SUPPLEMENTAL MATERIALS

MATERIALS AND METHODS

Materials

Ganglioside G_{M1}, G_{M2} and G_{D3} were purchased from WAKO Pure Chemical Industries, Ltd. (Osaka, Japan), and G_{M3} was from Matreya, Inc. (Pleasant Gap, PA). 2,2'-dioleoyl-*sn*-1,*sn*-1'-BMP was synthesized as described (1). G_{M1}(C1-alcohol) was obtained by the method described previously (2). Cholesterol, [4-^{14}C]-cholesterol and methyl-β-cyclodextrin (MβCD) were obtained from Sigma-Aldrich Co. (St. Louis, MO), American Radiolabeled Chemicals Inc. (St. Louis, MO) and Cyclolab R&D Lab. Ltd. (Budapest, Hungary), respectively.

Immunofluorescence

Cultured human skin fibroblasts were grown as described (3) in the presence or absence of G_{M1}. Cells were then fixed and permeabilized as described (4) and doubly labeled with anti-BMP (anti-LBPA) antibody (5) and Alexa 488-conjugated cholera toxin B-subunit (Molecular Probes, Eugene, OR).

Electron Microscopy

For negative staining images, the samples were absorbed to glow-charged formvar membrane on copper grids, and negatively stained by 2% sodium phosphotungstic acid. Low and neutral pH liposome were stained by different pH solution; one was adjusted to 4.2 and the other to 7.2, respectively. For freeze fracture images, the samples were frozen in liquid propane cooled by liquid nitrogen, fractured in a freeze-etching machine (Balzers BAF400T, Balzers, Liechtenstein) at -110ºC, and replicated by platinum/carbon. Replicated samples were immersed in household bleach to dissolve the lipids, washed in water, and then mounted on formvar-coated copper grids. Both specimens for negative staining and freeze fracture images were examined under a transmission electron microscope (JEOL 1200EX-II, Tokyo, Japan). Electron micrographs recorded on imaging plates were scanned and digitized by an FDL 5000 imaging system (Fuji Photo Film, Tokyo, Japan).

Small-angle x-ray scattering (SAXS)

Lipid film was formed from a chloroform solution of lipids under a stream of nitrogen gas, dried in high vacuum overnight, hydrated and vortexed with buffers. The employed buffers was 200 mM citrate buffer for pH 4.6, 20 mM MES buffer with 150 mM NaCl for pH 5.5 and 6.5, 20 mM HEPES buffer with 150 mM NaCl for 7.4, and 20 mM tricine buffer with 150 mM NaCl for pH 8.5. The lipid concentration of the samples was 10 mM. SAXS measurements were carried out at RIKEN Structural Biology Beamline I (BL45XU) at SPring-8 (6). The SAXS patterns were recorded with 60 s exposure by a beryllium-windowed x-ray image intensifier which is coupled with a cooled CCD camera (1000 × 1018 pixels). Detailed optical settings and data treatments were as described elsewhere (4,7). All the SAXS measurements were performed at 37ºC.

Cholesterol extraction

G_{M1}/BMP (1/1 mol/mol) vesicles containing 10 mol% cholesterol including trace amount of [4-^{14}C]-cholesterol were prepared essentially as describe for x-ray measurements, with the exception that the final concentration of lipids was 40 μM. Dispersions of G_{M1}/BMP/cholesterol at pH 4.6 and 7.4 were incubated with 25 mM MβCD at 37ºC for 30 min and pelleted with one hour centrifugation (4ºC, 53000 × *g*). The extracted cholesterol in the supernatant was measured with liquid scintillation counter. To avoid an artifact due to the difference in precipitation depending on the samples, all samples were pelleted in the absence of MβCD, and the ^{14}C cholesterol value in the supernatant was subtracted from the value obtained in the presence of MβCD.

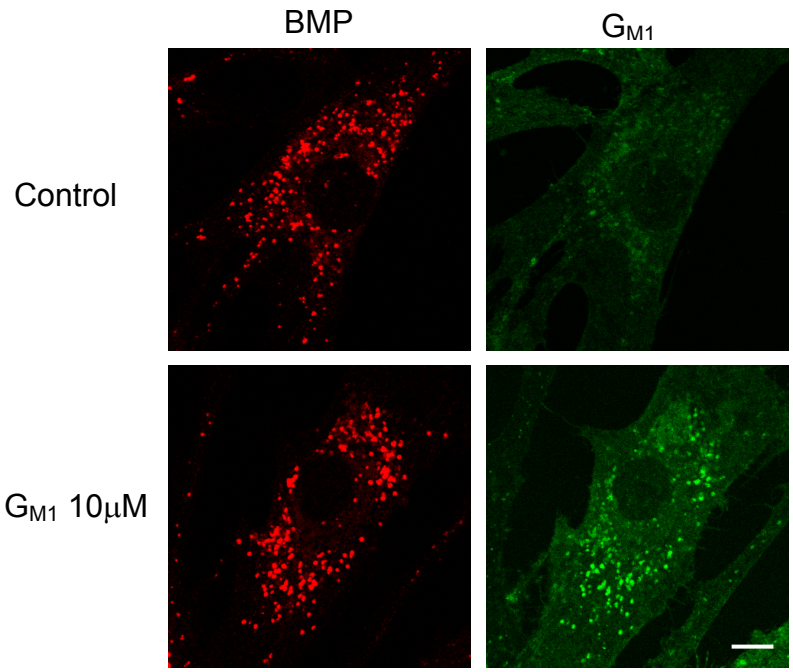

FIGURE 4
G_{M1} accumulates in BMP domains. Human skin fibroblasts were grown in the absence and presence of 10 μM G_{M1} as described in supplemented Materials and Methods. Cells were then fixed and the distribution of internalized G_{M1} and BMP-rich membrane domains was examined. Bar, 10 μm.

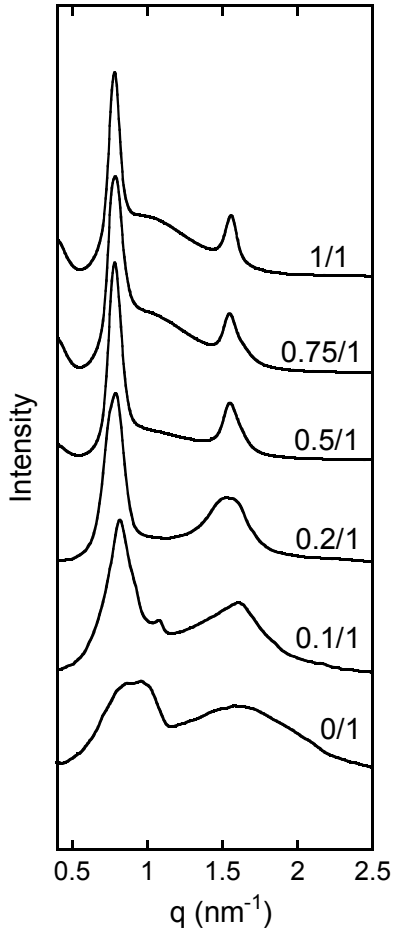

FIGURE 5
SAXS patterns of G_{M1}/BMP mixture with different G_{M1} content at pH 4.6. G_{M1}/BMP molar ratios for the respective SAXS pattern were shown in the figure. BMP alone forms diffuse lamellar structure with the lamellar repeat distance of ~7 nm. At G_{M1}/BMP = 0.1/1 (mol/mol), the first and second order lamellar peaks became more evident and shifted to the small angle region (q = 0.82 nm^{-1}) and another set of first and second lamellar peaks were observed at q = 1.08 nm^{-1} and 2.16 nm^{-1}, corresponding to the 5.82 nm lamellar repeat distance. The appearance of these two lamellar distances suggests that G_{M1} distributed heterogeneously in the membranes and G_{M1}-rich and -poor regions were phase separated. At molar ratio of 0.2/1, the lamellar peaks shifted further to the small angle region. The first lamellar peak was observed at q = 0.78 nm^{-1} which corresponds to 8.06 nm lamellar distance.

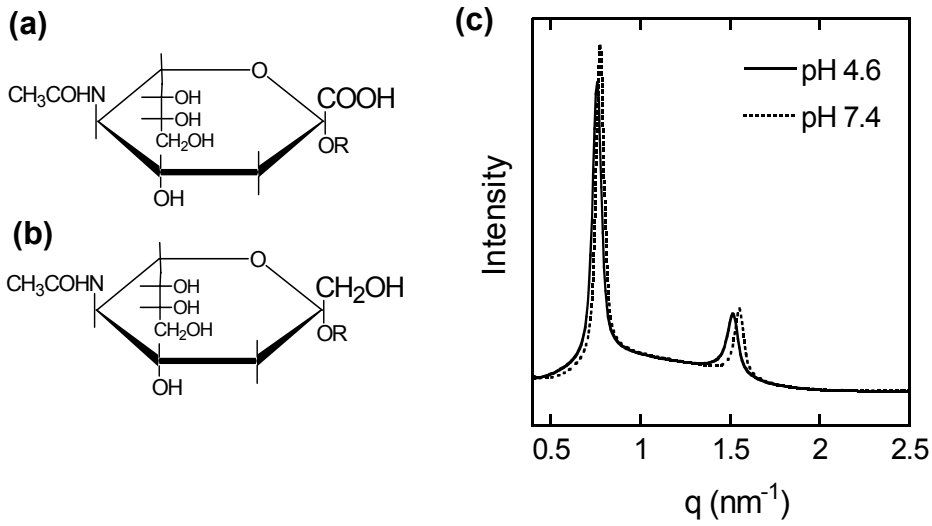

FIGURE 6
Structure of NeuAc (sialic acid) (a) and its derivative (b). For (b), carboxyl group attached to the C1-carbon was converted to the primary alcohol (2). (c), SAXS pattern of G_{M1}(C1-alcohol)/BMP = 1/1 (mol/mol) mixture at pH 4.6 and 7.4. At both pH conditions, diffraction peaks derived from the lamellar structure were observed with the lamellar repeat distance of 8.28 nm for pH 4.6 and 8.10 nm for pH 7.4.

REFERENCES IN SUPPLEMENTAL MATERIALS

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