A Supramolecular Strategy to Assemble Multifunctional Viral Nanoparticles

L. Chen

Xia Zhao
University of South Carolina - Columbia

Y. Lin

Y. Huang

Qian Wang
University of South Carolina - Columbia, wang@mail.chem.sc.edu

Follow this and additional works at: http://scholarcommons.sc.edu/chem_facpub

Part of the Biochemistry Commons, Immunology of Infectious Disease Commons, Medicinal-Pharmaceutical Chemistry Commons, Organic Chemistry Commons, Virology Commons, and the Virus Diseases Commons

Publication Info
Published in Chemical Communications, Volume 49, Issue 83, 2013, pages 9678-9680.
© Chemical Communications 2013, Royal Society of Chemistry
http://dx.doi.org/10.1039/C3CC45559A

This Article is brought to you for free and open access by the Chemistry and Biochemistry, Department of at Scholar Commons. It has been accepted for inclusion in Faculty Publications by an authorized administrator of Scholar Commons. For more information, please contact SCHOLARC@mailbox.sc.edu.
A supramolecular strategy to assemble multifunctional viral nanoparticles†

Limin Chen,*a Xia Zhao,a Yuan Lin,*a Yubin Huanga and Qian Wang*ab

Using a one-pot approach driven by the supramolecular interaction between β-cyclodextrin and adamantyl moieties, multifunctional viral nanoparticles can be facilely formulated for biomedical applications.

The development of multifunctional nanoparticles for biomedical applications is of great importance.1,2 For example, the development of theranostic nanoparticles loaded with therapeutic drugs and imaging probes for the combined therapy and diagnosis has been gaining wide interest.3,4 Recently, inspired by the hierarchical structures of biogenic viral particles, multifunctional virus-like nanostructures have been constructed from synthetic polymers.5,6 In the meantime, the application of viral nanoparticles (VNPs) derived from plants and bacteria in biomedical fields has also attracted great attention, primarily due to their well-defined, programmable, multi-valent and monodispersed structural features7–19 as well as their good biocompatibility.20,21 To make the best use of the inherent compositions and structures of VNPs, many multifunctional VNPs have been constructed by the combination of mutagenesis and bioconjugation approaches, by which a variety of targeting ligands, catalytic units, diagnostic probes and therapeutic cargos have been anchored on the surface or inside of the internal cavity of VNPs.12,22–29

On the other hand, supramolecular interactions have been extensively applied to constructing drug and gene delivery systems, as well as smart materials due to their modularity, reversibility and stimuli-responsiveness.30–36 For example, β-cyclodextrin (β-CD), a natural toroid-shaped cyclic oligosaccharide, is one of the most widely used host-systems in supramolecular chemistry thanks to its low cost, good water solubility and biocompatible properties.37 β-CD has a hydrophilic exterior surface and a hydrophobic interior cavity that can accommodate a broad range of guest molecules (e.g., adamantane, azobenzene, and ferrocene).38,39

In this work, the supramolecular interaction between β-CD and adamantyl (Ada) moieties was exploited to assemble multifunctional VNPs. Tobacco mosaic virus (TMV) was employed as a model VNP, which is a classic example of rod-shaped plant virus, 300 nm long and 18 nm in diameter, consisting of 2130 identical subunit proteins arranged helically around genomic single RNA strand. β-CD units could be grafted onto the exterior surface of TMV using efficient bioconjugation reactions; and folic acid (FA), rhodamine B (RhB), doxorubicin (Dox), and polyethylene glycol (PEG) (Mw 2000 Da) were selected to functionalize TMV particles upon derivatization with Ada moieties and sequential supramolecular assembly (Scheme 1).

As shown in Scheme 1A, β-CD was attached to the exterior surface of TMV by sequential diazonium-coupling and CuI-catalyzed azide-alkyne cycloaddition (CuAAC) reactions.40,41 β-CD-azide was synthesized using a reported protocol,42 and the CuAAC reaction was catalyzed with CuSO4 and sodium ascorbate (NaAsc).43

---


Scheme 1 (A) Synthesis of TMV-β-CD by diazonium-coupling and CuAAC reactions. (B) The structures of Ada derivatives. (C) Schematic demonstration of the formation of multifunctional TMV via the supramolecular interaction between β-CD and Ada moieties.
The formation of TMV-Alkyne was confirmed by UV-Vis spectroscopy (Fig. 1A) and MALDI-TOF MS (Fig. 1C). The UV-Vis spectrum of TMV-β-CD shows a new absorption peak at 510 nm in company with a significant decrease in absorbance at 330 nm compared to the spectrum of TMV-Alkyne, which can be attributed to the conjugative effect between the azobenzyl and 1,2,3-triazole moieties, implying a successful attachment of β-CD moieties by the CuAAC reaction. This can also be verified by the SDS-PAGE analysis (Fig. 1B). The grafting efficiency was roughly estimated to be about 50% based on band density analysis of TMV-β-CD subunit proteins versus TMV-Alkyne subunit proteins, indicating that every TMV particle has about 1000 β-CD units, which is consistent with the MALDI-TOF MS result (Fig. 1C). Increasing the concentration of β-CD-azide did not improve the grafting density, which saturated at ~50% (Fig. S1, ESI†). We hypothesize that the incomplete conjugation is due to the steric hindrance of the β-CD moieties. Finally, the integrity of the TMV particles upon conjugation was confirmed using transmission electron microscopy (TEM, Fig. 1D).

To test the supramolecular interaction between β-CD and Ada moieties, a series of Ada derivatives were synthesized by either esterification or amidation reactions following literature protocols (see ESI† for experimental details). As a typical protocol, TMV-β-CD was incubated with a 10-fold molar excess of Ada-Rhb relative to the subunit proteins for 30 min at 4 °C, followed by dialysis and ultracentrifugation affording TMV-β-CD/Ada-Rhb, which was confirmed by size-exclusion chromatography (SEC) analysis (Fig. 2A). The SEC diagram shows that the retention volume of TMV-β-CD/Ada-Rhb is identical to that of TMV-β-CD; however, a significantly enhanced absorbance at 568 nm is observed due to the encapsulation with Ada-Rhb. In comparison, the control experiment following the identical protocol but using unmodified TMV did not show any non-specific interactions with Ada-Rhb as confirmed by SEC analysis (Fig. S2A, ESI†). The results validated that TMV-β-CD can assemble with Ada-Rhb via the interaction between β-CD and Ada groups.

The eluted TMV-β-CD/Ada-Rhb fraction was subjected to UV-Vis spectroscopy assay (Fig. 2B), and the labeling efficiency was calculated based on typical absorbance of TMV-β-CD and Ada-Rhb. The yield was ~100%, indicating that all β-CD units were quantitatively filled with Ada moieties, i.e. each TMV-β-CD particle was complexed with ~1000 Ada-Rhb molecules (see ESI† for details). Furthermore, the integrity of TMV-β-CD/Ada-Rhb was confirmed using TEM (Fig. S2B, ESI†). It is noteworthy that the positively charged Ada-Rhb did not induce the aggregation and precipitation of TMV-β-CD particles. Following the identical procedure, TMV-β-CD/Ada-Dox (or Ada-FA, Ada-PEG-Rhb) can be readily achieved (Fig. S3–S5, ESI†). Each TMV-β-CD particle can accommodate ~1000 Ada-Dox and Ada-FA molecules but only ~700 Ada-PEG-Rhb molecules due to the steric hindrance.

To test the one-pot co-assembly behavior between TMV-β-CD and different Ada derivatives, TMV-β-CD/Ada-FA/Ada-Rhb was prepared following the typical protocol. The UV-Vis spectra show that the absorbance of Ada-FA (λ300nm) gradually increases in company with the decrease in the absorbance of Ada-Rhb (λ568nm) as various molar ratios of Ada-FA and Ada-Rhb from 1:9 to 9:1 were used (Fig. 2C). Based on the UV-Vis spectrum, the average number of Ada-FA and Ada-Rhb moieties per TMV-β-CD particle can be determined accordingly (see ESI†), and the results are given in Fig. S6C, ESI†. According to the TEM results (Fig. 1D and 2D), there were no significant changes in length and diameter of TMV particles after being grafted with β-CD and sequential assembly. TMV-β-CD/Ada-FA/Ada-Dox can also be prepared following the same protocol (Fig. S6, ESI†).

In vitro drug release kinetics showed that Ada-Dox released in a sustained manner due to the dissociation of the β-CD/Ada supramolecular structure (Fig. S7, ESI†), similar to the literature reports. To investigate the selective targeting ability of Dox-loaded TMV, HepG2 tumor cells (folate receptor positive) and NIH-3T3 fibroblast
cells (folate receptor negative) were treated with TMV-β-CD/Ada-Dox, TMV-β-CD/Ada-FA/Ada-Dox and free Dox. Cellular uptake and cell proliferation were evaluated by confocal laser scanning microscopy and cell viability assay, respectively. As shown in Fig. 3 and Fig. S8 (ESI†), the two types of Dox-loaded TMVs showed low red fluorescence in NIH-3T3 cells, while TMV-β-CD/Ada-Dox showed much stronger red fluorescence in HepG2 cells compared to TMV-β-CD/Ada-Dox. It indicated that the folate receptor-mediated endocytosis enhanced the intracellular drug delivery. As shown in Fig. S9 (ESI†), the two types of Dox-loaded TMV significantly decreased the cytotoxicity of Dox against NIH-3T3 cells, while TMV-β-CD/Ada-Dox showed much higher cytotoxicity against HepG2 cells than TMV-β-CD/Ada-Dox, which was comparable to that of free Dox. It demonstrated that Dox-loaded TMV with the aid of FA moieties showed noticeable antitumor selectivity compared to free Dox. The TMV-β-CD/Ada-FA/Ada-Dox particles afforded comparable therapy efficiency of free Dox towards HepG2 cells, confirming the potential for sustained release of Dox due to the dissociation of the β-CD/Ada supramolecular structure.31,32

In summary, we have demonstrated here a supramolecular strategy based on the interaction between β-CD and Ada moieties. Using this method, multifunctional TMV particles have been constructed in a facile manner to load with imaging agents, targeting ligands, and chemotherapeutic drugs, which could potentially be used in therapeutic and diagnostic applications. More importantly, based on this work, we expect that a broad range of stimuli-responsive supramolecular interactions can be combined with VNPs for versatile applications.

This work was supported by the National Natural Science Foundation of China (21128002 and 21104080).

Notes and references