VIP Photoluminescent Nanorings

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Preparation of Photoluminescent Nanorings with Controllable Bioreducibility and Stimuli-Responsiveness**

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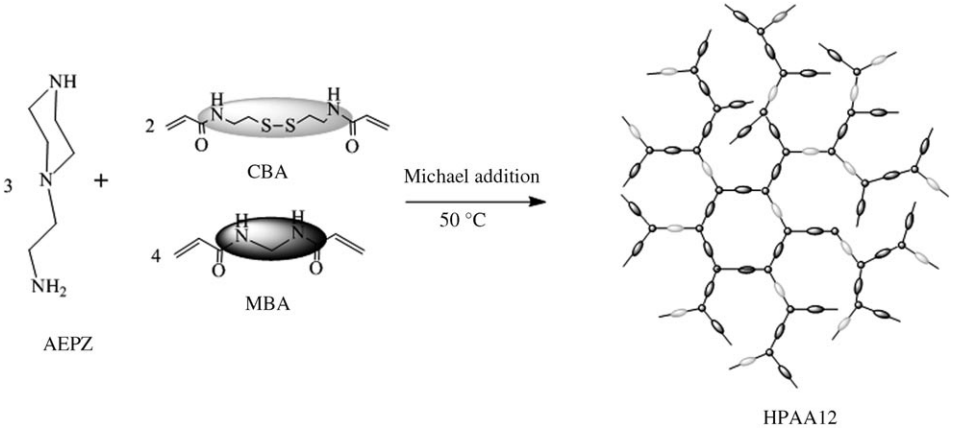
Nanoring materials with multiple functionalities have attracted significant attention owing to their potential applications in optical and electronic resonators, biological and chemical sensors, molecular imaging, and gene delivery.[1] Recently, several approaches have been proposed for fabricating nanoring materials, including synthesis of silver nanorings prepared from a one-pot procedure,[2] Au and Ag nanorings fabricated using the outer profile of silica nanoparticles as template,[3] and mesoscopic rings prepared by a method based on capillary force in the colloidal crystal.[4] Also, many interesting and unique properties of nanoring materials have been observed; for example, the toroidal structure of packed DNA is nature's most efficient morphology for transporting genetic information, done particularly well by viruses. Some researchers want to mimic this process by condensing DNA into a nanoring morphology to improve the effect of gene therapy. The DNA nanoring packed in viruses is multifunctional, but the nanoring materials fabricated to date are limited by a lack of the multiple functionalities. Thus, the preparation of nanoring materials with multiple functionalities of stimuli-responsiveness, biocompatibility, biodegradability, and photoluminescence will be very attractive.[5] We report herein an easy approach to fabricating multifunctional nanorings by the assembly of DNA with a novel multifunctional hyperbranched macromolecule.

It has been reported that multivalent cations can condense DNA into nanorings under suitable conditions.[1a,c,i] To prepare nanorings with multiple functionalities of stimuli-responsiveness, biodegradability, and photoluminescence, multivalent cations having these functionalities are prepared first and then used to condense plasmid DNA. Hyperbranched macromolecules are a special kind of macromolecule with three-dimensional structure. It is very easy to incorporate different functionalities into a single molecule of this type.[6,7] To develop photoluminescent nanorings with multiple functionalities, we prepared a novel disulfide-containing hyperbranched poly(amido amine) (HPAA) by Michael addition polymerization. Note that the syntheses of some linear disulfide-containing poly(amido amine)s have been reported, but these species easily condense plasmid DNA into nanoparticles; therefore, we prepared hyperbranched analogues.

The co-polyaddition reaction of 1-(2-aminoethyl)piperazine (AEPZ) with *N*,*N*′-cystaminebisacrylamide (CBA) and *N*,*N*′-methylenebisacrylamide (MBA; Scheme 1) was employed in the preparation of the HPAA containing a 1:2 molar ratio of the CBA unit to the MBA unit (HPAA12). This synthetic route was chosen for three reasons: 1) The disulfide-containing HPAA with a 1:0 molar ratio of CBA to MBA (HPAA10) obtained by polyaddition of AEPZ and CBA (at a molar ratio of 1:2) and the disulfide-containing HPAA with a 1:1 molar ratio of CBA to MBA (HPAA11) obtained by polyaddition of AEPZ with CBA and MBA at a 1:2 molar ratio of AEPZ to CBA + MBA are not water-soluble, and they have low buffer capacity. 2) HPAA with a 0:1 molar ratio of CBA to MBA (HPAA01) obtained by polyaddition of AEPZ and MBA (molar ratio 1:2) degrades very slowly, and it is not stimuli-responsive. 3) Disulfide-containing HPAA12 obtained by co-polyaddition of AEPZ with CBA and MBA at



Scheme 1. Synthesis of hyperbranched poly(amido amine) (HPAA12) by Michael addition polymerization.

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a 1:2 molar ratio of AEPZ to CBA + MBA (molar ratio of CBA to MBA is 1:2) has good water-solubility, good stability in buffer, low cytotoxicity, fast biodegradability, good biocompatibility, and excellent buffer capacity.[7] Most importantly, this kind of hyperbranched polymer can emit strong blue light owing to its special structure, as shown in Figure 1. The photoluminescence intensity increases with an increase of its molecular weight, and the photoluminescence becomes very weak after the polymer is reduced (see the Supporting Information).

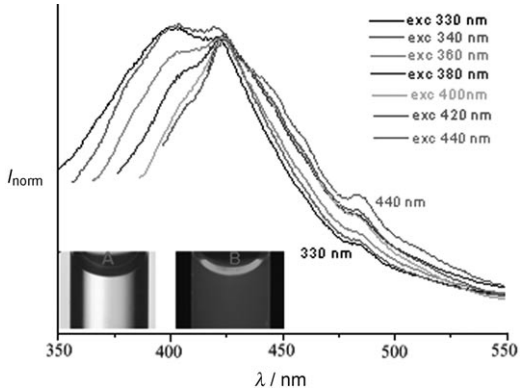
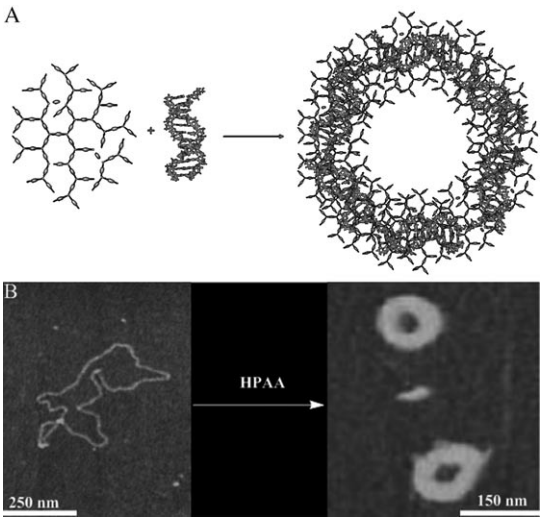


Figure 1. The luminescence emission spectra of HPAA12, the inset image (A) is the optical micrograph of HPAA12 solution without filter, and (B) is its optical micrograph with filter.

Nanomaterials composed of assembled DNA remain an active topic for experimental and theoretical research because of their importance in cell biology, polymer physics, and biotechnology.[8] The condensation of DNA is generally driven by an entropy increase associated with the release of counterions. The final condensate structures are determined by intermolecular forces including forces resisting condensation, such as entropy loss upon demixing of polymer and solvent and electrostatic repulsion among DNA chains, and favorable forces, such as correlated multivalent counterion fluctuation and cooperative hydration. The prepared HPAA12 (MW is 38 kDa) has many primary, secondary, and tertiary amines, which can bind and assemble DNA into nanorings. An assay excluding ethidium bromide shows that HPAA12 can bind strongly to DNA. Vortexing DNA solution in sodium acetate buffer with HPAA12 produces polyplex particles approximately 150 nm in diameter, as determined by dynamic light scattering (DLS) experiments. The zeta potential value of the formed polyplex nanoparticles is approximately 40 mV (see the Supporting Information).

To characterize the structure of the formed polyplex in detail, we transferred the nanoparticles onto mica. AFM images show that the polyplex particles have nanoring structure and are approximately 100 nm in diameter (Scheme 2 and Figure 2 A1). Furthermore, hyperbranched disulfide-containing poly(amido amine)s (MW of 15 and 58 kDa) also easily condense DNA into toroidal structures. However, linear disulfide-containing poly(amido amine)s were found to condense DNA into spherical nanoparticles approximately 150 nm in diameter when DNA solution was vortexed in sodium acetate buffer at an amine to DNA phosphate (N/P) ratio of 4:1 (as shown in Scheme 3). Therefore, the topology of disulfide-containing poly(amido amine)



Scheme 2. A) Schematic depiction of formation of the nanoring by the assembly of HPAA12 with plasmid DNA and B) AFM images of plasmid DNA before and after condensation with disulfide-containing HPAA12.

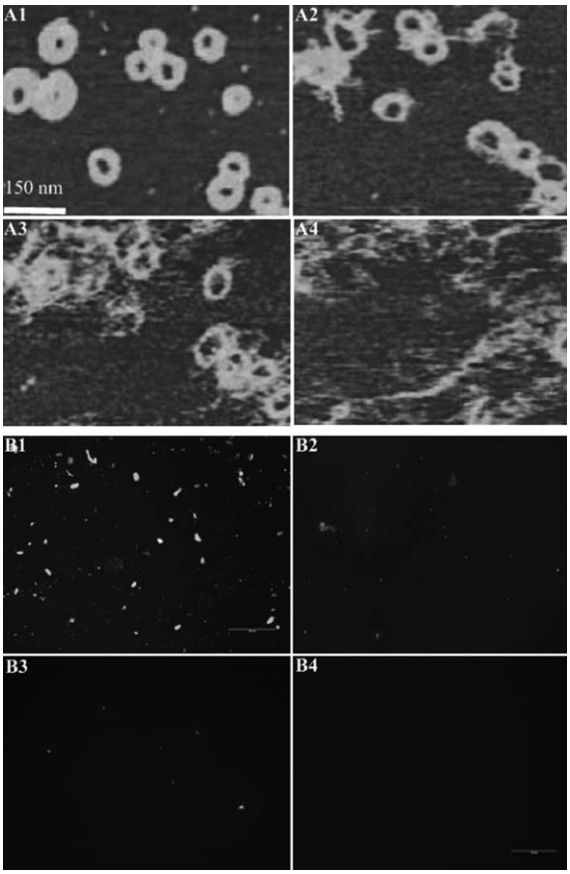
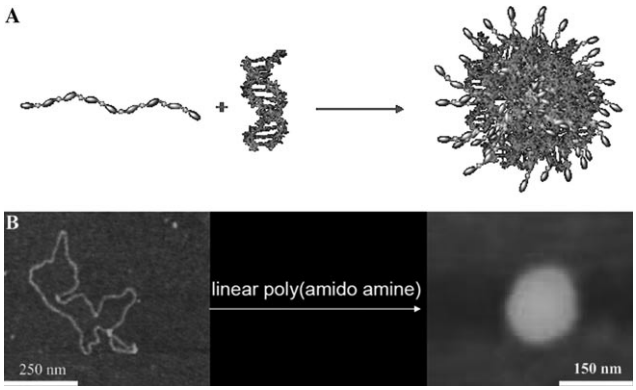


Figure 2. A) AFM images of the constructed nanorings before (A1) and after treatment with 20 mM DTT for 30 (A2), 45 (A3), and 60 min (A4). B) Fluorescent optical micrographs of the formed nanorings before (B1) and after treatment with 20 mM DTT for 30 (B2), 45 (B3), and 60 min (B4).



Scheme 3. A) Schematic depiction of the formation of spherical nanoparticles by the assembly of disulfide-containing linear poly(amido amine) with plasmid DNA and B) AFM images of plasmid DNA before and after condensation with disulfide-containing linear poly(amido amine).

determines the structure of the formed polyplex: hyperbranched poly(amido amine)s effectively condense plasmid DNA into nanoring-structured polyplexes, while some linear poly(amido amine)s easily condense DNA into spherical nanoparticles. The possible reason is that plasmid DNA is highly charged, which makes it rigid owing to the electrostatic repulsion among DNA chains, and so it is in a stretched structure; it becomes weakly charged and flexible after complexing with linear poly(amido amine)s, because the electrostatic repulsion among DNA chains becomes weak. Hence the DNA strand comes into a global structure and subsequently forms nanoparticles after further complexing with linear poly(amido amine)s. However, DNA is still highly charged after complexing with disulfide-containing HPAAs: some parts are highly positive, and some parts are highly negative; furthermore, the positive parts complex with the negative parts, which folds DNA strand (see the Supporting Information). Therefore, the disulfide-containing HPAAs easily condense DNA into toroidal structures, while some disulfide-containing linear poly(amido amine)s easily condense DNA into nanoparticles.

We have not obtained AFM images of the intermediates during the formation of the polyplex, but we used AFM to trace their disassembly in the presence of dithiothreitol. It is clear that the size of some nanorings increases while the size of the ring wall decreases, as some hyperbranched macromolecules are reduced in the presence of dithiothreitol and some folded strands unfold. During the disassembly of spherical nanoparticles, some random coil structures were observed (see the Supporting Information), which further verifies the presumed condensation mechanism. The N/P ratio and the molecular weight of disulfide-containing hyperbranched poly(amido amine) have some effects on the formation of nanoring structure: toroidal structures were observed at N/P ratio of approximately 5, but the spherical nanoparticles formed when the N/P ratio was over 10; toroidal structures were observed when the MW of HPAA is above 15 kDa, but spherical nanoparticles formed when the MW of HPAA was about 2 kDa. Of course, factors such as the pH of the buffer, the chemical structures of the poly(amido amine) chain, and incubation time also effect the morphology of the polyplex.[1,7] On the other hand, it is very interesting that these nanorings can emit strong blue and green light and weak red light when 330–385, 460–490, and 510–550 nm excitation filters were used, respectively. The nanorings were transferred onto a microscope slide, and the strong blue light was observed from the optical microscope after removing solvent (as shown in Figure 2 B1). The disulfide-containing poly(amido amine) is of very low cytotoxicity (see the Supporting Information), therefore the formed nanorings are photoluminescent with good biocompatibility.

The formed nanorings contain disulfide linkages in the ring wall. These disulfide linkages can be controllably bioreduced, and the nanorings will degrade if the disulfide linkages in the ring wall break. These nanorings are sensitive to dithiothreitol (DTT) and glutathione (GSH). After the nanorings were treated with 2 μM and 20 mM DTT, respectively, the solution was transferred onto microscope slides at different time intervals. There is almost no change in photoluminescence for the nanorings treated with 2 μM DTT even after 120 min; however, there is almost no photoluminescence for the nanorings treated with 20 mM DTT for 60 min, and the fluorescent optical micrographs show that the photoluminescence intensity decreased with time after treatment (Figure 2 B1–4), thus showing that these nanorings are stimulus-responsive. This stimulus-responsive photoluminescence may be used in molecular imaging.

The nanorings were also imaged in buffer solutions after DTT solution was injected. The DTT concentration was maintained at 20 mM in the cell, which is a similar level as the in vivo GSH concentration in the nucleus. AFM imaging ensued immediately after the introduction of DTT. The results show that the nanorings disassembled slowly and completely disassembled in approximately 60 min (Figure 2 A2–4). However, the ring structure remained unchanged after the nanorings were treated with 2 μM DTT for 120 min, which indicates that the disassembly of the formed nanoring is controllable. This controllable disassembly is very useful in gene and drug delivery, as the release of gene or drug can be controlled in cell. In the gene delivery experiment, it is found that the gene transfection efficiency using these stimuli-responsive delivery vectors is higher than that using non-stimulus-responsive delivery vector (see the Supporting Information).

In summary, a novel multifunctional disulfide-containing hyperbranched poly(amido amine) with stimuli-responsiveness, biocompatibility, biodegradability, and photoluminescence was developed, and this polymer can effectively assemble plasmid DNA into photoluminescent nanorings with disulfide linkages in the ring wall. These fabricated nanorings are not only photoluminescent but are also stimuli-responsive and biocompatible and can be controllably bioreduced, which will have potential applications in gene and drug delivery and molecular imaging.

Experimental Section

Synthesis of hyperbranched poly(amido amine) HPAA12: The synthesis of disulfide-containing bioreducible hyperbranched poly(a-

mido amine) by Michael addition copolymerization is similar to earlier reports.[7] In a typical experiment, AEPZ (129.0 mg, 1.0 mmol), CBA (173.6 mg, 0.67 mmol), and MBA (205.6 mg, 1.33 mmol; the molar ratio of AEPZ to CBA + MBA is 1:2) were added into a vial and dissolved in methanol/water (6.0 mL, 8:2 v/v). The reaction was allowed to proceed at 50 °C for 90 h, yielding hyperbranched polymer with vinyl end groups. Then AEPZ (180.0 mg) was added to terminate the vinyl units. The resulting bioreducible HPAA12 was obtained by precipitation in cool acetone and drying under vacuum for 2 h at room temperature. A 2 % solution of the prepared polymer was used in photoluminescence experiments; the quantum yield is 0.21.

Preparation of nanorings by assembly of DNA with hyperbranched poly(amido amine): DNA (50 mg L^{-1}) in 30 mM sodium acetate buffer (pH 5.1) was used to prepare nanoring-structured materials at an amine to DNA phosphate (N/P) molar ratio of 4:1. Then HPAA12 (2.0 mg mL^{-1}) solution was added to the DNA solution and mixed by vortexing at 3000 rpm for 60 s, and the solution was incubated at room temperature for 20 min. A 30 μL sample of nanoring particles was transferred onto approximately 1 cm^2 of freshly cleaved mica, and the surface was rinsed with deionized water.

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