Fluid and Air-Stable Lipopolymer Membranes for Biosensor Applications

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The behavior of poly(ethylene glycol) (PEG) conjugated lipids was investigated in planar supported egg phosphatidylcholine bilayers as a function of lipopolymer density, chain length of the PEG moiety, and type of alkyl chains on the PEG lipid. Fluorescence recovery after photobleaching measurements verified that dye-labeled lipids in the membrane as well as the lipopolymer itself maintained a substantial degree of fluidity under most conditions that were investigated. PEG densities exceeding the onset of the mushroom-to-brush phase transition were found to confer air stability to the supported membrane. On the other hand, substantial damage or complete delamination of the lipid bilayer was observed at lower polymer densities. The presence of PEG in the membrane did not substantially hinder the binding of streptavidin to biotinylated lipids present in the bilayer. Furthermore, above the onset of the transition into the brush phase, the protein binding properties of these membranes were found to be very resilient upon removal of the system from water, rigorous drying, and rehydration. These results indicate that supported phospholipid bilayers containing lipopolymers show promise as rugged sensor platforms for ligand−receptor binding.

Introduction

Supported phospholipid bilayers[1−4] could potentially serve as highly selective sensor devices for a variety of biological analytes.[5−7] Phospholipid membranes are attractive because of their two-dimensional fluidity,[8−11] which allows the individual molecular constituents to rearrange laterally just as they would on the surface of a cell membrane.[3,12−16] Moreover, supported bilayers are quite resistant to nonspecific protein adsorption and biofouling.[17] Unfortunately, such platforms have not been widely exploited for practical biosensors. One problem stems from their instability upon exposure to air, which causes the fluid lipid membrane to reorganize and/or delaminate from the surface.[18−25] Since the lipids are only held to the substrate by van der Waals forces,[26] the thin film can curl up and peel away from the aqueous/solid interface as air is introduced.[27] Moreover, any bilayer segments remaining behind at the solid/air interface probably reorganize to have their hydrophobic alkyl chains face toward the air.

In an effort to overcome these limitations we recently designed a supported membrane containing a close-packed protein layer linked to its outer surface.[27] This modification increased the bending elastic modulus[28,29] of the bilayer and thereby substantially raised the barrier to delamination. Also, the presence of the hydrophilic protein layer inhibited lipid reorientation after air exposure. When the system was pulled through the air/water interface, the bilayer remained intact on the surface and a thin water film could be visibly seen above the patterned bilayer regions. Furthermore, the supported membrane was not destroyed even when dried under a stream of flowing nitrogen. In fact, it remained two-dimensionally fluid in the presence of humid air as well as when it was reintroduced to bulk water. Unfortunately, such a system is unsuitable for sensor design because the tightly packed proteins leave no room for the binding of additional analyte species from aqueous solution when binding ligands are incorporated into the membrane. It would therefore be desirable to design a protective coating for the supported

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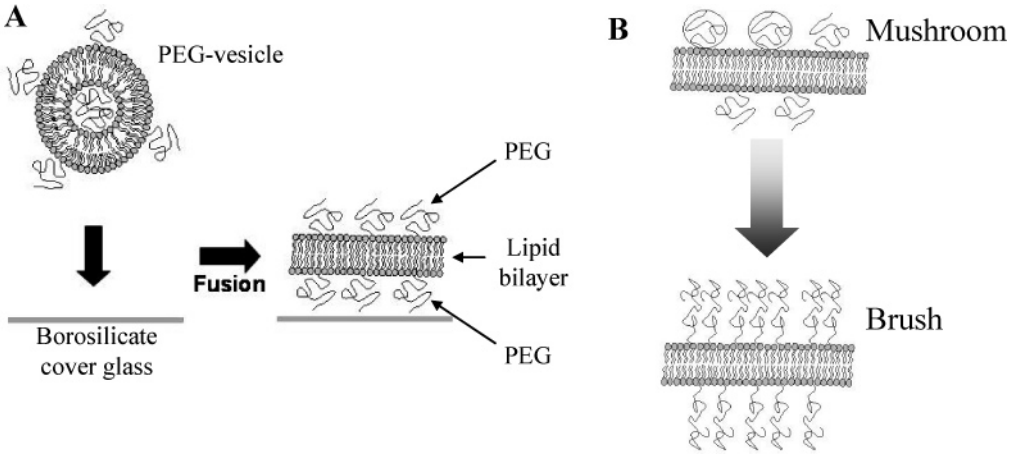

Figure 1. (A) Fusion of small unilamellar pegylated vesicles to a planar borosilicate substrate. The PEG-containing vesicles fuse to the substrate to form a supported bilayer. The polymer is presented in both leaflets of the membrane. (B) The mushroom-to-brush transition occurs as the PEG density increases. The interaction between the polymer chains leads to an elongation of the molecules known as the brush conformation.

bilayer that would afford air stability while still allowing target analytes to bind to ligands present in the membrane.

To achieve this goal, we have incorporated poly(ethylene glycol) phosphatidylethanolamine (PEG-PE) lipopolymers[30] into supported lipid membranes[31,32] (Figure 1A). An important property of PEG coatings is their conformational dependence on surface density.[33−35] At low surface coverage, PEG exists in a random coil or mushroom conformation.[36] Above a threshold density, the polymer chains begin to interact with one another and undergo a broad phase transition from the globular state to an extended or brushlike configuration (Figure 1B).[37] This transition, which depends on the polymer chain length,[38,39] is denoted as the mushroom-to-brush phase transition.[40]

The choice of a pegylated bilayer for stable biosensor design is inspired by the cell glycocalix,[41] which is a thick carbohydrate film consisting of oligosaccharides, polysaccharide chains, and adsorbed proteoglycans that can rise tens of nanometers above the plasma membrane.[42] There is some evidence in the literature that these sugar layers play a role in stabilizing cell membranes in the dry state.[43−45] On the other hand, when cells are fully hydrated, the carbohydrate films are presumably flexible enough to allow soluble proteins to diffuse through them and bind to underlying membrane constituents.[46] It has been proposed that a PEG coating can serve as a simple mimic of a glycocalix on phospholipid membranes.[47] We therefore reasoned that under the appropriate conditions the lipopolymer might protect the underlying SLBs against delamination and damage upon air exposure while still allowing proteins and other aqueous analytes to be facilely recognized by the appropriate binding ligands present in the phospholipid bilayer.

Herein we demonstrate that the surface density and chain length of PEG-PE are critical parameters in affording air stability. Specifically, the lipopolymer protects the membrane in the more tightly packed brush conformation, while delamination and damage occur in the mushroom state. Furthermore, PEG coatings do not prevent proteins such as streptavidin from binding with biotinylated lipids (biotin-PE) present within the membrane under the PEG surface densities that were tested. The results also indicate that pegylated bilayers in the brush conformation do not significantly change their ligand−receptor binding properties even after the supported membrane has been removed from bulk water, rigorously dried, and subsequently reintroduced to aqueous solution.

Experimental Section

Materials. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(poly(ethylene glycol))] (PEG-DPPE), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(poly(ethylene glycol))] (PEG-DSPE), and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(poly(ethylene glycol)] (PEG-DOPE) were purchased from Avanti Polar Lipids (Alabaster, AL) with PEG molecular weights of 550 and 2000. 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(cap biotinyl) (sodium salt) (biotin-cap-PE), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[amino-(poly(ethylene glycol))2000] (ammonium salt) (NH_2-PEG^{2000}-DSPE), and L-α-phosphatidylcholine from egg (egg PC) were also purchased from Avanti. *N*-(Texas Red sulfonyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Texas Red DHPE), Alexa Fluor-488-labeled streptavidin, and Alexa Fluor-594 succinimidyl ester were obtained from Molecular Probes (Eugene, OR). Purified water, which was acquired from a NANOpure ultrapure water system (Barnstead, Dubuque, IA), had a minimum resistivity of 18.2 MΩ·cm. This water was used in the

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preparation of all buffer solutions. Phosphate-buffered saline (PBS) was prepared using 10.0 mM sodium phosphate with the addition of 150 mM NaCl (Sigma-Aldrich). The pH was adjusted to 7.4 by the addition of NaOH (EM Science). Poly(dimethylsiloxane) (PDMS) was used to fabricate PDMS wells and microfluidic devices. The polymer and cross-linker were purchased from Dow Corning (Sylgard Silicone Elastomer-184, Krayden Inc.). Glass microscope slides were purchased from VWR International and were cleaned and annealed according to established procedures.[7]

Preparation of Pegylated Unilamellar Vesicles and Bilayer Formation. Small unilamellar vesicles[48] were prepared from egg PC, PEG-PE lipopolymers, and 0.5 mol % Texas Red DHPE, which was incorporated as a fluorescence probe. The desired mole fraction of PEG^{550}-DOPE or PEG^{2000}-DOPE was mixed with egg PC and dye-conjugated lipid in chloroform. The solvent was then evaporated under a stream of dry nitrogen followed by desiccation under vacuum for 4 h. Rehydration of the lipids was performed in PBS solution at pH 7.4. After 10 freeze−thaw cycles the large vesicles were extruded through a polycarbonate filter, which had an average pore size of 50 nm. Small unilamellar vesicles prepared by this method were 70 ± 10 nm in diameter as determined by dynamic light scattering using a 90Plus particle size analyzer from Brookhaven Instruments Corp. We found that the presence of the PEG moiety only had a minor effect on the size of the extruded vesicles. Moreover, the vesicles containing PEG seemed to be somewhat more stable than those without PEG as judged by light scattering measurements made two months after extrusion.

All PEG-PE-containing vesicles were delivered to the surface of planar glass microscope slides in a PDMS/glass microfluidic device format using previously described techniques.[43−45] It should be noted that previous studies indicate that free PEG in solution can help to promote vesicle fusion to oxide supports.[49−51] Furthermore, the covalently attached PEG moiety should increase the hydrophilic spacing between the lower bilayer leaflet and the substrate.[32,52] After a 30 min incubation period, the microchannels and microwells were thoroughly rinsed with the appropriate buffer or purified water. The samples were placed under an inverted epifluorescence Nikon Eclipse TE2000-U microscope and observed with a 10× objective. Images were obtained using a MicroMax 1024b CCD camera (Princeton Instruments), and data analysis was performed with MetaMorph software (Universal Imaging).

PEG Labeling. Dye labeling of NH_2-PEG^{2000}-DSPE was accomplished by first preparing small unilamellar vesicles of egg PC with various quantities of this lipopolymer (0.5−5 mol %). The labeling reaction was carried out by incubating the SUVs with Alexa Fluor-594 succinimidyl ester for 2 h at room temperature. The succinimidyl ester reacted with the primary amine to form a covalent linkage between the amine PEG chain and the fluorescent dye. Any unreacted dye was separated from the labeled vesicles via size exclusion chromatography. It should be noted that this procedure will label about half the amines present in the vesicles.

Fluorescence Recovery after Photobleaching (FRAP). FRAP curves[53] and NMR[54] measurements have been previously utilized to assay the lateral fluidity of polymer-tethered bilayer systems. Herein, FRAP curves were obtained by exposing the sample to laser irradiation from a 2.5 W mixed gas Ar^+/Kr^+ laser (Stabilite 2018, Spectra Physics). Planar bilayer samples were irradiated at 568.2 nm with 100 mW of power for times not exceeding 1 s. A 17.7 μm full width at half-maximum bleach spot was made by focusing the light onto the bilayer through the 10× objective. The recovery of the photobleached spot was monitored by time-lapse imaging. The fluorescence intensity of the bleached spot was then determined as a function of time after background subtraction and intensity normalization. All fluorescence recovery curves were fit to a single exponential to obtain both the mobile fraction of dye-labeled lipids and the half-time of recovery, $t_{1/2}$, following standard procedures.[55]

Drying Procedures for Bilayers. Before drying, the pegylated bilayers were thoroughly rinsed with purified water to remove salt from the buffer solutions. The excess water and the PDMS stamp were removed, and the supported membrane was dried under a stream of dry nitrogen. The bilayers were subsequently imaged to access damage caused by drying. The bilayers were stored for time periods up to 24 h in a dust-free container before rehydration. Rehydration was performed with either purified water or PBS buffer at pH 7.4. A FRAP curve was obtained to ascertain whether the lipid bilayers regained lateral mobility.

Ligand−Receptor Binding. Biotin−streptavidin binding was employed as a model of ligand−receptor interactions in the presence of the lipopolymer. Vesicles were prepared by adding 1 mol % biotin-cap-PE to a given lipid mixture. Binding was assayed by flowing 0.20 mg/mL Alexa Fluor-488 streptavidin over a biotinylated membrane and incubating the protein solution for 30 min followed by copious rinsing with PBS buffer at pH 7.4. The bilayers were imaged under a 10× objective, and the fluorescence intensity of the bound streptavidin was determined after background subtraction and image normalization.

Results

Vesicle Fusion with PEG-PE. Supported phospholipid bilayers containing various concentrations of PEG-PE in egg PC with 0.5 mol % Texas Red DHPE were characterized in a first set of experiments. The bilayers contained lipopolymer with either 12 (PEG^{550}-DOPE) or 45 (PEG^{2000}-DOPE) ethylene oxide repeat units. All supported bilayers appeared to be uniform down to the diffraction limit under fluorescence microscopy using a 100× objective. FRAP data were obtained for each system, and an example with 5 mol % PEG^{550}-DOPE is shown in Figure 2A. In this case, the mobile fraction of Texas Red DHPE was 98 ± 1% and the $\tau_{1/2}$ value was 23 s. The diffusion constant, D, could be obtained from the $\tau_{1/2}$ value by employing the following equation:

$$D = \frac{w^2}{4\tau_{1/2}\gamma_D}$$

where w is the full width at half-maximum of the Gaussian profile of the focused beam and γ_D is a correction factor that depends on the bleach time and the geometry of the laser beam.[55] For the data in Figure 2A the value of the diffusion constant, $D = (3.7 \pm 0.2) \times 10^{-8}$ cm^2/s, was obtained by using $w = 17.7$ μm and $\gamma_D = 1.1$. In the case of 5 mol % PEG^{2000}-DOPE (Figure 2B), the diffusion constant was $(4.0 \pm 0.2) \times 10^{-8}$ cm^2/s with 98 ± 1% recovery. Both values were identical within experimental error to data obtained with pure egg PC bilayers ($D = (4.0 \pm 0.2) \times 10^{-8}$ cm^2/s with 98 ± 1% recovery).

The effect of the lipopolymer density on the diffusion constant of the Texas Red-labeled lipids is shown in Figure 3. As can be seen, there is little if any dependence of the measured value of this constant on the PEG^{550}-DOPE density. Moreover, the mobile fraction of the probe remained at ∼98% for all concentrations employed. Analogous experiments were performed with PEG^{2000}-DOPE (Figure 3). In contrast to the case with PEG^{550}, the results for the highest lipopolymer densities did show a slowing of the diffusion constant. However, this only

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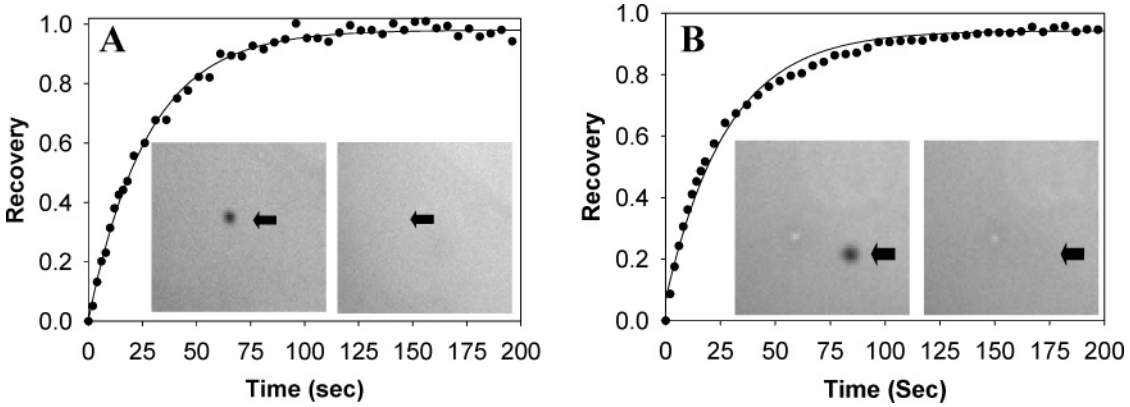

Figure 2. FRAP curves from PEG bilayers on planar borosilicate substrates: (A) recovery curve for a membrane containing 5 mol % PEG^{550}-DOPE in egg PC with 0.5 mol % Texas Red DHPE; (B) same conditions as in **(A)**, but with 5 mol % PEG^{2000}-PE. The mobile fraction of the dye moiety in the bilayers is 98 ± 1% in both cases.

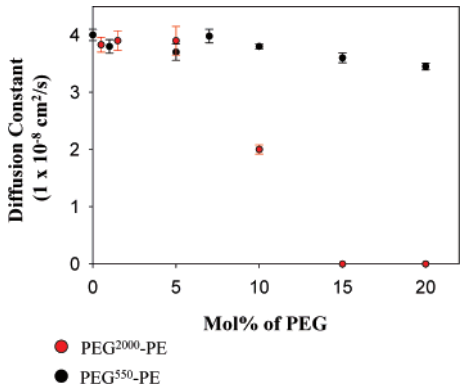

Figure 3. Texas Red DHPE diffusion constants in egg PC bilayers with various concentrations of PEG^{550}-PE (black dots) and PEG^{2000}-PE bilayers (red dots).

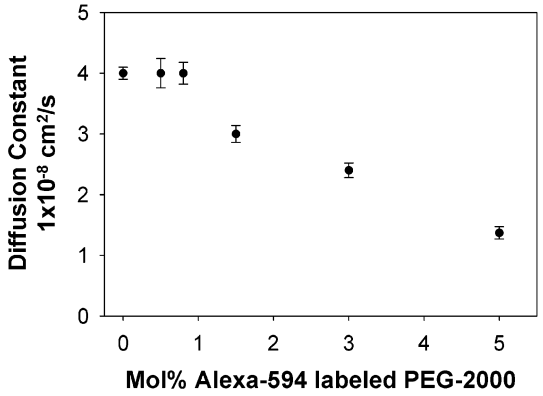

Figure 4. Diffusion constants of Alexa Fluor-594-labeled PEG^{2000}-DSPE in egg PC bilayers as a function of the lipopolymer density.

occurred at lipopolymer concentrations of at least 10 mol %. It should be noted that the onset of the mushroom-to-brush phase transition in liposomes occurs at 1.4 mol % for PEG^{2000}-PE.[30] Therefore, the initial drop in the diffusion constant occurred well into the brush transition. Additional FRAP experiments were performed with egg PC membranes containing various concentrations of PEG^{2000}-DPPE, PEG^{2000}-DSPE, and PEG^{550}-DPPE. The results showed that the absence of double bonds and the lengthening of the alkyl chains had no noticeable effect on either the diffusion constants or mobile fractions measured within experimental error.

To further characterize membrane fluidity, we investigated the diffusion of the PEG lipopolymer itself within the egg PC bilayer. This was accomplished by employing amine-terminated PEG^{2000}-DSPE (NH_2-PEG^{2000}-DSPE) that was labeled with Alexa Fluor-594. Supported lipid bilayers were formed on glass in the same fashion described above. Diffusion constants for the dye-labeled PEG^{2000}-DSPE were obtained as a function of density in the egg PC membranes, and the values obtained are plotted in Figure 4. At PEG densities below the onset of the mushroom-to-brush transition (1.4 mol % for a PEG^{2000}), uniform diffusion constants were observed that closely matched those of the dye-labeled lipids. At 1.5 mol % lipopolymer, however, a slight decrease in D to $(3.0 \pm 0.2) \times 10^{-8}$ cm^2/s was seen. This corresponds almost exactly to the onset of the mushroom-to-brush transition. The mobile fraction of the Alexa Fluor 594-labeled PEG^{2000}-DSPE decreased to 60 ± 5% at this PEG density. At higher PEG concentrations, the decrease in the diffusion constant continued. Such behavior is expected as the polymer molecules have increasing interactions with their neighbors as they are brought into ever closer proximity.

Air Stability of Pegylated Supported Bilayers. In the next set of experiments we wished to determine the ligand densities and oligomer repeat lengths of PEG that would confer air stability to the supported bilayers. A typical example of bilayer delamination in the absence of PEG-PE is shown on the left side of Figure 5. Much of the lipid material is transferred to the aqueous solution as the water phase retreats. This was verified by fluorescence analysis of the remaining solution. Additional fluorescently labeled material was left on the glass surface and formed a pattern which reflected the direction in which the coverslip was removed from solution. The introduction of either PEG^{550}-DOPE or PEG^{2000}-DOPE changed this situation remarkably. Indeed, even at low concentrations, the lipopolymer caused most of the bilayer to remain at the interface. As long as the PEG chain was in the mushroom state, however, significant damage to the bilayer could be clearly observed. Taking FRAP images after drying in air and subsequent rehydration confirmed the idea that some areas retained fluidity, while others did not. For the case of 5 mol % PEG^{550}-DOPE in egg PC, the fraction of the bilayer that returned to full fluidity after rehydration was ~40−50%. By contrast with the above results, performing experiments at lipopolymer concentrations above the onset of the mushroom-to-brush transition yielded supported membranes that showed excellent air-stability and lacked any obvious signs of damage or stress (Figure 5). This markedly improved air stability was imparted precisely at the same onset point of the mushroom-to-brush transition in liposomes, which

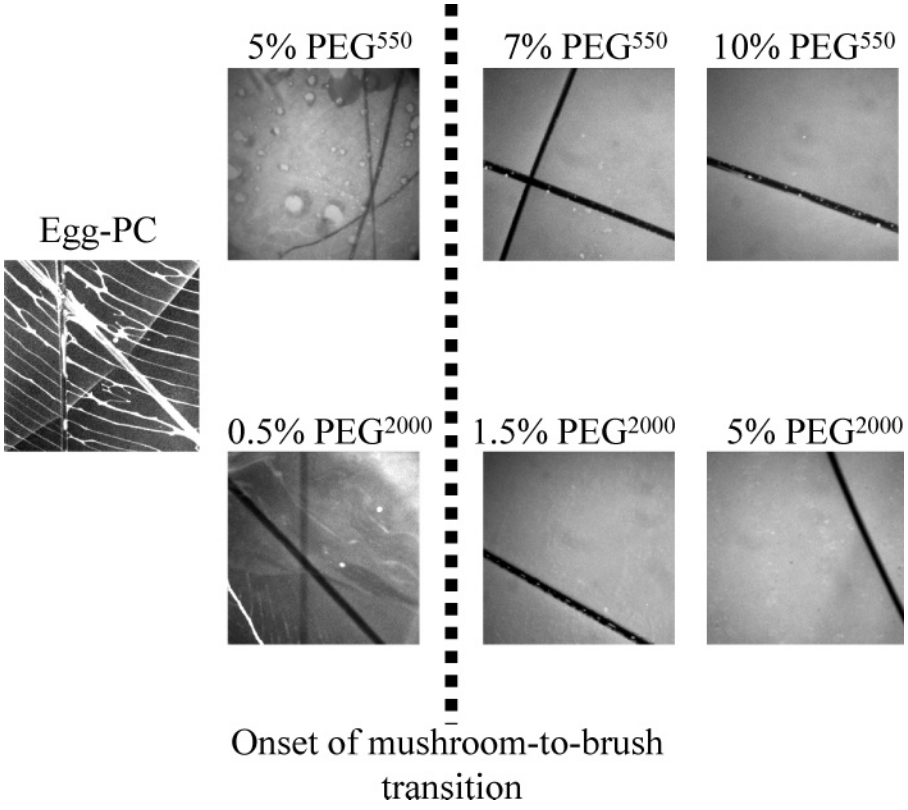

Figure 5. Fluorescence micrographs of planar supported phospholipid bilayers containing various concentrations of PEG-PE. The bilayers were imaged after removal from bulk aqueous solution and rigorous drying by blowing dry nitrogen over the sample. The black lines in the images are scratches that were intentionally made with a pair of metal tweezers for estimation of the background contribution to the measured fluorescence intensity.

occurs at 7 mol % for PEG^{550}-DOPE and 1.4 mol % for PEG^{2000}-DOPE as stated above.[30]

Control experiments were performed to test whether PEG-containing membranes in the brush phase could withstand multiple cycles of drying and rehydration. The results indicated that, as long as the membranes were not rigorously blown dry, they could easily withstand numerous cycles of removal from bulk water. On the other hand, membranes containing 5 mol % PEG^{2000}-DOPE showed evidence for modest damage after 10 cycles of rigorous drying and rehydration. In fact, the first indications of stress were noted after three drying cycles, and the fluorescence intensity began to drop as well. This indicates that the bilayers, while well protected by the PEG layer, nevertheless probably still sustain some finite damage upon each rigorous drying cycle. Such damage, however, is difficult to note by fluorescence microscopy after just one cycle. This is significant because most remote sensing applications would probably require fabricating a supported lipid bilayer device in one location, drying the platform for shipment, and rehydrating the system at a second location for use as a sensor. Thus, only one drying cycle should be typically necessary.

Effects of Poly(ethylene glycol) on Ligand–Receptor Binding. In a final set of experiments the ability of the pegylated membranes to bind proteins from the aqueous solution was determined when appropriate binding ligands were incorporated. To achieve this, supported membranes were prepared with biotin-cap-PE. Next, fluorescently labeled streptavidin was introduced into the bulk solution at 0.2 mg/mL and allowed to incubate for 30 min before being rinsed away with PBS solution. Three different membrane compositions were investigated: egg PC, egg PC with 5 mol % PEG^{550}-DOPE, and egg PC with 5 mol % PEG^{2000}-DOPE. For each of these cases the binding of Alexa Fluor-488 streptavidin was tested in the presence and absence of 1 mol % biotin-cap-PE in the membrane (Figure 6). As can be seen from the fluorescence micrograph of the microfluidic channels, the level of nonspecific adsorption of streptavidin was quite low in all cases in the absence of biotinylated lipids. On the other hand, streptavidin binding was substantially greater in those membranes where biotin was incorporated. Significantly, the presence of the pegylated lipids seemed to have little influence on the extent of protein binding under these conditions. This was the case for both the 5 mol % PEG^{550}-DOPE membrane and the membrane containing 5 mol % PEG^{2000}-DOPE. The polymer should be in the mushroom state in the former membrane, while it is well into the brush transition in the latter.

The experiments described above were repeated on membranes with the same six lipid chemistries, but after the supported bilayers were exposed to air, dried under nitrogen, left in ambient air for 24 h, and reintroduced to bulk PBS. This time only the membranes containing 5 mol % PEG^{2000} showed the same extent of streptavidin binding within experimental error as the corresponding membranes that were not exposed to air (Figure 7). The membrane that contained biotin and 5 mol % PEG^{550} showed roughly 75% as much binding, while the membrane that contained only egg PC (either with or without biotin) showed an amount of streptavidin binding that was consistent with nonspecific adsorption.

Discussion

Previous investigators have shown that the incorporation of PEG into bilayers increases the bending elastic

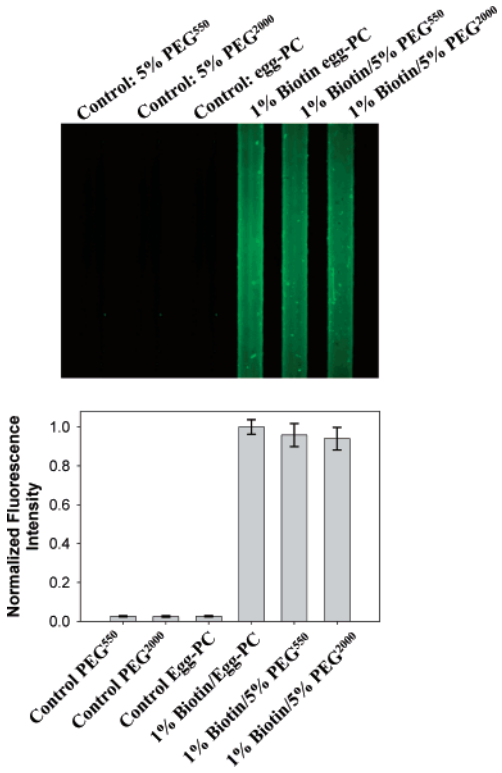

Figure 6. Effects of PEG-PE on streptavidin binding to supported membranes containing biotin-cap-PE. (top) Fluorescence micrograph of the microfluidic device containing various membrane chemistries. Alexa Fluor-488-labeled streptavidin was flowed over the bilayer-coated microchannels, incubated for 30 min, and rinsed away with PBS buffer before imaging. (bottom) A bar graph of the relative fluorescence intensity in each channel of the device. The intensity for streptavidin binding to the channel containing 1 mol % biotin-cap-PE in egg PC was normalized to 1.0.

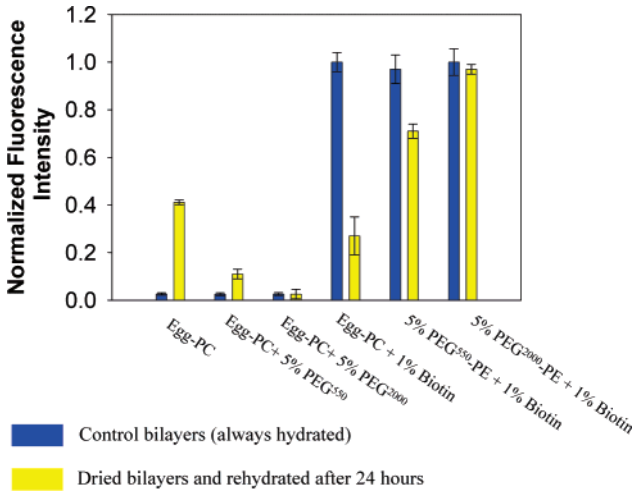

Figure 7. Bar graph of streptavidin binding in lipid bilayers containing biotin-cap-PE. A comparison is made between bilayers in which Alexa Fluor-488-labeled streptavidin is introduced over freshly prepared bilayers (blue bars) and after the bilayers have been dried in nitrogen, allowed to sit 24 h in air, and rehydrated with bulk water (yellow bars).

modulus of the membrane.[56−59] This PEG-induced stiffening varies as a function of grafting density and chain length. Other reports indicate that the degree of hydration of PEG molecules increases significantly when the polymer is in the brush conformation.[60] A PEG^{550}-PE molecule is associated with ∼36 water molecules in the mushroom state, whereas a PEG^{2000}-PE can bind ∼138 water molecules under similar conditions. In the brush conformation, the degree of hydration increases by ∼30% for both molecules. On the other hand, phosphatidylcholine in a lipid bilayer has only ∼2.5 water molecules per lipid headgroup.[61] The incorporation of PEG increases the degree of hydration of the membrane even when the larger footprint size of the PEG vs a regular phosphatidylcholine headgroup is taken into account. This, of course, occurs because the PEG thickens the hydrophilic layer. It is almost certainly this increase in the hydration layer thickness in combination with the increase in the bending elastic modulus which imparts stability upon air exposure. Such effects are reminiscent of the behavior of amorphous sugar glasses that can trap water at the surface of cell membranes as well as rigidify them.[62,63]

It should be pointed out that other mechanisms besides increasing the bending elastic modulus of the membrane can impart air stabilization. For example, employing a hybrid bilayer[24,64] (phospholipid monolayer/alkanethiol/Au) or using certain positively charged lipids[25] with PDMS can also allow membranes to retain their structure upon air exposure and rehydration. Even the substrate may play a role.[65] However, at least in the case of the hybrid bilayer, it is believed that rigorous drying causes the phospholipid leaflet to reorganize in air.[24] The lipid layer presumably reverts back to the original configuration after reintroduction to the aqueous solution. The mechanism by which the positively charged lipid bilayer operates is still unknown.

Poly(ethylene glycol) moieties are commonly employed at interfaces to resist nonspecific protein adsorption.[66−68] In the present work, however, these molecules had little if any effect on the extent of streptavidin binding to the lipid membranes (Figures 6 and 7). The key difference is that the PEG chains employed here were linked to a two-dimensionally fluid phospholipid bilayer, rather than grafted at fixed locations on the surface. Thus, the polymer chains are more flexible and able to reorganize to accommodate incoming protein molecules at least up to the size of streptavidin (66 kDa). On the other hand, the PEG film might somewhat alter the kinetics and thermodynamics of biotin−streptavidin binding. In particular, it is possible that the k_{on} and k_{off} values could be suppressed in the presence of the lipopolymer film. Furthermore, the size of the protein species may play a significant role in whether these physical constants are altered. In fact, it has been previously shown that particles of very large size, such as streptavidin immobilized on 2.8 μm polystyrene beads, do not readily bind to biotin moieties

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present on pegylated bilayers in a rolling-type assay.[69] Therefore, the PEG layers seem to allow modest sized proteins to penetrate through them while preventing the introduction of micrometer-sized objects.

In summary, pegylated phospholipid bilayers on planar solid supports can be employed as air-stable platforms for binding proteins. The lipopolymer layer remained flexible enough to accommodate protein binding, while dye-labeled lipid molecules were generally unaffected by the presence of PEG-PE up to moderate densities. It would therefore seem that poly(ethylene glycol) can imitate a cell glycocalix in a key respect. It protects the lipid bilayer from substantial damage upon exposure to air. It may also be the case that such films can act as size-selective filters. Thus, they might allow binding of smaller proteins while preventing the binding of larger complexes.

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