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Shape and Release Control of a Peptide Decorated Vesicle through pH Sensitive Orthogonal Supramolecular Interactions

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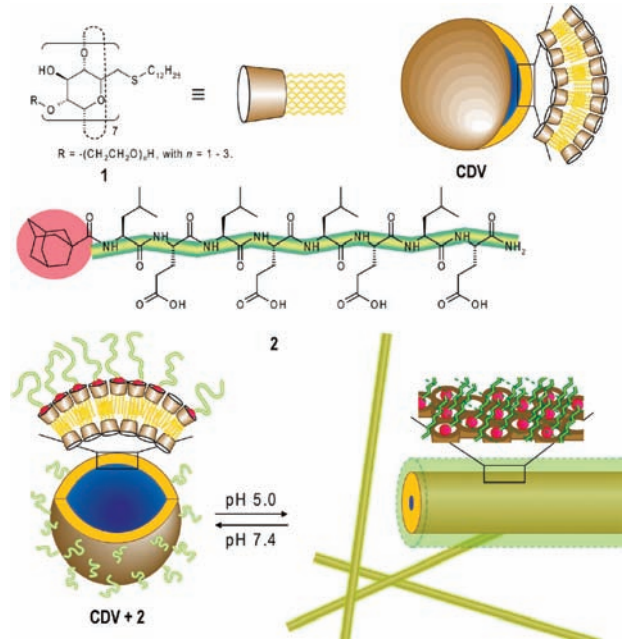
One of the main challenges in supramolecular chemistry is the design of structurally well-defined architectures with dynamic and stimulus-responsive properties that emulate biological systems. Dynamic and adaptive supramolecular materials that self-assemble from multiple components will find a range of applications in materials and biomedicine. A number of fundamental studies in the field of supramolecular chemistry have demonstrated that more intricate and functional materials can be engineered from noncovalent complexes by carefully combining several orthogonal interactions.[1,2] The use of a scaffold for self-assembly is a particularly interesting option, since a scaffold can guide the organization of weakly interacting molecules into well-defined self-assembled architectures.[3] In this paper, the pH sensitive β-sheet formation of an oligopeptide that self-assembles on the surface of a vesicle is used as a trigger for the release of contents through a reversible sphere-to-fiber transition of the vesicles.

In the present study we used a vesicle composed of an amphiphilic β-cyclodextrin (**1**)[4] as a scaffold for the formation of β-sheet domains composed of octapeptide (**2**). To decorate the surface of the cyclodextrin vesicles (CDVs) with β-sheet domains, we synthesized adamantane modified octapeptide **2**, which forms an inclusion complex at the surface of the CDVs.[5] In this two-component system three orthogonal interactions are combined: (i) hydrophobic interactions in the CDV bilayers; (ii) inclusion complex formation of β-CD and adamantane;[6] (iii) hydrogen bonding in β-sheet domains.[7] Using the reversible secondary structure transitions of the octapeptide from random coil to β-sheet domains as a function of pH,[8] a reversible morphological change of the multicomponent supramolecular complex from vesicles to fibers occurs (Scheme 1). Moreover, a pH-triggered release of the encapsulated contents is also apparent.

The interaction of β-CD and **2** was investigated using isothermal titration calorimetry. The binding constant (K) of the complex is $3.5 \times 10^4\ M^{-1}$, and the stoichiometry is close to 1:1.[9] These findings imply that the peptide moiety did not alter the inclusion of adamantane in the cyclodextrin cavity.

Peptide **2** does not adopt a β-sheet conformation in the absence of CDVs at either pH 7.4 or 5.0 as shown in circular dichroism (CD) spectroscopy (Figure 1). In the presence of CDVs at pH 7.4, the peptide does not form β-sheet domains. However, lowering the pH to 5.0 resulted in a random coil to β-sheet transition.[10] Note that the changes were reversible and repeatable. Further CD measurements with a range of ratios between **1** and **2** showed that the optimum molar ratio for β-sheet formation was ca. 2:1. This ratio implies that ~50% of the β-CD moieties are available for **2**

Scheme 1. Chemical Structures of the β-CD Derivative (**1**), Which Self-Assembles into Cyclodextrin Vesicles (CDVs), and the Adamantane Modified Octapeptide (**2**), Which Binds to the CDV[a]



[a] **2** adopts a random coil conformation at pH 7.4 while bound to the CDV. Upon acidification, **2** rearranges into a β-sheet which induces the morphological change from a sphere to a fiber.

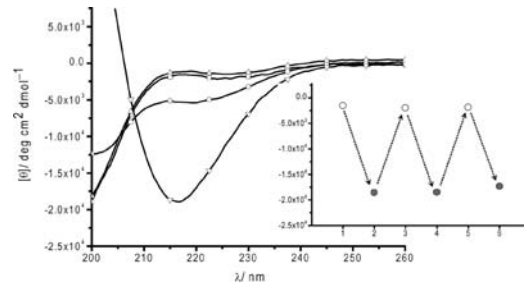


Figure 1. CD spectra of a mixture of **1** (1 mM) and **2** (0.5 mM), at pH 7.4 (□) and 5.0 (▽) and of a solution of **2** (0.5 mM) at pH 7.4 (Δ) and pH 5.0 (○). Insert: Intensity of the CD signal at 217 nm upon switching pH between 7.4 (○) and 5.0 (●).

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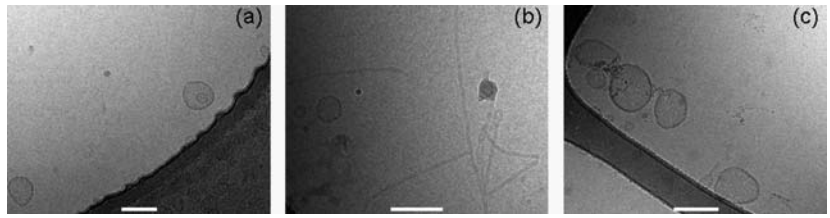

Figure 2. Cryo TEM images of a mixture of **1** and **2** at 1 and 0.5 mM, respectively: (a) The addition of **2** at pH 7.4 does not alter the shape of the CDVs (1 h after adding **2**). (b) Upon acidification the peptide adopts a β-sheet conformation, and the vesicles are transformed into fibers (1 h after lowering the pH to 5.0). (c) After changing the pH back to 7.4 spherical vesicles were observed again (1 h after raising the pH). Scale bars represent 100 nm.

to bind, suggesting that the CDVs preserve their bilayer structure and that **2** is unable to permeate into the vesicles.

The β-sheet formation of peptides is accompanied by a rearrangement of the peptides to acquire the optimal distance to form hydrogen bonds. Since **2** is bound to the CDVs, the formation of extended β-sheets is expected to affect the bilayer membrane and hence the structure of the assemblies. Indeed, the relaxation times (τ) obtained from dynamic light scattering showed a significant difference: at pH 7.4, τ were ca. 0.22 ms, while at pH 5.0, τ were ca. 0.32 ms. Again, the changes were reversible and repeatable.

The morphologies of the complex were investigated with cryo-TEM (Figure 2). The size and shape of the CDVs are not altered upon the addition of **2** at pH 7.4. However, after acidification to pH 5.0, the predominant structures were thin fiber-like aggregates with a thickness of ca. 8 nm and a length of up to several hundred nanometers. The reversibility of the system was shown by changing the pH back to 7.4, after which only spherical vesicles were observed again. This finding showed that the superstructure of the assemblies composed of CDV and **2** can be controlled through the secondary structure of the surface peptides (**2**).

To test whether the assembly can be used as a pH responsive capsule, we investigated the pH-triggered release of a cargo molecule by a quenching experiment:[11] tetrasodium 1,3,6,8-pyrene tetrasulfonate (Py) was added as a model component during the preparation of the CDVs. After addition of **2**, NaI was added to quench all of the nonencapsulated dye. In this manner, the observed signal is only due to encapsulated dye. The signal remained constant before changing the pH, showing that the vesicular capsule was not leaking.[4,6b] Lowering the pH induced the vesicle-to-fiber transformation, which was accompanied by a significant loss in Py fluorescence indicating Py was released from the assembly. After Py fluorescence stabilized, Triton X-100 was added to dissolve the complex, resulting in complete quenching. The obtained release profile is shown in Figure 3.

In conclusion, we have constructed a stimulus-responsive hierarchical supramolecular assembly from a cyclodextrin vesicle and an adamantane-modified octapeptide that features multiple orthogonal interactions. The dense packing of the peptide on the vesicle surface allows it to form β-sheet domains at pH 5.0. Furthermore, the reversible pH-triggered morphological change of the complex from a sphere to a fiber and the concomitant release of cargo molecules have been shown. This supramolecular system can be of use as a pH sensitive drug delivery system and a nanoscale switching device.

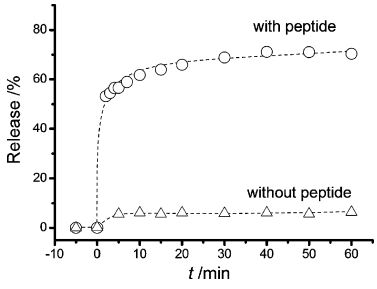

Figure 3. Fluorescent dye release from CDVs with (○) and without (Δ) octapeptide **2** at pH 5. The observed intensities before lowering the pH and after adding Triton X-100 were used as references for 0 and 100% release. The quencher was added at $t = -5$ min, and the pH was lowered at $t = 0$ min.

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Supporting Information Available: Full experimental details and ITC, CD, cryo-TEM, DLS, and fluorescence data. This material is available free of charge via the Internet at http://pubs.acs.org.

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