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VUVCD Measurements of dried proteins and its application to protein-membrane interaction study

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Proteins in the dry state are utilized in various fields such as pharmaceuticals and food science. It is known that the structural differences between the liquid and dry states are slight for globular proteins [1], but intrinsically disordered protein undergoes a structural alternation from non-regular to regular structures during the dry process, expressing some unique biological functions. Hence, the elucidation of the relationships between the structural alternation and the function expression is attractive targets. In this study, we measured some globular proteins in the dry state using a vacuum-ultraviolet circular dichroism (VUVCD) spectroscopy, which is the powerful tool for secondary structure analysis of proteins, to discuss the preparations methods of dried proteins. Further, we measured the VUVCD spectra of dried G3LEA protein, which has the structural alternation from disordered structure in liquid state to helical structure in the dry state, expressing a protective function of cell membrane [2].

Eight types of dried globular proteins with different secondary structures were prepared by a spin coating technique and a vacuum drop casting method. As expected, the spin coating can suppress the surface inhomogeneity of the samples compared the drop casting, depressing the artifact within the CD data due to linear dichroism, which were confirmed by the suppression of rotation and inversion CD dependence of dried samples. Dried G3LEA consisting of 22 residues were prepared by the spin coating technique and we confirmed the structural alternation from random structure in liquid state to α-helix structure in the solid state (Figure 1). The CD spectra of dried G3LEA protein were also measured in the presence of two types of liposomes which are composed of net neutral or negatively-charged phospholipids. As a result, the formation of α-helix structures was clearly observed in negatively-charged lipid liposome but not in neutral one. Furthermore, the formation of α-helix structures was further promoted in the lipid liposome of the gel phase, compared to that of the liquid crystal phase. These results indicated that the surface charge of liposome and its liposome fluidity influenced the structural alternations of dried G3LEA protein.

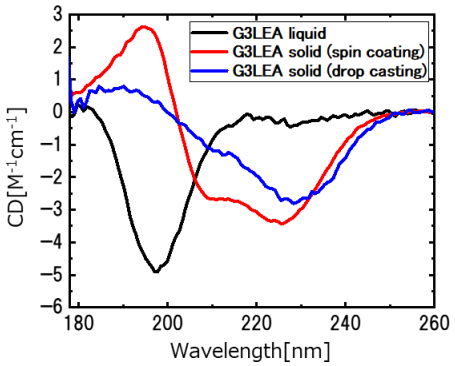

FIGURE 1 VUVCD spectra of G3LEA protein

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