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Interaction between lipid-liposome and amyloid-beta protein observed by time-resolved small-angle X-ray scattering

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1. Introduction

While the steady-state existence in the size and shape of liquid-ordered microdomains in cell membranes, so-called 'lipid rafts', still remain the subject of debate, glycosphingolipid-cholesterol rich regions in plasma membranes have been considered to have a function as platforms for signaling and sorting. In addition, recent spectroscopic studies show that the interaction between monosialoganglioside and amyloid beta (Aβ) protein promotes the transition of Aβ from the native structure to the cross-beta fold in amyloid aggregates. However, there is less evidence on the time-transient structural information of the formation of Aβ-membrane complex. We have carried out the time-resolved small-angle X-ray scattering measurements of the interaction between Aβ protein and G_{M1}-cholesterol-DPPC or DOPC mixed vesicles.

2. Experiment

Lipid-liposome measured was composed of monosialo-ganglioside (G_{M1}) from bovine brains, cholesterol and diplmitoyl phosphocholine (DPPC) or dioleoyl phosphocholine (DOPC). These lipids were purchased from Avanti Polar Lipids Inc. (USA) and used without further purification. Large uni-lamellar vesicle (LUV) solutions were prepared by an extrusion method using the LiposoFast Basic extruder system (Avestin, Canada) with a polycarbonate filter (pore diameter of 50 nm from Nucleopore, Plesanton, USA). The samples were subjected to ~40 passes through the filter. The molar ratio [G_{M1}]/[cholesterol]/[DPPC or DOPC] of the mixtures was 0.1/0.1/1, where the phospholipid concentration was fixed at 1 % w/v. Amyloid β protein (1-40) purchased from Wako Chem. Co. was used without further purification. The detail of LUV preparation was described elsewhere [1, 2]. The solvent condition was 10mM Hepes, pH7.4, 50mM NaCl, 5mM KCl, 2mM $MgCl_2$, 2mM $CaCl_2$. SAXS measurements were performed by using the synchrotron radiation small-angle X-ray scattering spectrometer installed at BL10C beam port of PF at KEK, Tsukuba, Japan. The X-ray wavelengths was 1.4 Å, and the sample-to-detector distances was 190 cm. X-ray scattering intensity was recorded by an imaging plate system of R-AXIS IV from RIGAKU Co. The exposure time was 60 seconds. Sample cells composed of a pair of thin-quartz windows with 1 mm path length were used.

3. Results and Discussion

Fig. 1 shows the time evolution of distance disrtibution function $p(r)$ after addting Aβ protein in LUV solutions, which were obtained by the Fourier transform of the observed SAXS curves. In Fig. 1, A and B correspond to G_{M1}-cholesterol-DPPC LUV and G_{M1}-cholesterol-DOPC LUV, respectively. The small humps appearing in the $p(r)$ function at short distance below ~50 Å reflect the intra-bilayer structure of LUV [1]. The broad peaks with the maximum at ~400 Å result from the globular structure of LUV. The time-evolution of the $p(r)$ function after the addition of Aβ in Fig. 1 only shows a minor change in the time range from 0 to 400 minites. The present SAXS results suggest that the interaction between Aβ and raft-region (G_{M1}-cholesterol rich region, so-called lipid rafts, on the outer-leaflet of LUV [3. 4]) proceeds without an evident deformation of LUV structure. To analyse some local change of the bilayer structure, We are planning to execute wide-angle X-ray scattering experiments. The detailed results and discussion will be shown elsewhere.

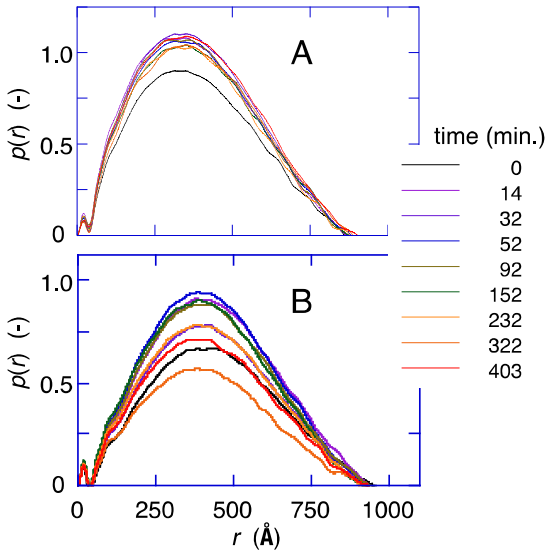

Fig. 1: Time evolution of distance disrtibution function $p(r)$ after addting Aβ protein in LUV solutions, where A and B correspond to G_{M1}-cholesterol-DPPC LUV and G_{M1}-cholesterol-DOPC LUV, respectively.

References

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