

Title	Dynamic transformation of a cell-sized liposome containing ganglioside
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Citation	2011 International Symposium on Micro-NanoMechatronics and Human Science (MHS): 461-465
Issue Date	2011-11
Type	Conference Paper
Text version	author
URL	http://hdl.handle.net/10119/10864
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Description	

DYNAMIC TRANSFORMATION OF A CELL-SIZED LIPOSOME CONTAINING GANGLIOSIDE

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Abstract

It is important to understand the physicochemical mechanisms that are responsible for the morphological changes in the cell membrane in presence of various stimuli such as osmotic pressure. Role of micro domains such as lipid rafts in cellular processes are now beginning to unfold. In this study, we examined how constituent molecules affect the dynamical motion of liposome. We used cell-sized lipid vesicles to enable the direct observation of these changes¹. We observed the effect of GM1 ganglioside to osmotic transformation in a homogeneous and heterogeneous liposome. Interestingly it was observed that for the formation of spherostomatocyte their exist a particular critical cut off concentration. Also in the case of heterogeneous liposome it was observed that at 10 % of GM1 almost all domains were pinched out from the vesicles, forming their own homogeneous liposome's. This finding provides a possible mechanism of apoptosis induction by GM1. Incorporation of GM1 into membrane leads to an increase of the line tension. Thus, necessary proteins can find themselves in one common raft and start the corresponding cascade of reactions.²

1. INTRODUCTION

Regulation of lipid membranes is critical for many cellular processes. It is important to understand the physicochemical mechanism that governs the morphological changes or responses in a cell membrane structure to various external or internal stimuli. Hence, we use cell-sized (>10 μm) synthetic liposome that mimic natural cell structures. The detailed study of membrane dynamics in the presence of gangliosides can be helpful to understand the mechanism of membrane dynamics of actual living cells. Here we prepared liposome's containing GM1, monosialotetrahexosylganglioside which is a component of membrane raft and has an important role in signal transduction. Rafts are dynamic clusters composed largely of cholesterol and sphingolipids, which are rich in highly ordered saturated acyl chains³.

In this decade, the formation of lipid micro domain in mammalian plasma membrane, so-called a lipid raft, has been attracting intensive interests since lipid rafts are assumed to have functions as platforms of membrane-associated events such as signal transduction, cell adhesion, lipid/protein sorting and so on. Although the steady-state existence, size, and shape of lipid rafts (liquid ordered micro domains) in plasma membranes still remain the subject of debate, lipid rafts can be considered essentially as a dynamic assembly of a variety of lipids and proteins. As knowledge about lipid raft

functions in cellular signalling has been accumulated, the present agreement has been reached on the fact that raft domains coalesce upon cross-linking to form signalling and sorting platforms. A common feature of lipid rafts is their peculiar lipid composition, being rich in glycosphingolipids (GSLs), sphingomyelin and cholesterol. Gangliosides are major components of GSLs, Functions of lipid rafts are assumed to relate closely to the peculiar features of GSL molecules both in ceramide and oligosaccharide portions that can form complex hydrogen bonding networks (hydrogen bond donor and acceptor)⁵

GM1 refers to the GM1 ganglioside, one of the glycosphingolipids widely distributed in all tissues, but occurring in highest concentrations in the central nervous system. It is primarily located in the outer surface of the mammalian cells plasma membrane and in synaptic membranes of the CNS. GM1 ganglioside modulates a number of cell surface and receptor activities as well as neuronal differentiation and development, protein phosphorylation and synaptic function⁴.

In our proposed model GM1 clusters in a homogeneous liposome in the presence of 1mM osmotic pressure leads to the formation of small spherostomatocyte. Especially at 10 %

concentration of GM1 this transformation is observed in maximum number and further decreasing for higher and lower concentrations of GM1. Also for heterogeneous liposome it was observed that all domains separated and formed a homogeneous liposome when introduced to osmotic pressure of 1mM in the presence of 10 % GM1.

2. MATERIALS AND METHODS

2.1 Materials

Unsaturated phospholipids, dioleoyl L- α phosphatidylcholine (DOPC), a saturated phospholipids, dipalmitoyl Lphosphatidylcholine (DPPC) and cholesterol were purchased from Avanti Polar Lipids. Bovine brain ganglioside GM1 ammonium salt was purchased from Calbiochem. Fluorescent dyes, *N*-(rhodamine red-X)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine triethylammonium salt (rhodamine red-X DHPE) ($\lambda_{ex} = 560$ nm, $\lambda_{em} = 580$ nm) and Alexa Fluor 488 conjugate cholera toxin subunit B (CtxB-488) ($\lambda_{ex} = 495$ nm, $\lambda_{em} = 519$ nm) were obtained from Invitrogen. All other reagents were purchased from Nacalai Tesque and were of analytical grade. Deionised water obtained from a Millipore Milli Q purification system was used to prepare buffers and reagents.

2.2 Preparation Of Giant Unilamellar Vesicles (GUV) and observation using an Optical Microscope

Giant liposome's were prepared using the natural swelling method from a dry lipid film; the lipid mixture dissolved in 1:2(v/v) chloroform/methanol along with rhodamine red-XDHPE and D(+)-glucose in a glass test tube were dried under vacuum for 3 hrs to form thin lipid films⁶. Next, the films were hydrated with deionised water at 37⁰C for an hour before being combined with a CtxB-488 solution. Then, the liposome was incubated for more than several hours at room temperature (24⁰C) before microscopic observations. The final concentration were 0.2mM lipids (DOPC/DPPC/Cholesterol=4:4:2, 2:5:3, 5:5) with 10% GM1 and 0.2 % rhodamine red-X DHPE and CtxB-488. Additionally, homogeneous liposome without a raft phase was also prepared using the same swelling method.

2.3 Microscopic Observations.

Confocal fluorescence images of GUV's were acquired with an inverted laser confocal microscope. Immediately before the confocal microscopy experiment, the GUV suspension was diluted with equal volume of mixture of 2 mM glucose. This concentration difference induced osmotic pressure. Images were collected and stored on the computer hard disk for further analysis.

2.4 Treatment With osmotic Stress

Lipid vesicles and 2mM glucose solution were poured into a test tube and gently mixed by tapping. Observation of the vesicular dynamics was within 2 min of glucose solution introduction to the lipid vesicles.

3.RESULTS AND DISCUSSIONS

Figure 1A shows examples of the effect of environmental stimuli, glucose osmosis on spherical single phase liposome of DOPC without raft domains. As shown in figure1 the spherical liposome begins to undulate and to assume an ellipsoid shape due to osmosis. It is well known that the decrease in aqueous volume due to osmotic pressure results in a transformation from spherical to various asymmetric shapes.⁷ Thus in case of homogeneous liposome, the external stimuli led to large deformations of the entire membrane surface. (Fig 1B)

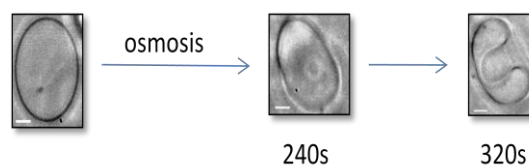


Figure1A. Phase contrast microscopic image of the transformation of homogeneous DOPC liposome after addition of high osmolarity glucose.

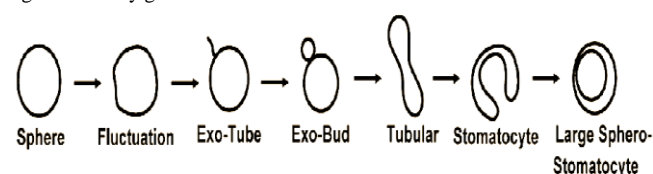


Figure1B

3.1 Low contents of GM1 In Homogeneous Liposome

Interestingly GM1 incorporation exerted an effect on GUVs in a particular manner. In Fig.2 a typical

image of a GUV containing 1 mol% GM1 after introduction to osmotic stress is shown. Also Fig.3 shows the image of GUV containing 5 mol% GM1 after introduction to osmotic stress. Both the images show that in most of the cases the GUV undergoes either exo-budding or tubular formation. The formation of small sphero-stomatocytes is seen

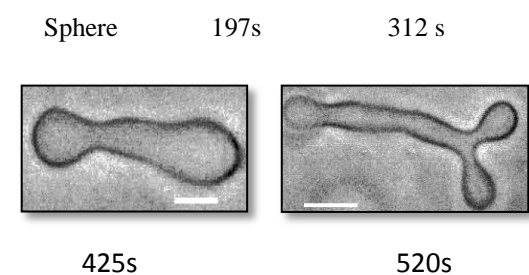
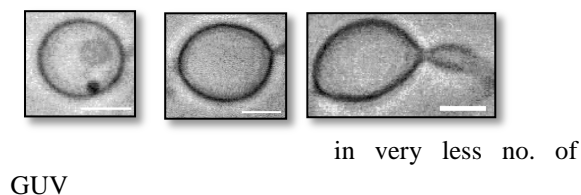


Figure 2. Phase contrast image of the transformation of homogeneous DOPC liposome containing 1 mol% GM1 after the addition of glucose.

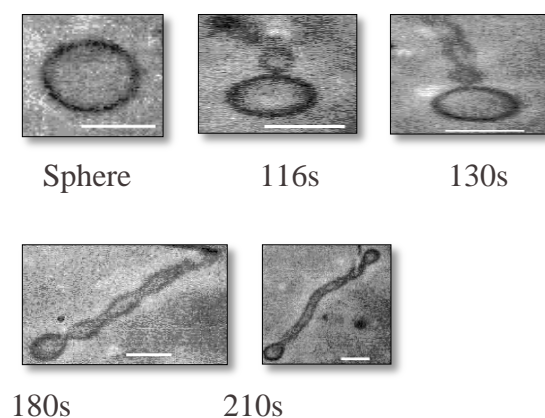


Figure 3. Phase contrast image of the transformation of homogeneous DOPC liposome containing 5 mol% GM1 after the addition of glucose.

3.2 Moderate contents of GM1 In Homogeneous Liposome

Further addition of GM1 to the GUVs increases the tendency of GUVs to form small sphero stomatocytes. The addition of 10 mol% GM1 to the GUV leads to the formation of sphero stomatocytes in most of the case. Fig 4. Shows a typical example of a GUV containing 10 mol% GM1 under osmotic stress.

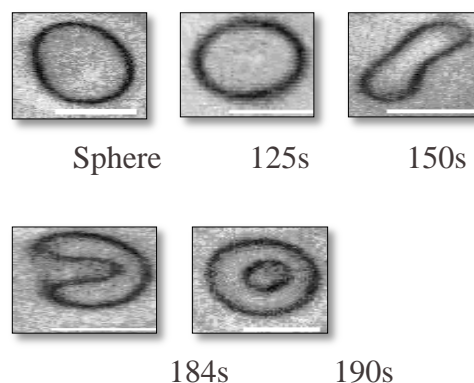


Figure 4. Phase contrast image of the transformation of homogeneous DOPC liposome containing 10 mol% GM1 after the addition of glucose.

3.3 High contents of GM1 In Homogeneous Liposome

On increasing the concentration of GM1 further from 10 mol% to 25 and 50 mol% shows a vast deviation from the above shapes. The formation of small sphero stomatocyte is rarely observed in any case, whereas the number of liposomes undergoing endo budding, tubular formation and fluctuations increased tremendously. Fig 5 and Fig. 6 show typical microscopic images of GUVs containing 25 and 50 mol% GM1 under osmotic stress.

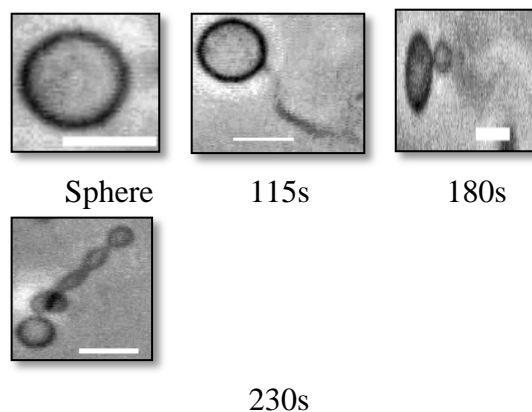
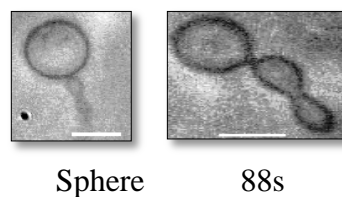
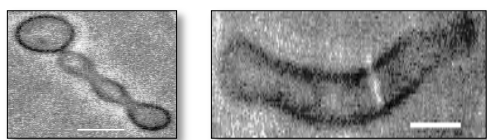


Figure 5. Phase contrast image of the transformation of homogeneous DOPC liposome containing 25 mol% GM1 after the addition of glucose.



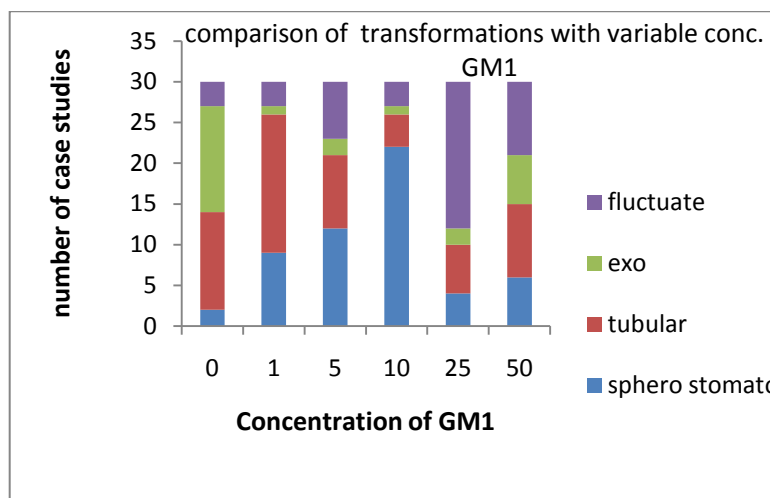


140s

180s

Figure 6. Phase contrast image of the transformation of homogeneous DOPC liposome containing 50 mol% GM1 after the addition of glucose.

3.4 Comparison Of the nature of liposome containing different concentrations of GM1 under osmotic pressure



At 10mol% concentration of GM1 we observed the transformations for three type of heterogeneous liposome;

- 5:5 (DOPC:DPPC)
- 4:4:2 (DOPC:DPPC:Cholesterol)
- 2:5:3 (DOPC:DPPC:Cholesterol)

For 5:5 type of liposome, it was observed that there is no separation of phases under osmotic stress, but continuous fluctuations are observed in such type of liposome. Fig.9 clearly depicts the example of such a type of liposome.

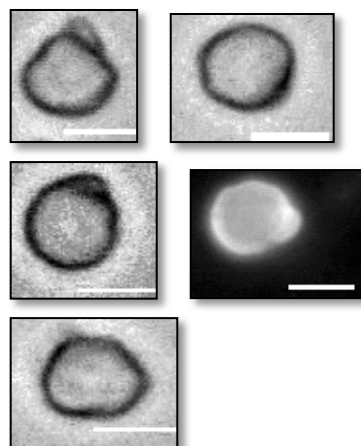
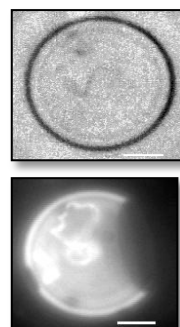


Figure 9. Heterogeneous liposome of 5:5 types under osmotic stress

For 4:4:2 type of liposome it was observed that there takes place a separation of both the phases on exposure to osmotic stress. Based on the observations illustrated by Fig. 10 we can conclude that at such high concentrations of GM1, Lo domains tend to separate from GUVs forming their own vesicles.



3.5 Moderate Concentration Of GM1 In Heterogeneous Liposome

Typical images of GUV containing 10 mol% GM1 are shown in Fig.8. The vesicles are large(about 10 micrometers in diameter or larger) and well formed, easily distinguishable by confocal microscopy. We then added pure water to GUVs, thus generating hypotonic conditions and causing osmotic stress. This osmotic pressure caused swelling of the GUVs that increased the lateral tension of their membranes.⁸

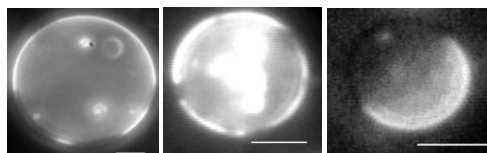


Figure 8. Fluorescence microscopic images of Heterogeneous Liposome containing 10%GM1

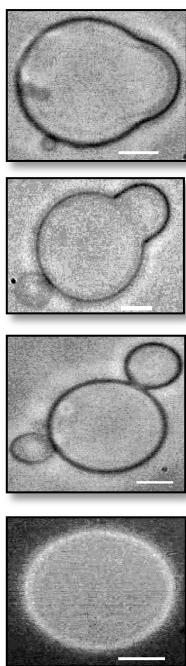


Figure 10 phase contrast images of liposome containing 10 % GM1 under osmotic stress

In case of 2:5:3 type of liposome it was observed the same case that of 4:4:2 where the Lo domain separates from GUVs forming their own domains as shown above. But for the cases where the Lo phase domains or the Ld phase domains are very small they did not take any separation of the domains.

3.6 Discussion

The transformations in response to the stimuli can be attributed to an increase in excess surface area. When the liposome are subjected to high osmolarity, the water efflux across the membrane reduces the inner aqueous volume.⁹ The resulting excess membrane allows transformations in variety of vesicular morphologies, as reported. The lateral tension induced by osmotic stress results in increase of the line tension at the Lo domain boundary. This suggests that the addition of GM1 also results in the increase of line tension at the Lo/Ld phase boundary which is sufficient enough to produce the lateral tension, exceeding the rupture limits of the membranes.⁸

4. Conclusion

In the present study, using optical confocal microscopy, we studied the influence of ganglioside GM1 on the properties of Lo domains in GUVs. It was found that for homogeneous liposome, at 10 mol% concentration of GM1 the formation of small spherostomatocyte is most favoured and can be attributed as the critical cut off concentration for such a transformation. For heterogeneous type of liposome it was observed that at 10 mol% concentration of GM1 the tendency of the domains to separate from the GUVs was same for different compositions of liposomes.

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