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Stimuli responsive charge-switchable lipids: Capture and release of nucleic acids

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ABSTRACT

Stimuli responsive lipids, which enable control over the formation, transformation, and disruption of supramolecular assemblies, are of interest for biosensing, diagnostics, drug delivery, and basic transmembrane protein studies. In particular, spatiotemporal control over a supramolecular structure can be achieved using light activated compounds to induce significant supramolecular rearrangements. As such, a family of cationic lipids are described which undergo a permanent switch in charge upon exposure to 365 nm ultraviolet (UV) light to enable the capture of negatively charged nucleic acids within the self-assembled supramolecular structure of the lipids and subsequent release of these macromolecules upon exposure to UV light and disruption of the assemblies. The lipids are composed of either two different tripeptide head groups, Lysine–Glycine–Glycine (KGG) and Glycine–Glycine–Glycine (GGG) and three different hydrocarbon chain lengths (C_6, C_{10}, or C_{14}) terminated by a UV light responsive 1-(2-nitrophenyl)ethanol (NPE) protected carboxylic acid. The photolysis of the NPE protected lipid is measured as a function of time, and the resulting changes in net molecular charge are observed using zeta potential analysis for each head group and chain length combination. A proof of concept study for the capture and release of both linear DNA (calf thymus) and siRNA is presented using an ethidium bromide quenching assay where a balance between binding affinity and supramolecular stability are found to be the key to optimal nucleic acid capture and release.

1. Introduction

Lipids self-assemble into a wide range of supramolecular structures including uni-lamellar and multi-lamellar micelles, vesicles, and membranes (Dan and Danino, 2014; Nikolelis et al., 1999). In this role, lipids serve as the interface or boundary between aqueous environments, a means to compartmentalize different biologics, as well as a relatively hydrophobic medium for anchoring of surface proteins. The specific supramolecular assemblies formed are dictated by the size and charge of the hydrophilic head group(s), as well as the length of the hydrophobic chain of the lipid (Vill and Hashim, 2002; Nguan et al., 2010; Hashim et al., 2012). Examples of lipid compositions synthesized include those bearing phosphate, saccharide, nucleoside, quaternary amine, or polypeptide head groups and varying chain configurations (e.g., unsaturated vs. saturated or branched vs. linear) (Fahy et al., 2005; Bhattacharya and Bajaj, 2005; Gissot et al., 2008; Ratnayaka et al., 2012).

Stimuli-responsive lipids represent a unique class of functional lipids. These lipids or amphiphiles are employed to induce transitions between supramolecular states or dissolution of the assembly via the application of an external or internal stimulus. For example, temperature (Kurisawa et al., 2000; Hinrichs et al., 1999; Kono,, 2001; Türk et al., 2004; Ma et al., 2010; Zhang et al., 2011; González-Henríquez and Sarabia-Vallejos, 2015), pH (Wang and Huang, 1989; Budker et al., 1996; Legendre and Szoka, 1992; Shi et al., 2002; Simões et al., 2004; Xiong et al., 2007), enzymatic (Zhang et al., 2011), chemical (Byk et al., 2000; Wetzer et al., 2001; Abbott et al., 2005; Liu et al., 2005; Jewell et al., 2006; Liu et al., 2008; Jewell et al., 2008; Oumzil et al., 2011), mechanical (ultrasound) (Wells,, 2010; Yoon and Park, 2010), and light (Nagasaki et al., 2000; Nagasaki et al., 2003; Sasaki et al., 2006) responsive systems are described. From an application perspective, these stimuli responsive amphiphiles are used as triggers of supramolecular assembly into biosensing membranes (Liu et al., 2012; Uto et al., 1990; Szymańska et al., 2001), tunable hydrogels (Moreau et al., 2004; Ji et al., 2004; Menger and Peresypkin, 2003;

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Yang et al., 2012), carriers for drug delivery (Ganta et al., 2008; Mura et al., 2013; Torchilin, 2014; Liu et al., 2014), and as membranes for the study of transmembrane proteins (Bhattacharya and Bajaj, 2005; Folgering et al., 2004). For example, Liu et al. developed a phospholipase D responsive biosensor by creating a phospholipid-graphene nanoassembly that mimicked a cellular membrane and provided enhanced fluorescence due to the improved efficiency of fluorescence energy transfer between fluorophores and the nonoxidized graphene. By incorporating a fluorescently labeled phospholipid into this nanoassembly, the activity of phospholipase D was quantified over a wide dynamic range and down to a very low detection limit (0.01 U/L) in real time as the enzyme cleaved the fluorescent phospholipids from the nanoassembled construct.

Among the many possible stimuli, light-activated systems offer a high degree of spatiotemporal control. In fact, photo-caging of small molecules has facilitated the study of enzymes and metabolites. In this strategy, a light activated protecting group is conjugated to the biomolecule effectively "caging" it from an active state allowing for the controlled addition of these molecules to a biologic system upon exposure to the appropriate wavelength of light (Kaplan et al., 1978; Adams and Tsien, 1993). The first use of a photo-caging system was reported by Kaplan et al. to study the activity of a Na, K-ATPase in red blood cells by caging ATP into an inactive form using a 1-(2-nitro) phenylethyl phosphate and providing active ATP under light activated control (340 nm) (Kaplan et al., 1978). While these caging events occur on the individual molecule level, strategies employing similar chemistries to alter or disrupt a supramolecular assembly can be used to release a large number of biomolecules simultaneously or induce a change in conformation within a biosensing membrane (Nagasaki et al., 2000; Nagasaki et al., 2003; Sasaki et al., 2006; Folgering et al., 2004). As such, there is a need for the development of stimuli responsive amphiphiles which respond to a specific external stimuli.

Herein, we describe the synthesis of a family of ultraviolet light-activated amphiphiles which capture and release anionic biopolymers such as DNA and siRNA. The approach relies on electrostatic interactions between a cationic amphiphile and the negatively charged biopolymer to capture it, and then release it upon switching the amphiphile charge from cationic to anionic using UV light (Fig. 1). Specifically we report: 1) a family of four new lipids with two different cationic tripeptide head groups (KGG or GGG) and three different hydrocarbon chain lengths (C_6, C_{10}, or C_{14}) terminated by a UV light responsive nitrobenzyl protected carboxylic acid; 2) the quantification of the deprotection rate via UV light; and 3) the efficiency of the capture and release of DNA and siRNA.

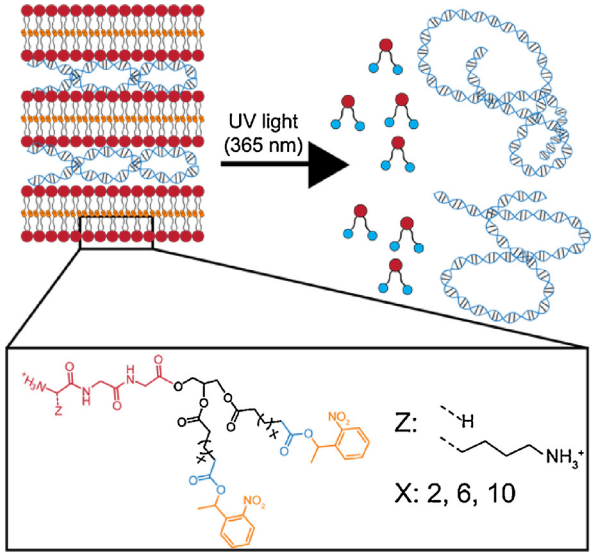

Fig. 1. Schematic diagram of cationic lipids forming supramolecular structures with anionic nucleic acids sandwiched between the hydrophilic tripeptide head groups (red) and separated by the hydrophobic alkyl chains (black). The photoactive NPE protecting group (orange) is cleaved by 365 nm UV light exposing a free carboxylic acid (blue), which destabilizes the assembly resulting in nucleic acid release. Not to scale. (For interpretation of the references to color in legend, the reader is referred to the web version of this article.).

2. Experimental

2.1. General materials and procedure for synthesis

All chemicals were purchased from Aldrich. Solvents used during synthesis were dried and distilled prior to use. All reactions were done in dry conditions using nitrogen. NMR spectral analysis was performed using a Varion Mercury spectrometer operating at 300 MHz, unless otherwise noted.

2.2. Synthesis of O,O'-(3-{2-[2-(2,6-diaminohexanamido) acetamido] acetoxy}propane-1,2-diyl) bis[1-(2-nitrophenyl) ethyl] diadipate (KGG-C_6-NPE). 6-[1-(2-nitrophenyl) ethoxy]-6-oxohexanoic acid (1a)

Adipic acid (6 g, 41.1 mmol), was dissolved in minimal DMF, DCM was added (1:1 vol), and the solution was cooled to 0 °C. 1-(2-nitrophenyl) ethanol (2.3 g, 13.7 mmol) and cat. DMAP were added. Last, EDCI (3.9 g, 20.5 mmol) was added to the mixture and the reaction was stirred overnight warming up to RT. The solution was taken up into EtOAc (200 mL) and washed with water, ^{1}N HCl, and sat. NH_4Cl solution. The organic layer was dried over Na_2SO_4. The volatiles were evaporated and the residue was purified on silica gel chromatography (gradient EtOAc:hexanes, 1:2–1:1), affording product **2a** as a white thick oil in 66% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.58–1.72 (m, 7 H), 2.31–2.40 (m, 4 H), 6.32 (q, 1 H, J = 6.6 Hz), 7.41–7.47 (m, 1 H), 7.62–7.68 (m, 2 H), 7.93 (d, 1 H, J = 8.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.9, 23.9, 24.1, 33.6, 33.8, 68.0, 124.4, 127.2, 128.4, 133.6, 137.8, 147.7, 172.2, 179.5. HRMS: m/z calcd for $C_{14}H_{17}NO_2$ $[M + Na]^+$: 318.0954; found: 318.0966.

2.2.1. O,O'-{3-[bis(4-methoxyphenyl) (phenyl) methoxy]propane-1,2-diyl} bis[1-(2-nitrophenyl) ethyl] diadipate (2a)

3-[Bis(4-methoxyphenyl)(phenyl) methoxy] propane-1,2-diol (200 mg, 0.51 mmol), compound **1a** (389 mg, 1.32 mmol), and cat. DMAP were dissolved in DCM (10 mL) and the solution was cooled to 0 °C. EDCI (340 mg, 1.77 mmol) was added to the mixture and the reaction was stirred overnight warming up to RT. The solution was washed with sat. $NaHCO_3$ and sat. NH_4Cl solutions. The organic layer was dried over Na_2SO_4. The volatiles were evaporated and the residue was purified by silica gel chromatography (gradient hexanes:EtOAc, 4:1 to 1:1 with 1% TEA). The product 2a was obtained as white thick oil in 35% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.49–1.55 (m, 14 H), 2.15–2.25 (m, 8 H), 3.13 (d, 2 H, J = 3.9 Hz), 3.68 (s, 6 H), 4.09–4.28 (m, 2 H), 5.16–5.18 (m, 1 H), 6.23 (q, 2 H, J = 6.0 Hz), 6.73 (d, 4 H, J = 8.4 Hz), 7.11–7.33 (m, 11 H), 7.47–7.53 (m, 4 H), 7.81 (d, 2 H, J = 7.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.9, 24.1, 24.2, 33.5, 33.8, 55.2, 61.9, 63.0, 68.0, 70.6, 86.1, 113.1, 124.4, 126.9, 127.2, 127.8, 128.0, 128.4, 130.0, 133.6, 135.6, 137.9, 144.5, 147.7, 158.5, 172.0, 172.5, 172.8. HRMS: m/z calcd for $C_{52}H_{56}N_2O_{15}$ $[M + Na]^+$: 971.3578; found: 971.3577.

2.2.2. O,O'-(3-hydroxypropane-1,2-diyl) bis[1-(2-nitrophenyl) ethyl] diadipate (3a)

Compound **2a** (100 mg, 0.11 mmol) was dissolved in DCM (3.5 mL) and the solution was cooled to 0 °C. To the solution was