Desiccation-Induced Conformational Change of Group 3 LEA Protein in the presence of Membrane Characterized by Vacuum-Ultraviolet Circular Dichroism Spectroscopy

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Keywords: circular dichroism, G3LEA protein, membrane, phase transition, secondary structure, trehalose, water

Elucidating the molecular mechanisms of cell desiccation tolerance is necessary for engineering the biological stability of mammalian cells in dry state and for developing countermeasures against foodborne illnesses caused by dry foods. Recent studies have reported that organisms, including sleeping chironomid, survive severe dehydration by accumulating saccharides and proteins, such as trehalose and group 3 late embryogenesis abundant (G3LEA) protein, inside their cells [3]. Although the trehalose protects cell structure and function via the well-understood mechanisms of water replacement and glass formation, the molecular mechanism of desiccation protection by the G3LEA protein remains to be further explored. In this study, to characterize the desiccation protection mechanism of G3LEA protein, we measured vacuum-ultraviolet circular dichroism (VUVCD) spectra of two tandem repeats of 11-mer consensus motif of G3LEA protein in the presence of lipid membranes under aqueous and dry conditions. We also investigated the conformation of G3LEA peptide in the presence of trehalose to reveal the synergistic effect of G3LEA protein and trehalose on the desiccation protection.

G3LEA peptide was synthesized in GL Biochem (Shanghai, China). 1,2-Dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC), 1,2-dimyristoyl-sn-glycero3-phosphatidylglycerol (DMPG), and 1-palmitoyl-2-oleoyl-snglycerol-3-phosphatidylcholine (POPC) lipids were purchased from Avanti. D-(+)-trehalose was obtained from Sigma. Each molecule was dissolved in 10 mM phosphate buffer (pH 7.4). Liposome suspension was prepared using extrusion technique with 100 nm polycarbonate filter, and then mixed with peptide solution at lipid-to-peptide molar ratio of 10. A 100 µl droplet was dried on a CaF_2 window in a vacuum overnight. The VUVCD spectra of G3LEA peptide were measured at HiSOR BL-12. The artefacts from linear dichroism and linear birefringence in VUVCD spectra of dry samples were eliminated by averaging the VUVCD spectra measured at four rotation angles (θ, ϕ) = (0°, 0°) (0°, 180°), (90°, 0°), and (90°, 180°), where θ is the angle around axis parallel to the incident light and φ is the angle around axis vertical to the incident light. The content of secondary structures of G3LEA protein was estimated by using SELCON3 software and VUVCD dataset.

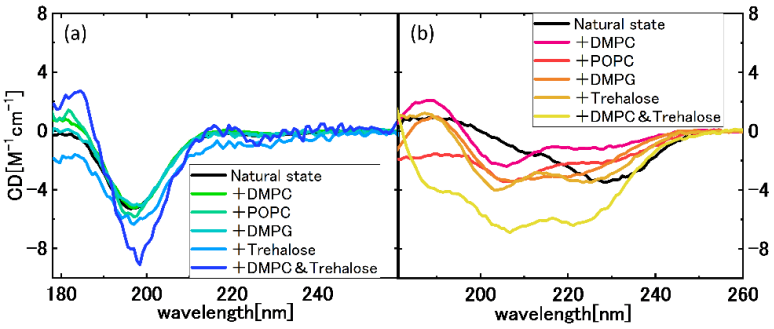

FIGURE 1. VUVCD spectra of G3LEA peptide under (a) aqueous and (b) dry conditions at 25° C.

Figure 1 (a) shows the VUVCD spectra of G3LEA peptide in the presence and absence of DMPC, DMPG, and POPC liposomes under aqueous condition. Native G3LEA peptide shows a negative peak CD at 200 nm, which is characteristic for random coil structure of proteins. The spectral shapes of G3LEA peptide in DMPC, DMPG, and POPC liposomes were consistent with that of native G3LEA peptide, indicating that G3LEA peptide did not interact with liposomes in aqueous solution. Figure 1 (b) shows the VUVCD spectra of G3LEA peptide in the presence of DMPC, DMPG, and POPC liposomes under dry condition. G3LEA peptide in DMPC liposome in dry state shows two negative peaks around 222 and 208 nm and a positive peak at 190 nm, which are characteristics for α-helix structure of proteins. This indicates that the formation of α-helix in G3LEA peptide contributes to the function to preserve the membrane from desiccation. Further, the peak intensities of VUVCD spectra of G3LEA peptide in DMPG liposome are about 2-fold greater than that in DMPC liposome, implying the importance of electrostatic interaction between positively charged residues of G3LEA peptide and negatively charged headgroups of phospholipid membrane. We also found that the spectral intensities of G3LEA peptide in DMPC liposome in the presence of trehalose was 4-fold greater than that in the absence of trehalose (Figure 1 (b)). These results suggest that G3LEA protein protects cell structure by interacting with the headgroups of phospholipid membranes, and that trehalose enhances the function of G3LEA protein to protect membrane.

REFERENCES

1. Juliana Sakamoto Yoneda, Andew J.Miles, Ana Paula Ulian Araujo, and B.A. Wallace , Differential dehydration effects on globular proteins and intrinsically disordered proteins during film formation , Protein Sci Vol.26 pp718-726(2017)
2. Daixi Li ,and Xiaoming He ,Desiccation Induced Structural Alterations in a 66-Amino Acid Fragment of an Anhydrobiotic Nematode Late Embryogenesis Abundant (LEA) Protein ,Biomacromolecules Vol.10 No.6 pp1469-1477(2009)
3. Takao Furuki, Takahiro Watanabe, Tadaomi Furuta, Kiyoshi Takano, Ryo Shirakashi , and Minoru Sakurai, The Dry Preservation of Giant Vesicles Using a Group 3 LEA Protein Model Peptide and Its Molecular Mechanism, Bull.Chem.Soc.Jpn. Vol.89 No.12 pp1493-1499(2016)