2-ALKYL-2-OXAZOLINE LIPOPOLYMERS FOR THE CONSTRUCTION OF POLYMER TETHERED LIPID BILAYERS

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Introduction

Solid-supported lipid bilayers are used as model biomimetic systemsfor the study of membrane associated processes of cell membranes. On hydrophilic substrates a thin water interlayer of about 10-25 Å is intercalated between the lipid bilayer and the substrate surface.1 In many cases, this interlayer is sufficient to enable diffusion of lipids or small molecules within the lipid bilayer. However, often the water layer is too thin for the conservative incorporation of e.g. large transmembrane proteins which irreversibly adsorb and denature on the solid substrate. Ringsdorf2 and Sackmann3 introduced the concept of an intermediate, soft polymer cushion or tether between the lipid bilayer and the solid substrate to overcome the problem of surface roughness or defects, slower lateral diffusion within the bilayer and resulting inactivity of incorporated transmembrane proteins. Since then, different approaches for the construction of polymer tethered/supported lipid membranes were studied. For Example, Tamm et al.4 used a linear polyethylene glycol (PEG) with a lipid head group (lipopolymer) and a triethoxysilane end group. They varied the concentration of the lipopolymer tether, but limited the study to only one kind of polymer. Another approach is reported by Frank et al.,5 who used poly(2-ethyl-2-oxazoline)s with a lipid head group. The lipopolymers were bond to a self-assembled monolayer (SAM) of benzophenone via a photochemical reaction with the alkyl moieties of the polymer. Since the surface coupling reaction is unspecific, the morphology of the polymer cushion may vary strongly. To have full control over the polymer tethering, the interaction between the lipopolymer and the membrane lipids as well as the fixation, we developed the direct synthesis of silane end-functionalized lipopolymers. The living character of the cationic ring-opening polymerization of the 2-alkyl-2-oxazolines results in linear polymer chains, with an adjustable degree of polymerization, low polydispersity, and quantitative end-functionalization at both ends of the polymer (**Figure 1**). The hydrophilic/lipophilic balance (HLB) can be fine tuned by the choice of the monomer, the degree of polymerization and the lipid head group.

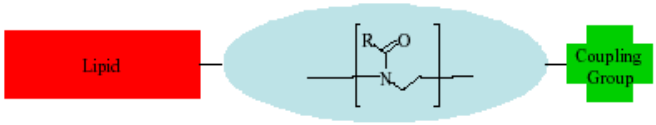

Figure 1. Cartoon of the 2-alkyl-2-oxazoline lipopolymer with a lipid head group, a hydrophilic polymer spacer and a silane coupling end function.

This strategy allows us to fine tune the polymer architecture to construct stable and functional biomimetic membranes and to overcome the present limitations.

Experimental

Materials. All glassware was heated in the vacuum and purged with dry nitrogen to remove traces of water from the glass surface. All reactions, polymerization and purification were carried out in a dry nitrogen atmosphere using the Schlenk technique. Phythol (**1**) (Fluka), 1,2-*O*-dioctadecyl-*sn*-glycerole (**3**) (BACHEM) and trifluoromethanesulfonic anhydride (Fuka) where used without further purification. Methanol was distilled, dry diethyl ether and dry benzene where purchased from Fluka. Dichloromethane (Aldrich), chloroform (Fluka) and 2-methyl-2-oxazoline (Aldrich) where dried over CaH2 and freshly distilled prior to use. The 3-aminoproyltrimethoxysilane (ABCR) was freshly distilled prior to use. The resulting lipopolymers where kept under anhydrous condition to avoid hydrolysis and condensation of the trimethoxysilane groups.

Instrumentation. 1H NMR (300 MHz) spectra were recorded in CDCl3 using an ARX 300 (Bruker, Karlsruhe, Germany). Gel permeation chromatography (GPC) was carried out on a Waters Liquid Chromatograph GPC 510 with refractive index detector and Waters Ultrasyragel column (pore size 103, 104, 105 Å), with CHCl3, using polystyrene standard for calibration. Mass measurements were performed using a Bruker BIFLEX III MALDI TOF mass spectrometer equipped with a nitrogen Laser (LSI, 337 nm, 3 ns pulse length) and two detectors.

Synthesis of Phytanol (3,7,11,15-Tetramethyl-hexadecan-1-.ol) (2).6 Raney nickel (15 g) was washed and suspended in 150 mL of methanol. 21.30 g (71.8 mmol) 3,7,11,15-tetramethyl-2-hexadecen-1- ol (**1**) were added and reduced at room temperature under a hydrogen gas atmosphere for 72h. The catalyst was removed with a PTFE filter (0.45 µm; Sartorius) and methanol was removed under reduced pressure. The residue (**2**) (21.43 g, 71.8 mmol, 100%) was used without further purification. 1H NMR (in CDCl3) d (ppm): d 0.8-0.9 (b, -CH3, 15 H); d 1.0-1.6 (b, -(CH1,2)14-, 24 H); d 3.5-3.7 (b, -CH2-OH, 2 H).

Synthesis of 3,7,11,15-Tetramethyl-hexadecyltrifluormethansulfonate (3). 1.40 g (4.97 mmol) trifluoromethanesulfonic anhydride is drop wise added to a precooled (0°C) solution of 0.99 g (3.32 mmol) (**2**) and 1.83 g (13.24 mmol) anhydrous potassium carbonate in 40 mL of dichloromethane. The reaction mixture is stirred over night (0°C to room temperature). The solution is filtered, concentrated under reduced pressure and freeze dried (benzene) to give 1.43 g (3.32 mmol, 100%) (**3**) as a brown oil. 1H NMR (in CDCl3) d (ppm): d 0.8-0.9 (b, -CH3, 15 H); d 1.0-1.6 (b, -(CH1,2)14-, 24 H); d 4.5-4.7 (b, -CH2-OTf, 2 H).

Synthesis of 1,2-*O*-Dioctadecyl-*sn*-glyero-3-trifluormethanesulfonate (5). (**5**) was synthesized analog to (**3**) using 0.50 g (0.84 mmol) 1,2-*O*-Dioctadecyl-*sn*-glyerol (**4**), 0.46 g (3.36 mmol) anhydrous potassium carbonate, 0.35 g (1.26 mmol)trifluoromethanesulfonic anhydride in 30 mL of dichloromethane. Filtration and lyophilization gave 0.61 g (0.84 mmol) (**4**) as a colorless powder. 1H NMR (in CDCl3) d (ppm): d 0.9 (b, CH3-CH2-, 6 H); d 1.1-1.4 (b, -(CH2)15-, 60H); d 1.5 (b, -CH2-CH2-O, 4H); d 3.4-3.7 (b, CH2-O, CH-O, 7H); d 4.5-4.7 (b, CH2-OTf, 2H).

General Polymerization Procedure. At 0°C the corresponding amount of 2-methyl-2-oxazoline (0.85 g, 10 mmol for n=10; 1.70 g, 20 mmol for n=20; 3.40 g, 40 mmol for n=40) was added to a solution of 1 mmol of the alkyl triflate in 50 mL chloroform and the flask was sealed. The reaction mixture was transferred to a preheated oil bath (60°C). The reaction time varies with the degree of polymerization (30h for n=10; 40h for n=20 and 50h for n=40).

Termination. The reaction mixture is cooled to 0°C and 1.79 g (10 mmol) 3-aminoproyltrimethoxysilane are added and stirred over night at room temperature. Most of the chloroform was removed under reduced pressure and the polymer was precipitated in 300 mL of dry diethyl ether at 0°C. After filtration the polymer was dissolved in 10 mL CHCl3 and stirred with 1 g of potassium carbonate over night. The mixture was filtered, reprecipitated (CHCl3/diethyl ether) and freeze dried (benzene), to give a colorless powder. The yield, molar masses and polydispersities of the resulting polymers are given in **Table1**.

Table 1. Synthesized Lipopolymers, Molar Masses And Yield After Work Up.

Lipopolymer	GPC				MALDI TOF			Yield
	Mn	Mw	Mp	PDI	Mn	Mp	PDI	
Phy-PMOx$_{10}$-Si	1407	1582	1863	1.12	1305	1447	1.11	54%
Phy-PMOx$_{20}$-Si	2111	2440	2678	1.16	2086	2272	1.09	78%
$(C_{18})_2$-PMOx$_{10}$-Si	855	933	1056	1.09	1469	1608	1.09	63%
$(C_{18})_2$-PMOx$_{20}$-Si	1191	1582	1243	1.33	2255	2289	1.01	86%
$(C_{18})_2$-PMOx$_{40}$-Si	1496	1950	2385	1.30	2697	2843	1.05	75%

$(C_{18})_2$-PMOx$_n$-Si: ^{1}H NMR (in $CDCl_3$) d (ppm): d 0.6 (b, CH_2-Si(OMe)$_3$, 2 H); d 0.86 (t, CH_3-CH_2-, 6H); d 1.1-1.4 (b, $(CH_2)_n$); d 2.0-2.2 (b, CH_3-CO-N); d 3.3-3.6 (b, CH_2-N-CH_2).
Phy-PMOx$_n$-Si: ^{1}H NMR (in $CDCl_3$) d (ppm): d 0.6 (b, CH_2-Si(OMe)$_3$, 2 H); 0.8-0.9 (b, -CH_3, 15 H); d 1.0-1.6 (b, -$(CH_{1,2})_{14}$-, 24 H); d 2.0-2.2 (b, CH_3-CO-N); d 2.7-2.9 (b, $C_{19}H_{39}$-CH_2-N, 2 H); d 3.3-3.7 (b, CH_2-N-CH_2).

Results and Discussion

The cationic ring-opening polymerization of 2-alkyl-2-oxazolines is well established. This polymerization leads to homogenous polymers with good control of the chain length at low polydispersities. A wide variability of the 2-substituent of the 2-oxazoline and a fast initiation with alkyl triflates has been reported.7 Using alkyl triflates (**3**,**5**) as initiators for the polymerization, lipid end groups can be introduced quantitatively into the polymer (**Figure 2**). The introduction of the silane coupling group can be achieved by a quantitative termination reaction with nucleophilic primary amines.8 In this case we used a trifunctional silane coupling group to ensure an efficient and fast coupling reaction to the silanol functions at SiO2 surfaces.

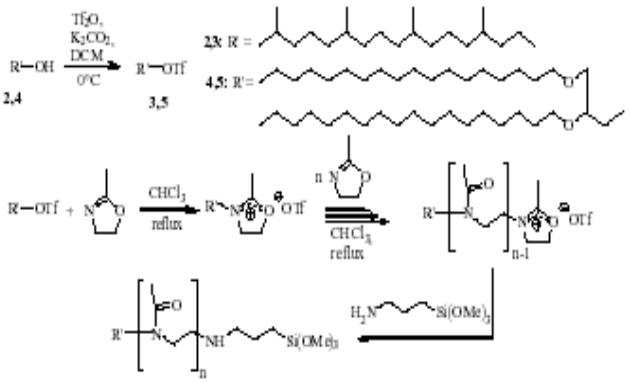

Figure 2. Reaction scheme for the preparation of poly(2-methyl-2-oxazoline)s with lipid headgroups and silane coupling group introduced via a quantitative termination reaction.

Earlier studies showed that both 2-methyl- and 2-ethyl-oxazoline give hydrophilic polymers and unexpectedly both polymers show an almost identical swelling behavior. A strong dependence on the degree of polymerization of the grafted polymer brushes in equilibrium swelling experiments and under osmotic shock were investigated in detail.9 On the other hand an amphiphilic behavior of the ethyl group in block copolymers containing a 2-ethyl-2-oxazoline block where observed during Langmuir-Blodgett experiments at the air water interphase.10 Regarding this results we synthesized 2-methyl-2-oxazoline lipopolymers with different polymer chain length to control the HLB, as well as the thickness of the polymer tethering. The polymerization of the 2-alkyl-2-oxazoline is limited due to chain transfer reactions at higher degrees of polymerization as reported by Litt et al.11 Since one of the most important properties of lipid membranes is the fluidity, we synthesized lipopolymers with different lipid head groups. The double chained C18 lipid might crystallize, whereas the phytanoyl has a melting point far below room temperature. The preparation of first polymer tethered model membranes resulted in very promising systems. Therefore, a lipopolymer/lipid monolayer was transferred to the solid substrate by Langmuir-Blodgett technique. The second layer was formed through a vesicle fusion. The resulting polymer tethered lipid bilayers were stable for weeks and had an almost free lateral diffusion (**Figure 3**).12

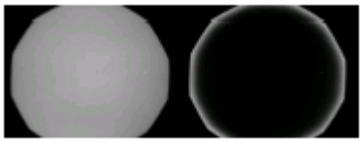

Figure 3. Fluorescence microscope pictures of the fluid homogenous lipid membrane before (left) and while (right) continous beaching (aperture size 200 μm).

The left image reveals the existence of a homogenous membrane over a large area. During the bleaching the system comes to a steady state, where unbleached chromophores diffuse into the bleached region, forming a corona.

Conclusions

The direct synthesis of linear, silane functionalized lipopolymers in a controlled manner could be achieved. With this strategy we have a synthetic pathway to fine tune the properties of lipopolymers for the application in tethered lipid bilayers at suitable ranges of the molecular weight. First polymer tethered membranes could be prepared, showing lateral diffusion similar to free lipid membranes. Via a silane coupling group the polymer can covalently bind to semiconductor surfaces. This increases the stability of the membranes, necessary for the application in biophysical detection devices.

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