

タンパク質内包リポソームの構造と熱安定性 Structure and thermal stability of liposome entrapping proteins

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By using synchrotron radiation wide-angle and small-angle X-ray scattering (SR-SWAXS), we studied the structural characteristics and stability of liposomes entrapping proteins. The purpose of this study is to clarify the structural stability of a protein in a confined environment that mimics an intracellular condition. The liposomes used were lipid-mixtures composed of glycosphingolipid, cholesterol and phospholipid lipids (egg-PC (L- α -phosphocholine), DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), or DPPC (1,2-dipalmitoil-sn-glycero-3-phosphocholine)). The protein occluded in liposomes was myoglobin from horse skeletal muscle. Liposome samples were prepared by a sequential combination of the following methods. The lipid mixtures dissolved in protein solutions (1 wt% ~ 5 wt%) in 10 mM HEPES buffer (at pH 7.5) were subjected to natural swelling (at R.T.), ultra-sonic dispersion (using a high-power probe-type ultrasonicator at 50 W), freeze-throw, extrusion (using an extruder system with a polycarbonate filter (pore diameter of 50 nm or 100 nm)) and spin-filtration (Mt. cut-off 50 k dalton), successively. SR-SWAXS experiments were done by using the BL10C spectrometer at PF and the BL-40B2 spectrometer at SPring-8. Due to the combination of different types of sample preparation, we succeeded in confining the protein within the liposome at high concentrations, and its structure and stability were examined. We have found an evident improvement of the thermal stability of liposome. Further experimental results and analyses will be shown.