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Lamellar structures of ternary mixtures of GM3/SM/cholesterol.

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Introduction

It has been recognized that sphingolipid enriched microdomains are present in cell plasma membranes. In these microdomains sphingomyeline(SM) and cholesterol are enriched as well as glycosphingolipid. These microdomains has been observed also in the model membrane consisting of a fluid phosphatidylcholine, SM and cholesterol. In this report, to investigate whether cholesterol affects the structure and biological function in the sphingolipid enriched microdomain, x-ray diffraction measurements of ternary mixtures of GM3/SM/ cholesterol was carried out and the results were compared with those in binary mixtures of GM3/SM.

Materials and Methods

Ganglioside GM3(bovine brain) was purchased from Alexis Corp.(San Diego, USA). Sphingomyeline(SM) and cholesterol were purchased from Sigma Chemical (St.Louis,MO,USA). To prepare multibilayer vesicles, mixtures of GM3, SM and cholesterol (27mol%) dissolved in chloroform-methanol were dried under vacuum and then were hydrated with phosphate buffer at 55°C that is above the main transition temperature.

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 spacings due to the lamellar repeat distance as a function of GM3 in ternary mixtures of GM3/SM/cholesterol and in binary mixtures of GM3/SM in the L_α phase. From 2 to 6 mol% of GM3 content lamellar repeat distance rapidly increased and above 6 mol% gradually increased. The dependence of lamellar repeat distance on GM3 content was not affected by the presence of cholesterol.

Fig. 2 displays x-ray diffraction profiles of ternary mixtures of GM3/SM/cholesterol in the L_α phase. There observed the 1st-order, the 2nd-order and the 3rd-order (above 6 mol%GM3) diffraction peaks due to the lamellar structure. At 4 mol% GM3, there observed a shoulder in the x-ray diffraction profile suggesting two distinct lamellar structures with different lamellar repeat distance. It is possible that phase separation of collective GM3 structures takes place. In GM3/SM system two distinct lamellar structures were not observed , however, the peak width of x-ray diffraction profile was large at 4 - 5 mol% GM3 content. This may suggest that the collective GM3 structures forms also in GM3/SM system.

By the experiment of precipitation of unilamellar vesicles (diameter 100 nm) by anti-GM3 antibody (M2590), the threshold for the reactivity of M2590 in GM3/SM/cholesterol system was 6 mol%. This corresponds to the GM3 content where lamellar repeat distance was saturated as shown in Fig.1 and is similar to that in SM/GM3 system. Thus, the formation of the structure recognized by the antibody was not affected by the presence of cholesterol.

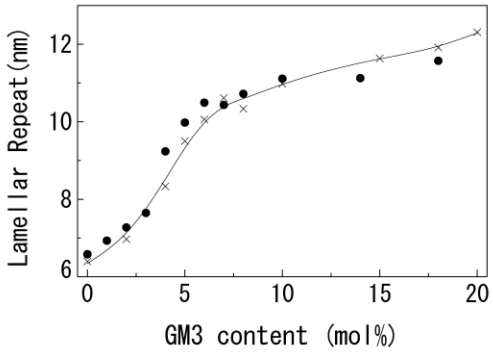

Fig. 1 Lamellar repeat distance of ternary mixtures of GM3/SM/cholesterol (●) and binary mixtures of GM3/SM(×) as a function of GM3(bovine brain) in the L_α phase. Line represents smoothed spline with binary mixtures of GM3/SM.

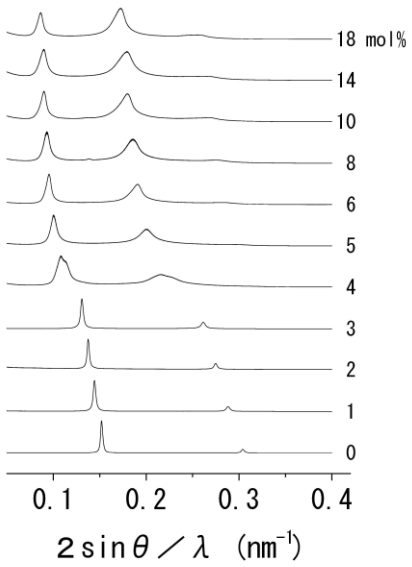

Fig. 2 X-ray diffraction profiles of multibilayer vesicles of ternary mixtures of SM/cholesterol containing different molar ratios of GM3(bovine brain) in the L_α phase(48°C).