

Rapid Communication

Temperature Responsive 3D Structure of Rod-like Bionanoparticles Induced by Depletion Interaction^{*}

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Abstract Herein, we show that rod-like tobacco mosaic virus (TMV) can self-assemble into 3D bundled structures in aqueous solution using azobenzene modified Pluronic F127 block copolymers (Azo-F127) to induce depletion. The structures are characterized by both transmission electron microscopy and dynamic light scattering. The self-assembly process can be controlled reversibly by temperature. The formation of Azo-F127 hard spheres during the self-assembly is the main cause of this phenomenon.

Keywords: Tobacco mosaic virus; Pluronic; Depletion interaction; Self-assembly.

INTRODUCTION

Self-assembly has been proved to be a universal way to form complex three dimensional materials with great structural diversities^[1, 2]. Inspired by natural systems, various artificial complexes have also been developed using different assembly methods, such as DNA base pairing^[3], interfacial^[4] or electrostatic interactions^[5]. It is very crucial to precisely design and control the assembly pathway in order to achieve multilevel hierarchical structures which have potential applications in many fields, such as photonics, electronics and catalysis^[6].

Recently, the depletion interaction has been proved to be a simple and effective method to control the self-assembly of nanoparticles^[7, 8]. Depletion interactions arise when non-adsorbing additives, such as polymers or micelles, are present in colloidal solutions^[9, 10]. The additives lead to osmotic pressure and push the particles together^[11, 12]. Many parameters, such as temperature and particle rigidity, can affect the balance of attractive and repulsive forces in the system. Therefore, it remains a challenge to effectively tune and control the assembly behavior of nanoparticles by the depletion interaction.

The rod-like tobacco mosaic viruses (TMVs) have been proved to be ideal anisotropic nanoparticles to explore their assembly behavior through the depletion interaction^[13]. Native TMV particles consist of 2130 identical protein subunits arranged helically around genomic single-stranded RNA. TMV is 300 nm long and 18 nm in diameter, with a 4 nm cylindrical cavity along the central core^[14, 15]. Li *et al.* reported that interparticle distances of TMV superlattice could be controlled by temperature through sol-gel transition of rigid homopolymer methyl cellulose^[7]. In this study, we show that by introduction of rigid molecule at the end of

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Pluronics F127 block copolymer, the formation of Azo-F127 micelles can produce depletion during the self-assembly process, which is the driving force of formation of TMV 3D bundled structure. Furthermore, because of the thermosensitive characteristics of F127^[16, 17], the self-assembled 3D bundled structure of TMV can be controlled reversibly in response to temperature.

MATERIALS AND EXPERIMENTS

TMV was isolated according to previously reported method^[4]. Ferritin from equine spleen and Pluronics F127 were purchased from Sigma-Aldrich Co. 4-(Phenylazo) benzoic acid, dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were purchased from TCI Co. All the other reagents were commercially available and used as supplied without further purification.

Azo-F127 was prepared as follows: F127 (1.0 mmol), 4-(phenylazo) benzoic acid (2.2 mmol), DCC (2.2 mmol), and DMAP (0.2 mmol) were dissolved in 20 mL of anhydrous methylene chloride, and the reaction was performed at room temperature for 48 h. The byproduct dicyclohexylcarbodiurea (DCU) was removed by filtration. Then precipitated the solution into diethyl ether, and repeated the dissolving-precipitation three times, the obtained product was dried in vacuum. ¹H-NMR (400 MHz, CDCl₃): δAzo-F127 = 4.21 (m, 2H, CH₂OCO) 3.75 (s, (OCH₂CH₂)_n), 1.0 (d, CH₃ of PPO), 6.88–6.98 (m, 2H, in azobenzene), 7.24–7.46 (m, 3H, in azobenzene), 7.74–7.87 (m, 4H, in azobenzene).

In a typical assembly experiment, purified TMV was dispersed in potassium phosphate buffer (10 mmol/L, pH 7.8) and TMV solution was slowly added to Azo-F127 aqueous solution. The final concentration of TMV and Azo-F127 was 0.1 mg/mL and 2.5 mg/mL, respectively. TMV/Azo-F127 solution was shaken strongly with mixer. Ferritin/Azo-F127 solution was prepared using the similar method.

Characterization: Absorption spectra of TMV solution were measured using a UV-Vis spectrophotometer (UV-3900, Hitachi, Japan) to calculate the concentration of TMV. The visualized TEM images of the assemblies were obtained from a JEM-2100F electron microscope (JEOL, Tokyo, Japan) with an accelerating voltage of 120 kV. DLS data was collected on a DynaPro NanoStar instrument (Wyatt Technology) with a He-Ne laser (λ = 659 nm) operated at 10 mW.

RESULTS AND DISCUSSION

Azo-F127 was synthesized by the similar method in the literature (Fig. 1a)^[18]. The molecular structure was confirmed by NMR. Moreover, the introduction of azobenzene did not affect the thermosensitive characteristics of F127. In our experiment, we found that when Azo-F127 was mixed with TMV in solution at 25 °C, as illustrated in Fig. 1(b), a clear solution was obtained. However, if the temperature increased to 35 °C, the sample became opaque. As the temperature decreased to 25 °C, the solutions turned into clear again. And this behavior could be repeated many times.

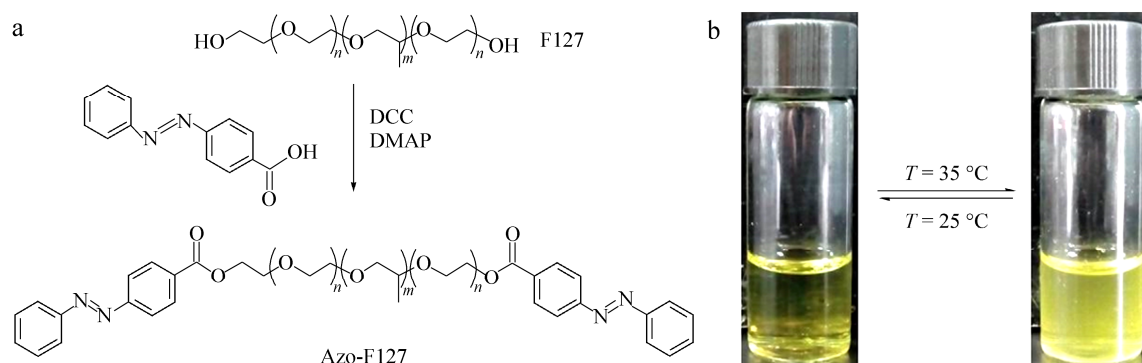


Fig. 1 (a) Synthetic route for Azo-F127; (b) Optical images of TMV/Azo-F127 at different temperatures

In our previous study, by varying the temperature, we demonstrated that Pluronic F127 was able to assembly on the surface of TMV and formed a core-shell structure^[19]. However, a different phenomenon was observed when the azo-F127 was used instead of F127 at the temperature above the critical micelle temperature. As shown in Fig. 2(b), TMV formed a big bundled structure, in which TMVs were closely packed in a side by side manner (Fig. 2c). This result confirmed that the turbidity was due to the formation of TMV bundles. When the temperature was decreased to 25 °C, TMV restored to its original appearance (Fig. 2a) as shown in Fig. 2(d). The result indicated that TMV particles did not form aggregates at 25 °C, which was in good agreement with the clear solution shown in Fig. 1(b).

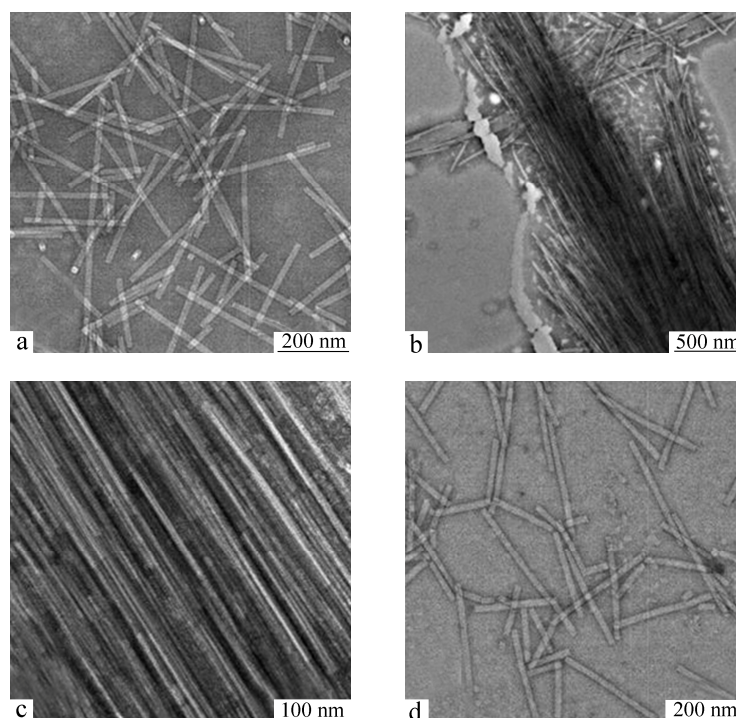


Fig. 2 TEM images of (a) TMV and Azo-F127 at 25 °C, (b) TMV and Azo-F127 at 35 °C, (c) a zoom-in image of Fig. 2(b) and (d) TMV and Azo-F127 after decreasing temperature back to 25 °C

To probe the initial self-assembly behavior of TMV under different conditions, dynamic light scattering (DLS) experiments were performed. As shown in Fig. 3(a), in the case of mixing TMV with Azo-F127 ($c_{\text{TMV}} = 0.1 \text{ mg/mL}$, $c_{\text{F127}} = 2.5 \text{ mg/mL}$) at 25 °C, the small peak of Azo-F127 unimer at a radius of about 2 nm and the peak of TMV at a radius of about 70 nm could be visualized. While at 35 °C, the aggregation of Azo-F127 led to the formation of micelles. The peak at radius about 10 nm could be attributed to the micelles of F127. The rigid molecules at the surface of F127 micelles could effectively block the approach of EO chain onto the surface of TMV. Therefore, the “hard sphere” of Azo-F127 micelles could be used as depletants to push TMV together. The peak at a radius of about 600 nm compared with 70 nm for TMV alone and the broader distribution provided evidence of the formation of the TMV aggregates. After lowering the temperature to 25 °C, the micelles of F127 disassembled into individual unimers in aqueous solution again. The disappearance of “hard sphere” induced the restoration of the original appearance of TMV and the average hydrodynamic radius of TMV transferred back to around 70 nm. And this phenomenon could also be repeated several times (Fig. 3b). Therefore, the self-assembly process can be controlled by adjusting the temperature (Fig. 4).

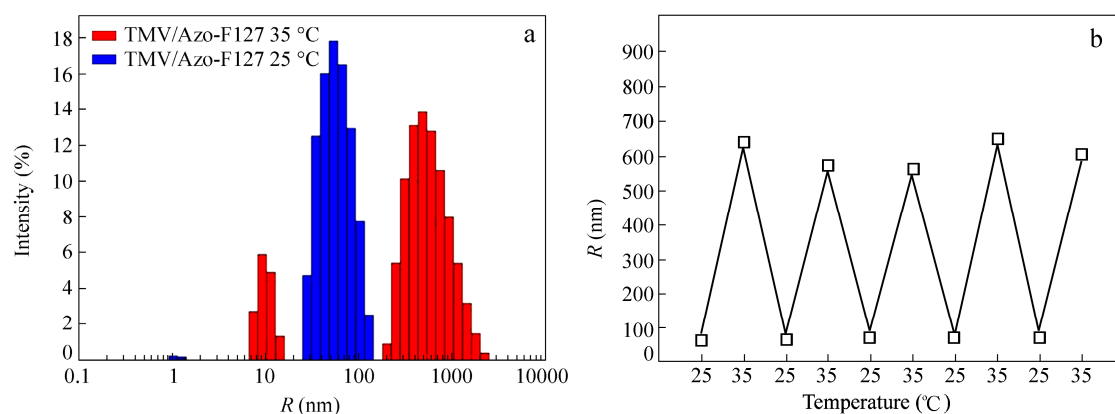


Fig. 3 (a) Hydrodynamic radius measured by DLS in the solution of TMV/Azo-F127 at 25 °C and 35 °C respectively ($c_{\text{F127}} = 2.5$ mg/mL, $c_{\text{TMV}} = 0.1$ mg/mL); (b) Changes of averaged hydrodynamic radius over a number of temperature cycles

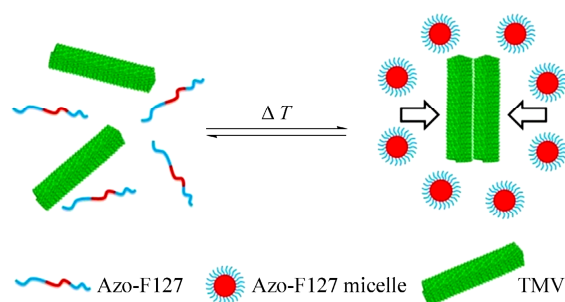


Fig. 4 Schematic representation of the formation of TMV bundles

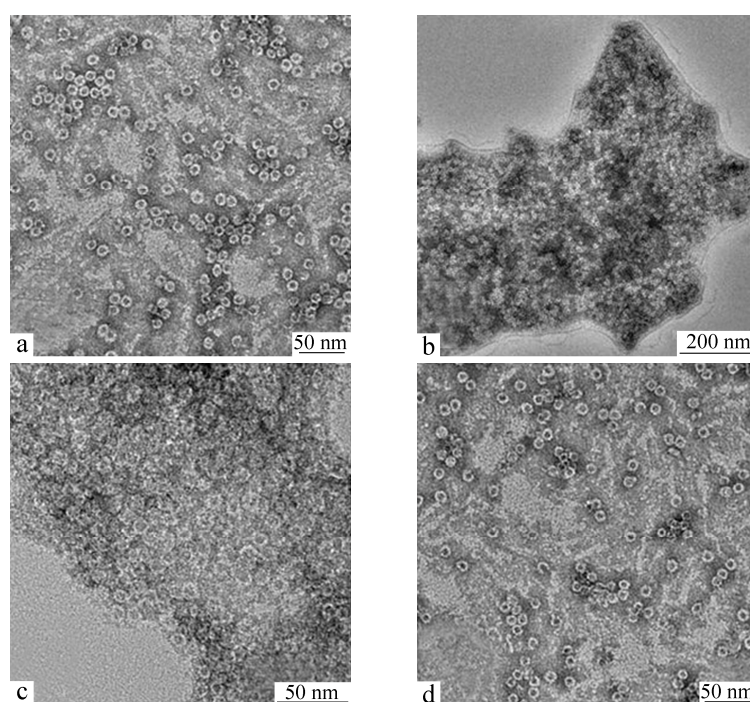


Fig. 5 TEM images of (a) ferritin and Azo-F127 at 25 °C, (b) ferritin and Azo-F127 at 35 °C, (c) a zoom-in image of Fig. 5(b) and (d) ferritin and Azo-F127 after decreasing temperature back to 25 °C

To extend this method to a different protein-based nanoparticle, Azo-F127 was also found to induce the self-assembly of spherical ferritin nanoparticles. TEM results confirmed the assembly and disassembly behavior of ferritin could be controlled reversibly in response to temperature (Fig. 5). This behavior suggests that Azo-F127 could act as a potential temperature-responsive medium to trigger the assembly or disassembly of protein based bionanoparticles.

CONCLUSIONS

To conclude, we have shown that Azo-F127 can be used to assemble bionanoparticles into temperature-responsive three-dimensional structures in solution. The formation of Azo-F127 micelles induced depletion force is the main cause of this phenomenon. This simple, effective method enables the production of temperature responsive materials and helps us to understand the interaction between polymers and biological molecules for potential applications in many fields.

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