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Lamellar structures of binary mixtures of GM3 and SOPC.

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Introduction

It has been known that ganglioside GM3-enriched domains formed in some kinds of cell membranes contain sphingomyeline(SM) and cholesterol. The acyl chains of these sphingomyelines are both saturated, whereas phospatidylcolines outside of GM3-enriched domains include unsaturated acyl chains. Thus the structure of binary mixtures of GM3 and 1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine (SOPC) which includes an unsaturated acyl chain was studied by x-ray diffraction to investigate how unsaturation of acyl chain affects the formation of the microdomains. These results were compared with reactivity of monoclonal antibody that recognizes the collective GM3 structures in the bilayer membranes.

Materials and Methods

Ganglioside GM3(bovine brain) was purchased from Alexis Corp.(San Diego, USA). 1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine (SOPC) was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). To prepare multibilayer vesicles, mixtures of GM3 and SOPC dissolved in chloroform-methanol were dried under vacuum and then were hydrated with phosphate buffer at room temperature that is above the main transition temperature (6-10°C).

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).

The reactivity of M2590, antibody towards GM3 was measured using large unilamellar vesicles (LUV) of GM3/SOPC prepared by extruder (Avanti Polar Lipids, Inc) .

Results

Fig. 1 displays x-ray diffraction profiles of binary mixtures of GM3(bovine brain) and SOPC in the L_α phase. There observed the 1st- and the 2nd-order diffraction peaks due to lamellar structures. Coexistence of two lamellar structures observed below 2 mol% may be due to buffer because the sample hydrated by distilled water showed almost one component of lamellar structure.

Fig. 2 displays spacings due to the lamellar repeat distance as a function of GM3 in GM3/SOPC system in the L_α phase. Lamellar repeat distance increases as increasing GM3 content and was saturated near 12 mol% GM3 content. In GM3/SM system lamellar repeat distance was saturated about 7 mol% GM3(see Fig. 2).

M2590 precipitated LUV of SOPC containing 12 mol% GM3 but not in the case of 10 mol% GM3. Thus the threshold for the reactivity of M2590 with SOPC/GM3 located near 10 - 12 mol%. In SM/GM3 system, the threshold for the reactivity of M2590 was 6-7 mol%. Thus, collective structures of GM3 that react with M2590 are not formed easily in the GM3/SOPC compared with in the GM3/SM, probably because of unsaturation of acyl chains. In both systems, the threshold of antibody reactivity corresponds to the GM3 content where lamellar repeat distance was saturated as shown in Fig.2. This indicates that the formation of collective GM3 structures affect the lamellar-lamellar interaction.

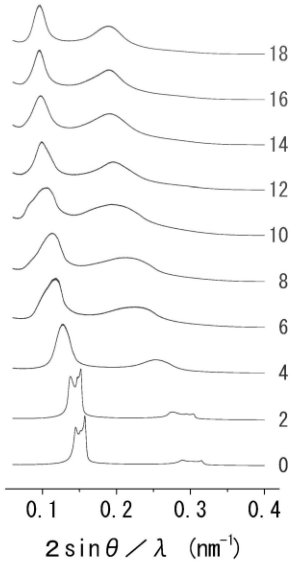

Fig. 1 X-ray diffraction profiles of multibilayer vesicles of SOPC containing different molar ratios of GM3(bovine brain) in the L_α phase(18°C)

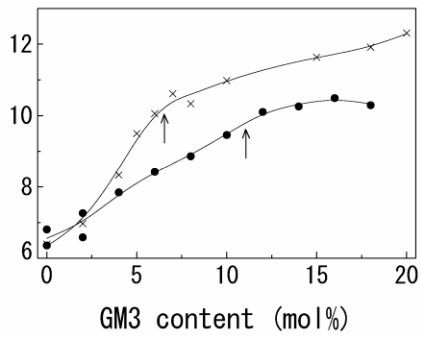

Fig. 2 Lamellar repeat distance of binary mixtures of GM3/SOPC (•) and GM3/SM(×) as a function of GM3(bovine brain) in the L_α phase. Arrows represents the threshold of reactivity of M2590 to LUV.