

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

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A B S T R A C T

Supported phospholipid bilayer membranes on polysaccharide-based cationic polymer beads (cationic group: $-[\text{OCH}_2\text{CH}(\text{OH})\text{CH}_2]_2\text{N}^+(\text{CH}_3)_3 \cdot \text{X}^-$, 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.

Keywords: FRAP; membrane fluidity; phospholipid; polysaccharide; supported lipid membrane.

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 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: – $[\text{OCH}_2\text{CH}(\text{OH})\text{CH}_2]_2\text{N}^+(\text{CH}_3)_3 \cdot \text{X}^-]$ 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-phosphatidyl-ethanolamine (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 μ mol) 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 $\mu\text{g/mL}$ -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, and the phospholipid-Q Sepharose complexes were prepared 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 ($54.8 \mu\text{m}^2$) 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 $50 \pm 10\%$ 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 $f(t) = \text{FI}_0 / (\text{FI}_0 - \text{FI}_t) = a \times t + b$ (FI_0 : 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_0 was calculated from the ratio of the intercept (b value) to slope (a value) of the linear plot of t versus $\text{FI}_0 / (\text{FI}_0 - \text{FI}_t)$. 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 $54.8 \mu\text{m}^2$).

3. Results and discussion

The PS/PC-Q Sepharose complexes were prepared by a previously reported similar procedure [20,22]; the small unilamellar vesicle suspensions from phosphatidylcholine (PC) and phosphatidylserine (PS) were mixed with the Q Sepharose beads at a temperature in which the PC and PS are in lamella states. By using this methodology, we expected the

adhesion of the liposomes onto the surface of the Q Sepharose beads by electrostatic attractive forces, and subsequent liposomal membrane fusion and restructuring into the continuous phospholipid bilayer membrane structure. The zeta potential and particle diameter of the liposomes used for the preparation of the phospholipid-bead complexes are summarized in Table 1. All the liposomal vesicles were in the colloidal particle size range from 50 to 80 nm. The magnitude of the zeta potential, a measure of the mobility of colloidal particles in an electric field, increased with an increase in the proportion of anionic PS in the liposomes. To estimate the binding amount of the phospholipids, the liposomal suspensions before and after mixing with the Q Sepharose beads were subjected to a colorimetric determination of phosphorous from the phospholipids. After acid digestion of the phospholipid vesicle suspensions, the contents of the phosphorous bound to the complexes were colorimetrically determined on the basis of the complexation of the phosphate ion with the vanadium (V) ion [24]. With the increasing fraction of PS in the liposomes, the amount of phosphorous in the phospholipids-Q Sepharose complexes also increased (Table 2). This indicates that the electrostatic attractive interaction between the anionic PS and cationic Q Sepharose plays a role in the formation of the phospholipids-Q Sepharose complexes. The smaller occupied area of PS molecule could give a possible explanation for the observed difference in the phosphorous content; the surface density of the phospholipid molecules in the complexes with high PS molar fraction could be higher than that with high PC molar fraction.

To analyze the location of the phospholipid bilayer membranes bound to the Q Sepharose beads, a fluorescent membrane probe, NBD-PE (0.1 mol%), was spiked into the liposomal vesicles before the formation of the complex, and then the resultant was subjected to confocal laser scanning fluorescence microscopic observations. In the cross-sectional fluorescence images of the PS(25)/PC(75)-Q Sepharose complex, a single circular fluorescence from the NBD-PE was clearly found along the outer surface of the spherical Q Sepharose beads, while

no fluorescence was detected from any inside regions of the beads (Fig. 1 A). Similar localization trends in the fluorescence from the NBD-PE probe molecules were also observed for the other three PS/PC-Q Sepharose complexes (Figs. 1B–D). No fluorescence was observed for the phospholipid-free Q Sepharose when it was treated with the same concentration of NBD-PE. We also tried to prepare a similar PS(50)/PC(50) or PS(100)/PC(0) complex by the same procedure using a spherical glass bead (105–125 μm in diameter) instead of Q-Sepharose beads, and then observe the glass beads by the confocal fluorescence microscopy. No membrane probe NBD-PE fluorescence was observed in any regions of the glass beads, indicating that the PS/PC molecules were not able to form vesicular structures on the glass beads. This fact suggests that the electrostatic attractive interaction between the PS/PC molecules and Q-Sepharose plays an essential role in the formation of the phospholipid bilayer membranes on the surface of the Q-Sepharose bead (data not shown). Based on these fluorescence microscopic analyses, the continuous phospholipid bilayer membrane structures without any remarkable defects and/or edges were thought to reconstitute around the Q Sepharose beads. In the PS/PC-Q Sepharose complexes, the PS molecules would preferentially reside in the inner leaflet of the membranes due to its anionic character rather than the neutral PC molecules. Similarly, the PS also normally localize in the inner leaflet of the plasma membranes of living cells. Our preparation method may provide a better mimic of the actual plasma membrane in comparison to the conventional liposomes.

The fluidity and/or the mobility of the individual phospholipid molecules are distinctive features required for biomimetic membrane systems. In fact, the membrane fluidity is critical in many living cell processes, such as the functions of membrane proteins and the signal transduction [27]. Many SLM systems are known to retain the fluidity and lateral mobility of the phospholipid bilayer membranes [6,28]. This is thought to be due to a thin layer of water

(~1–2 nm) between the bilayer membrane and the supporting material that acts as a lubricant and allows for the diffusion of the phospholipid molecules in both membrane leaflets [8]. Fluorescence recovery after photobleaching (FRAP) using fluorescence membrane probes is a technique to measure the membrane fluidity, and the extent to which phospholipid diffusion in the bilayer membranes might be constrained [29]. The recovery of the fluorescence within the photobleached region is due to random motion or diffusion of the unbleached fluorescent lipid molecules from the surroundings over time. The fluorescence intensity-time profiles of the PS(25)/PC(75)-, PS(50)/PC(50)-, PS(75)/PC(25)- and PS(100)/PC(0)-Q Sepharose complexes in the FRAP measurements are shown in Fig. 2. The fluorescence intensities of the photobleached regions in the four complexes similarly recovered, which demonstrates that the phospholipid bilayer membranes formed on the Q Sepharose surfaces retain the fluidity to some extent. The time required for the photobleached fluorescence to reach 50% of the complete recovery value ($t_{1/2}$) and the apparent lateral diffusion coefficient (D) were calculated from the FRAP measurement results (Table 3). The observed D values for all the PS/PC-Q Sepharose complexes were one-third or less than that reported for the 1-palmitoyl-2-oleoyl-PC giant unilamellar vesicles without supporting solid materials [$33 \pm 18 (\times 10^3, \mu\text{m}^2/\text{s})$] [30]. If the Q Sepharose surfaces mostly adsorb intact liposomal vesicles without losing their integrity, the FRAP of the PS/PC-Q Sepharose complexes may generate comparable D values. Thus, the D values for these complexes imply that the phospholipid molecules are apparently not in the forms of intact liposomal vesicles used for the preparation of the complexes. Such less-fluid membrane structures in these complexes would be responsible for the immobilization of the phospholipid bilayer membranes by the electrostatic attractive forces between the PS and Q Sepharose. A higher PS fraction of the liposomes can provide a higher number of electrostatic interaction sites for the phospholipid membranes formed on the surface of the Q Sepharose. Among the four complexes, the D value for the

PS(100)/PC(0)-Q Sepharose complex with the highest PS fraction was lower than those for the other three complexes with lower PS fractions. These results indicated that the phospholipid bilayer membranes are immobilized on the surface of the Q Sepharose bead with some structural constraints due to the electrostatic attractive forces.

To verify the effects of the supporting material on the fluidity of the phospholipid membrane, the FRAP profile of the PS(50)/PC(50)-Q Sepharose complex was compared to that of the SA11A complex prepared from the liposomal vesicles with the same phospholipid composition. SA11A is a polystyrene-based quaternary ammonium-type organic polymer bead with a 350–550 μm diameter. Its ammonium nitrogen content (0.85 mmol/mL-bead in wet state) is nearly fourfold higher than that of the Q Sepharose. Thus, the surface density of the ammonium groups of the SA11A is higher than that of the Q Sepharose. Shown in Fig. 3 are the fluorescence intensity-time profiles of the PS(50)/PC(50)-Q Sepharose and PS(50)/PC(50)-SA11A complexes in the FRAP measurements. The fluorescence recovery of the PS(50)/PC(50)-Q Sepharose complex was much faster than that of the PS(50)/PC(50)-SA11A complex, even though these two complexes have the same phospholipid composition. Such a faster fluorescence recovery of the PS(50)/PC(50)-Q Sepharose complex can also be seen from the actual fluorescence images; the fluorescence of the photobleached region surrounding the circle almost recovers in the (A) 4 image, but not in the (B) 4 image of the PS(50)/PC(50)-SA11A complex. The surface adhesion energy is thought to be the dominant factor, largely dependent on electrostatics and van der Waals forces [19, 31], although the rupture mechanism is still a subject of active research. When the adhesion force is higher, the phospholipid bilayer membrane can strongly adhere to the surface and lose lateral fluidity [16]. This appears to be the case for the PS(50)/PC(50)-SA11A complex, since the adhesion force becomes high due to the high density of the electrostatically interactive ammonium groups on the surface of the SA11A. The interaction energy could also increase to allow

vesicle rupture and fuse by mixing charged phospholipids [32,33]. Overall, the higher fluidity of the phospholipid bilayer membrane formed on the Q Sepharose is definitely attributable to the low ionic group density, a highly hydrated state provided by the polysaccharide backbone structure and the small particle diameter. This fact suggests that the membrane fluidity of the ionic polymer bead-supported phospholipid systems is controllable by selecting the appropriate supporting material.

Liposomal vesicles in the suspension state easily tend to aggregate and/or fuse to each other, followed by phospholipid precipitation within several days after their preparation. When the PS(50)/PC(50)-Q Sepharose complex was kept in 50 mM Tris-HCl buffer (pH 8.5) at ambient temperature for one month, no significant changes in its appearance were observed at all under a light microscope. The fluidity of the phospholipid bilayer membrane formed on this complex was then periodically followed by the FRAP measurement (Table 4). The parameters $t_{1/2}$ and D for the PS(50)/PC(50)-Q Sepharose complex at day 10 were comparable to those just after the preparation of the complex. No significant changes in the two parameters were observed for at least ten days after the preparation, demonstrating that the phospholipid bilayer membranes formed on the Q Sepharose is fairly stable in comparison to the conventional liposomes without solid supporting materials. At day 14, the $t_{1/2}$ value became higher and the D value decreased to less than one-fourth that at day 10, which suggests that even longer-term storage finally causes structural changes in the phospholipid bilayer membranes formed on the Q Sepharose.

4. Conclusions

In this study, we prepared the phospholipids-polysaccharide-based cationic bead complex by varying the phosphatidylserine and phosphatidylcholine composition using a reconstitution

method based on the electrostatic attractive forces. Confocal microscopic images using fluorescent membrane probes revealed that the phospholipid bilayer membranes were located on the outer surface of the Q Sepharose bead without any remarkable discontinuity. Based on the FRAP experiment results, the lateral diffusion coefficients (D) for the PS/PC-bead complexes were lower than that for PC 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 the PS and the beads. The D values for the 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 beads were much more fluid than those on a polystyrene-based cationic bead. Furthermore, the polysaccharide-based cationic bead could possibly provide stable and fluid phospholipid bilayer membranes. The appropriate combination of an ionic polymer bead and the oppositely charged lipid molecule could possibly provide an SLM system with a fluid bilayer membrane structure that can mimic biomembranes.

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Table 1

Zeta potential and particle diameter of liposomes used for the preparation of the phospholipid-Q Sepharose complexes.

Phospholipid composition ^a (mol%)	Zeta potential (mV)	Particle diameter (nm)
PS(25)/PC(75)	-24.60 ± 5.89	78.50 ± 12.43
PS(50)/PC(50)	-38.80 ± 3.38	72.75 ± 10.36
PS(75)/PC(25)	-48.05 ± 4.08	66.20 ± 10.19
PS(100)/PC(0)	-61.45 ± 1.52	54.50 ± 5.26

^a PS: phosphatidylserine and PC: phosphatidylcholine.

Data expressed mean ± standard error ($n = 9$).

Table 2

Phosphorous content of the phospholipid-Q Sepharose complexes with varying lipid composition of liposomes.

Phospholipid composition ^a (mol%)	Phosphorous content (µg/mL-Q Sepharose)
PS(25)/PC(75)	38.6 ± 5.2
PS(50)/PC(50)	67.4 ± 8.1
PS(75)/PC(25)	116.2 ± 15.4
PS(100)/PC(0)	244.9 ± 32.8

^a PS: phosphatidylserine and PC: phosphatidylcholine.

Data expressed mean ± standard error ($n = 9$).

Table 3

Fluorescence recovery after photobleaching parameters of PS/PC-Q Sepharose complexes with various phospholipid compositions.

Phospholipid composition ^a (mol%)	$t_{1/2}$ ^b (s)	D ^c ($\times 10^3$, $\mu\text{m}^2/\text{s}$)
PS(25)/PC(75)	651.2 \pm 190.2	9.81 \pm 2.18
PS(50)/PC(50)	409.7 \pm 49.8	13.02 \pm 2.60
PS(75)/PC(25)	378.8 \pm 55.3	12.99 \pm 1.77
PS(100)/PC(0)	791.5 \pm 154.4	6.08 \pm 1.45

^a PS: phosphatidylserine and PC: phosphatidylcholine.

^b Time required for the photobleached fluorescence to reach 50% of complete fluorescence recovery. Relation coefficients for linear regression analyses of the plots [$FI_t / (FI_0 - FI_t) = a \times t + b$] were greater than 0.9524.

^c Apparent lateral diffusion coefficient.

Data expressed mean \pm standard error ($n = 9$).

Table 4

Changes in fluorescence recovery after photobleaching parameters of PS(50)/PC(50)-Q Sepharose complex after preparation.

Time after preparation (d)	$t_{1/2}$ ^a (s)	D ^b ($\times 10^3$, $\mu\text{m}^2/\text{s}$)
0 (just after preparation)	373.1 \pm 61.8	13.01 \pm 1.45
1	421.3 \pm 80.4	11.77 \pm 2.04
3	522.1 \pm 151.9	11.67 \pm 2.63
5	497.2 \pm 83.7	10.55 \pm 2.04
7	457.5 \pm 97.4	12.28 \pm 2.36
10	421.8 \pm 64.8	11.72 \pm 1.36
14	1829.0 \pm 234.9	2.46 \pm 0.29

^a Time required for the photobleached fluorescence to reach 50% of complete fluorescence recovery. Relation coefficients for linear regression analyses of the plots $[\text{FI}_0 / (\text{FI}_0 - \text{FI}_t) = a \times t + b]$ were greater than 0.9891.

^b Apparent lateral diffusion coefficient.

Data expressed mean \pm standard error ($n = 9$).

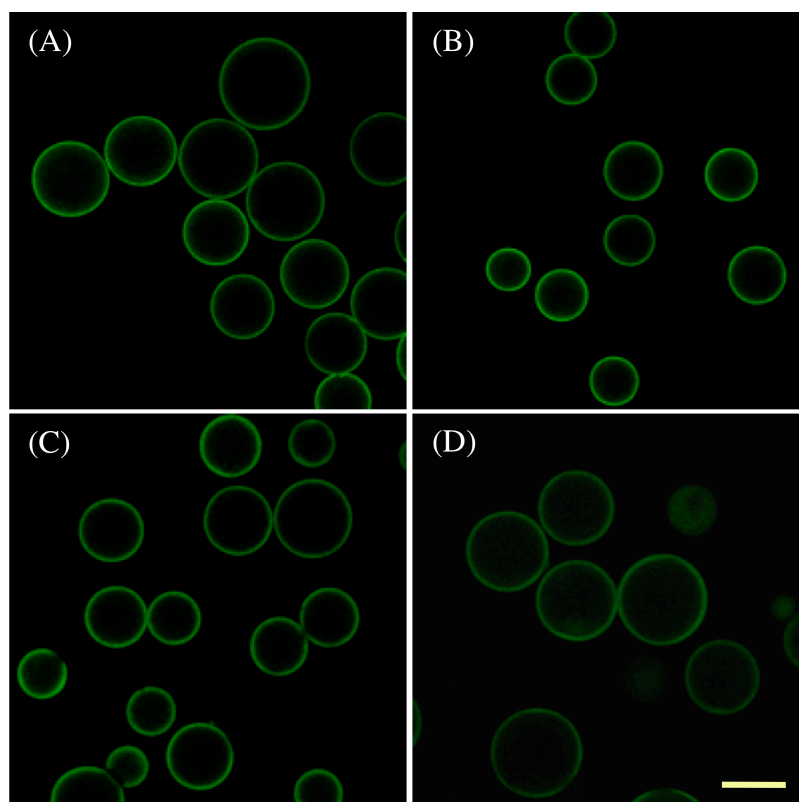


Fig. 1. Cross-sectional fluorescence images of NBD-PE-labeled phospholipid-Q Sepharose complexes. Phospholipid composition used for the preparation of the complexes (PS/PC by mol%): (A) PS(25)/PC(75), (B) PS(50)/PC(50), (C) PS(75)/PC(25) and (D) PS(100)/PC(0). Pale yellow scale bar in the panel represented 100 μm . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

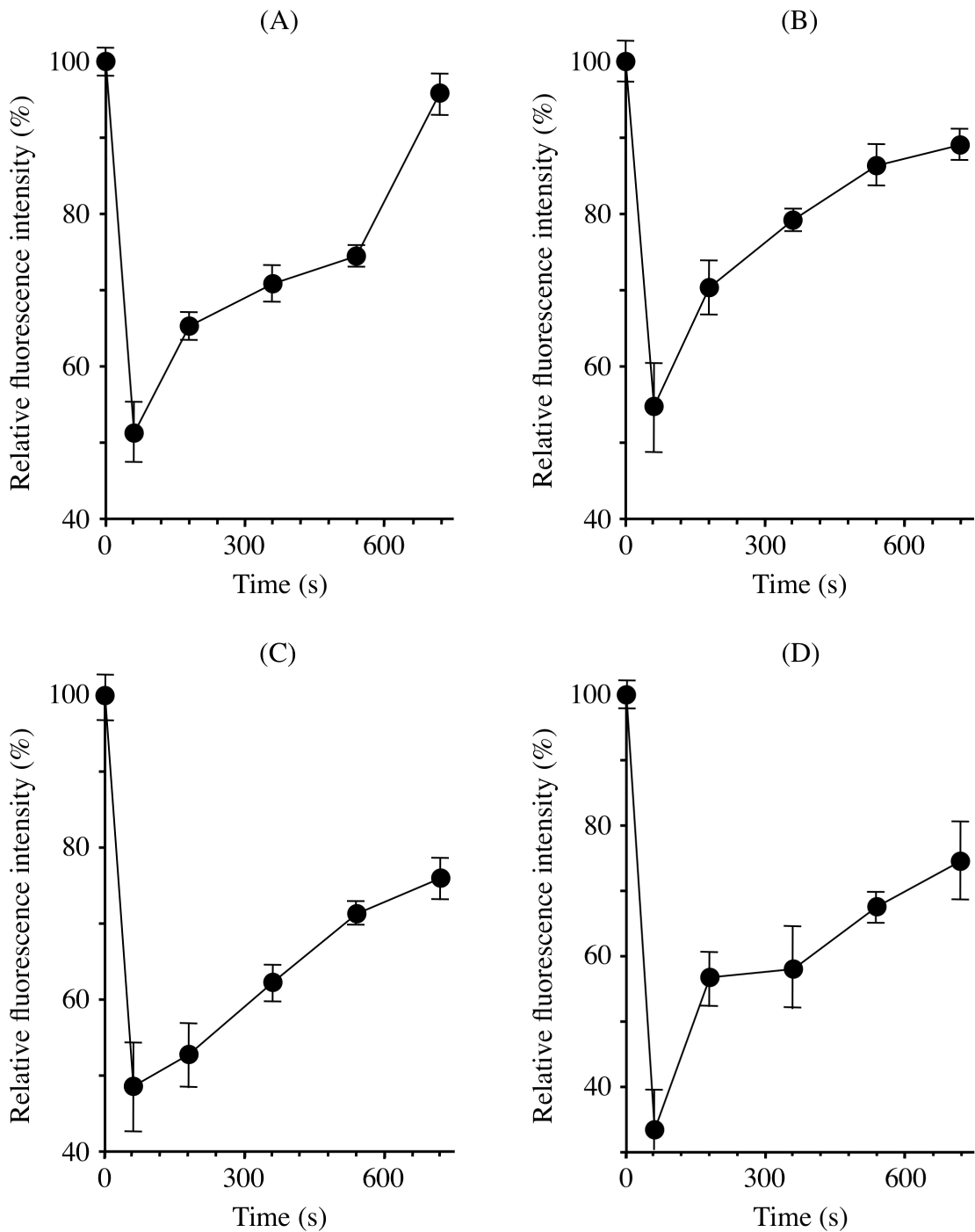


Fig. 2. Fluorescence intensity-time profiles of NBD-PE labeled phospholipid-Q Sepharose complexes in FRAP measurements. Phospholipid composition used for the preparation of the complexes (PS/PC by mol%): (A) PS(25)/PC(75), (B) PS(50)/PC(50), (C) PS(75)/PC(25) and (D) PS(100)/PC(0). The fluorescence intensity of the region before photobleaching (FI_0) was defined as 100%. Data expressed mean \pm standard error ($n = 9$).

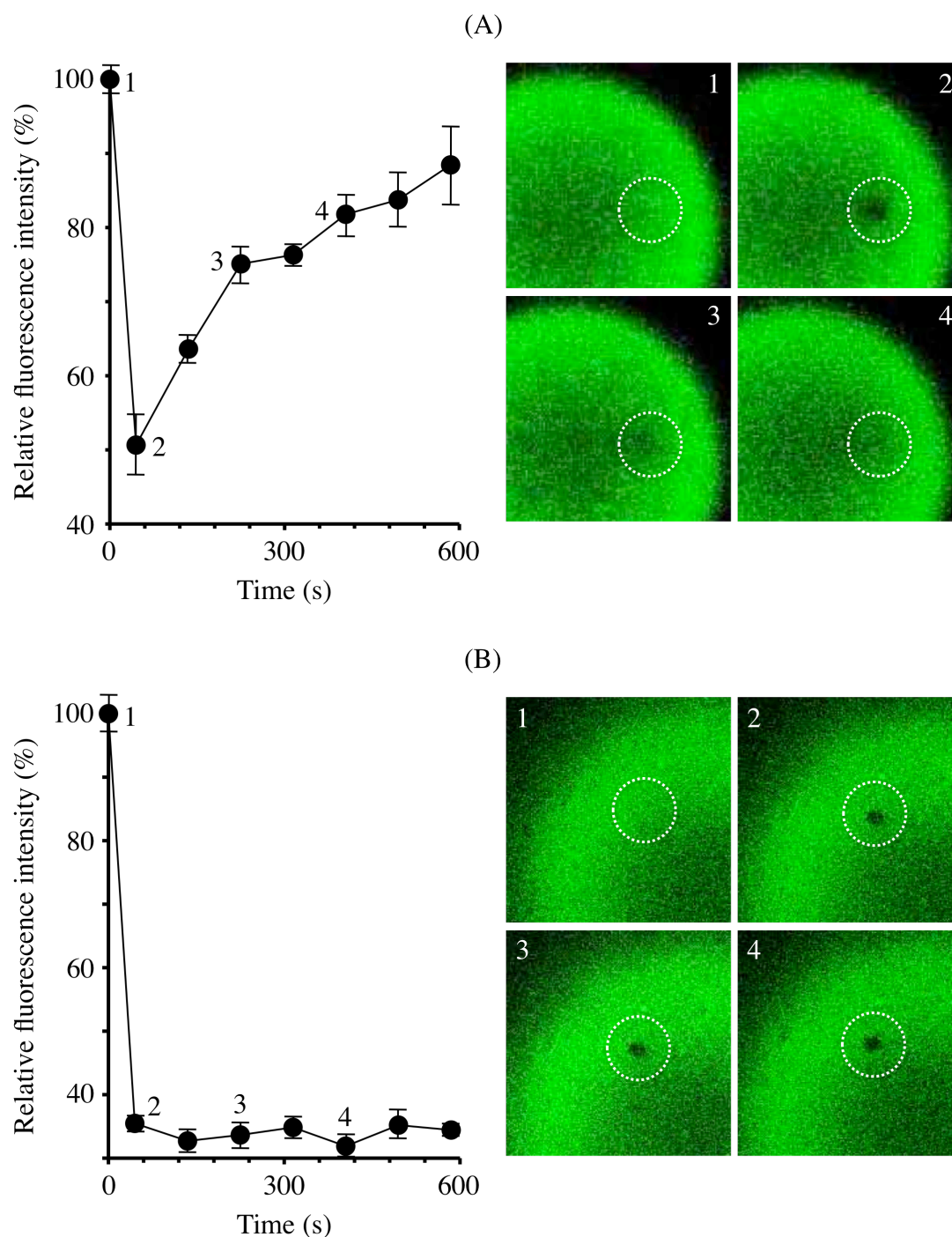


Fig. 3. Fluorescence intensity-time profiles of NBD-PE labeled phospholipid-Q Sepharose (A) and -SA11A (B) complexes in FRAP measurements. Phospholipid composition used for the preparation of the complexes (PS/PC by mol%): PS(50)/PC(50). The fluorescence intensity of the region before photobleaching (FI_0) was defined as 100%. Pictures were the actual fluorescence images of specimens at appropriate times, and numbers given in the pictures corresponded to those in the fluorescence intensity-time profile panels (left).

Diameter of circular photobleached region: 17.45 μm . White dotted lines in the pictures surrounded the outside the photobleached regions. Data expressed mean \pm standard error ($n = 9$).