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Phase behaviors of binary mixtures of GM3 and Sphingomyeline.

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Introduction

Glycosignaling domain (GSD) in B16 melanoma has been known to play an important role in cell adhesion concerning metastasis and act as the target of antibody towards melanoma. It has been reported that the main component of GSD is GM3 and sphingomyeline(SM). Thus, the investigation of structures and phase behaviors of binary mixtures of GM3 and SM will give important information about the above physiological phenomena. We report the phase behaviors of GM3/SM system observed by x-ray diffraction.

Materials and Methods

Ganglioside GM3 was purchased from Alexis Corp.(San Diego, USA) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Chicken egg yolk sphingomyeline(SM) which primarily containing palmitic acid was purchased from Sigma Chemical Co.(St.Louis, USA),. Mixtures of GM3 and SM dissolved in chloroform-methanol were dried under vacuum and then were hydrated with phosphate buffer at 55 ^{o}C to prepare multibilayer vesicles.

X-ray diffraction measurements were carried out at BL-15A. The diffraction patterns were detected by imaging plates (Type BAS-III, Fuji Photo Film Co., Ltd., Japan).

Results

Fig. 1 displays x-ray diffraction profiles of binary mixtures of GM3 purchased from Alexis Corp. and SM in the L_α phase. There observed the 1st and the 2nd order diffraction peaks due to lamellar structure. Below 25 mol% GM3 content the diffraction peak seems to consist of single component, however in the 28.3 mol% the another peak appears in the wider angle region as a shoulder. The lamellar repeat distances obtained from the diffraction profiles as a function of GM3(Alexis) content are displayed in Fig.2a. The region where the lamellar repeat abruptly increases (up to 6 mol%) were defined as region A. Above 6 mol% the lamellar repeat gradually increases (denoted as region B).

Fig.2b shows the results using GM3 purchased from Wako Pure Chemical Industries. As in the case of GM3(Alexis) the regions A and B were observed. However, the new phase (denoted as C-phase) of which lamellar repeat is smaller than that in the region B appeared above 13 mol% and become dominant above 17 mol%. It may be possible that the shoulder observed 28.3 mol% GM3(Alexis)/SM is the C-phase. These indicate phase behaviors depend on GM3 samples, but were basically similar. Based on experiments of differential scanning calorimetry, we expect that chain length difference of GM3 molecules results in differences of phase behaviors but further experiment is necessary.

From these multibilayer vesicles large unilamellar vesicles (LUV) were prepared by extruder (Avanti Polar Lipids, Inc.) and were mixed with M2590 that is monoclonal antibody towards GM3. In result the LUV prepared from GM3/SM in the region B and in the C-phase caused aggregation but not in the region A. This exhibits that M2590 react with vesicles in regions B and the C-phase .

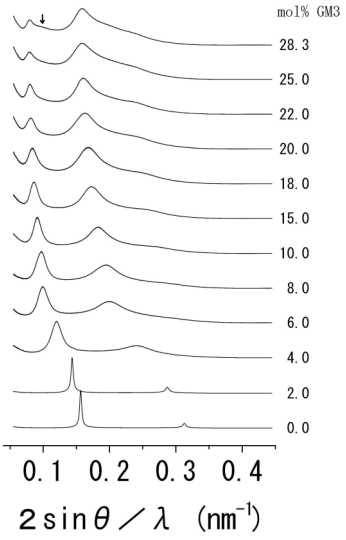

Fig. 1 X-ray diffraction profiles of multibilayer vesicles of SM containing different molar ratios of GM3(Alexis) in the L_α phase. An arrow represents shoulder.

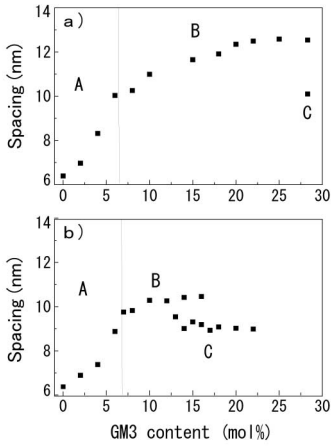

Fig. 2 Lamellar repeat distance of binary mixtures of GM3/SM as a function of a) GM3 (Alexis) and b) GM3(Wako) contents in the L_α phase.