Supramolecular assemblies of DNA with neutral nucleoside amphiphiles†

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A neutral uridine-based amphiphile is described which condenses plasmid DNA. AFM studies show that the three distinct structural components of the amphiphile (*i.e*, nucleobase, alkyl chains, and poly(ethylene glycol)) are required for the formation of DNA–amphiphile supramolecular assemblies on a mica surface.

Amphiphiles play important roles in diverse applications including building blocks for nanotechnology, tools for biophysical membrane studies, and carriers for drug delivery.[1–13] The supramolecular structures formed in those systems are a consequence of non-covalent interactions (*e.g.*, electrostatic, hydrophobic, H-bonding, *etc.*) and vary in size from a few nanometers to millimeters. We are interested in determining the molecular design criteria required for an amphiphile to have selective interactions with biological macromolecules; such systems are likely to advance our understanding, ability to manipulate biological systems, and capability to form supramolecular assemblies. For example, the binding of cationic amphiphiles with biological macromolecules, such as DNA, to form assemblies is of widespread interest and clinical importance.[14–17] However, the electrostatic interaction between the cationic amphiphile and anionic DNA is non-specific and therefore these amphiphiles can also bind a number of proteins at pH = 7.4, since many proteins have a low isoelectric point (*e.g.*, albumin). Herein, we describe a neutral nucleoside amphiphile for condensing plasmid DNA on a surface.

The design principle of this neutral amphiphile relies on cooperative non-covalent interactions, such as the molecular forces that hold nucleic acid helices together (Watson–Crick/Hoogsteen hydrogen-bonding and base π-stacking), and hydrophobic chain–chain interactions present in lipid bilayers.[18–20] Consequently, this amphiphile possesses a DNA nucleobase, a hydrophobic region (*e.g.*, alkyl chains), and a poly(ethylene glycol) (PEG) segment as shown in Fig. 1 (structure **1**). Amphiphiles **1–4** were synthesized and studied to determine the possible role of and interplay between the nucleobase, alkyl chains, and PEG of the amphiphile structure in the formation of DNA–amphiphile assemblies (Fig. 1).†

Given the amphiphilic structure of these compounds, it is likely that micelles will form in aqueous solution. Previous results with PEG derivatized phospholipids showed spherical micelle formation with critical micelle concentrations (CMCs) in the μM range.[21,22] Micelles are formed with amphiphiles **1** and **2** with CMC values of 6.4×10^{-5} and 4×10^{-4} M, respectively. The lower CMC observed with amphiphile **1** compared to **2** is consistent with amphiphile **1** possessing increased intermolecular interactions.

The ability of compounds **1** through **5** to condense plasmid DNA on a mica surface was investigated using atomic force microscopy (AFM). DNA is well known to undergo an elongated coil-to-globular transition in the presence of amphiphilic cationic polymers. Several AFM studies have described the toroidal structures formed in DNA–polymer condensates.[23,24] Fig. 2 shows the atomic force micrographs of plasmid DNA (pBR322 plasmid DNA *E. coli* strain RII) in the presence of **1** and **2**. All the studies were performed above a concentration of 5×10^{-4} M (*i.e.*, above the CMCs for the amphiphiles). Only amphiphile **1** condenses plasmid DNA. The micrographs at this amphiphile concentration (1 mg mL^{-1}) show more than 80% of the structures on the surface are of a collapsed state. The AFM images of DNA in the presence of **3**, **4**, or **5** are very similar to the micrographs of **2** or DNA without an amphiphile. Upon examining a series of micrographs of DNA (0.5 μg mL^{-1}) with varying amphiphile **1** concentrations from 0.5 to 5 mg mL^{-1} we observed a range of collapsed states from the partial collapse to the condensed supramolecular structure, a toroid. The AFM image of a toroid at higher magnification is shown in Fig. 3. The toroid has an outer and inner diameter of approximately 100 nm (av. 106 ± 22 nm) and 20 nm (av. 21 ± 11 nm), respectively, and is similar to structures previously observed with cationic DNA condensation agents.[24] Under similar conditions toroids are not observed with compound

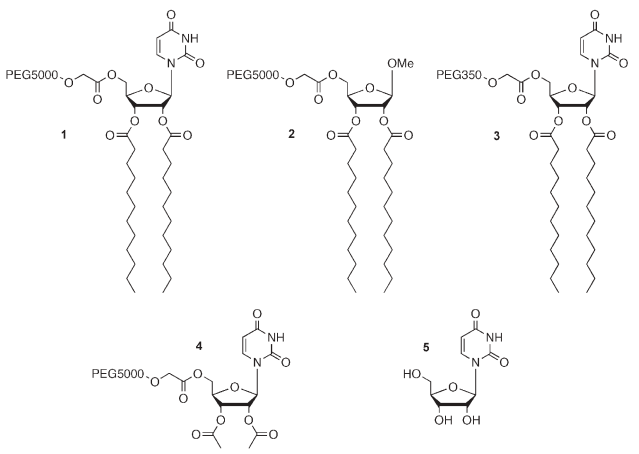

Fig. 1 Nucleoside amphiphiles and structural analogs.

† Electronic supplementary information (ESI) available: detailed experimental information for the synthesis of the amphiphiles. See http://www.rsc.org/suppdata/cc/b4/b412670j/

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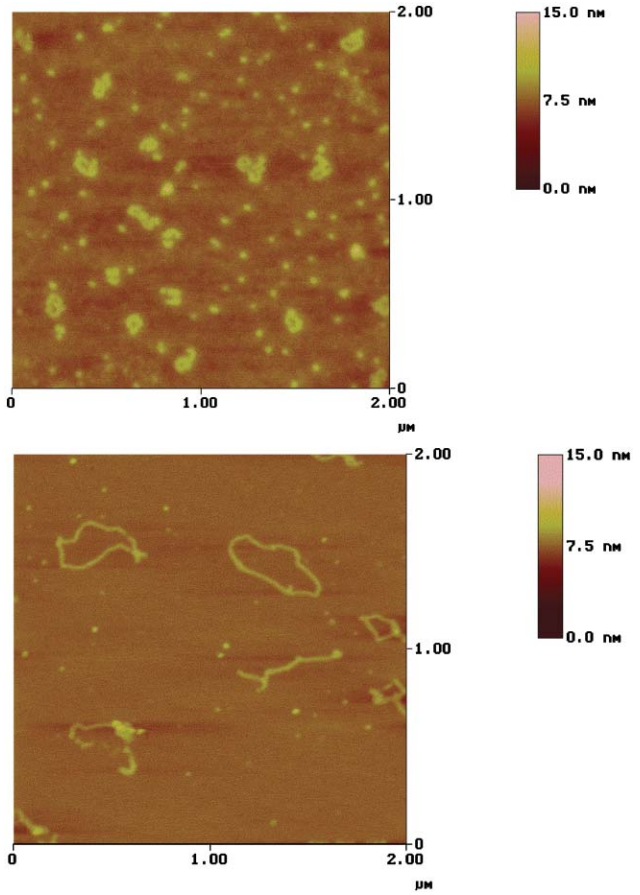

Fig. 2 AFM images of plasmid DNA in the presence of (top) amphiphile **1** and (bottom) amphiphile **2**.

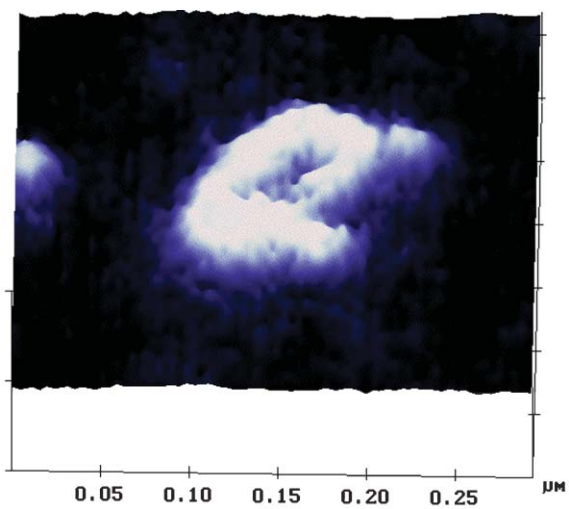

Fig. 3 3D AFM image of a toroid formed between amphiphile **1** and DNA at higher magnification (outer diameter of the toroid is approximately 100 nm). Image is a 300 nm^2 with a *z* range of 10 nm.

2 which lacks the nucleobase, confirming the requirement of the uridine in the amphiphile structure. Since the uridine but not the OMe analog condenses DNA, it is reasonable to propose that the nucleobase weakly interacts with the plasmid DNA *via* H-bonding and/or π-stacking interactions. The condensation of DNA is not observed with **4** or **5**, highlighting the significance of the hydrophobic chains. Finally, toroid formation is not observed with **3** or **5**, indicating the need for a large molecular weight PEG chain.

All three structural components in the amphiphile (*i.e*, nucleobase, alkyl chain, and PEG) are important for DNA condensation. For example, the binding constant for just a single U–U base–base interaction in water is small. In a non-hydrogen bonding organic solvent (*e.g.*, $CHCl_3$), the binding constant is estimated to be less than 10^2 M^{-1} and orders of magnitude weaker than known cationic amphiphiles like DOTAP (1,2-dioleoyloxy-3-(trimethylammonio)propane; 10^7 M^{-1}) binding to DNA in water.[25,26] Thus, it is unlikely that the nucleobase itself is responsible for DNA condensation, consistent with the experimental results – compound **1** but not **5** affords toroids. The hydrophobic alkyl chain–chain interactions play a significant role, as observed in liposomes,[20,27] and contribute to favorable enthalpic energies for the condensation, given that **1** but not **4** gives toroids. The role of the high molecular weight PEG chain is multifaceted. First, the PEG increases the limited solubility of the hydrophobic, long-chain acylated nucleoside in aqueous solution. Second, the PEG and alkyl chains are required for micelle formation. Third, it has been shown that very high concentrations of PEG in aqueous solution will precipitate DNA.[28] This effect is manifested through a loss of water hydration of the DNA *via* increased interactions between the DNA and PEG. The PEG concentrations used in our experiments are significantly lower, and DNA condensation is not observed with PEG 5000 Mw under these experimental conditions. Thus, we propose that **1** condenses plasmid DNA through an organized macromolecular crowding mechanism.[29,30] In solution, the micelles composed of the uridine amphiphiles interact with each other as well as with the plasmid DNA, and condensation occurs when the local micelle concentration becomes high near the DNA. This is consistent with the observation that amphiphiles **1** and **2** form micelles, but only **1**, the amphiphile that possesses the uridine, condenses DNA.

In summary, a non-cationic amphiphile for the condensation of DNA is described. With the objective of mimicking a nucleosome, a recent report describes a glycocluster amphiphile which binds DNA through interactions with the phosphate backbone.[7] These results provide further evidence for the use of non-catonic interactions to manipulate DNA. The importance of multiple non-covalent weak interactions working in unison to form supramolecular structures cannot be overstated. The development of amphiphiles possessing affinity to a biological macromolecule (*e.g.*, protein, mRNA, DNA, *etc.*) through more specific interactions, as opposed to more general electrostatic forces, will broaden their use in the areas of biological chemistry, biomaterials, and biotechnology.

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