Supramolecular Structures of Novel Carbohydrate-Based Phospholipids

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Conventional glycerol-based phospholipids such as phosphatidylcholines self-assemble in water as spherical three-dimensional self-closed structures known as liposomes.[1−4] The potential applications of liposomes are numerous and include their uses as drug delivery vehicles, gene transfection agents, ultrasound phase contrast agents, carriers for dyes and fragrances, as well as models and tools for membrane structure and function studies.[5−12] Current modifications of phospholipid structure are limited to the hydrophobic tails, hydrophilic headgroups, and structural variations of the glycerol backbone.[12−20] Consequently, alteration of the conventional glycerol backbone by complete substitution provides new opportunities for (1) assessing supramolecular structure formation and (2) attaching macromolecules or ligands for biological targeting. Herein we report the synthesis and physical characterization of a novel carbohydrate phospholipid, that is an analogue of 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC) (A) (Figure 1), where glycerol is replaced by ribose (B). These new phospholipids spontaneously form three-dimensional supramolecular structures in aqueous solution.

Methyl-2,3-di-*O*-lauroyl-*β*-D-ribo-5-phosphocholine, **7** (DLRPC), was synthesized as shown in Scheme 1. The first step involved protecting the primary hydroxide of **1** using trityl chloride. Purification of **2** was accomplished by silica gel column chromatography in 87% yield with an eluent of 3% methanol/chloroform. Next, a DCC coupling with DMAP and lauric acid in DMF afforded compound **3**. The compound was purified on a silica gel column with the eluent of 9/1 hexane/ethyl acetate, and then immediately dissolved in aqueous acetic acid to remove the trityl protecting group. Compound **4** was subsequently purified by silica gel column chromatography (eluent 7/3 hexane/ethyl acetate) with an overall yield of 41% from **2** to **4**. The synthesis of compound **5** was accomplished by first reacting **4** with 2-cyanoethyl diisopropylchlorophosphoramidite followed by addition of choline chloride. The phosphorus(III) compound was subsequently oxidized to phosphorus(V) by I_2. Finally, the cyanoethyl protecting group of **6** was removed by dissolving the mixture in 0.14 M (aq) TEA and stirring for 3 h. at room temperature. Compound **7** was isolated after alumina (65/25 chloroform/methanol; 65/25/4 chloroform/methanol/H_2O), Sephadex G-10 size exclusion (50/50 chloroform/methanol), and reverse-phase C-18 Sep Pak chromatography (10/90 methanol/chloroform). The overall yield for these three steps (d−f) was 33.5%.[21]

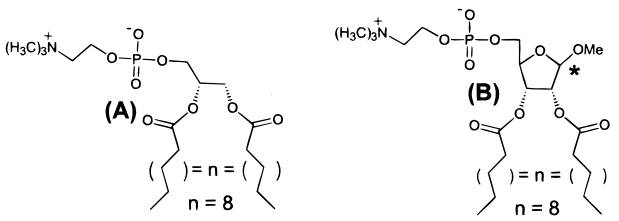
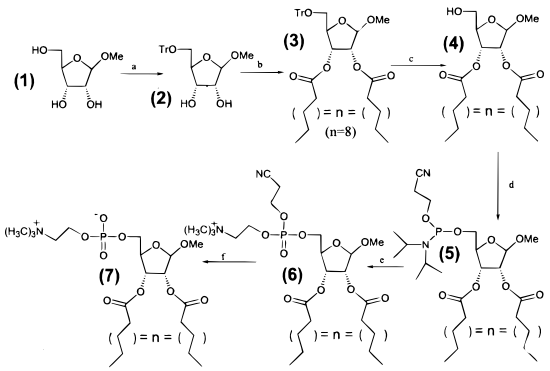


Figure 1. 1,2-Dilauroyl-*sn*-glycero-3-phosphocholine (DLPC) (A) and methyl-2,3-di-*O*-lauroyl-*β*-D-ribo-5-phosphocholine (DLRPC) (B).

Scheme 1. Synthetic Scheme for DLRPC[a]



[a] a) TrCl, C_5H_5N, 3 h, 120 °C, b) DCC, DMAP, lauric acid, DMF, 48 h, 60 °C, c) acetic acid, H_2O, 12 h, 50 °C, d) 2-cyanoethyl diisopropylchlorophosphoramidite, DiPEA, CH_2Cl_2, 2 h, 22 °C, e) tetrazole, choline chloride, I_2, ACN, 3 h, 22 °C, f) TEA (aq), 3 h, 22 °C.

These molecules self-assemble into liposome-like structures in aqueous solution; we call these supramolecular structures "carbohydrosomes" since the structures are carbohydrate analogues of glycerol-based liposomes.[22] A light micrograph of carbohydrosomes budding off of a thin film of DLRPC is shown in Figure 2 (bar = 20 μm). We are able to prepare carbohydrosomes with sizes ranging from 0.2 to 15 μm. A typical procedure for 0.2 μm vesicles involves forming a thin film of DLRPC in a 100 mL round-bottom flask by dissolving 5 mg of **7** in chloroform and subsequently removing the solvent by rotoevaporation.[23] Phos-

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(21) The final product was characterized by HR-FAB mass spectrometry ($(M-H)^+$ theoretical = 694.4659, observed = 694.4653) as well as ^{1}H NMR, ^{13}C NMR, and ^{31}P-NMR. Complete experimental details are found in the Supporting Information.

(22) Carbohydrosomes or carbohydro-liposomes are 3-dimensional structures formed from zwitterionic, cationic, or anionic carbohydrate-based lipids.

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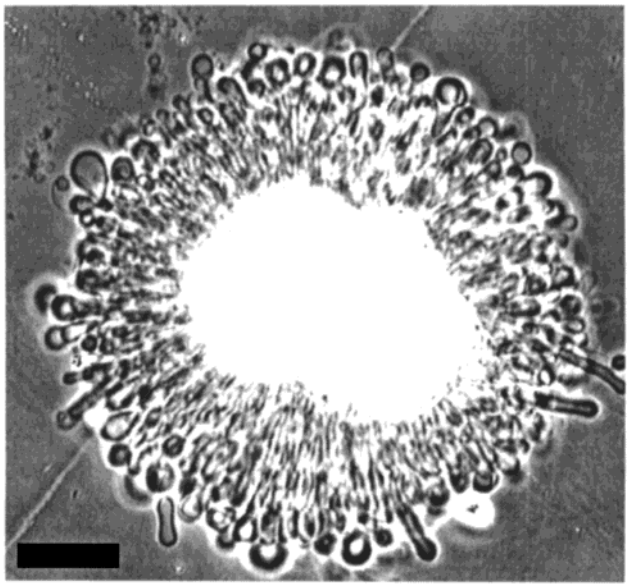

Figure 2. Light micrograph of hydrated DLRPC (bar = 20 μm).

phate buffer (1 mL) is next added to the flask after drying under vacuum overnight, and multilamellar vesicles are formed by agitating the film with a stirbar at 21 °C. After 20 min of agitation, the carbohydrosomes are extruded through a polycarbonate filter (200 nm) five times at 21 °C. Particle sizing using a Brookhaven Instruments Corporation Zeta Plus Potential Analyzer shows supramolecular structures of 197 nm (fwhm = 68.7 nm).

The phase transition temperature (T_m) of the carbohydrosomes formed by DLRPC is determined by Modulated Differential Scanning Calorimetry (MDSC). A melting temperature of 15.75 ± 0.05 °C is observed which is approximately 16 °C higher than the phase transition of DLPC (−1 °C).[24] This increase in T_m indicates a more efficient packing of the bilayer below the T_m. Supramolecular structures with a single transition temperature between −1.00 and 15.75 °C can be formed by simply varying the DLPC/DLRPC ratios illustrating DLRPC mixes with DLPC to form mixed lipid bilayers.

To further characterize the carbohydrosomes, X-ray diffraction data are obtained on dispersions of DLRPC. At 20 °C X-ray patterns show two sharp reflections that indexed as orders of a lamellar phase of repeat period 63 Å, as well as a broad wide-angle band centered at 4.5 Å. Such patterns are typical of bilayers in the disordered or liquid-crystalline (L_α) phase.[25] Moreover, the lamellar spacing is similar to that found for DLPC in its L_α state. Preliminary micropipet experiments to assess the mechanical properties of the DLRPC L_α state indicate the bilayer to be weaker and more fragile compared to other glycerol-based phospholipids.[26,27] At 10 °C, X-ray patterns consisted of two orders of a lamellar periodicity of 55 Å and several sharp wide-angle reflections at 7.7, 6.1, 4.9, 4.6, and 3.9 Å, indicating the presence of bilayers with their hydrocarbon chains crystallized in the plane of the bilayer.[28] Such crystalline hydrocarbon chain packing is not typically observed with glycerol-based phospholipids below their phase transition temperature.[24] The presence of this crystalline chain packing and the relatively high phase transition temperature indicate a more stable bilayer packing arrangement within the carbohydrosome below the phase transition temperature than is found with glycerol phospholipids such as DLPC.

Supramolecular self-closed structures are formed with DLRPC demonstrating that the geometry and ampiphilicity of **7** are sufficient for bilayer formation even though DLRPC possesses (1) a larger backbone that increases the spacing between the head and tail groups, (2) an increase in hydrophobicity, and (3) a decrease in backbone flexibility. The structural and mechanical property studies indicate that below the phase transition DLRPC forms a lamellar crystalline phase and above the T_m a fragile lamellar fluid state exists. Work is currently in progress to chemically alter the fatty acid chain length, zwitterionic headgroup, and ribose to further understand the factors that control bilayer and supramolecular structure formation. This novel carbohydrate lipid, DLRPC, expands the current repertoire of supramolecular monomers, (which includes diblock polymers,[29] triple-chain ampiphiles,[30] "defective" phospholipids,[16] proteins,[31] bola-amphiphiles,[32] pseudoglyceryl dimeric lipids,[33] and ladder-like polymers[17]) that are available to synthesize and engineer vesicles for specific biotechnological and biomedical applications.

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Supporting Information Available: Complete experimental details, ^{1}H NMR, and HR-FAB MS (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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