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Interaction of amyloid-β protein with raft-model liposome under mimic environment of cell

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1 Introduction

It is now widely accepted that the amyloid β -peptide (Aβ), which exists in fibrillar forms as a major component of senile plaques, is central to the development of Alzheimer's disease (AD). Aβ–membrane interactions have been extensively investigated to elucidate the molecular mechanisms of the Aβ-induced cellular dysfunctions underlying the pathogenesis of AD. Particularly, according to accumulating evidence using spectroscopic methods, the importance of ganglioside clusters in the fibrillization of Aβ is emphasized. Gangliosides, major components of glycosphingolipids (GSLs), are acidic lipids and rich in central nervous system. GSL-cholesterol formed microdomains in cell membranes, so-called lipid rafts, are suggested to function as platforms of various membrane-associated events such as signal transduction, cell adhesion, lipid/protein sorting and so on. The interaction between mono-sialoganglioside (G_{M1}) and Aβ is reported to promote the conformational change of Aβ to its cross-beta structure and the G_{M1}-bound form seeds in the process of Aβ polymerization to amyloid fibril [1]. Recently, we reported that that the interaction between the Aβ proteins and the model membrane at the liquid-crystal phase significantly suppresses a bending-diffusion motion, suggesting the possibility of non-receptor-mediated disorder in signaling through a modulation of a membrane dynamics induced by the association of amyloidogenic peptides on a plasma membrane [2].

2. Experimental

In the present study, we have investigated the Aβ-membrane interactions under a mimic intracellular environment by using the time-resolved small- and wide-angle X-ray scattering (SWAXS) 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 were 1.0 Å and 1.5 Å. The sample-to-detector distances were 50 cm and 300 cm. X-ray scattering intensity was recorded by a hybrid pixel detector, PILATUS3 2M (Dectris). The exposure time was 60 seconds. Sample cells composed of a pair of thin-quartz windows with 1 mm path length were used. The membrane used was the small unilamellar vesicle (SUV) composed of G_{M1}, cholesterol, and phospholipid. 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 solvent condition was 10mM Hepes, pH7.4, 50mM NaCl, 5mM KCl, 2mM $MgCl_2$, 2mM $CaCl_2$. The mimic intracellular environment, so-called crowding environment, was realized by the addition of the high-molecular weight neutral polymer (polyvinyl-pyrrolidone, PVP).

3. Results and Discussion

Fig. 1 shows the time evolution of SWAXS curve of the SUV (G_{M1}-cholesterol-DPPC) after addting Aβ protein. The present results are summarized as follows. Under non-crowding environment, the interaction between SUV and Aβ protein proceeds mostly without affecting the SUV membrane structure. While, under crowding environment, the presence of crowders induces the deformation of the SUV shape and the interaction between SUV, and Aβ protein causes further aggregation to form the lamellar stacking. The time-course of the lamellar distance and the lamellar-peak height suggest the preferential cohesion or intercalation of Aβ proteins in the inter-bilayer region.

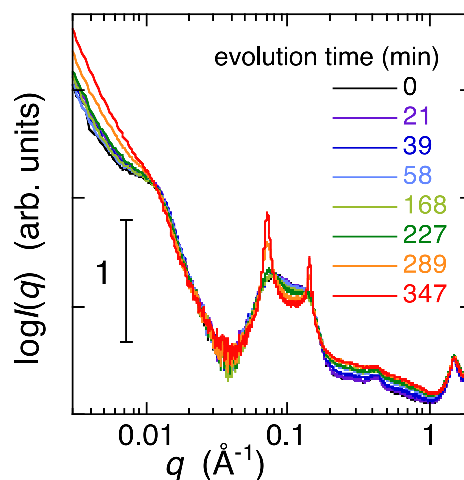

Figure 1. Time-evolution of SAWXS curve of SUV after Aβ addition

References

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