Membrane-Mimetic Films of Asymmetric Phosphatidylincholine Lipid Bolaamphiphiles

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Membrane-spanning phospholipid bolaamphiphiles either alone or as a constituent of a multicomponent lipid membrane may prove to be facile building blocks for generating robust bioactive membrane-mimetic assemblies. We have previously reported the synthesis of asymmetric dialkyl phospholipid bolaamphiphiles that contain ester linked phosphatidylincholine and amine functionalities at opposite chain ends. In this report, we describe the synthesis of phospholipid bolaamphiphiles that are conjugated to biotin via the terminal amine with or without a poly(ethylene oxide) spacer arm of varying chain length. The behavior of biotinylated bolaamphiphiles as a self-assembled monolayer at an air—water interface was characterized by epi-fluorescence microscopy and revealed that domain structure and π—A isotherms were substantially influenced by linker type and size. Substrate bound assemblies were produced by Langmuir—Blodgett deposition onto planar substrates coated with an avidin derivatized polyelectrolyte multilayer. Significantly, external reflectance infrared spectroscopy confirmed the fabrication of bolaamphiphile thin films that display extended stability in vitro.

Introduction

The fabrication of supported bilayer lipid membranes that mimic cell surfaces have attracted considerable attention due to their potential application as tools to probe cellular and molecular interactions and as bioactive coatings for biosensor or medical implant applications.1–2 In most studies, phospholipids differ in chemical composition, degree of saturation, and size have been utilized as the primary building blocks of lipid membrane-based structures because of their capacity to self-assemble into lamellar systems of high packing density that exhibit limited nonspecific protein adsorption.3–6 However, since the major driving forces for the assembly of lipid membranes or membrane mimics on solid supports are relatively weak hydrophobic interactions, film instability remains a major obstacle for many long-term applications of this technology. Prior studies from our group and others have demonstrated that the polymerization of a planar lipid assembly provides a feasible strategy for enhancing membrane stability.7–10 Nonetheless, a limitation of polymerization approaches includes the potential of the initiating species or growing polymer chain to inactivate or otherwise alter membrane-associated bioactive proteins or carbohydrates. Further, the cost of an increase in membrane stability is a concomitant loss of local molecular mobility. That is, the creation of a surface composed of either multiple lipid based polymer chains or a cross-linked networks of chains restricts the movement of nonlipid components within the film.

Archaebacterial lipids are naturally occurring double chain lipid bolaamphiphiles, which are members of a family of branched hydrocarbons that contain isoprenoid phytolan groups connected to glycerol backbones via an ether linkage (Figure 1A). These lipid constituents allow archaebacteria and related synthetic systems to maintain membrane integrity at high temperature (~90 °C) and low pH (1–1.5).20,21 Although several structural features of archaebacterial lipids are responsible for enhanced membrane stability, the principle of tail-to-tail lipid coupling has been recognized as a critical attribute.22 As a result of these

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observations, we have postulated that membrane-spanning phospholipid bolaamphiphiles either alone or as a constituent of a multicomponent lipid membrane would provide a starting point for generating membrane-mimetic films that exhibit long-term stability while facilitating lateral mobility of membrane associated guest species (Figure 1C).

As an initial step toward this goal, we have previously reported the synthesis of asymmetric bolaamphiphiles that contain ester linked phosphatidylcholine and amine functionalities at opposite chain ends and demonstrated their capacity to form multilamellar bilayers. It is noteworthy that, although a large variety of single or double-chain lipid-based bolaamphiphiles have been synthesized, nearly all double-chain bolaamphiphiles are symmetric compounds with identical headgroups that are coupled to a lipophilic core through ether-linkages. The presence of the amine group provides a convenient means for introducing a probe group or surface linker. Avidin (streptavidin)/biotin-based surface engineering has the advantage of being rapidly completed in a mild aqueous environment, with simple washing and purification steps. Thus, potential damage to candidate surface ligand groups due to the conjugation process is limited and any moeity that can be biotinylated can be immobilized onto an avidin/streptavidin surface. As such, biotinylated lipid-based monolayers have been investigated widely.

In this report, we describe the synthesis of phospholipid bolaamphiphiles that are conjugated to biotin via the terminal amine functionality with or without a spacer arm (water interface and as a substrate bound assembly produced by Langmuir–Blodgett deposition).

Materials and Methods

General Methods. Thin-layer chromatography was performed on Whatman silica gel aluminum-backed plates (F254, 250 mm thickness) and detected by fluorescence quenching or phosphomolybdic acid (20 wt % in ethanol) with an Rf value around 0.2–0.4 for all intermediates and final compounds at the column chromatography elution conditions. Column chromatography was performed using silica gel (EM Science, 230–400 mesh) or Sephadex LH-20 (Sigma). 1H NMR spectra were recorded at 300 MHz with a Varian Mercury 300 NMR spectrometer in CDCl3, CD3OD (internal Me4Si, δ = 0) at the sample concentration of 5 mg/0.6 mL. Mass spectra (FAB, ES) were measured using a JEOL JMS-SX102/SX102/AE mass spectrometer. Infrared spectra were acquired using a BioRad FTS-60 Fourier Transform Infrared (FT-IR) spectrometer equipped with a wide band MCT detector and UV–vis spectra acquired using a Varian Cary 4E UV–visible spectrophotometer. Fluorescence micrographs were taken with a confocal laser scanning microscope (Zeiss LSM 510) equipped with a 40 × oil immersion objective.

Synthesis of Biotinylated Phospholipid Bolaamphiphiles. The asymmetric dialkyl bolaamphiphile I was synthesized as previously reported. Bola-Biotin (2). Triethylamine (0.1 mL, 0.71 mmol) was added to a solution of I (10 mg, 0.0081 mmol) in DMF (2 mL). After the solution was stirred for 30 min, biotin–PEG–NH2 poly(t-lysine) (PLL, ~30 kDa), algininate (Alg), avidin, and avidin–FITC (AF) were purchased from Sigma (St. Louis, MO). Biotin–PEG–SPA 3400 was purchased from Nektar Therapeutics (Huntsville, AL). Texas Red–X succinimidyl ester (NHS) and N-(2-aminoethyl)biotin amide was purchased from Molecular Probes (Eugene, OR).

Scheme 1. Synthesis of biotin derivatized phospholipid bolaamphiphiles.

2. R = Biotin
3. R = CO2CH2CH2OPEG2-OCH2CH2NH2
4. R = CO2CH2CH2COOH
5. R = CO2CH2CH2CONHCH2OPEG2-OCH2CH2NH2

Conditions: a. Biotin-NHS, Et3N/DMF, 45% of 2; Biotin-PEG-SPA 3400, Et3N/DMF, 74% of 3; succinimidyld ester, Py (CHCl3), 91% of 4.

b. Biotin–PEG–NH2, DCC, DMAP/CH2Cl2, 35% of 5.

Figure 1. Design of a phospholipid-based asymmetric bolaamphiphile.
8.0 Hz), 4.13 (m, 2 H), 4.00 (m, 2 H, \( H - C - \)), and C\(_2\)-biotin), 4.94 (d, 2 H, J = 5.6 Hz), 3.87 (m, 2 H), 3.55–3.49 (m, 8 H), 3.10 (s, 9 H, \(-\text{N(CH}_3\text{)}_3\)), 3.02 (dd, 1 H, J = 7.6, 11.2 Hz), 2.40 (m, 8 H, \(-\text{COCH}_2\text{-}\)), 2.17 (m, 2 H, \(-\text{COCH}_2\text{-}\)), 1.53 (m, 8 H, \(-\text{CH}_2\text{-}\)), 1.20 (m, 34 H, \(-\text{CH}_2\text{-}\)), 0.81 (t, 6 H, J = 4.4 Hz, \(-\text{CH}_2\text{CH}_3\text{-}\)).

**Bola-PEG\(_{\text{co}}\)-Biotin (3).** Triethylenamine (0.1 mL, 0.71 mmol) was added to a solution of I (10 mg, 0.0081 mmol) in DMF (2 mL). After the solution was stirred for 30 min, biotin-PEG\(_{\text{SPA}}\), 3400 (32 mg, 0.0244 mmol) in CHCl\(_3\) (2 mL). The reaction mixture was stirred for 24 h at room temperature and then concentrated in a vacuum to give a residue, which was purified by Sephadex LH 20 column chromatography using MeOH:CH\(_2\text{C}_2\text{H}_4\text{OH}\) (65:25:4) as eluent to afford 4 (30 mg, 91%). \( R_f \) value: 0.40 (TLC, CH\(_2\text{C}_2\text{H}_4\text{OH} :\text{MeOH} 3:1\)). 1H NMR (CD\(_3\)OD, MeOH:CD\(_3\)OD 95:5), 8.0 Hz, 4.10 (m, 2 H), 4.00 (m, 2 H, \(-\text{CH}_2\text{CH}_3\text{-}\)), 1.48 (m, 12 H, \(-\text{CH}_2\text{-}\)), 0.81 (t, 6 H, J = 4.4 Hz, \(-\text{CH}_2\text{CH}_3\text{-}\)), \(-\text{CH}_2\text{CH}_3\text{-}\), bola).

**Bola-PEG\(_{\text{COOH}}\)-Biotin (4).** Pyridine (3.94 mL, 0.0488 mmol) and succinic acid (4.88 mg, 0.048 mmol) were added to a solution of I (30 mg, 0.0244 mmol) in CHCl\(_3\) (2 mL). The reaction mixture was stirred for 18 h at room temperature and then concentrated in a vacuum to give a residue, which was purified by silica gel column chromatography using CH\(_2\text{C}_2\text{H}_4\text{OH}\) (1:1, 1:1:1:1) as eluent to afford 5 (30 mg, 91%). \( R_f \) value: 0.075 (TLC, CH\(_2\text{C}_2\text{H}_4\text{OH} :\text{MeOH} 3:1\)). 1H NMR (CD\(_3\)OD : CHCl\(_3\)), d: 5.15 (m, 1 H, \(-\text{H}\)), 5.03 (m, 1 H, \(-\text{H}\)), 3.35 (m, 2 H, 2.40 (d, 2 H, J = 2.0, 8.0 Hz), 4.00 (dd, 1 H, J = 4.8, 8.0 Hz), 3.15 (s, 9 H, \(-\text{NCH}_3\)), 2.53 (m, 1 H, J = 4.4 Hz, \(-\text{COCH}_2\text{CH}_2\text{CO-Su})\), 2.39 (m, 1 H, J = 4.4 Hz, \(-\text{CH}_2\text{CH}_3\text{CO-Su})\), 2.25 (m, 8 H, \(-\text{COCH}_2\text{-}\)), 1.53 (m, 8 H, \(-\text{CH}_2\text{-}\)), 1.20 (m, 34 H, \(-\text{CH}_2\text{-}\)), 0.81 (m, 6 H, J = 4.4 Hz, \(-\text{CH}_2\text{CH}_3\text{-}\)).

**Bola-PEG\(_{\text{coOH}}\)-Biotin (5).** DCC (3.6 mg, 0.0171 mmol), DMAP (1 mg), and biotin-PEG\(_{\text{COOH}}\)-NH\(_2\) (5 mg, 0.0073 mmol) were added to a solution of 1 (11 mg, 0.0088 mmol) in DMF (2 mL). The reaction mixture was stirred for 24 h at room temperature and then filtered through Celite. The filtrate was concentrated in a vacuum to give a residue, which was purified by Sephadex LH 20 column chromatography using MeOH:CH\(_2\text{C}_2\text{H}_4\text{OH}\) (1:1) as eluent to afford 5 (3 mg, 35%). \( R_f \) value: 0.75 (TLC, CH\(_2\text{C}_2\text{H}_4\text{OH} :\text{MeOH} 3:1\)). 1H NMR (CD\(_3\)OD : CHCl\(_3\)), d: 5.15 (m, 1 H, \(-\text{H}\)), 5.03 (m, 1 H, \(-\text{H}\)), 3.35 (m, 2 H, 7.80 (d, 1 H, J = 4.4 Hz), 7.28 (m, 2 H), 6.74 (m, 1 H), 6.39 (d, 1 H, J = 6.7 Hz), 6.16 (m, 1 H), 5.80 (d, 1 H, J = 4.4 Hz). Biotin ethyl linker: 4.18 (t, 4 H, J = 2.4 Hz, \(-\text{N}-\text{CH}_2\text{-}\text{CHOH})-\text{N}\)), biotin, 4.08 (m, 2 H, H-), 4.0.

**Preparation of a Polyelectrolyte Multilayer on a Planar Substrate.** Multilayer films were prepared by layer-by-layer (LbL) deposition of a series of polyelectrolytes of opposite charge on Au-coated glass slides. Glass slides were cleaned by O\(_2\) etching for 5 min in a plasma etcher (Plasmatic Systems, Inc., North Brunswick, NJ) and sequentially coated with 100 Å Ti and 2000 Å Au using a Thermionics VE-100 electron beam evaporator (Modesto, CA). Substrates were cleaned by immersion in a H\(_2\text{SO}_4\)/H\(_2\text{O}_2\) (7:3) bath for 1 h followed by rinsing in distilled water. Slides were then immersed in a solution of PLL (1 mg/mL) in PBS for 5 min to deposit the first layer of PLL. After rinsing with deionized water, the slide was immersed in an alginate solution (1 mg/mL in PBS) for 5 min and rinsed with deionized water. The procedure was repeated until six layers of (PLL/Alg\(_3\)) were deposited. The slide was then immersed in a solution of PLL (1 mg/mL) in PBS for 10 min and dried. A solution of PLL (1 mg/mL in PBS) for 5 min and rinsed extensively in deionized water to yield a final film structure consisting of (PLL/Alg\(_3\))/PLL/avidin.

To confirm the deposition of PB, slides were immersed in a solution of avidin-FITC (0.1 mg/mL in PBS) for 30 min. After extensive rinsing with deionized water, films were assessed by UV/vis absorption spectroscopy. Likewise, to confirm avidin deposition on thin films of (PLL/Alg\(_2\))/PLL/peptide, substrates were immersed in a solution of biotin-Texas Red (2 mg/mL) in PBS for 30 min and rinsed extensively in deionized water to yield a final film structure consisting of (PLL/Alg\(_2\))/PLL/Biotin.

**Characterization of Bolaamphiphile Films at an Air–Water Interface.** Surface Balance Methods and Epi-Fluorescence Microscopy. A custom-designed Langmuir-Wilhelmy film balance (Nima, Coventry, England) interfaced to an epi-fluorescence microscope was used to study film behavior as a function of surface area at room temperature (22.0 ± 0.3 °C). The epi-fluorescence microscope (AxioTech Vario, Carl Zeiss, Jena, Germany) was equipped with a Zeiss optical filter set consisting of a 450–490 nm excitation band-pass filter, a 510 nm beam splitter, and a 515 nm


long-pass emission filter. Sample illumination was accomplished using a 100 W Hg lamp. Fluorescence imaging measurements utilized the fluorescent lipid probe BODIPY-PC (2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoyl)-1-hexadecanoyl-sn-glycero-3-phosphocholine—β-BODIPY FL, C_{12}-HPC, Molecular Probes, Eugene, OR) that was added to the bolaamphiphiles at a concentration of 1 mol%. Real-time fluorescence detection utilized a low-light level camera (SIT camera C2400—08, Hamamatsu, Japan). Images were directly stored into computer memory via an on-line image processor (Argus 20, Hamamatsu, Japan). Digital resolution exceeded 3 pixels/μm, yielding an optical resolution of \(\sim 1\) μm.

The trough of the film balance measured 60 cm \(\times\) 8 cm and surface pressure was monitored during compression with a pressure sensor utilizing a Wilhelmy plate. The temperature of the trough and subphase was controlled by a recirculating bath (9100 Series, Polyscience, Niles, IL). Isotherms were acquired at a film compression ratio (initial area to final area) of 5.6, resulting in a compression rate of 88 min/compression or 1.58 Å²/molecule/min. The film balance was placed on a computer-controlled x–y stage (Aerotech, Pittsburgh, PA) to compensate for film drift, and the entire assembly was isolated from building vibrations by an active vibration isolation table (Halcyon, Switzerland).

Imaging studies were conducted using deionized water as the subphase at 22 °C. Temperature-dependent studies were performed on water and 0.2 M K₂SO₄ (buffered at pH 7 using KH₂PO₄/NaOH) at 23, 35, and 40 °C. Solutions of biotinylated bolaamphiphiles (bola-biotin, bola-PEG10-biotin and bola-PEG70-biotin) were prepared at 1 mg/mL in CHCl₃ :CH₃OH (v/v; 3:1). The trough of the film balance measured 60 cm \(\times\) 8 cm and surface pressure was monitored with a pressure sensor utilizing a Wilhelmy plate, and the temperature of the subphase was maintained at 22 °C.

Avidin coated gold slides were immersed in the subphase (120 mM NaCl, pH 7) and allowed to equilibrate for 20 min. Biotinylated bolaamphiphiles were deposited at the air–water interface and the system equilibrated for 20 min before compressing at a rate of 20 cm²/min to the target pressure of 20 mN/M. The substrate was then rinsed vertically from the subphase at a linear rate of 1 mm/min while maintaining the monolayer at the target pressure. Transfer ratios were calculated and deposited films stored in water at 4 °C.

Polarization Modulation Infrared Reflection–Absorption Spectroscopy. Polarization modulation IR reflection absorption (PM-IRRAS) spectra of deposited amphiphile films onto Au-coated slides were recorded using a Digilab FTS 7000 (Randolph, MA) spectrometer equipped with a step scan interferometer. The step-scan interferometer was operated using a phase modulation frequency of 88 min/compression or 1.58 Å²/molecule/min. The IR beam exiting the interferometer was \(\sim\) p-polarized. This \(\sim\) p-polarized light was passed through a ZnSe photoelastic modulator (PEM-80, Hinds Instruments, Hillsboro, OR) operating at 37 kHz before reflecting off the sample at a grazing incidence angle of \(\sim 80°\). Reflected light from the sample was directed onto the sensing chip of a liquid-nitrogen-cooled photovoltaic HgCdTe detector (Infrared Associates, Inc., Stuart, FL) using an on-axis refractive (ZnSe) optics. An optical lowpass filter (cut off \(\sim\) 3950 cm⁻¹) was used in the optical path to restrict the wavenumber range reaching the detector and ensure the correct undersampling ratio. Spectra were recorded at a resolution of 8 cm⁻¹. PM-IRRAS spectra were calculated from the measured modulated step-scan interferograms using a Digital Signal Processing (DSP) algorithm incorporated in the Digilab Win-IR Pro 3.3 software, as previously described.1,42

Results and Discussion

Phosphatidylcholine derived asymmetric bolaamphiphilic lipid 1 with an amine terminus was synthesized as previously reported.23 Amine site biotinylation was performed by direct amidation of 1 with commercially available N-hydroxysuccinimido-biotin or biotin-PEG-SPA (Mw 3400) to afford either a nonspacer biotinylated bolaamphiphilic lipid 2 or a biotinylated bolaamphiphilic lipid 3 with an intervening long PEG spacer arm, respectively. A biotinylated bolaamphiphilic lipid 5 with an intermediate sized PEG spacer arm was synthesized by adding amine bolaamphiphilic 1 to succinic acid derivative 4 followed by amidation with commercial biotin PEG10 amine. All intermediates and biotinylated bolaamphiphiles were characterized by \(^1\)H NMR spectroscopy (see Materials and Methods).

Surface Area—Pressure Isotherms. The \(\pi–A\) isotherms for bola-biotin, bola-PEG10-biotin, and bola-PEG70-biotin monolayers on water at 23 °C are presented in Figure 2. Isotherms of bola-biotin display well-defined expanded and condensed phases that occur at 125 and 75 Å²/molecule, respectively. The phase transition occurs at \(\sim\) 7 mN/m, which corresponds to structural changes observed by epi-fluorescence microscopy (Figure 3). These data are consistent with the notion that the observed optical contrast represents a liquid-expanded—liquid-condensed (LE−LC) phase transition. The LC domains only appear with the onset of the phase transition from a clear bright LE region, and they were absent at lower pressures. In contrast, optical contrast due to dye immiscibility is characterized observed even at low surface pressures when the monolayer is expanded. The observed domain structure is similar to the ‘snow-flake’ patterns reported for naturally occurring archaeobacterial lipids.43

At \(\sim\) 30 mN/m, a plateau region is observed in the bola-biotin isotherm, marked by the decrease in the molecular area without further increase of surface pressure. Similar behavior has been observed by Kim et al.,44 Yamauchi et al.,45 and Kitano et al.46 during studies of other bolaamphiphiles having a similar diglycerotetraethyle type framework. This isotherm property was

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suggested to be an indicator of a U-shaped molecular conformation adopted by the bolaamphiphiles, with the lipid headgroups in the subphase and the hydrophobic moieties in air during compression. Upon further decrease of barrier area, the bipolar lipid molecules were extruded from the monomolecular film at the interface and accumulated above the membrane, resulting in the plateau region. Further compression leads to the collapse of the bola-biotin amphiphile. Although the area at collapse of 50 Å²/molecule is typical of a dialkyl amphiphile, the surface pressure at which collapse occurs is considerably lower than that normally observed for nonbolaform dialkyl phospholipids (~70 mN/m). However, low collapse pressures have also been observed for other bolaform ether lipids such as 2,2′-dioctyl-1,1′-O-hexadecylmethylene-rac-diglycerol-3,3′-diphosphoric acid.

The isotherms of bolaamphiphiles 3 and 5 containing poly(ethylene oxide) spacer arms are more expanded than that observed for bola-biotin 2 and do not display a clear secondary transition to a condensed phase (Figure 2). In general, the molecular areas for bolaamphiphiles 3 and 5 are much larger than those observed for the other bolaamphiphiles. For example, at a surface pressure of 8.5 mN/m, the molecular areas 91, 110, and 170 Å²/molecule are measured for bola-biotin, bola-PEG10-biotin, and bola-PEG70-biotin, respectively, consistent with the presence of a spacer arm that likely limits close packing of the amphiphiles. Unlike the bola-biotin 2, the bola-PEG-biotin amphiphiles 3 and 5 did not exhibit a plateau region, which was

Figure 3. Epiflourescence images of biotin-bolaamphiphile 2 at an air–water interface during the course of film compression at 23 °C.

Figure 4. Epiflourescence images of biotin-PEG10-bolaamphiphile 5 at an air–water interface during the course of film compression at 23 °C.

expected because of the introduction of the soluble PEG spacers. Fluorescent images of bola-PEG10-biotin reveal scattered domains with increasing surface pressure, but these domains lack the structure and shape noted for bola-biotin (Figure 4). No appreciable domain formation was observed for bola-PEG70-biotin throughout the surface pressure range investigated (Figure 5).

The presence of ions in the subphase (120 mM NaCl) had little effect on $\pi$-$A$ isotherms. The lower critical solution temperature (LCST) of poly(ethylene oxide) in water is approached at elevated temperature and high salt content (e.g., 0.2 M K$_2$SO$_4$ and 60 °C). Therefore, we postulated that chain repulsion could potentially be reduced and, as a consequence, packing density for pegylated bolaforms increased under subphase conditions that approached or exceeded the LCST. Given the limitations of our film balance, 40 °C was the maximum subphase temperature that could be maintained during the course of $\pi$-$A$ studies. This approach was assessed using 0.2 M K$_2$SO$_4$ at 23, 35, or 40 °C. An increase in the collapse pressure of bola-PEG70-biotin was observed, but a substantial enhancement in packing density or transition to a condensed phase was not achieved.

**Substrate Supported Films of Bola-Biotin and Bola-PEG10-Biotin.** UV/vis absorption spectra of polyelectrolyte multilayer-coated glass slides confirmed binding of avidin-FITC and the peak at 585 nm is attributed to Texas Red-X. Biotinylated PLL (PB) can bind avidin and, in turn, biotin-Texas Red-X. In the absence of avidin, a peak that can be attributed to biotin-Texas red is not observed, which suggests limited nonspecific binding.

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**Figure 5.** Epifluorescence images of biotin-PEG70-bolaamphiphile 3 at an air–water interface during the course of film compression at 23 °C.

**Figure 6.** UV/vis absorption spectra of polyelectrolyte multilayer-coated glass slides. The peak at 500 nm is attributed to FITC and the peak at 585 nm is attributed to Texas Red-X. Biotinylated PLL (PB) can bind avidin and, in turn, biotin-Texas Red-X. In the absence of avidin, a peak that can be attributed to biotin-Texas red is not observed, which suggests limited nonspecific binding.

**Figure 7.** Confocal fluorescence images of glass slides coated with (PLL/Alg)$_3$/PB/biotin-Texas Red-X (A) or (PLL/Alg)$_3$/PB/avidin/biotin-Texas Red (B). Without an interlayer of avidin, fluorescence is weak consistent with limited nonspecific binding of biotin-Texas Red-X. Without an interlayer of avidin, fluorescence is weak consistent with limited nonspecific binding of biotin-Texas Red-X.

Confocal fluorescence images provide further evidence for the specific binding between PB and avidin with limited nonspecific adsorption (Figure 7). Without an interlayer of avidin, fluorescence is weak consistent with limited nonspecific binding of biotin-Texas Red-X. Of note, repeated confocal fluorescence imaging of (PLL/Alg)$_3$/PB/biotin-Texas Red LbL films stored in water at 4 °C did not reveal loss of film components, despite transfers through an air–water interface.

Bola-biotin and Bola-PEG10-biotin were transferred onto avidin-coated polyelectrolyte multilayers by Langmuir–Blodgett (LB) deposition at 20 mN/m. When the film transfers were performed at constant pressure, there was a decrease in barrier area during the transfer, as expected for the LB process. However, even in absence of an LB transfer, a small but constant decrease
in trough surface area was needed to maintain a constant surface pressure for the bola-biotin and bola-PEG10-biotin monolayers. This biphasic behavior was likely due to some degree of instability in the monolayer induced by the presence of the hydrophilic biotin and poly(ethylene glycol) components. Ideally, the decrease in trough surface area should only occur during a transfer; however, in the case of these two compounds, there was a surface area decrease even in the absence of the transfer. Therefore, while calculating the transfer ratios, the decrease due to monolayer instability was subtracted from the decrease in area due to the transfer. Assuming that film transfer occurred on both sides of the coated planar substrate, the estimated transfer ratios were 120% and 28% for bola-biotin and bola-PEG10-biotin, respectively. The compromised LB transfer of the pegylated compound can likely be attributed both to monolayer instability and limits imposed upon bolaamphiphile packing density at the air–water interface induced by the PEG linker.

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Infrared Spectroscopy of Substrate Supported Phospholipid Bolaamphiphile Films. The PM-IRRAS spectra of the bola-biotin and the bola-PEG10-biotin bulk samples on a gold surface are shown in Figure 8, and the principle absorption bands observed for these samples are summarized in Tables 1 and 2, respectively. Of note, the ν(C–H) stretching region between 2750 and 3000 cm$^{-1}$ and the 1738 cm$^{-1}$ band due to the carbonyl group are more intense for bola-biotin than for bola-PEG10-biotin.

Infrared spectra of films of bola-biotin and bola-PEG10-biotin deposited onto avidin-coated substrates revealed similar
features to the original compounds (Figures 9 and 10). Obtaining accurate alkyl chain orientation was not feasible since the CH region would have contributions from the PEG linker, as well as from bolaamphiphile alkyl chains. However, all characteristic absorption bands observed in the bulk compounds were present in the related supported membrane-mimetic films. The films also appeared to be quite stable when subjected to repeated studies over a three-week incubation period in deionized water. No major changes were observed in the spectral signature other than slight changes in the band shapes (Figures 9 and 10). Admittedly, the potential exists for bolaamphiphile LB film transfer without selective biotin–avidin binding. However, if a significant proportion of bolaamphiphile was bound to the substrate through nonspecific interactions, it would be anticipated that loss of bolaamphiphile would be observed over the several weak incubation period in water. Although spectroscopic characterization of these films did not permit precise determination of bolaamphiphile surface density or packing, substantial change in film structure was not evident.

Conclusions

Bolaamphiphile lipids continue to generate interest as stabilizing elements in the fabrication model membrane systems formulated either as vesicular assemblies or as supported films. In this report, we have described the synthesis of dialkyl phosphorylcholine lipid bolaamphiphiles conjugated to biotin with or without a poly(ethylene oxide) spacer arm. π–π isotherms and epi-fluorescence microscopy reveal that the properties of these compounds at the air–water interface, including domain structure and packing density, are profoundly influenced by linker type and size. This behavior, in turn, influenced the effectiveness of film transfer by Langmuir–Blodgett deposition onto a substrate supported polyelectrolyte multilayer. Significantly, biotin provided a convenient anchoring group, as external reflectance infrared spectroscopy confirmed the fabrication of bolaamphiphile thin films that displayed extended stability in vitro. However, the formation of closely packed, surface supported, membrane-mimetic thin films will likely require the use of bolaamphiphiles that have been designed with a relatively short spacer between the surface anchor and the lipid unit.

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Supporting Information Available: 1H NMR spectra for compounds 1–5. This material is available free of charge via the Internet at http://pubs.acs.org.

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