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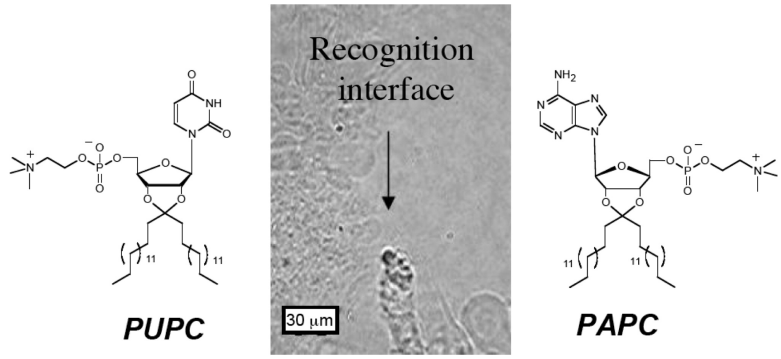
J. Am. Chem. Soc., **2008**, 130 (44), 14454-14455 • DOI: 10.1021/ja805974g • Publication Date (Web): 14 October 2008



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Published on Web 10/14/2008

Real Time Imaging of Supramolecular Assembly Formation via Programmed Nucleolipid Recognition

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The synthesis of supramolecular systems using weak noncovalent chemical bonds to assemble molecules is an important and increasingly successful strategy.[1] These supramolecular assemblies represent diverse structures and span length scales from a few nanometers to millimeters, with examples reported such as DNA scaffolds,[2] nucleolipoplexes,[3] peptide nanofibers,[4] reversible polymers,[5] dendritic assemblies,[6] and nanotubes.[7] Elucidation and control of the factors that govern the recognition events at the molecular level represent a significant advantage to construct functional or intricate systems. To this end, our interest is in using increasingly complex molecules capable of forming programmable supramolecular systems. Nucleolipids possessing both nucleic acid recognition and lipophilic chains components are emerging as building blocks for constructing such assemblies because these amphiphiles possess a diversity of functional groups capable of cooperative noncovalent interactions combined with specific base−base recognition.[8] We have used such nucleolipids to form nanofibers and gels as well as supramolecular complexes with nucleic acids for gene transfection.[9] Herein, we report the formation of self-assemblies resulting from the interactions of complementary ketal-based adenosine and uridine nucleolipids (Scheme 1). The dynamics of the recognition and supramolecular assembly formation can be visualized in real time at the micrometer scale and is dependent on the nucleobase complementary recognition and the hydrophobic ketal chains.

Uridine and adenosine phosphocholine amphiphiles **3** and **6**, respectively, were synthesized starting from the aliphatic symmetrical palmitone and the appropriate nucleoside (see SI for complete details). Briefly stated, uridine and palmitone were refluxed in THF containing triethylorthoformate under acid catalysis. The acetonide derivative **2** was then reacted successively with 2-chloro-1,3,2-dioxaphospholane-2-oxide and trimethylamine in THF to give the final uridine nucleolipid **3** (2′,3′-*O*-16-hentriacontanyliden-uridine-5′-phosphocholine, **PUPC**). For preparation of the adenosine analogue, the dimethoxy ketal of palmitone **4** was synthesized by refluxing the ketone **1** in THF containing triethylorthoformate, methanol under acid catalysis conditions. Next, condensation of **4** with adenosine followed by reaction with 2-chloro-1,3,2-dioxaphospholane-2-oxide and trimethylamine in THF afforded the adenosine nucleoamphiphile **6** (2′,3′-*O*-16-hentriacontanyliden-adenosine-5′-phosphocholine, **PAPC**). The phase transition temperature (T_m) and phase transition enthalpies (ΔH) of the nucleoside phosphocholine were similar for the uridine (**PUPC**, T_m = 14.1 °C, ΔH = 2.0 ±0.1 Kcal/mol) and adenosine (**PAPC**, T_m = 14.5 °C, ΔH = 1.7 ±0.1 Kcal/mol) nucleolipids. The T_m values are lower than the corresponding acyl analogues[9] as well as for the natural diacylglycerol phosphocholine.[10] Equimolecular mixtures of **PAPC** and **PUPC** exhibit a single-phase transition temperature (T_m = 14.5 °C). However, enthalpies of the transition are higher for the mixture (2.6 ± 0.1 Kcal/mol) than that of the individual ketals. Such enhanced enthalpies confirm that there is a mixing of the two lipids. The increased amount of energy needed for the main phase transition also indicates that the PAPC/PUPC supramolecular system is stabilized compared to pure nucleolipids because of complementary A/U recognition events.

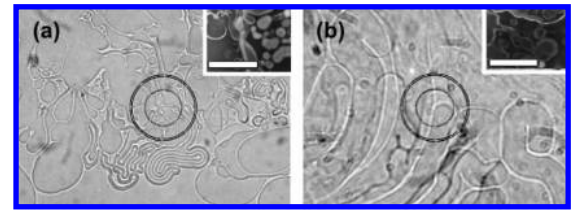
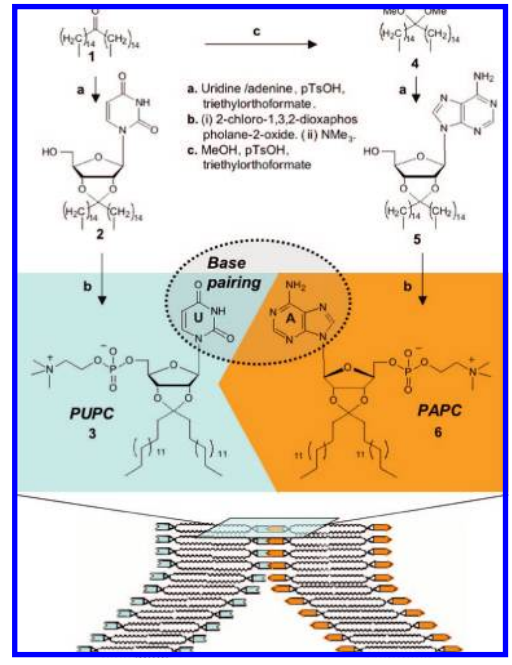


Figure 1. (i) Light micrograph of **PUPC** (a) and **PAPC** (b) supramolecular organizations upon hydration (internal circle diameter = 14 μm) at room temperature ($T > T_m$). (ii) Insets, TEM image of a typical lamellar system of **PUPC** (a) and **PAPC** (b) in aqueous solution (5% w/w, $T > T_m$, bars = 1 μm).

Scheme 1. Synthetic Scheme and Proposed Assembly for the Ketal Nucleolipids



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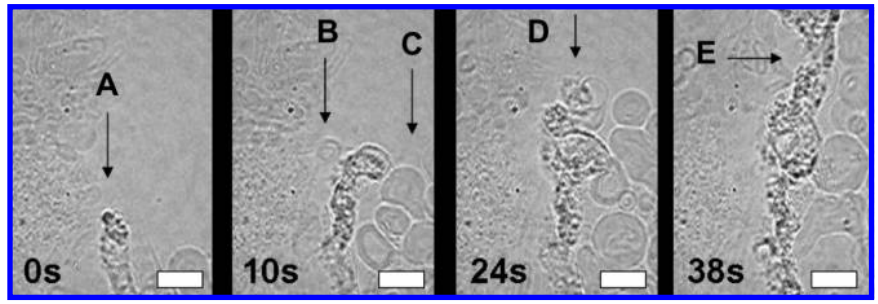

Figure 2. Photographs of vesicular formation at the interface of **PUPC** and **PAPC** upon hydration. Arrows show (A) the **PUPC/PAPC** interface, (B, C) **PUPC** and **PAPC** dispersions are moving toward the recognition interface, (D, E) "wall extension" at a rate of about 5 μm per second (bars = 30 μm).

Next, we investigated the supramolecular structures formed by these ketal-based nucleolipids. Below their T_m values and at a concentration above 4% w/w, both nucleolipids form hydrogels. Above their T_m values, we observed the formation of large lamellar systems upon hydration with no major differences observed between **PUPC** and **PAPC** lipids (Figure 1). TEM images show heterogeneous vesicles for both **PUPC** and **PAPC** samples (Figure 1, insets, and SI).

The formation of supramolecular assemblies in the presence of these two nucleolipids was investigated. Separately lipids **PUPC** and **PAPC** exist in a fluid state at room temperature and, as noted earlier, organize into lamellar systems upon hydration. However, when the two complementary lipids are hydrated in close proximity to each other a new supramolecular structure spontaneously forms at the interface in a few seconds. For this experiment, solid **PUPC** and **PAPC** are loaded on a glass slide opposite each other and separated by a distance of 0.5 mm. Real time photographs show that upon hydration the two lamellar systems approach each other and upon contact form a wall of vesicles at the interface (Figure 2; See movie in SI). Numerous small vesicles (<50 μm) are present in this wall of vesicles as well as fusions with larger ones. The molecular recognition and vesicular formation event occur rapidly and we can estimate a vesicle wall extension rate of about 5 μm per second. Similarly in solution, the mixture of **PUPC** and **PAPC** gives spontaneously a macroscopic assembly at a temperature higher than T_m, whereas control samples made of **PUPC/PUPC** or **PAPC/ PAPC** mixtures do not. TEM experiments confirm that the **PUPC/PAPC** assembly is a lamellar system composed of a mixture of closely associated heterogeneous vesicles with numerous very small vesicles and/or buds, which are not observed for pure samples. When the mixing experiment (in solution or on a glass slide) is repeated with analogues of the nucleolipids lacking the ketal chains or the complementary base pairing, assembly formation is not evident demonstrating the important role of the hydrophobic ketal chains and base pair recognition.

To further understand the supramolecular recognition event, we performed Monte Carlo simulations on the ketal nucleoamphiphiles using an Amber force field in all atom mode in virtual solvation (Born−Oppenheimer, water solvant).[11] The studies suggest that the ketal functionality locks this bicyclic ribonucleoside in the southern conformation (C2′ endo) restricting the conformation to favor base-pair recognition between the self-organized nucleolipids (see Figure 3 and SI).

In summary, we have prepared new uridine and adenosine phosphocholine amphiphiles. Upon hydration at room temperature the individual nucleolipids form lamellar systems, whereas in the presence of each other microsized vesicles are formed. The vesicle formation is dependent both on the presence of the ketal hydrophobic chains and the complementary nucleobases. The visualization of this the **PUPC/PAPC** molecular recognition and vesicle formation in real time at the micrometer scale is striking as typically we must use physicochemical methods such as UV, IR, or NMR techniques to observe such phenomenon.[12,13] Continued research is this area will provide key design requirements and understandings toward synthesizing specific lipid-based supramolecular assemblies via programmed or encoded small molecules.

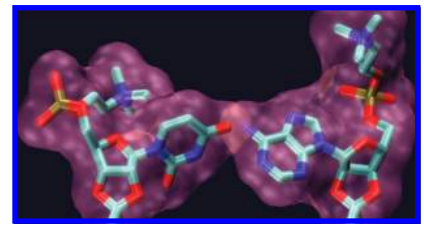

Figure 3. Molecular models of the proposed amphiphile structures in the south conformation (C2′ endo conformation of U and A derivatives left and right, respectively).

Acknowledgment. L.M. and P.B. acknowledge financial support from the Army Research Office. M.W.G. acknowledges support from BU. N.T. acknowledges support from "Association Française contre les Myopathies" (AFM).

Supporting Information Available: Synthesis of compounds **3** and **6**, transmission electron micrographs, and molecular modeling details and movie. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA805974G