Carbohydrate-coated nanocapsules from amphiphilic rod–coil molecule: binding to bacterial type 1 pili†

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Stable carbohydrate-coated nanocapsules designed as multivalent nanoscaffolds for selective interactions with receptors are able to encapsulate guest molecules within their interior and to bind efficiently to FimH adhesin of bacterial type 1 pili.

The development of artificial multivalent carbohydrate-coated objects has received a great deal of attention due to their strong and specific interactions with the receptor proteins as a result of carbohydrate cluster effects.[1] Higher binding affinities are achieved through synergistic multivalent protein–carbohydrate interactions. Presentation of multivalent carbohydrate ligands to various receptors has been achieved using a number of scaffolds including glycoproteins,[2] linear polymers,[3] glycoclusters,[4] shell cross-linked polymer micelles,[5] block copolymers,[6] and dendrimers.[7] Multivalent carbohydrate ligands can also be constructed by self-assembly processes of amphiphilic molecules containing carbohydrate moieties on their hydrophilic end, because they generate carbohydrate-coated aggregates in aqueous solution.[8] Block molecules that mimic lipid amphiphilicity have been proved to be promising scaffolds for nanometre-sized capsule-like aggregates that can be used to effectively encapsulate guest molecules in aqueous solution.[9] Introduction of a rigid rod segment into a self-assembling system has been reported to enhance aggregation stability.[10] In addition to stability, another important issue regarding the preparation of capsule-like aggregates is their capability to interact with biological receptors. To obtain stable capsule-like aggregates with specific biological functions, however, the more elaborate design of corresponding building blocks containing a bioactive moiety is required. Accordingly, we synthesized an amphiphilic rod–coil molecule consisting of tetra(*p*-phenylene) and oligo(ethylene oxide) with a mannose terminal unit that can endow aggregates with stability and strong biological receptor binding ability.

In this communication, we report the formation of stable nanocapsules coated with mannose units, artificial multivalent carbohydrate-coated objects, from the self-assembly of rod–coil molecules. The resulting capsules are able to encapsulate guest molecules within their interior and to bind efficiently to FimH adhesin of bacterial type 1 pili (Fig. 1). The rod–coil molecule that forms the mannose-coated capsule-like aggregates was obtained in a multiple synthesis from commercially available starting materials.[11]

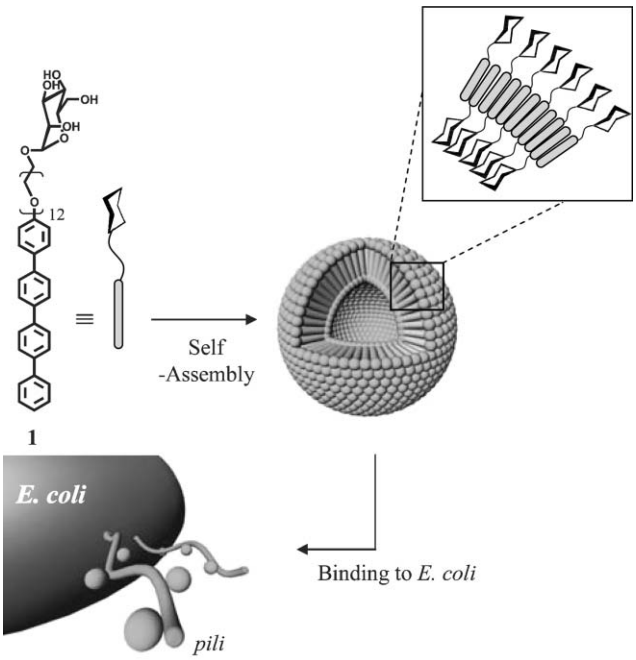

Fig. 1 Schematic representation of binding of carbohydrate-coated nanocapsules with *Escherichia coli*.

The mannose terminated rod–coil molecule was synthesized by glycosylation of a hydroxy terminated rod–coil molecule with peracetylated bromo-D-mannose and subsequent deprotection of the acetyl protecting groups with sodium methoxide (see ESI†). The resulting rod–coil molecule **1** was characterized by ^{1}H- and ^{13}C-NMR spectroscopy, elemental analysis and MALDI-TOF mass spectroscopy, and shown to be in full agreement with the structures presented.[12]

Dynamic light scattering studies of aqueous solutions showed that the rod–coil molecule self-assembles into aggregates of uniform size (Fig. 2a). The average diameter of the aggregates was observed to be approximately 36 nm. The measured diameter exceeded the corresponding extended molecular length (approximately 6 nm), suggesting that these aggregates are rather vesicular entities than simple micelles. Further evidence for the formation of the vesicles was provided by TEM experiments. As shown in Fig. 2b, the images revealed that there is obvious contrast between the periphery and center in the sphere, characteristic of the projection images of hollow spheres. The formation of spherical aggregates of the molecule was also confirmed by FE-SEM experiments (Fig. 2c). The micrograph showed spherical aggregates that are approximately 40 nm in diameter and are thus

† Electronic supplementary information (ESI) available: experimental details; MALDI-TOF mass spectroscopy, DSC, small-angle X-ray diffraction, TEM. See http://www.rsc.org/suppdata/cc/b4/b419258c/

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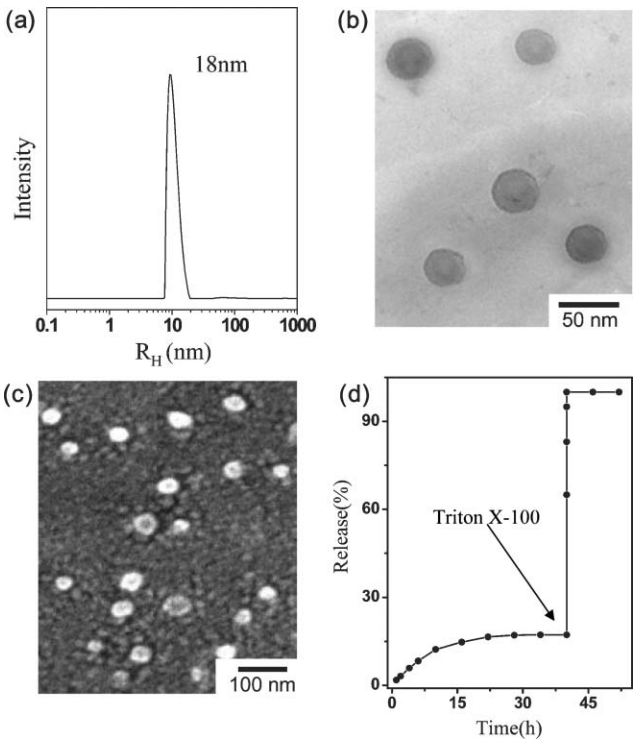

Fig. 2 (a) Dynamic laser light scattering study, (b) transmission electron micrograph, and (c) scanning electron micrograph of the aggregates formed by molecule **1** in the solution; (d) release profile of calcein from the vesicle as a function of time.

consistent with the results obtained from dynamic light scattering and TEM experiments. It should be noted that the objects preserve their spherical morphology even after their isolation from the solution under high vacuum as confirmed by TEM and SEM experiments, indicating that the capsule-like aggregates formed in aqueous solution are stable.

The vesicular nature was further confirmed by performing encapsulation experiments of the fluorescence dye calcein. Calcein, as a hydrophilic fluorescent guest, was encapsulated at a sufficiently high, self-quenching concentration and free calcein was removed by filtration over a Sephadex column. Release of calcein from the inside of a vesicle was accompanied by an increase in fluorescence emission as the free calcein in solution was dequenched.[13] As shown in Fig. 2d, very slow release of entrapped calcein was observed over periods of 40 h, indicating that the vesicles are highly stable toward leakage. Exposure of the calcein-loaded vesicles to Triton-X 100 resulted in a rapid and complete release of encapsulated calcein, demonstrating that the dye molecules are effectively encapsulated within the interior of a capsule.

More important, the capsules appeared to efficiently bind to FimH adhesin of bacterial type 1 pili in *Escherichia coli* (*E. coli*), suggesting that the capsule exterior is coated by bioactive mannose units. Type 1 pili are heteropolymeric mannose binding proteinacious fibers that protrude from the surface of many Gram-negative bacterial cells.[14] The bioactive binding ability of the capsules was then evaluated by interactions of the nanocapsules with bacterial cells *via* TEM imaging. ORN 178 *E. coli* strain was used in experiments to confirm the binding of the

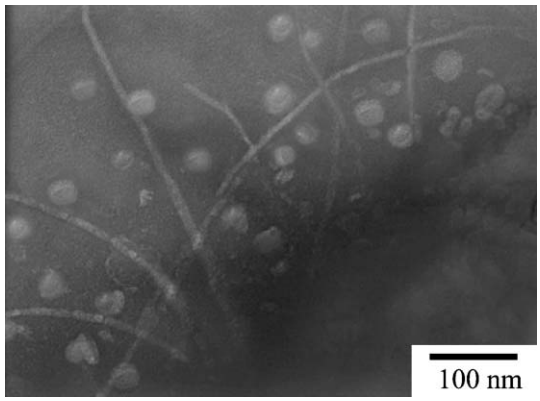

Fig. 3 TEM image of sectioned area of pili of the *E. coli* ORN 178 strain bound with nanocapsules of **1**. A portion of an *E. coli* is shown in lower right.

nanocapsules to FimH.[15] After incubation of the bacterial strain with the nanocapsules, the suspensions were centrifuged to precipitate the cells that were washed further to remove unbound capsules. As shown in Fig. 3, a number of aggregates were clearly observed to be located along the fibers, indicative of strong binding of the nanocapsules to FimH strain. Notably, the shape and size of the capsules was shown to be retained even after binding to the bacterial pili, indicative of high stability of the capsules. The strong binding between the capsules and FimH seems to be attributed to recognition of multivalent mannose ligands on the surface of a capsule by the receptors located on the bacterial pili.[1] In order to confirm the selective binding to FimH, ORN 208 *E. coli* cells with a lack of the FimH protein[15] were incubated with the nanocapsules. In contrast to the ORN 178 strain, no capsules were observed to be imaged through TEM, indicating that the bacterial pili of the ORN 208 strain are unable to mediate mannose selective binding. These results demonstrate that stable carbohydrate-coated nanocapsules designed as multivalent nanoscaffolds for use in enhanced receptor binding can be constructed from the self-assembly of rod–coil molecules. The resulting capsules are able to encapsulate guest molecules within their interior and to bind efficiently to FimH adhesin of bacterial type 1 pili.

In summary, we have demonstrated that rational design of a self-assembling molecule based on a rigid rod building block allows stable mannose-coated nanocapsules to be produced. The capsules were shown to be able to encapsulate guest molecules within their interior, as confirmed by dye-encapsulation experiments. More importantly, incubation with *E. coli* showed that the mannose-coated capsules selectively bind to the bacterial pili of the ORN 178 strain, demonstrating efficient binding of the nanocapsules to FimH. This recognition indicates the possibility of multivalent interactions between the mannose-coated capsules and mannose receptors to achieve a carbohydrate cluster effect. Carbohydrate-coated nanocapsules that are able to encapsulate guest molecules and to selectively bind to bacterial type 1 pili, have attractive potential uses in a wide variety of applications ranging from target selective nanocarriers, labelling specific protein on the cell surface, to sensing of a range of pathogens.

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