BL-10C/2011G160

Interaction between lipid-raft model membrane LUV and amylioid beta protein

Mitsuhiro Hirai[1,*] Kazuki Takeuchi[1,] and Ryota Kimura[1,]
[1]Graduate School of Engineering, Gunma University, 4-2 Aramaki, Maebashi, 371-8510, Japan.

Introduction

Plasma membranes are dynamic assemblies of a variety of lipids and proteins. In this decade, it has been revealed that glycosphingolipid (GSL) and cholesterol assemble into microdomains, so-called 'lipid rafts', in plasma membranes which have functions as platforms (rafts) for membrane-associated events such as signal transduction, cell adhesion, lipid/protein sorting, and so on. As knowledge about lipid raft functions in cellular signaling accumulates, abnormalities in microdomains and associated molecules have been found in various diseases, including atherosclerosis, muscular dystrophy and neurodegenerative disorders such as Alzheimer's disease. Gangliosides, major components of GSLs, are acidic lipids composed of a ceramide linked to an oligosaccharide chain containing one or more sialic acid residues, which are rich in central nervous systems. In addition, the interaction between Aβ protein and monosialoganglioside (G_{M1}) was shown to promotes the transition of Aβ from its native structure to cross-beta fold to amyloid fibril mainly by using spectroscopic methods. However, in spite of the biological significance of 'lipid rafts', there exists few evidence on their physicochemical properties, especially, on the structure and dynamics. Therefore, we have carried out small-angle X-ray scattering (SAXS) experiments to clarify the effect of the amyloid beta protein on the structure raft-model membrane.

Experimental

SAXS measurements were also performed by using the synchrotron radiation small-angle X-ray scattering spectrometer installed at BL10C beam port of the 2.5 GeV synchrotron radiation source (PF) at the High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. The X-ray wavelength used was 1.49 Å and the sample-to-detector distances were 80 cm. The Raft-model membrane measured was large uni-lamellar vesicles (LUV) composed of monosialo-ganglioside (G_{M1}) from bovine brains, cholesterol and phospholipids (DOPC, or DPPC). These lipids were purchased from SIGMA Chemical Co. (USA) and from Avanti Polar Lipids Inc. (USA). Amyloid β protein (1-40) purchased from Wako Chem. Co. was used without further purification. The Amyloid β and LUV were dissolved in 10 mM Tris buffer at pH 7.4. After mixing Aβ protein solution with the LUV solution at appropriate molar ratios of [Aβ]/[G_{M1}], time-resolved measurements were started at 37 °C.

Results and Discussion

Figure 1 shows the time course of the SAXS curve of LUV (molar ratio of [G_{M1}]/[cholesterol]/[DOPC] = 0.1/0.1/1) + Aβ mixture, where DOPC concentration was 0.5 % w/v, the molar ratio of [Aβ]/[G_{M1}] = ~3/1. After the mixing, the SAXS curve has changed gradually. The measurement conditions were selected to observe the oligomerization process Aβ proteins. Therefore, the molar ratio of [Aβ]/[G_{M1}] was set to be relatively high. The broad-rounded peak of LUV at ~0.1 $Å^{-1}$ reflects the lipid bilayer structure of LUV membrane. The peak at ~0.64 $Å^{-1}$ corresponds to the pleated-sheet stacking of the formed cross-β structure of Aβ proteins. The broad peak at ~0.14 $Å^{-1}$ indicates the presence of oligomers of Aβ proteins. The increase of the scattering intensity at small-q region below 0,05 $Å^{-1}$ is attributable to the growth from oligomers to protofibrils to fibrils of Aβ proteins. The present results suggest the kinetics of the amyloid transition of Aβ proteins is relatively slow process. The detail of the results and discussion will be shown elsewhere.

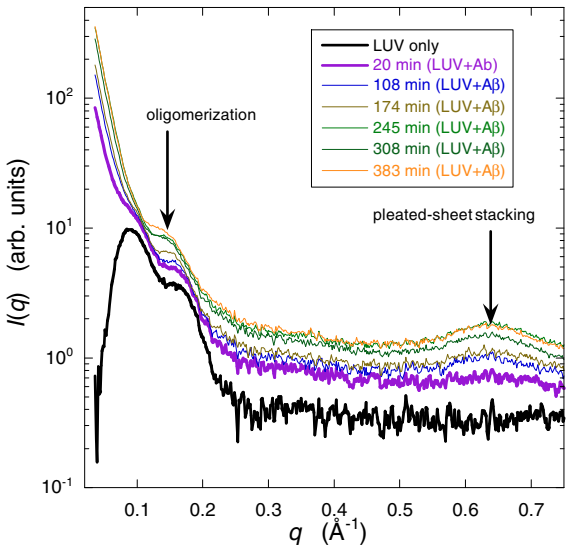

Fig. 1. Time-resolved SAXS curve after mixing of raft-model LUV and Aβ protein solutions.

* mhirai@fs.aramaki.gunma-u.ac.jp