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Interaction of Cholera Toxin B-subunits with ganglioside/cholesterol/phospholipid mixture

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Introduction

Structure and function of mammalian cell membrane domains (GSLs), so-called rafts, have been attracting a huge interest and are one of the current hot topics in cell biology since these domains are postulated to have a significant function as a molecular device for localization of specific proteins and to be involved in important membrane-associated events such as signal transmission, cell adhesion and lipid/protein sorting. Gangliosides, major components of GSLs, are acidic lipids composed of a ceramide linked to an oligosaccharide chain containing one or more sialic acid residues. In addition monosialogangliosides (G_{M1}) are known to be receptor of cholera toxin B-sub units. In this study we have studied the intraction between cholera toxin B-sub unit and G_{M1} /cholesterol/ phosphatidylcholine (PC) mixed vesicle.

Sample Preparation

Ganglioside used was monosialoganglioside, G_{M1}, from bovine brain purchased from SIGMA Chemical Co., which was used without further purification. Cholera toxin B-sub unit from Vibrio choletate was purchased from SIGMA Chemical Co. Cholesterol and phosphatidylcholine (L-α-lecithin) were purchased from Avanti Polar Lipids Co. G_{M1}, cholesterol and phospholipid were separately dissolved in the chloroform/methanol mixture solvent (1/1 (v/v)) and mixed with the appropriate molar ratios. After mixing, to remove the organic solvent, the G_{M1}-DPPC mixture solutions were dried under a nitrogen stream and annealed *in vacuo* for overnight at 45 °C. The dried mixtures were suspended again in 50 mM Hepes buffer (pH 7.0), warmed to 50 °C, and vortexed at 50 °C for ~20 minutes. For preparing SUV, the mixtures were sonicated for 5 minutes by using a high-power probe-type ultrasonicator (Model UH-50 of SMT Co.) at 50 W. The molar ratios [G_{M1}]/[cholesterol]/[PC] of the mixture were 0/0.1/1, 0.1/0.05/1, 0.1/0.1/1, 0.1/0.2/1, where the PC concentration was fixed at 1 % w/v. Cholera toxin B-sub unit was added to each mixed vesicle with varing the molar ratio [B-sub]/[G_{M1}] from 1/127 to 1/8.

SAXS measurements were carried out by a SAXS spectrometer installed at the BL-10C beam line of PF at KEK. The X-ray wave length, the sample-to-detector distance, and the exposure time were 1.49 Å, 198 cm, and 480 seconds respectively.

Results and Discussion

Figure 1 shows the change of the scattering curves depending on [B-sub]/[G_{M1}] molar ratio. With increasing B-sub units, G_{M1} micellar structure significantly changed, whereas, the structures of G_{M1}/PC mixed vesicle and G_{M1}/cholesterol/PC mixed vesicle are resistant to the addition of B-sub units. For vesicles, the ripple profiles around q range from 0.03-0.07 Å-1 appear. The detailed results will be shown in elsewhere.

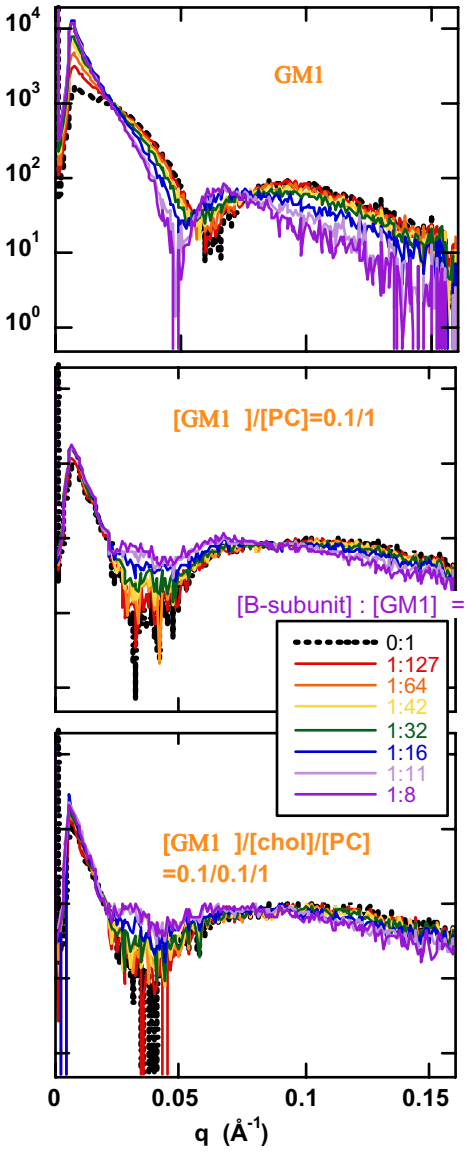

Figure 1. Effect of the addition of cholera toxin B-sub units on GM1 micelle, GM1/PC and GM1/cholesterol/PC mixed vesicles.