 s for the interaction of LysoDMPG and between 0.1-90 s for the interaction of SDS. The global fitting analysis was conducted for all CD values from 235 to 205 nm and then two rate constants were obtained for each membrane interaction, indicating the existence of at least one intermediate state. Further the CD spectra of intermediate states in each membrane interaction were also estimated from the all TR spectra considering the two rate constants. Secondary structural contents and positions of native, intermediate, and membrane-bound states are obtained using SELCON3 program and VUVCD-NN method, revealing the step-by-step conformation changes in the both membrane interactions. TR-CD system is an excellent experiment tool to disclose the conformation dynamics of protein during the interaction. In addition to the static observations of concentration- and temperature-dependence, the observations of structural dynamic of proteins from TR system would be helpful for elucidating the unique and important functions of membrane-bound proteins.

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