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Improved membrane fluidity of ionic polysaccharide bead-supported phospholipid bilayer membrane systems

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ABSTRACT

Supported phospholipid bilayer membranes on polysaccharide-based cationic polymer beads (cationic group: $-[OCH_2CH(OH)CH_2]_2N^+(CH_3)_3 \cdot X^-$, 45–165 μm in diameter) were prepared using small unilamellar vesicles from mixtures of phosphatidylserine (PS) and phosphatidylcholine (PC). Confocal fluorescence microscopic observations with a fluorescent membrane probe (*N*-4-nitrobenzo-2-oxa-1,3-diazole-phosphatidylethanolamine) revealed that the phospholipid molecules in the phospholipid-bead complexes were along the outer surface of the beads. The fluidity of the phospholipid bilayer membranes in the PS/PC-bead complexes was investigated by the fluorescence recovery after photobleaching (FRAP) technique. The lateral diffusion coefficients (*D*) for the PS/PC-bead complexes were lower than that for the 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine giant unilamellar vesicles without solid supports. Such less fluid membranes in the complexes appeared to be due to the immobilization of the phospholipid bilayer membranes by electrostatic attractive forces between PS and the bead. The *D* values for the PS/PC-bead complexes were dependent on the phospholipid composition; the PS(100 mol%)/PC(0 mol%)-bead complex had the least fluid membranes among the PS/PC-bead complexes tested in this study. The phospholipid bilayer membranes formed on the polysaccharide-based cationic polymer beads were much more fluid than those on a polystyrene-based one. Furthermore, such fluid phospholipid bilayer membranes formed on the polysaccharide-based cationic polymer bead were maintained for 10 days, even though the complex sample was stood in plain buffer (pH 8.5) at ambient temperature.

1. Introduction

Liposomal membrane systems have been widely used as the most simplified model for biomembranes in the fields of chemical and/or physical biology [1–3]. However, their poor mechanical stability due to a structure without a solid support often causes problems for their use as model biomembranes. Thus, several model systems have been developed to improve such drawbacks of the liposomes. A supported lipid membrane (SLM) system on microparticulate materials, such as silica and glass beads, is a better example of these models [4–8]. The SLM systems have contributed to the elucidation of many membrane processes since their discovery; for instance, the in vitro studies of membrane–cytoskeleton interactions using biomimetic membranes turned out to be helpful to obtain mechanistic insights into the dynamics of these processes [9,10]. Due to the mechanical strength provided by the planar and microparticle supports, the surface of the SLMs also allow the use of modern surface sensitive analytical techniques, such as a quartz crystal microbalance and attenuated total reflection Fourier transform infrared spectroscopy [11,12].

Most of the SLM systems are prepared by adding a hydrophilic or hydrated supporting material to a suspension of small unilamellar vesicles (SUVs, <100 nm in diameter). The lipid vesicles initially adsorb onto the surface of the supporting material, and at a high coverage, they rupture and fuse to form a flat lipid bilayer membrane. The mechanism and parameters that govern the SLM formation have been systematically studied [13–15] and include the nature of the supporting materials (its surface charge, chemical composition and roughness), the lipid vesicles (their composition, the charge and geometry of the lipids) as well as the buffer being used (its composition, pH and ionic strength). The presence of divalent cations, especially calcium, strongly promotes the vesicle rupture on the surface of the supporting materials. For the design of the SLM systems, the control of the interaction between the surface of the supporting materials and lipid molecules is regarded as an

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important issue. It is so far unclear why the transformation from a vesicle to a flat bilayer for egg-yolk phosphatidylcholine vesicles is limited to a small set of hydrophilic surfaces (silica and mica) and why similar surfaces, such as alumina [16], gold [17], platinum [18] and titanium (IV) oxide [19], adsorb the intact vesicles but do not cause rupture of the vesicles. Conversely, the phospholipid bilayer membranes formed on the surfaces of chromium and indium tin oxide microparticles are reported to lose their lateral fluidity due to very high adhesion forces [16]. The continuity and fluidity of the phospholipid bilayer membranes appear to largely depend on the structural features of the supporting materials.

We have already reported a novel methodology for the preparation of SLM systems on ionic polymer beads in the range from 10 to 500 μm in diameter [20–23]; the electrostatic attractive forces between ionic lipids and the oppositely charged organic polymer beads appear to promote the formation and stabilization of lipid bilayer membranes formed on the beads. So far, we have used hydrophobic polystyrene-based cationic spherical polymer beads as the supporting material for the preparation of the SLM systems. The physico-chemical property of the ionic polymer bead (structure of backbone polymer, ionic group structure and density on the surface, etc.) could be due to other structural factors affecting the membrane fluidity of the complexes. In this study, the SLM systems were prepared using hydrophilic polysaccharide-based cationic polymer beads and naturally occurring phospholipids. Confocal laser scanning fluorescence microscopic techniques characterized the resulting phospholipid bilayer membrane structures on the cationic polymer beads.

2. Materials and methods

2.1. Materials

Dioleoylphosphatidylserine (PS) and egg-yolk phosphatidylcholine (hydrogenated) (PC) were purchased from the NOF Corporation (Tokyo, Japan). Q Sepharose [particle size: 45–165 μm in the wet state, capacity: 0.18–0.25 mmol/mL, functional group: $-[OCH_2CH(OH)CH_2]_2N^+(CH_3)_3{\cdot}X^-$] was obtained from the Sigma Co. (St. Louis, MO, U.S.A.). DIAION SA11A was from the Mitsubishi Chemical Co., which is a nonporous quaternary ammonium type anion-exchange polymer bead with a 350–550 μm diameter and its ammonium nitrogen content is 0.85 mmol/mL polymer in the wet state. *N*-4-Nitrobenzo-2-oxa-1,3-diazole-phosphatidylethanolamine (NBD-PE) was from Molecular Probes Inc. (Eugene, OR, U.S.A.). The water used in this study was generated using a Milli-Q Biocel system (Millipore Corp., Billerica, MA, U.S.A.). All other chemicals were of commercial reagent grade and were used as received.

2.2. Preparation of phospholipid-bead complexes

The phospholipid-bead complexes were prepared by the vesicle shaking method. Phospholipid mixtures (25 μmol) with varying ratios of PS and PC placed in a round bottom flask (100 mL) were dissolved by adding 5 mL of chloroform. The chloroform was then gently evaporated under reduced pressure. After the addition of 50 mL of 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris–HCl) buffer (pH 8.5) to the flask, the mixture was vortexed for 1 h. The obtained milky suspension was further sonicated by a probe-type sonicator 250D (Branson, Danbury, CT) at 80 W for 15 min. The zeta potential and particle diameter of the liposomes were determined by an ELS-7500 (Otsuka Electronics Co. Ltd., Osaka, Japan) and a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK), respectively. Ten milliliters of the liposomal suspensions were shaken with 1 mL of Q Sepharose in the Cl^- form at 55 °C for 30 min. The Q Sepharose or the SA11A beads had been previously immersed in 50 mM Tris–HCl buffer (pH 8.5) at room temperature for 24 h before mixing with the liposomal suspensions. The obtained phospholipid-bead complexes were thoroughly washed with 50 mM Tris–HCl buffer (pH 8.5), and kept in the washing buffer at room temperature until used.

2.3. Determination of the phosphorous content of the complexes

After wet digestion of the phospholipid vesicle suspensions, using a 4:1 mixture of nitric acid and perchloric acid, the phosphorous content was determined based on vanadium (V) that is capable of forming a yellow complex with the phosphate ion [24]. The color produced by the complexation was monitored at 440 nm. The phosphorous amount of the beads was calculated from the difference in the phosphorous concentration of the liposomal suspension before and after preparation of the phospholipid-Q Sepharose complexes. The phosphorous amount of the phospholipid-free Q Sepharose beads was less than 0.01 μg/mL-Q Sepharose.

2.4. Confocal fluorescence microscopic observation of the complexes

For the fluorescence microscopic observation, the liposomal suspensions from the mixtures of PS and PC were spiked with 0.1 mol% NBD-PE for the phospholipids just before sonication, as described in Section 2.2. Confocal laser scanning fluorescence microscopic observations were performed using an LSM 710 equipped with ZEN 2008 operation software (Carl Zeiss Inc., Tokyo, Japan). The specimens of the complexes that were immersed in the washing buffer were mounted on the microscopy stage. An objective with a 10-fold magnification and a 0.3 numerical aperture was used to detect the fluorescence emission excited by an Ar 458 nm laser beam (25 mW). All the fluorescence images were collected using a 500–600 nm filter set in a 1024 × 1024 image size at 12 bits and a scan speed of seven units.

The FRAP measurements were carried out for three separately prepared phospholipid-Q Sepharose complexes. Fluorescence from the NBD-PE in the phospholipid-Q Sepharose complexes was collected using the same LSM 710 confocal microscope as described in Section 2.3. A specific area of the selected imaging circle region (54.8 μm^2) in the top of the complexes was photobleached using 80 iterations of a 100% intensity Ar laser (λ_{ex} 458 nm at 25 mA tube current) to obtain 50 ± 10% bleaching compared to the prebleach intensity. The photobleaching started just after the initial scan during a time series at 3 min or shorter intervals. The fluorescence intensity data were normalized to the fluorescence in the first prebleach image and corrected for loss during the recovery imaging by adding back the fluorescence lost from an adjacent unbleached structure. Data from three assays were averaged and plotted versus time in seconds in Figs. 2 and 3.

The lateral diffusion coefficients were calculated according to the procedure described by Yguerabide et al. [25,26]. The analysis was based on the observation that a plot of the reciprocal function $f(t) = FI_O/(FI_O - FI_t) = a \times t + b$ (FI_O: the fluorescence intensity of the circular region before photobleaching, FI_t: the fluorescence intensity at time t after photobleaching) was linear over 30 min when (a) recovery involves a single diffusion coefficient and (b) there is no membrane flow. The time ($t_{1/2}$) required for the photobleached fluorescence to reach 50% of the complete recovery value FI_O was calculated from the ratio of the intercept (b value) to slope (a value) of the linear plot of t versus $FI_O/(FI_O - FI_t)$. The lateral diffusion coefficient (D) was calculated using the equation, $t_{1/2} = \omega^2/4D$, where ω is the effective radius of the photobleached region (area 54.8 μm^2).