RAPID PAPER

Conformational Change of Ganglioside G_{M1} with Surface Pressure, Related to Signal Transduction?

Shoko YOKOYAMA*,1, Tadahiro TAKEDA*1, Yumiko OHTA*2, Tomohiro IMURA*2, Aritomo YAMAGUCHI*2, Hideki SAKAI*2,3 and Masahiko ABE*2,3

*1 Kyoritsu College of Pharmacy
(1-5-30, Shibakoen, Minato-ku, Tokyo 105-8512, JAPAN)
*2 Faculty of Science and Technology, Science University of Tokyo
(2641, Yamazaki, Noda-shi, Chiba 278-8510, JAPAN)
*3 Institute of Colloid and Interface Science, Science University of Tokyo
(1-3, Kagurazaka, Shinjuku-ku, Tokyo 162-0825, JAPAN)

Edited by H. Shimasaki, Teikyo, Univ., and accepted July 23, 2001 (received for review July 11, 2001)

Abstract: Gangliosides participate in cellular interactions and signal transductions. An initial step in these processes is glycolipid interaction. We observed the conformational change of ganglioside G_{M1} (GM1) at the membrane surface by means of atomic force microscopy (AFM), and revealed the following changes of GM1 with increasing surface pressure: a uniform pattern at 29 mN/m, a swelling pattern at 33 mN/m, and a return to a uniform pattern at 40 mN/m. This behavior is thought to be related to specific cell recognition or signal transduction, and may prove useful for elucidating the functions of glycolipid microdomains in biological membranes.


Key words: ganglioside G_{M1}, atomic force microscopy, conformational change, surface pressure, phase transition

1 Introduction

Gangliosides participate in cellular interactions, differentiation and transformation (1-4). Specific gangliosides have been shown to be highly antigenic (5), while certain others play a role in receptor function (6). Increasing evidence that gangliosides are able to modulate cell membrane receptors, membrane ion pumps and ion channels (4,5) has given further impetus to research on these compounds. New evidence that interactions between glycolipids form an initial step in cell recognition has also been published (7).

Atomic force microscopy (AFM) is a surface imaging technique capable of nanometer-scale lateral resolution, which operates by measuring the forces acting between a probe and the sample (8). AFM images of mixed phospholipid membranes have already been reported (9-11), however, the change in ganglioside G_{M1} (GM1) monolayers with surface pressure has not yet been shown. Gangliosides form microdomains at the cell surface, and signal transduction occurs through the microdomains (12,13). As such, the ganglioside monolayer is regarded as a model of the biological membrane. We studied the characteristics of GM1 membranes deposited under various surface pressure conditions, and observed the conformational changes by AFM. The finding that the conformation of GM1 changes with surface pressure is new, and may assist in elucidating the functions of GM1 (cell recognition, signal transduction, etc.). In addition, we obtained surface pressure-area curves for GM1 monolayers at various temperatures and clarified the phase transition of GM1, for which detailed data has not as yet been reported from the surface pressure measurements of other researchers (14,15).

Corresponding author: Shoko YOKOYAMA
E-mail: yokoyama-sk@kyoritsu-ph.ac.jp
2 Experimental

GM1, Gal β1→3Gal NAc β1→4Gal β1 (3→2 αNANA)→4Glc β1→1Cer, was purchased from Sigma Chemical Co. GM1 was dissolved in a mixed solvent of chloroform/methanol (9:1), giving a 1 mM GM1 solution. After the GM1 solution was spread on the water without surface disturbance using a microsyringe, the system was allowed to stand for 10 min. The surface pressure of the GM1 monolayer at the air/liquid interface was determined at 23 to 50°C±0.5°C by the Wilhelmy plate method using a surface pressure meter (HBM-A, Kyowa Interface Science Co., Ltd.). The compression rate was 20 mm/min.

Single-layer LB films of GM1 for AFM were obtained at 30°C using a vertical dipping method onto freshly cleaved mica. Deposition proceeded at 5 mm/min, under surface pressures of 7, 21, 29, 33 and 40 mN/m. Images were captured using an SPI 3800 (Seiko Instruments Co., Ltd.) atomic force microscope. The AFM probe was a Micro Cantilever (SN-AF01-A, Olympus Optical Co., Ltd.) made of Si3N4 and coated with Au. The AFM probe had a spring constant of 0.11 N/m, a length of 100μm, and a thickness of 4000 Å.

3 Results and Discussion

The surface pressure (π) vs. area (A) isotherms at 23-50°C of GM1 monolayers at the air/liquid interface are shown in Fig. 1.

The phase transition of GM1 from the liquid-expanded film to the liquid-condensed film was observed in the range 23-40°C, whereas only the liquid-expanded phase was observed at above 45°C. The phase transition temperature of GM1 was estimated to be 40-45°C. The thermotropic phase transition of GM1 micelles occurs at 40-50°C (16). The phase transition temperature of GM1 monolayers observed in this study is consistent with the previous results (16), although there is a difference between monolayers and micelles. The surface pressure at which the phase transition from the liquid-expanded film to the liquid-condensed film was observed at about 18, 24, 29 and 32 mN/m at 23, 30, 35 and 40°C, respectively.

GM1 monolayers deposited under a range of deposition surface pressures were observed by AFM. Deposition was performed at a constant temperature of 30°C, at which the phase transition can be clearly observed in the π-A curve. Figure 2 shows the AFM images of GM1 monolayers deposited at surface pressures of 7, 21, 29, 33 and 40 mN/m.

At lower surface pressure, defects like a needle-point were observed in the AFM images (Figs. 2a and 2b). The black points in Fig. 2 are mica. The density of defects decreased with increasing surface pressure up to 29 mN/m, at which a uniform layer was obtained. At lower pressure, the oligosaccharide residues of GM1 molecules would stand up from the surface. At 29 mN/m, just above the phase transition pressure, the oligosaccharide residues lie flat and interact with each other, thus allowing the GM1 molecules to arrange uniformly on the membrane surface. This process fixes the defects in the film. Furthermore, the flattened state of the oligosaccharide residue appears conducive to signal transduction. At 33 mN/m, a swelling pattern was observed (bright area in the images indicate topographic highs). This swelling pattern is thought to be related to specific cell recognition. This surface changeability of GM1 is considered to be the result of the conformational flexibility of the oligosaccharide chains, which may contribute to the promotion of various physiological surface events in biomembranes. The films became uniform again when the surface pressure was increased further to 40 mN/m. Surface pressures in biological membranes range from 30 to 45 mN/m (17-19). When cells or signals approach the GM1 (or glycolipid) microdomains, the surface pressure at the GM1 microdomain should rise. Therefore, the phenomena seen in the AFM images seem reasonable. Iwabuchi et al. (12) proposed from biochemical point of view the schematic illustration of glycosphingolipid-enriched microdomain.
Fig. 2  AFM Image of GM1 Monolayer for Various Deposition Surface Pressures.
Surface pressures: (a) 7, (b) 21, (c) 29, (d) 33 and (e) 40 mN/m.
Temperature: 30 °C.
The scan area was 10 μm × 10 μm.

(GEM) function in cell adhesion coupled with signaling: they suggested that stimulation of glycosphingolipids (GSLs) in GEM by GSL ligands induces conformational change of GEM and transducers, which triggers signal transduction. Our AFM observation (Figs. 2c, 2d and 2e) at 29-40 mN/m seems to correspond to their schematic illustration.

This is the first report of the visual observation of the conformational change of GM1 membrane with increasing surface pressure, and it is expected that these results will aid in the elucidation of molecular recognition and signal transduction in glycolipid microdomains in biological membranes.

References