Biological Science

10C/2006G410

Structure of SUVs of lipid mixtures composed of glycosphingolipid, cholesterol and phospholipid depending on lipid molar ratio and salt concentration

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Introduction

By using optical and electron microscopes phospholipid vesicles were reported to form neuron-like tubes with addition of gangliosides under some solvent condition. Gangliosides, major components of glycosphingolipids, are acidic lipids composed of a ceramide linked to an oligosaccharide chain containing one or more sialic acid residues, which are known to be rich in central nervous system and to form lipid-microdomains, called rafts, on mammalian plasma membrane surface. Rafts are assumed to play as a platform of important biological functions such as signal transduction, cell adhesion and lipid/protein sorting. Such functions of rafts relate to the peculiar features of gangliosides. We have studied both effects of addition of salts and ganglioside on the vesicles composed of ganglioside, cholesterol, phospholipid by using a samall-angle X-ray scattering (SAXS) method.

Experimental

SAXS experiments were performed by using a spectrometer installed at BL-10C. The sample-to-detector distance was 80 or 180 cm. The X-ray wavelengths used were 1.5 Å. Gangliosides used were monosialo-ganglioside (G_{M1}), disialo-gangliosides (G_{D1a}, G_{D1b}), and trisialoganglioside (G_{T1b}) from bovine brain. Phospholipids used were dipalmitoyl phosphocholine (DPPC), distearoyl phosphocholine (DSPC), dioleoyl phosphocholine (DOPC), palmitoyl oleoyl phosphocholine (POPC), and L-a-phosphocholine (PC). These lipids were purchased from SIGMA Chemical Co. (USA), and used without further purification. For the preparation of the vesicles, required quantities of ganglioside cholesterol and phospholipid were dissolved in a 2:1 (v/v) mixture of chloroform and methanol. After removing organic solvent under a stream of nitrogen gas, and the samples were dried at 45 °C in vacuo for overnight. The dried samples were suspended in 10 mM HEPES buffer adjusted to pH 7.0 to become 0.5 wt % lipid concentration. Small uni-lamellar vesicles (SUVs) were prepared by using a high-power probe-type ultrasonicator (Model UH-50 of SMT Co.) at 50 W for 5 minutes.

Results

Figure 1 shows the structural change of the $[G_{T1b}]$/[choresterol]/[DOPC] vesicle depending on both salt concentration and the lipid composition, where (a), (b), (c), and (d) correspond to the SUVs of $[G_{T1b}]$/[choresterol]/[DOPC] = 0.1/0/1, 0.1/0.05/1, 0.1/0.1/1, and 0.1/0.2/1, respectively. Up to the molar ratio of cholesterol 0.1, the SUV vesicle shows similar response against the increase of Ca concentration. The detailed analyses are now proceeding.

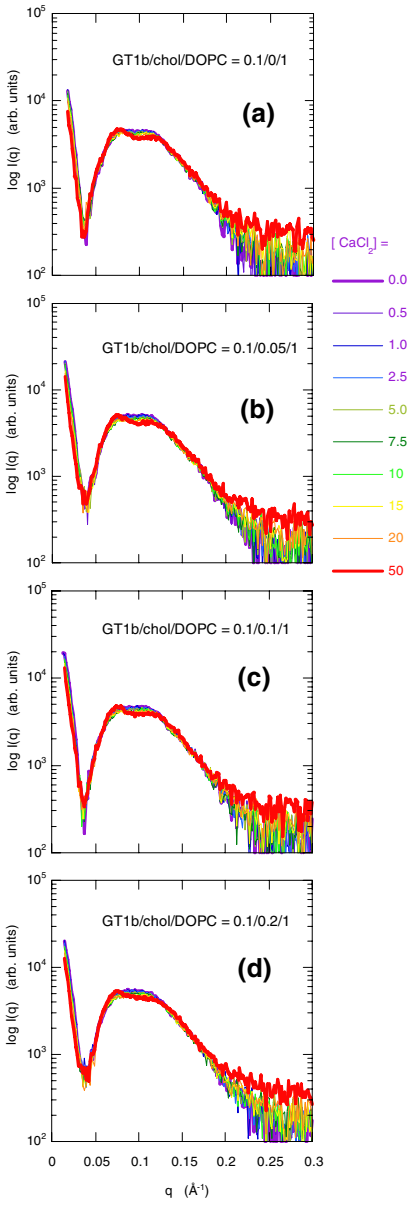

Fig. 1 SUV structure depending on Ca salt and cholesterol concentration.