RECONSTITUTION OF G-PROTEIN COUPLED RECEPTORS (GPCRS) INTO GIANT LIPOSOME ARRAY

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ABSTRACT

This paper describes a method to reconstitute GPCRs into an array of giant liposomes in a buffer solution. We first achieved the formation of giant liposomes in a buffer solution by adding fructose to a lipid film; normally such giant liposomes are formed by using only pure waters (MilliQ). Moreover, we successfully reconstituted adrenergic receptors (AR) of GPCRs into the giant liposome array by a baculovirus-liposome fusion method. The specific binding of fluorescent dye-conjugated agonists was detected on AR-giant liposomes.

KEYWORDS: Liposome, GPCR, adrenergic receptor, array

INTRODUCTION

Giant liposomes are composed of phospholipid membranes similar to those commonly found in living cells. Giant liposome is typically ~10 μm in diameter, which is large enough to observe using optical microscopy. Therefore, such an artificial liposomal system has played an important role in the biochemical and biophysical studies such as the interactions of lipid membranes with cytoskeletal proteins, and the microencapsulation of gene expression systems [1]. An assay based on a giant liposome may help to clarify biochemical and biophysical reactions; the use of an array of uniform-liposome is an attractive investigation approach. Our recent study has shown that uniform-size giant liposomes array is easily prepared by a selective lipid-patterning with an electrospray deposition technique (Fig.1) [2,3]. However, this method could not be applied for practical uses; for instance, liposomes were unable to form under physiological buffer solutions.

Membrane proteins are crucial for sophisticated biological functions such as signal transduction, energy production and cellular communication. Recently, membrane proteins have been are made to function as nanodevices in biomedical applications such as drug delivery system and tissue engineering. However, their use is limited because of difficulties in isolating and purifying functional membrane proteins. Proteoliposomes are membrane protein-reconstituted lipid vesicles, and membrane proteins can be reconstituted in the bilayer membranes of liposomes, and can thus facilitate studies on membrane protein functions. Proteoliposomes are usually prepared by the detergent removal method, in which the detergents are removed from mixed micelles containing lipids and membrane proteins. The main limitation of this method is that one can obtain only small (0.1–0.2 μm) reconstituted proteoliposomes. Giant proteoliposomes are still difficult to prepare directly by this method. Yoshimura's group proposed a baculovirus expression-liposome fusion method for preparation of proteoliposomes (Fig.2) [4,5]. The membrane fusion phenomena between liposomes and glycoprotein 64 (gp64) displayed on recombinant budded viruses (BVs) of baculovirus under acidic conditions were applied. The human nicotinic acetylcholine receptor a-subunit (AChRa) and, the connexin, which formed cellular gap junction, were successfully prepared by this method. The advantage

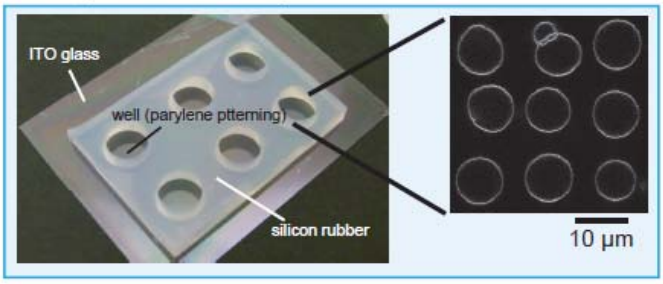

Fig. 1 *Image of uniformly-sized liposome array device and fluorescent image of liposomes prepared using this array device [3].*

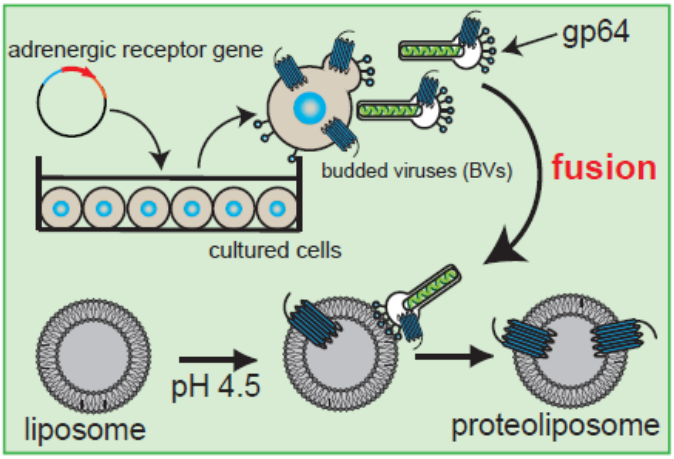

Fig. 2 *Illustration of baculovirus–liposomes membrane fusion method. Recombinant adrenerigic receptor (AR) budded viruses (BVs) were fused with giant liposomes. BVs have the fusogenic envelope glycoprotein (gp64), which gets actived under acidic conditions. BVs fused with liposomes in the presence of a weak acid (pH4.5-5.5), and proteoliposomes are obtained.*

of this fusion method is retention of the original orientation and conformation of the membrane proteins after their incorporation into the liposome.

In this work, we focused on the improvement in an array of uniform-liposome which formed under physiological buffer solutions. The lipid deposit on indium-tin-oxide (ITO)-glass surface was improved by electrospraying of the phospholipids solution mixed with fructose and a giant liposome array was formed in a buffer solution. This giant liposome array is a useful device, plays an important role in the biochemical and biophysical studies under physiologic condition (ionic strength, pH). Furthermore, we demonstrated the reconstitution of a G protein-coupled receptor (GPCR) protein into the buffer-hydrated liposomes by a baculovirus-liposome fusion method. The specific binding of an agonist to the GPCR on the liposome surface was examined.

EXPERIMENTAL

Figure 3 shows the preparation process of giant liposomes by using a selective lipid-patterning device [1]. Lipid patterning was performed on a poly (choroid-p-xylene) (parylene)-masked surface by electrospraying of 0.25 mg/mL DOPS, 0.25 mg/mL DOPC, 0.0025 mg/mL of a fluorescent (NBD)-conjugated DPPE and 3.8 mg/mL fructose in chloroform (voltage, 1.45 kV/cm; duration, 2.5 min). The film was then hydrated by adding a phosphate buffered saline (PBS) buffer, resulting in immediate giant liposome formation.

Adrenergic receptor BVs were harvested as follows: an insect cell line Sf9 derived from the fall armyworm *Spodoptera frugiperda* was cultured in culture flasks (75 cm^3) containing Sf-900 III medium each until about 80% of each of the flasks was covered. A suspension of adrenergic receptor (AR) BVs was spread in each flask at a multiplicity of infection (MOI) of 1. Flasks were incubated at 27^{o}C for 94 h. AR-BVs were purified by sucrose density gradient centrifugation. Finally, an AR-BV solution was infused to the buffer-hydrated giant liposome array at pH 4.5 or 7.0 (i.e., the baculovirus-liposome fusion method) to reconstitute the AR protein into the liposomes. The agonist-binding assay of AR on giant liposomes was conducted as described below. A fluorescent dye-conjugated AR agonist was mixed with the array at a final concentration of 100 nM and this was incubated for 30 min. The AR on the giant liposomes was visualized by a confocal laser scanning microscopy (LSM). The fluorescence intensity values were measured from LSM images.

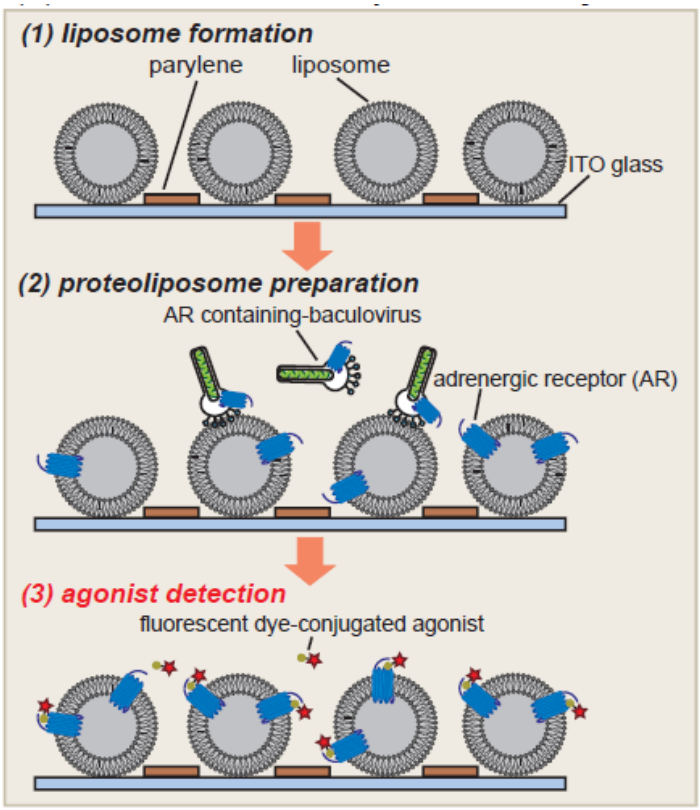

Fig. 3 *The agonist against AR was detected using this AR-reconstituted giant liposome array.*

RESULTS AND DISCUSSION

To obtain buffer-hydrated giant liposomes, lipid films were prepared from mixed DOPS/DOPC/fructose by electrospraying. The fructose-mixing lipids were patterned onto the ITO-glass surface. Confocal laser scanning microscopic observation showed that giant liposomes were formed from lipid films of DOPS/DOPC/fructose than from those of DOPS/DOPC in PBS condition. These giant liposomes had large diameters (about 10 μm) and were dome-shaped (Fig.4). The formation of giant liposomes from fructose-mixing lipid films may have a similar beneficial effect as PEG-conjugated lipids in the formation of giant liposomes under solutions with physiologic conditions.

AR-proteoliposomes were prepared by the fusion of recombinant AR-BVs and giant liposomes. A giant liposome consisting of acidic/neutral phospholipids (DOPS/DOPC 1:1w/v) was mixed with recombinant AR-BVs at pH 4.5 and pH 7.0. A fluorescent dye-conjugated AR agonist was mixed into the array at a final concentration of 100 nM and incubated for 30 min. Figure 5 shows the fluorescence (red) of AR agonists on the liposomal membrane at pH 4.5. The results indicated that the recombinant AR-BVs were successfully fused with the giant liposomes only at pH 4.5 because of the low pH-triggered activity of gp64 [4, 5]. The fluorescence intensity values of the agonists on the giant liposomes (diameter, 10-15 μm) were measured from the LSM images (Fig.5). The fluorescence intensity with ARs was 17.2±7.3 (SD) in arbitrary units (au) while the intensity without ARs was 0.64±0.60 (SD). This result suggested that the folding of AR protein was maintained on the

giant liposomes even after the fusion and reconstitution.

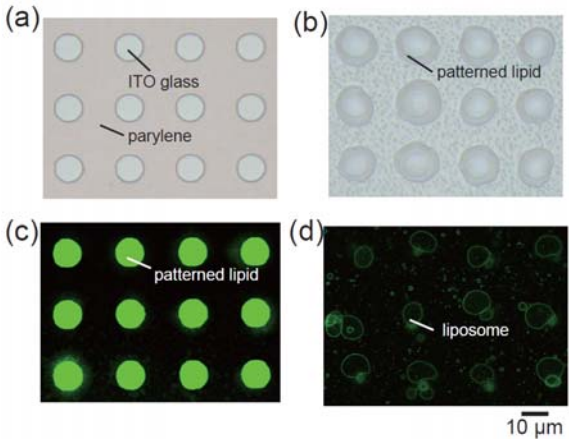

Fig. 4 *These images of buffer-hydrated liposomes preparation by uniformly-sized liposome array device. Microscopy image of a patterned parylene of this device (a). Microscopic (b) and fluorescent microscopic (c) images of fructose-mixing lipid pattern by the electrospray deposition (ESD) method. Fluorescent image showed patterned lipids were hydrated with buffer solution, and giant liposomes were formed (d).*

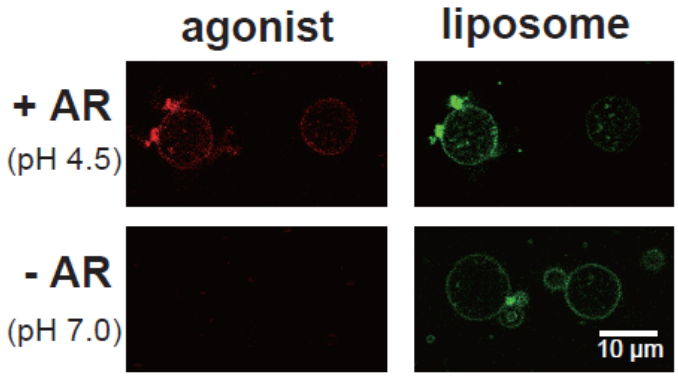

Fig. 5 *Typical LSM fluorescent images of agonist binding to adrenergic receptor (AR) on giant liposomes. Giant liposomes (+AR) were mixed with AR-BVs at pH 4.5, and giant liposomes (-AR) were mixed with AR-BVs at pH 7.0. Green fluorescence represents liposome membrane and red fluorescence represents agonist against AR.*

CONCLUSION

We succeeded in producing an improved array of giant liposomes of uniform size under physiological buffer solutions. The giant liposome formation was stimulated by fructose. Furthermore, we demonstrated the reconstitution of an adrenergic receptor into giant liposomes using the baculovirus-liposome fusion method. The fluorescent dye-conjugated agonists could be successfully detected on an AR-reconstituted liposome. This buffer-hydrated giant liposome array that can be used as a research tool will create new opportunities in biochemical and biophysical studies.

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