pH-dependent formation of membranous cytoplasmic body-like structure of ganglioside G(M1)/bis(monoacylglycero)phosphate mixed membranes.

Tomohiro Hayakawa, Asami Makino, Motohide Murate, Ichiro Sugimoto, Yasuhiro Hashimoto, Hiroshi Takahashi, Kazuki Ito, Tetsuro Fujisawa, Hirotami Matsuo, Toshihide Kobayashi

To cite this version:

pH-dependent Formation of Membranous Cytoplasmic Body-Like Structure of Ganglioside G_{M1}/Bis(Monoacylglycero)Phosphate Mixed Membranes

Tomohiro Hayakawa,* Asami Makino,† Motohide Murate,‡ Ichiro Sugimoto,‡ Yasuhiro Hashimoto,‡ Hiroshi Takahashi,† Kazuki Ito,§ Tetsuro Fujisawa,§ Hirotami Matsuo,*, and Toshihide Kobayashi*†‡

*Lipid Biology Laboratory and †Supra-Biomolecular System Research Group, RIKEN, Saitama, Japan; ‡Department of Physics, Gunma University, Gunma, Japan; §RIKEN SPring-8 Center, Hyogo, Japan; *School of Pharmacy, Shujitsu University, Okayama, Japan; and †INSERM U585, INSA-Lyon, Villeurbanne, France

ABSTRACT Membrane structures of the mixtures of ganglioside G_{M1} and endosome specific lipid, bis (monoacylglycero) phosphate (BMP, also known as lysobisphosphatidic acid) were examined at various pH conditions by freeze-fracture electron microscopy and small-angle x-ray scattering. At pH 8.5–6.5, a G_{M1}/BMP (1:1 mol/mol) mixture formed small vesicular aggregates, whereas the mixture formed closely packed lamellar structures under acidic conditions (pH 5.5, 4.6) with the lamellar repeat distance of 8.06 nm. Since BMP alone exhibits a diffuse lamellar structure at a broad range of pH values and G_{M1} forms a micelle, the results indicate that both G_{M1} and BMP are required to produce closely stacked multilamellar vesicles. These vesicles resemble membranous cytoplasmic bodies in cells derived from patients suffering from G_{M1} gangliosidosis. Similar to G_{M1} gangliosidosis, cholesterol was trapped in BMP vesicles in G_{M1} and in a low pH-dependent manner. Studies employing different gangliosides and a G_{M1} analog suggest the importance of sugar chains and a sialic acid of G_{M1} in the pH-dependent structural change of G_{M1}/BMP membranes.

Received for publication 2 October 2006 and in final form 12 October 2006.
Address reprint requests and inquiries to Toshihide Kobayashi, Tel.: 81-48-467-9612; Fax: 81-48-467-8693; E-mail: kobayasi@riken.jp.

A characteristic feature of endosomes along with the degradative endocytic pathway is the accumulation of vesicles within the organelle (1,2). Recently, it has been shown that the unconventional phospholipid bis(monoacylglycero) phosphate (BMP, also known as lysobisphosphatidic acid, LBPA) can induce the formation of multivesicular liposomes that resemble multivesicular endosomes (3). BMP is a structural isomer of phosphatidylglycerol with characteristic sn-1, sn-1′ glycerophosphate stereoconfiguration (4,5). This lipid is highly enriched in the specific internal membrane domains of multivesicular late endosomes where the lipid comprises >70% of the total phospholipids (6,7). It has been reported that late endosomes/lysosomes change their organization from multivesicular to multilamellar membranes under different pathological conditions and by treatment with certain drugs. These multilamellar vesicles, in which membranes are tightly stacked, are called membranous cytoplasmic bodies (MCB). Although the involvement of BMP domains in late endosomes (8) and lipid-protein interaction (9) have been suggested, the mechanism of the formation of MCB is not well understood. Recently we have shown that a drug that induces multilamellar endosomes alters BMP liposomes from swollen and loosely packed lamellar vesicles to closely stacked multilamellar structures at low pH (10).

Sphingolipidosis is a genetic disease defective in the proteins involved in sphingolipid metabolism (11). Accumulation of MCBs is a characteristic feature of this disease. Different sphingolipids are accumulated depending on the defect. These lipids, such as sphingomyelin and galactosylceramide, themselves form multilamellar structures in aqueous solution. In contrast, in G_{M1} gangliosidosis, micelle-forming lipid G_{M1} is extensively accumulated and still MCBs are formed. Therefore, it is of interest to investigate the conditions in which the accumulation of G_{M1} induces the formation of closely stacked membranes. In our study, we examined the membrane structure of ganglioside/BMP mixture in neutral and acidic pH conditions, the latter of which resembles the lumen of late endosomes/lysosomes.

First, we examined whether the accumulated G_{M1} colocalize with the BMP-rich membrane domains in intact cells. The addition of exogenous ganglioside to cultured cells mimics the behavior of the cells from gangliosidosis (12). Diffuse fluorescence was observed when cultured human skin fibroblasts were fixed, permeabilized, and labeled with fluorescently labeled cholera toxin, which recognizes G_{M1} (see Fig. 4 of the Supplementary Material). In contrast, intracellular compartments were brightly labeled with cholera toxin when cells were grown in the presence of 10 μM G_{M1}. The fluorescence was colocalized with that labeled with anti-BMP antibody. The result suggests the presence of BMP and G_{M1} in the same membrane domains. We next examined the...
membrane structure of BMP/GM<sub>1</sub> complex. 2,2'-Dioleoyl-
-sn-1,sn-1'-BMP is a major molecular species of naturally
occurring BMP (7,13). We chemically synthesized 2,2'-diol-
eoyl-sn-1,sn-1'-BMP (14) and measured the structure of
the membranes in the presence of GM<sub>1</sub> by using electron
microscopy and small-angle x-ray scattering (SAXS). Fig. 1
shows freeze-fracture electron micrographs of the GM<sub>1</sub>/BMP
(1:1 mol/mol) mixture at pH 7.4 and 4.6. The particles
observed at pH 7.4 were mainly unilamellar vesicles, as
demonstrated in cross-fracture images, whereas the results at
pH 4.6 indicated structures filled with multiple layers or large
multilamellar vesicles. Each layer was closely stacked, and
the distance between the adjacent layers was <10 nm. The
size of vesicles at pH 7.4 was ~100–300 nm diameter in
contrast to ~300 nm–3 μm diameter at pH 4.6. Similar re-
sults were observed by negative-staining electron micros-
copy (data not shown). In Fig. 1, pH dependence of the
SAXS patterns of the GM<sub>1</sub>/BMP (1:1 mol/mol) mixture are
also shown. At pH 8.5–6.5, the SAXS profiles displayed
similar curves, exhibiting an evident minimum at \(q = 0.55\)
nm<sup>-1</sup> and a broad bell-shaped peak at \(q = 1\) nm<sup>-1</sup>. These
are characteristics of a scattering curve from an assembly of
identical small particles. It is reported that dioleoyl BMP
forms a diffuse lamellar structure at a pH range of 3.0–8.5
(10,15), whereas GM<sub>1</sub> forms a stable micellar structure at a
pH range of 3.6–8.0 (16). Considering the negatively charged
bulky headgroup of GM<sub>1</sub>, which gives a high curvature when
inserted into the membrane, it is expected that the GM<sub>1</sub>/BMP
mixture formed such compact aggregates. At pH 5.5,
however, the SAXS pattern exhibited two small peaks at
\(q = 0.78\) and 1.56 nm<sup>-1</sup> in addition to the broad peak at
\(q = 1\) nm<sup>-1</sup>. These two peaks correspond to the first- and
second-order diffraction peaks from a lamellar structure with
an 8.06 nm repeat distance. At pH 4.6, the first- and second-
order peaks became much more evident, indicating that the
acidic pH condition transformed the GM<sub>1</sub>/BMP mixture from
small aggregates to a planar lamellar structure. The dose
response of GM<sub>1</sub> indicates that the alteration of the mem-
brane structure was inducible by the addition of as low as
10% of GM<sub>1</sub> (see Fig. 5 in the Supplementary Material) at
low pH.

One of the consequences of the storage of sphingolipids in
MCBs, including GM<sub>1</sub>, is the accumulation of cholesterol. It
is proposed that the preferential association of sphingolipids
and cholesterol causes the accumulation of cholesterol in
MCBs (8). We investigated whether the GM<sub>1</sub>/BMP mem-
brane traps cholesterol in a pH-dependent manner (Fig. 2).
Methyl-β-cyclodextrin (MβCD) extracts cholesterol from
the membrane. Extraction of cholesterol from BMP and
GM<sub>1</sub>/BMP membranes by MβCD was investigated at pH 7.4
and 4.6. Cholesterol was equally extracted from the BMP
liposomes irrespective of pH. The presence of GM<sub>1</sub> did not
affect the extraction at pH 7.4. In contrast, the extraction of
cholesterol was significantly reduced in the presence of GM<sub>1</sub>
at pH 4.6. The addition of 10 mol % cholesterol did not alter
the gross structure of the GM<sub>1</sub>/BMP membranes (data not
shown). This result suggests that the formation of the closely
packed multilamellar structure of GM<sub>1</sub>/BMP in an acidic
environment prevents the cholesterol extraction by MβCD.

Fig. 3 shows the examination of the effects of various
gangliosides on the membrane structure of BMP at pH 4.6.
Similar to GM<sub>1</sub>/BMP, lamellar diffraction peaks were ob-
served in GM<sub>2</sub>/BMP membrane. However, the GM<sub>3</sub>/BMP
and GD<sub>3</sub>/BMP mixtures did not exhibit clear lamellar peaks,
suggesting that a branched carbohydrate chain is required for

---

**FIGURE 1** (Left) Freeze-fracture electron micrographs of GM<sub>1</sub>/BMP (1:1 mol/mol) mixture at different pH. (Right) SAXS patterns of GM<sub>1</sub> at pH 4.6 and GM<sub>1</sub>/BMP mixture at different pH.

**FIGURE 2** Cholesterol extraction from BMP/cholesterol and GM<sub>1</sub>/BMP/cholesterol (10 mol % cholesterol) membranes at different pH.
suggest that BMP and a low pH are additional players in cholesterol accumulation in MCBs.

SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at http://www.biophysj.org.

REFERENCES and FOOTNOTES


