J|A|C|S COMMUNICATIONS

Published on Web 05/19/2010

Virus-like Particles Templated by DNA Micelles: A General Method for Loading Virus Nanocarriers

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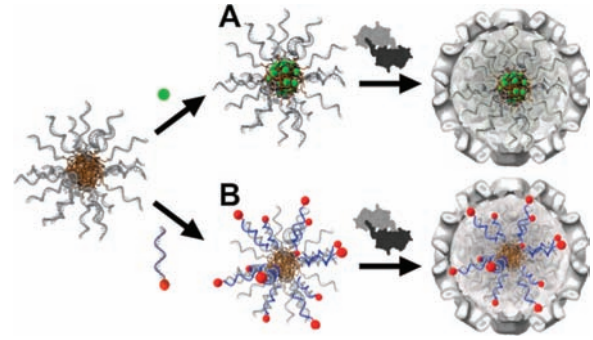
Virus capsids (VCs) or virus-like particles are a relatively new class of natural biomaterials with great potential for materials science and nanotechnology. They form precisely defined stable cage structures, permit coat protein (CP) manipulation through mutagenesis or chemical modification,[1] and can be easily produced. Such exceptional characteristics make them particularly strong candidates for applications in biomedicine.[2−7] The natural container-like properties of viruses, as well as their ability to specifically target individual cells, have been attractive for gene delivery and are now being harnessed for therapeutic delivery. While some VCs have been investigated and chemically modified to probe targeting behavior,[8] scant work has been dedicated to loading these nanocontainers.[9] An excellent model system in this regard is the Cowpea Chlorotic Mottle Virus (CCMV). As with other VCs, CCMV evolved to encapsulate and transport RNA; in its natural state it consists of 180 identical CPs, which self-assemble around the central RNA into 28 nm icosahedral particles. These can be described according to the Caspar and Klug T (triangulation) number as $T = 3$ particles.[10] It has been shown that such capsids can also be readily made to self-assemble around large polyanions, resulting in smaller $T = 1$ icosahedral particles of 18 nm.[11,12] CCMV is unique in that capsid assembly can be induced in acidic conditions even in the absence of nucleic acids, allowing the possibility of loading more diverse cargos such as enzymes.[13] Nonetheless, the porous walls of the shell severely complicate the loading and retention of small molecules. A still more severe limit is imposed by solvent incompatibilities, which prevent the loading of hydrophobic drugs except through covalent modification of the protein or complexation with polyanions.[14] The success of engineered virus nanoparticles as delivery vehicles will hinge in large part on the resolution of these issues and the development of efficient loading strategies for small molecules and macromolecular entities.

We report here a strategy for the facile self-assembly and loading of CCMV capsids using DNA amphiphiles. These structures aggregate into micelles with a hydrophobic core and an anionic DNA corona. The negatively charged particles induce capsid formation, allowing the entrapment of a large number of small oligonucleotides (ODNs) as a constituent part of the micellar template. Furthermore, preloading of the micelles with hydrophobic entities in the core or hydrophilic entities by sequence-specific hybridization enables encapsulation of various small molecules inside VCs.

Scheme 1. DNA Micelle-Templated VC Formations[a]



[a] (A) Loading of hydrophobic molecules (green) into the core. (B) Equipping moieties (red) attached to complementary DNA by hybridization. Coat proteins encapsulate the micelle by a simple mixing process at neutral pH.

Two classes of DNA amphiphiles known to self-assemble into larger aggregates should be distinguished, though representatives of both were investigated in this study. The first class consists of low molecular weight hydrophobic molecules that are attached to ODNs.[15] Here a lipid-DNA 11mer (**UU11**) containing two 5-dodec-1-ynyluracil nucleobases at the 5′- end was synthesized (Scheme S1). The other class of DNA amphiphiles involves linear DNA block copolymers (DBCs) in which a nucleic acid sequence is covalently connected to a hydrophobic organic polymer via complementary end groups.[16] This study employed two DBCs containing polypropylene oxide blocks of the same molecular weight ($M_W = 6800$ g/mol) but different lengths of ODNs, namely an 11mer (**P11**) and a 22mer (**P22**) sequence. See Figure S2 for the DNA sequences and key chemical structures.

All three amphiphiles formed micellar structures at room temperature, and in some cases the micelles were additionally loaded with model cargo molecules (SI.5). Particle sizes were characterized by dynamic light scattering (DLS) and AFM, and all fell in the range 7−11 nm (Figures S4 and S5).[15]

To assess the ability of these DNA particles to template VC formation both components were combined through a simple mixing procedure. In most experiments DNA amphiphiles were mixed with CP in a 1:2.3 molar ratio at pH 7.5 and incubated for 30 min at 4 °C. It should be stressed that under these conditions VC formation can only be attributed to the organizing role of the micelles. The resulting materials were isolated by fast protein liquid chromatography (FPLC). Transmission electron microscopy (TEM) analysis revealed successful envelopment of all DNA micelle species by the CCMV capsid protein. Particles eluted at 1.3−1.4 mL (Figure

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 10.1021/ja101444j

S7A) and exhibited a size of 19.9 ± 3.1 nm (Figure 1A). This size suggests that the objects have $T = 2$ symmetry. Also, a small proportion of the particles formed a $T = 1$ architecture. The TEM micrographs provide additional evidence of VC loading, since empty cores would appear dark under the negative staining conditions (inset Figure 1A).[13] Moreover, a fraction that eluted at 1.6 mL could be clearly identified as the remaining **UU11** micelles from its size distribution, 8.1 ± 1.6 nm (Figure 1B), in agreement with DLS measurements.

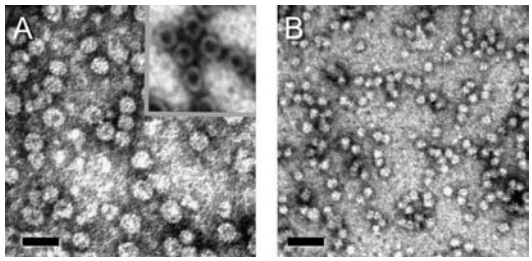

Figure 1. TEM micrographs, stained by uranyl acetate, of **UU11**-VC FPLC fractions eluted at (A) 1.4 mL, VCs, and (B) 1.6 mL (see Figure S6B), **UU11** micelles. The inset shows empty VCs for comparison. Scale bars are 40 nm.

After confirming the loading of VCs with DNA amphiphile aggregates, these scaffolds were further exploited for incorporation of other moieties. Since hydrophobic compounds are known to accumulate within the hydrophobic core of DBC amphiphiles, the fluorescent aromatic compound pyrene was introduced into **UU11** aggregates as a model small hydrophobic compound. Templated assembly of VCs was subsequently carried out as described above. During the FPLC elution of pyrene-loaded **UU11**-VC (Figure S7A), a fraction eluted at ~1.3 mL showed clear pyrene absorption. The fluorescence spectrum of the chosen fraction showed sharp and well-resolved pyrene emission bands demonstrating the presence of the fluorophore within the **UU11** aggregates and thus also inside the VCs (Figure S7B). Similar measurements were carried out using micelles loaded with a membrane staining dye, DiI (Figure S6B). The hydrophobic dye-loaded VCs were further examined by silver-stained gel electrophoresis (Figure S8) and TEM, which corroborated the analysis above for pristine micelle-loaded VCs.

Finally the challenge of loading small hydrophilic compounds was addressed. DNA micelles can be equipped by hybridization with almost any compound if conjugated by means of a cDNA sequence. Many functionalities are commercially available or can be easily synthesized. As a proof of concept, **UU11** micelles were labeled with ROX, a hydrophilic fluorescent dye, by hybridization with a ROX-DNA conjugate. VCs were formed employing the same general encapsulation procedure. Again FPLC analysis confirmed successful envelopment of the functionalized micelles (Figure S6A). With the help of the ROX label and Dylight functionalized CP, the aggregation number Z of micelles as well as the DNA content within the VC could be calculated. It turned out that Z amounted to 25 ± 2, which is in very good agreement with a geometrical calculation (SI.4). Assuming a $T = 2$ configuration, the ODN content to capsid ratio was found to be 6% by weight.

Short ODNs have already been combined with virus-like particles. VC networks were formed by hybridization employing virus-like particles that were chemically modified with nucleic acid sequences at the outside.[17] Incorporation of pristine ODNs into VC was demonstrated for the polyomavirus. However, the loading procedure was cumbersome because packaging required osmotic shock treatment as well as an acidic pH.[18] The approach presented herein is much simpler since a high number of ODNs are preassembled by the attached hydrophobic units, i.e. alkyl chains or polymers, acting as an efficient soft matter template and avoiding the need of altering the assembly conditions. Moreover, copackaging of various small compounds can be achieved by either hybridizing them onto the micelles or incorporating them into the core. Our novel loading approach therefore marks a significant step toward virus-based targeted therapeutics. This is especially true for hydrophobic drugs, which are difficult to couple chemically to water-soluble capsid proteins due to solvent incompatibilities. With the general loading strategy presented here, it is now possible to fully explore diverse applications, especially the potential of these nanocarriers as high-impact drug delivery systems.

Acknowledgment. M.K., A.J.M., and A.H. were supported by the EU (ERC starting grant, ECCell), The Netherlands Organization for Scientific Research (NWO-Vici), the Nuffic, the DFG, and the Zernike Institute for Advanced Materials. R.J.M.N. and J.J.L.M.C. acknowledge the NWO-CW, the Royal Netherlands Academy for Arts and Sciences (KNAW), and the European Science Foundation (ESF) for financial support.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA101444J