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Mannosylated self-assembled structures for molecular confinement and gene delivery applications

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ABSTRACT

This paper reports self-assembly of a lysine conjugated with a biantennary mannose to form spherical structures. These supramolecular structures are found to be hollow in nature and they afford effective encapsulation of alkaline phosphatase enzyme, plasmid DNA and a GFP reporter gene, which was transfected in COS-7 cells. Loaded hollow structures also get disrupted upon mild sonication, releasing encapsulated molecules thereby illustrating their potential for confinement and delivery applications.

Self-assembled hollow structures have attracted considerable attention due to their potential applications as supramolecular containers [1,2] and as delivery vehicles [3,4]. Such applications are feasible as the empty core of hollow spheres can encapsulate guest molecules and also act as reaction chambers by bringing two or more reactants into close proximity [5,6]. In addition, several reports also describe covalent decoration of such structures by multiple carbohydrate residues to elicit interactions with specific cell surface receptors [7–11].

We have been interested in studying peptide-based soft structures from the viewpoint of generating diverse morphologies and to discover enabling mechanisms that can trigger disruption of such soft structures. As a result of our investigations, we have discovered peptide-based supramolecular ensembles where some of them respond to colchicine, physiologically relevant cations, and covalently attached structure modifiers [12–15].

In this report, we demonstrate that bis-mannosylated lysine (**3**) (Fig. 1A) self assembles in aqueous medium to reveal formation of hollow spheres which can complex/or encapsulate guest molecules. These soft structures disrupt when ultrasonicated and release guest molecules. Remarkably, these structures complex pEGFP-N1 expression construct, encoding the green florescent protein, and upon transfection with this complex, a number of mammalian cells exhibit nuclear fluorescence.

Materials and methods

Bis-mannosylated lysine (**3**) was prepared by a slightly modified previously reported procedure [16,17] via Scheme 1 [18]. Sample Preparation for different microscopy analysis, i.e., AFM, TEM, SEM, fluorescence microscopy, and optical microscopy, was done by dissolving 1 mM of **3** in deionized water for 16 h at 37 °C. It was necessary to incubate samples for 16 h because **3** did not show instantaneous self assembly and required 16 h for formation of stable self assemblies. Enzyme assays were done by adding 5 U of CIAP to 1 mL of 1 mM solution. AFM and gel electrophoresis was done by adding 20 μL of DNA to 180 μL of 1 mM of **3** dissolved in autoclaved water for 16 h at 37 °C. Compound **3** (1 mM) dissolved in autoclaved water was filter sterilized and then incubated with DNA (1 μg) at 37 °C for 16 h for cell transfection experiments. Details of all procedures are given in supporting information [18].

Results and discussion

Bis-mannosylated lysine (**3**) thus synthesized was fully characterized by various spectroscopic methods which showed it to be in full agreement with molecular structure of **3**. Several glycosylated lysine and lysine containing peptides are reported in the literature [19–21] and more specifically, the interaction of **3** and its galactose analog with macrophages and HepG2 cells has been described [22,23]. This background provided us an impetus to investigate self-assembly properties of mannosylated **3**.

Scanning electron microscopy (SEM) and atomic force microscopy (AFM) confirmed formation of spherical structures (Fig. 1B

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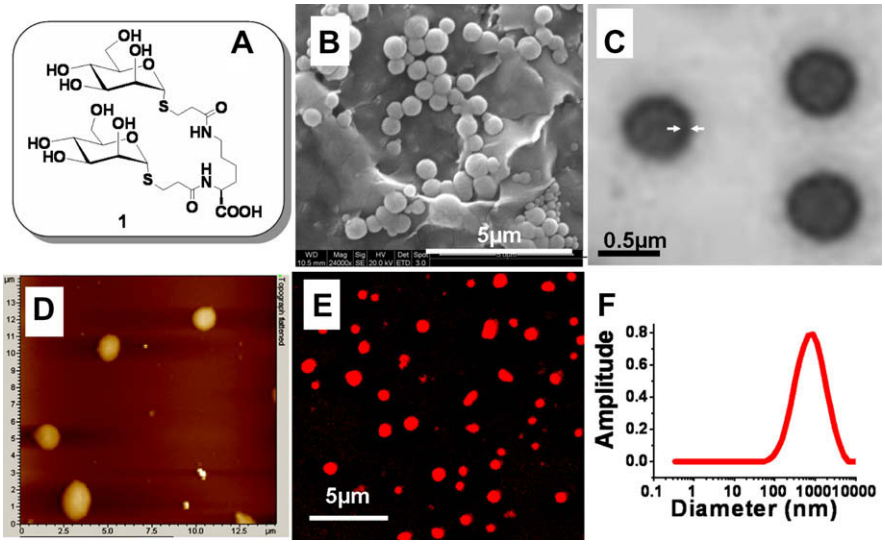

Fig. 1. (A) Molecular structure of **1**. (B) SEM of **1** (1 mM, aqueous solution) after 16 h incubation, (C) TEM image showing a contrast between periphery and core of spherical structures (marked by two arrows), (D) AFM, (E) fluorescence micrograph, (F) DLS analysis.

and D). Further evidence was obtained from transmission electron (TEM) (Fig. 1C) which interestingly revealed a contrast between the periphery and core of spherical structures, a typical characteristic of the projection images of hollow spheres [24].

It was possible to stain these structures with rhodamine B and fluorescence microscopy image once again afforded brightly stained spherical aggregates (Fig. 1E). Size distribution of these spherical aggregates was determined by dynamic light scattering (DLS) measurements which revealed a broad peak (Fig. 1F) with an average size of ~700 nm, which was in accordance with the size measured by other microscopy methods. Notably, gross spherical structure was observed in all the microscopy studies confirming an inherent preference of this morphology.

Looking at the molecular structure of **3**, it can be proposed that more hydrophilic mannose groups will prefer being displayed at the outer surface of the spherical structures for a favourable interaction with water. We decided to confirm whether mannose appendages are indeed projected outwards through an assay exploiting carbohydrate–lectin interactions. It is known that mannose–Concanavalin A (Con A) interaction leads to aggregation, thus enhancing turbidity of the solution [25]. Therefore, a turbidimetric assay was employed to study the interaction of **3** with Con A. Increase in the absorbance with respect to time was observed at 400 nm which indicated aggregation of Con A by mannose-coated spherical structures, thus providing an indirect proof of mannose display at the surface of these soft structures [18].

We decided to probe whether these structures could be disrupted by ultrasonication to expand application of these spherical structures. Interestingly, we observed that these structures could be completely disrupted by ultrasonication over a 5 min exposure in an ultrasonic bath (Fig. 2). Taken together with the possibility of guest molecule interaction, a non-invasive release method appeared promising enough for us to further investigate these soft structures for complexation, confinement and delivery of biological molecules of interest.

Calf intestine alkaline phosphatase (CIAP) and plasmid DNA pBR322 was used for confinement studies. CIAP (5 U, 1 μM) was incubated with **3** (1 mM) in (10 mM Tris buffer, pH 7.9) for 16 h in the aqueous medium. To confirm complete encapsulation (or complexation) of the enzyme, *p*-nitrophenol phosphate (pNPP) was added to this solution. Enzymatic activity was not observed as judged by the lack of detection of the yellow color corresponding to hydrolysis product *p*-nitrophenolate (pNP) anion, even after 4 h of addition of substrate (Trace 3, Fig. 3).

This suggests that either the enzyme has simply complexed with **3** or it got encapsulated inside hollow spherical particles, thus rendering the enzyme ineffective towards pNPP hydrolysis. However, the enzymatic action of alkaline phosphatase was restored when the solution of enzyme complexed or loaded spherical structures were ultrasonicated for 5 min, as evidenced by the appearance of the yellow color (Trace 5, Fig. 3). In control experiments, alkaline phosphatase was added to preformed spherical structures

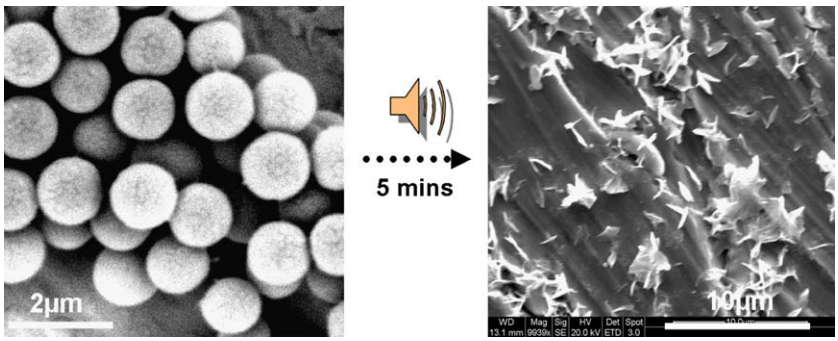

Fig. 2. SEM images of self-assembled structures of **3** before and after 5 min sonication.