Selective Encapsulation of Dye Molecules in Dendrimer/Polymer Multilayer Microcapsules by DNA Hybridization

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Introduction

The controlled encapsulation of biomolecules in microcapsules is of great interest for application in different areas.[1] The encapsulation was often performed on the microcapsules prepared by layer-by-layer (LbL) technique, which can produce hollow micro or nanocapsules with a precise control over the capsule wall thickness. Only a few reports deal with the encapsulation of uncomplexed DNA within the interior of polyelectrolyte capsules.[2] Until now, DNA encapsulation in the microcapsules made with polycationic dendrimers and polyanionic polymers was not reported yet so far. The use of dendrimers (PAMAM) and polyanionic polymers was reported by Caruso et al., but these authors did not describe encapsulation of DNA within these microcapsules (only dyes with no high molecular weight).[3]

In this work, the microcapsules made with polycationic dendrimers and polyanionic polymers will be prepared by LBL[4] and the encapsulation of Cy5 dye molecules in the microcapsules through DNA hybridization will be reported. To do this, ssDNA probes were firstly deposited in capsules prepared by LBL assembly of cationic phosphorus dendrimers of the fourth generation (G_4^+) (s-Fig.1) and polystyrenesulfonate (PSS). The addition of Cy5 ("cargo") dye labeled ssDNA targets ("vehicle") solution on the prepared capsules was then followed. With the specific interaction between ssDNA probe and ssDNA complementary target, the selective encapsulation of Cy5 dye molecules in the capsules could be achieved.

Experimental section

Materials. Cationic phosphorous dendrimers were synthesized as reported in reference.[5] 11-mercaptoundecanoic acid (MUA) and polystyrenesulfonate (PSS; *M*w ~ 7000 g/mol) were purchased from Aldrich and used as received. ssDNA probes (P2) and Cy5 labeled ssDNA targets were from MWG-biotech AG in Germany. 80mer ssDNA probe (P2): 5'- $(TTT)_{21}$ TT TGT ACA TCA CAA CTA-3'; Cy5 labeled 80 mer P2: 5'Cy5- $(TTT)_{21}$ TT TGT ACA TCA CAA CTA-3'; amino group functionalized P2: 5'NH_2-$(TTT)_{21}$ TT TGT ACA TCA CAA CTA-3'. Cy5 labeled 15mer ssDNA complementary (Cy5T2): 5'-Cy5-TAG TTG TGA TGT ACA-3'; Cy5 labeled 15mer ssDNA total mismatch (Cy5Tmm): 5'Cy5TTT TTT TTT TTT TTT3'.

Suspensions of monodispersed weakly cross-linked melamine formaldehyde particles (MF particles) were purchased from Microparticles GmbH (Berlin, Germany).

Capsule Preparation. MF (10 wt% dispension) particle suspersion (50 μL) was mixed with 10 μL P2 (100 μM) and 940 μL Milli-Q water (18.2 Ωm). The solution was sharked after adding 1 mg Sp in the solution for 30 min. Further LBL assembly of G_4^+/PSS shell was carried out with 1 mg/mL PSS (0.5 M NaCl) and G_4^+ solutions, respectively. The microcapsules were obtained by dissolving the MF template in HCl at pH 1.3 and washing with Milli-Q water three times. DNA hybridization between P2 and Cy5T2 or Cy5Tmm was performed by applying Cy5T2 or Cy5Tmm PBS solution (pH 7.4; 100 nM) for 30 min.

Result and Disscussion

To prepare capsules containing ssDNA probes, the weakly cross-linked melamine formaldehyde (MF) templates with 3.5 μm diameters were used as cores. MF particle suspension was mixed with 80 mer ssDNA probes (P2, 26.4 kDa) (100 μM) and Milli-Q water. The solution was stirred after adding 1 mg spermidine (Sp) for the formation of complex with P2. Further LBL assembly of G_4^+/PSS shells was carried out with 1 mg/mL PSS and G_4^+ in aqueous solution, respectively (Fig. 1). The mean increase in the thickness of the multilayer system was determined by surface plasmon resonance spectroscopy to be $\Delta d \approx 2.1$ nm for an additional PSS layer and $\Delta d \approx 4.1$ nm for an additional G_4^+ layer. Therefore, about 6.2 nm thick can be obtained after depositing each G_4^+/PSS bilayer.

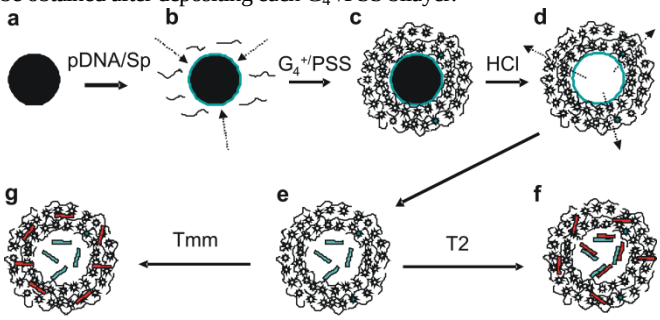

Figure 1. Schematic illustration of P2 deposition and Cy5 labeled ssDNA targets encapsulation. (a,b) Controlled precipitation of P2/Sp complex on the surface of template particles. (b,c) LBL assembly of protective G_4^+/PSS shells. (c,d) Template dissolution. (d,e) P2/Sp complex dissolution. (f) Adding Cy5 labeled ssDNA complementary (Cy5T2). (g) Adding Cy5 labeled ssDNA total mismatch (Cy5Tmm)

The encapsulation of Cy5 molecules in the capsules by DNA hybridization was studied. For this, Cy5 labeled 15 mer ssDNA complementary targets (Cy5T2; 4.95 kDa) were selected. The highly permeable properties of G_4^+/PSS capsules makes it possible that the low molecular weight Cy5T2 can diffuse through the capsule shells in the aqueous solution by spontaneous deposition approach reported by Gao's group.[6] The coiled Cy5DNA can recognize its structure and penetrate through a tiny pore on the wall alike a snake due to the interaction between the amino-containing positively charged G_4^+ with negatively charged Cy5DNA.[6]

Figure 2a shows the fluorescence microscopy image after applying Cy5T2. Clearly, the fluorescent signal distributed in the whole volume of the capsules was observed, which suggested that Cy5T2 molecules were successfully encapsulated in the capsules. Hence, the presence of Cy5T2 molecules in the internal volume of the capsules mainly depends on the existence of P2. Typically, the formation of double strand DNA after hybridization made Cy5T2 bind to the high

molecular weight P2 molecules. This resulted in the difficulty for the encapsulated Cy5T2 to diffuse outside the capsules again. Another possibility concerning the missing leaching of DNA might be due to the fact that the interior of phosphorus dendrimers is hydrophobic while the surface is hydrophilic, which maybe hinders or decreases the speed of the leaching of DNA. In addition, the network of dendrimers may be imbricated that only relatively small molecules are able to enter into the microcapsules. On the other hand, if a part of the Cy5T2 is a little bit inside the microcapsule it will obligatory meet the complementary one and therefore the driving force for the entire encapsulation (hybridization) is very strong and the resulting complex can be too big to go outside.

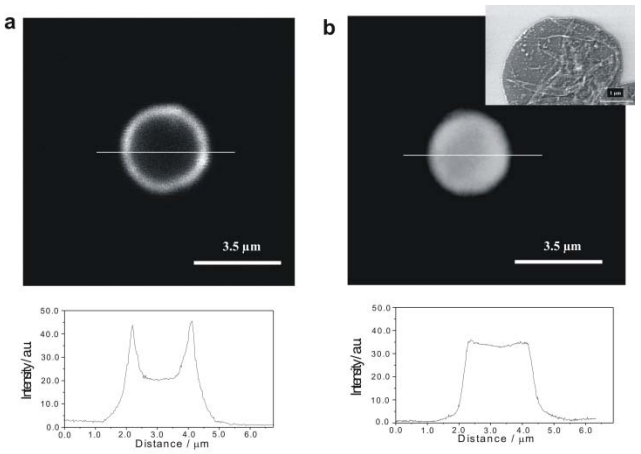

Figure 2. Fluorescence microscopy images of the P2 deposited capsules composed of four G_4^+/PSS bilayers after removing MF template particles with (a) adding Cy5T2 and (b) adding Cy5Tmm. The below demonstrates the fluorescence profile for both.

Finally, encapsulation efficiency of Cy5T2 in the capsules was determined by the adsorption at 260 nm using a Perkin Elmer Lambda 25 UV spectrophotometer. Fig. 3a shows UV absorption of the capsules in 300 μL PBS solution before ssDNA targets. Cy5T2 or Cy5Tmm (1 μM) were applied in the solution for 30 min and measured (Fig.3b and c). The increase of absorption intensity was achieved after encapsulation of Cy5T2 in the capsules and much less increase was only detected after adding Cy5Tmm. Using simple equation,[7] the encapsulation efficiency of Cy5T2 in the capsules is found around 20% and encapsulation efficiency of Cy5Tmm is about 4%. This result is compatible with that of polymersome used as capsules for encapsulating DNA.[8] At the same time, the result about the successful encapsulation of Cy5T2 is also in agreement with the data above.

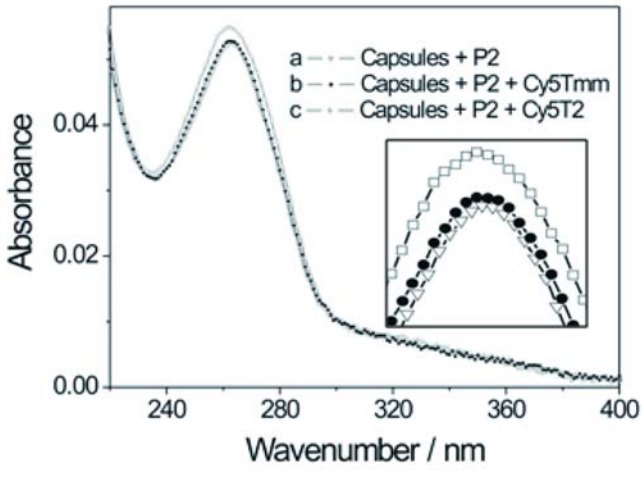

Figure 3. a) UV absorption of the capsules before adding ssDNA targets; the peak at 260 nm is from the encapsulated P2 in the capsules; b) UV absorption of the capsules after adding Cy5Tmm; c) UV absorption of the capsules after adding Cy5T2.

Conclusion

Using ssDNA target as "vehicle" and Cy5 molecule as "cargo", a method to selectively encapsulate Cy5 dye molecules by DNA hybridization in LBL based microcapsules has been developed. The dye to be encapsulated is covalently bond to complementary DNA by conjugation chemistry. This is the cargo approach and a standard approach for sensing purposes. This is certainly a useful approach for encapsulating molecules as reporters. The approach for encapsulation can be applied for a broad range of low molecular weight molecules, which are difficult to be encapsulated by some methods due to the easy diffusion of small molecules through capsule shells.[9] Secondly, the encapsulation of molecules into the well shaped dendrimer based capsules is possible without adjusting shell permeability by solvents, chemical oxidation, ionic strength, or pH,[10] which may increase the chance to destroy the shell or denature encapsulated biomolecules. In this experiment, the hybridized double strand DNA can be further dehybridized by an external stimulus (e.g. SDS or NaHPO4),[11] which would be a means to control the release of Cy5. The work is under the progress in our group.

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7 Cy5T2 or Cy5Tmm (1 μM) were applied in the solution for 30 min. Using centrifugation, the microcapsules were collected and rinsed by PBS for 3 times. The UV adsorption of the capsules were then measured. Efficiency = $(x-a)/(a+(15/80)a)$, Here, x is measured UV absorption intensity of the capsules after adding Cy5T2; a is the UV absorption intensity of the capsules before adding Cy5T2; $(15/80)a$ means the maximium increased UV intensity after complete hybridization between Cy5T2 (15 mer) and P2 (80 mer).

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