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Facially amphiphilic polyionene biocidal polymers derived from lithocholic acid

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ABSTRACT

Bacterial infections have become a global issue that requires urgent attention, particularly regarding to emergence of multidrug resistant bacteria. We developed quaternary amine-containing antimicrobial poly(bile acid)s that contain a hydrophobic core of lithocholic acid in the main-chain. Interestingly, by choosing appropriate monomers, these cationic polymers can form core-shell micelles. These polymers exhibited biocidal activity against both Gram-positive and Gram-negative bacterial species. It is demonstrated that the micelles can deliver hydrophobic antibiotics that functionally have dual antimicrobial activities. Cytotoxicity assays against HeLa cells showed dosage-dependent toxicity for polymers with longer linkers.

1. Introduction

Microbial infections, especially those caused by resistant bacteria have become problematic due to increasing ineffectiveness of conventional antibiotics [1]. The overuse of antibiotics in health care settings and agriculture, coupled with the slow pace of new antimicrobial discoveries and approvals over the last few decades, have resulted in many new resistant bacterial strains that complicate the well-being of humans, food security, and societal development [2,3]. Therefore, there is an increased interest by the scientific community to explore new avenues to circumvent the problem with resistant bacteria. Bacterial membrane anionic lipids are considered attractive targets to design novel antibacterial agents [4]. In general, antimicrobial polymers are a class of hydrophilic cationic macromolecules that can selectively destroy microorganisms such as bacteria, fungi or protozoans with little or no cytotoxicity to mammalian cells [5,6]. Most antimicrobial polymers contain quaternary ammonium centers as the cations, while others possess cations such as phosphonium, sulfonium or metal centers [7–13].

We have developed several antimicrobial macromolecules utilizing pine tree-derived natural resin acids (or rosins) that have relatively good antimicrobial activities [14–17]. Cationic charges were implemented as pendent groups from polymer backbones. However, antimicrobial agents such as antimicrobial peptides and antimicrobial polymers with facially amphiphilic orientation show better antimicrobial properties due to a local balance of amphiphilicity [18]. We noted bile acids as potential candidates for preparing effective antimicrobial polymers. Bile of mammals and other vertebrates is rich in bile acids, which are amphiphilic steroidal acids. They typically stay conjugated with taurine or glycine in the liver forming bile salts that serve as surfactants to solubilize dietary lipids and fats by the formation of micelles allowing digestion of food. Bile acids have been utilized in many areas including gene delivery [19,20], drug delivery [21], sensing [22], polymeric gels [23], antimicrobial agents [24–26] and other biological applications [27].

The 5β framework of bile acids or the cis A-B ring junction imparts a curvature to the ring system resulting in two faces with dramatically different properties [28]. Hydroxyl groups of bile acid molecules are positioned in the α-face while their methyl groups

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are in the β-face, thereby creating facial amphiphilicity (Fig. 1A). The steroidal nucleus with four fused rings provides the hydrophobic core that can preferentially embed into cell membranes. The presence of hydroxyl and carboxylic acid groups offers hydrophilic chemical functionalization to achieve robust molecular designs and architectures to investigate key determinants of its surface activity and the ability to selectively interact with membrane lipids [29,30].

Recent advances on bile acid in macromolecular research include a variety of structures having bile acids as repeating units in the polymer backbone, as pendant groups along the polymer chain in block, or statistical polymers and chain end-functional polymers [31]. Notable advances on bile acid polymers have been carried out by Zhu and coworkers [32,33]. Controlled polymerization methods such as ATRP, RAFT and ROMP have been used to make side chain bile acid-containing polymers. Polymers containing bile acids in the main chain have been prepared using step-growth polymerization via incorporating a variety of linkers, such as esters, anhydrides, triazoles, β-amino esters and sulfide [34–38]. Polycondensations are useful to prepare highly efficacious and inexpensive antimicrobial polymers for numerous applications [39]. A unique class of polyelectrolytes known as ammonium polyionenes can be prepared by step-growth polymerizations to have cations in the main chain at regular and specific sites [40]. They are generally prepared from a reaction between ditertiary amines and dihalides via Menschutkin reaction [41,42]. These polymers exhibit strong and fast acting antimicrobial activities [43–45]. However, little is known regarding the preparation of hydrophobic polyionenes that incorporate natural product-derived chemicals targeted for antimicrobial applications.

We envisioned the possibility to develop dihalides monomers from bile acids such as lithocholic acid that can be used to prepare quaternary ammonium polyionenes. Water soluble cationic polymers with degradable ester linkages can be easily developed using lithocholic acid in the main chain. In this study, we developed difunctional monomers from lithocholic acid, and used them to make cationic polymers that contain quaternary ammonium groups along the polymer backbone. These polyionene antimicrobial polymers formed micelles in water. The antimicrobial activity, cytotoxicity, and drug delivery applications were then demonstrated.

2. Experimental section

2.1. Materials

Lithocholic acid (95%, Aldrich), lithium aluminum hydride (95%, Aldrich), 2-bromoacetyl chloride (95%, Aldrich) and 6-bromohexanoyl chloride (97%, Aldrich) were used as received. *N,N,N′,N′*-tetramethyl-1,2-ethanediamine (99%, TCI) and *N,N,N′,N′*-tetramethyl-1,6-hexanediamine (99%, Aldrich) were distilled before use. Tetrahydrofuran (THF) and *N*, *N*-dimethylformamide (DMF) were dried over drying columns. Ampicillin was purchased from VWR as the pure form. All other reagents and solvents were from commercial resources and used as received unless otherwise mentioned. Spectrum Spectra/Por® 3 Dialysis Membranes with MWCO 3500 were purchased from VWR. All other reagents used for biological assays were purchased from Thermo Fisher Scientific or Sigma Aldrich and will be mentioned in the respective sections.

2.2. Characterization

^{1}H NMR (300 MHz) spectra were recorded on a Bruker Avance III HD 300 with deuterated chloroform or dimethyl sulfoxide as solvents. The molecular weight of polymers was determined by size exclusion chromatography (SEC) using Agilent 1200 (pump, autosampler), Wyatt DAWN HELEOS-II multiangle light scattering detector (MALS) (λ = 662 nm), Wyatt Optilab T-rEX (dRI) with Viscotek columns (Model- I-MBMMW-3078), exclusion limit (PS)-200 kDa, max pore size 10,000 Å. The mobile phase was DMF with 0.05 M LiBr. The samples were prepared at 5.0 mg/mL in DMF. The molecular weights were determined using the light scattering detector response with Astra V (Wyatt Technologies, Santa Barbara, CA) software. The polymer dn/dc was estimated from the mass recovery, and values ranged from 0.0062 to 0.0332 mL/g. The morphology of polymer micelles was recorded by Field-Emission Scanning Electron Microscopy (FE-SEM, Zeiss UltraPlus). A solution containing 1.0 mg/mL was prepared in water and 10 μL drop was added on to 1 cm × 1 cm plasma cleaned Si wafers. Drop-cast films were air dried for 12 h and sputtered with gold before imaging. Films were observed using an acceleration voltage of 5.00 kV. Imaging was done under in-lens secondary electron detector with a working distance of 3.00 mm or less during the acquisition of images. The steady-state fluorescence spectra were recorded at room temperature using a PTI QM-400 fluorometer. UV–vis spectra were recorded on a Shimadzu UV 2450 spectrophotometer.

2.3. Synthesis of 3α,5β-cholane-3,24-diol

A suspension of $LiAlH_4$ powder (2.0 g, 0.053 mol, 2.0 equiv.) was added to dry THF 100 mL in a round-bottom flask slowly and carefully in an ice bath. While maintaining cold temperature, lithocholic acid (10 g, 0.027 mol, 1.0 equiv.) was added to the

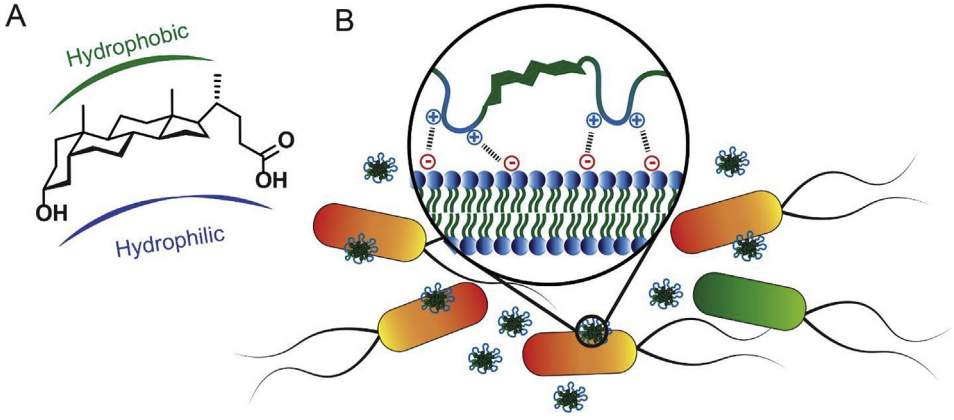

Fig. 1. (A) Facial amphiphilic structure of lithocholic acid; and (B) the functional importance of this structure in antimicrobial polymers for conferring destructive membrane interactions promoting antimicrobial activity.