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Controlled and reversible binding of positively charged quantum dots to lambda DNA

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1. ABSTRACT

Biomacromolecules/Nanomaterials bioconjugate complexes have many applications in the interdisciplinary research fields. Accessible and easy synthesis methods of these complexes are the key roles for these applications. High quality water-soluble surface-charged quantum dots (QDs) were successfully prepared via surface modification by amphiphilic surfactants. The positively charged QDs can interact with deoxyribonucleic acid (DNA) molecules to form QDs/DNA bioconjugates via self-targeting electrostatic force. The stability of these QDs/DNA bioconjugates is influenced by ionic strength and concentration of negative or neutral surfactants in the solution. High ionic concentration or ca. 10^{-3} mol/L surfactants can break the interaction between the QDs and DNA molecules (Lambda DNA/Hind III Marker segments) and controllably release DNA molecules from these bioconjugates. The conformation of DNA molecules has little change during the binding and releasing process. The condensation of lambda DNA molecules can be induced by positively charged QDs. High resolution transmission electron microscopy experiments have revealed the different stages of DNA condensation process, showing the fine structures of QDs/DNA bioconjugates at biomolecular scale. A long chain DNA molecule starts to self-enwind and condense to a porous globule when it is exposing to positively charged QDs but there is no direct interaction between QDs and DNA at early stages of condensation. After the DNA molecule becomes a compact globule, QDs stick onto its surface via electrostatic force. The coil conformation of the DNA molecules can be recovered from globule structure after DNA molecules are controllably released from bioconjugate complexes. These QDs/DNA bioconjugates have great potential applications for gene delivery and at the same time the fluorescence of QDs can be utilized to monitor the DNA releasing process.

2. INTRODUCTION

Quantum dots (also called semiconductor nanocrystals) are novel functional nanomaterials with outstanding spectral and electronic properties. In the past decade, much attention was focused on their applications in biological analysis and nanodevices (1-5). Since the two original research papers on QDs' applications in biological labeling and cell imaging by Bruchez et al. (6) and Chan et al. (7) were published, many more creative and interesting results in this research field have appeared in the literature (8-14). Due to their extraordinary fluorescent properties, QDs have the potential to replace traditional organic dyes and be widely used for biological analysis. To date, the well defined nanostructures of QDs/biomacromolecules bioconjugates have been popularly investigated because they have great potential applications in diagnostic and environmental analysis. Based on fluorescent resonance energy transfer (FRET), Goldman developed QDs/antibody conjugates as a kind of nanosensor for specific detection of trinitrotoluene (TNT) in aqueous solution (15); Chen conjugated QDs to peptides which carried nuclear localization signal (NLS) and transfected these bioconjugates into living cells and made long-time nuclear targeting come true (16). Some interesting experiments about bioconjugates of QDs and DNA molecules and their applications have also been reported. Our group used QDs-labeled specific DNA sequences for fluorescent *in situ* hybridization (FISH) in microorganism Escherichia coli (17). Up to now, for the preparation methods of QDs/biomacromolecules bioconjugates, the chemical covalent bonding and metal-sulfur coordination are popularly used. But the disadvantages of these two methods are obvious and hard to overcome, such as complicated and time consuming process, low yield, and difficult access to staring materials for chemical covalent bonding method and unstable final product, low yield, low fluorescent quantum yield for metal-sulfur coordination method.

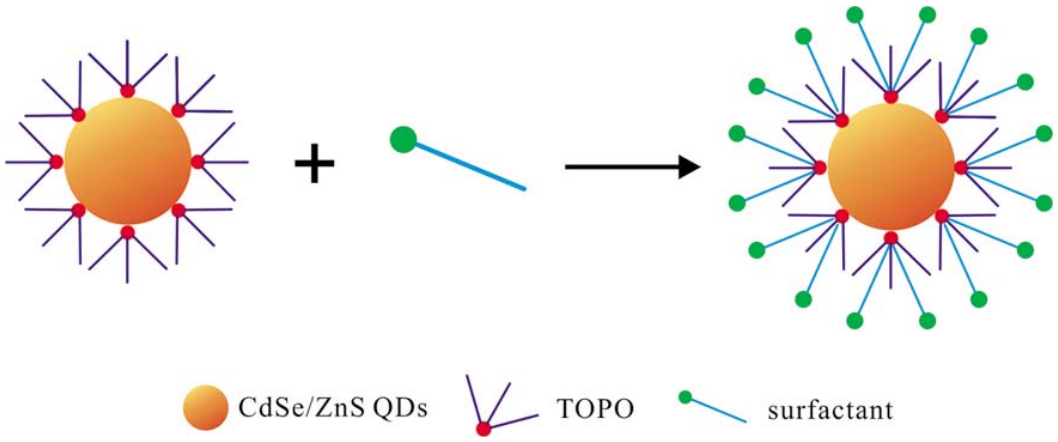

Figure 1. Preparation of high-quality water-soluble surfactant-modified QDs.

DNA condensation has attracted great research interest in recent years for its importance in biomedical applications especially in gene delivery and gene therapy. It can be induced by cationic materials, such as surfactants (18), chitosan (19), polymers (20), dendrimers (21) and so on. The compact conformation protects DNA from nuclease, reducing the degradation or damage of DNA during the uptake by cells. Therefore, the investigation of the entire process of DNA condensation is very crucial in gene delivery. To our knowledge, there is no literature to investigate the fine structure at molecular scale of DNA during its condensation process induced by nanoparticles. Electrostatic force is a kind of helpful interaction used in designing nanostructure of nanomaterials and biomolecules due to its high efficiency and self-targeting selectivity (22).

In this work, bioconjugates of DNA molecules and hydrophilic positively charged QDs were constructed. First, cationic amphiphilic surfactant cetyltrimethylammonium bromide (CTAB) as a modification material was adopted to prepare high quality, water-soluble, positively charged QDs based on the hydrophobic interaction between the hydrophobic end of CTAB and trioctylphosphine oxide (TOPO) ligands of TOPO-capped hydrophobic CdSe/ZnS QDs. The results show that these positively charged QDs can reversibly bind to lambda DNA through strong electrostatic force to form QDs/DNA bioconjugates, and the DNA condensation process can be induced by such surface-charged QDs.

3. MATERIALS AND METHODS

3.1. Materials

High quality hydrophobic CdSe and CdSe/ZnS quantum dots were prepared according to a literature method (23). CTAB was A.R. grade reagent (China Medicine (Group) Shanghai Chemical Reagent Corporation). Sodium dodecyl sulfate (SDS) and Triton X-100 were purchased from Fluka. Sodium hydroxide was purchased from Sigma. Float A Lyzer (MWCO 1000) was purchased from Spectra/Pro. Lambda DNA/Hind III Marker and lambda DNA were purchased from SinoBio Company. Ultrapure deionized (18.2 MΩ) water was used in all experiments. All reagents were used as received without further purification.

3.2. Preparation of water-soluble quantum dots by amphiphilic surfactants

CTAB or SDS (150 mg) was placed in a carnelian mortar, ground and mixed thoroughly. A 0.5 mL CdSe or CdSe/ZnS QDs solution in n-hexane (~6.4×10^{-6} mol/L) was added into the mortar and mixed with surfactant powder. The mixture was ground continuously until the hexane had been evaporated completely. Another 0.5 mL QDs hexane solution was added and the same procedure for grinding and evaporating was carried out. The process was repeated for three times. Figure 1 is the cartoon showing the phase transferring process of QDs from oil phase to aqueous phase based on strong hydrophobic interaction between surfactants and TOPO ligands on the surface of QDs. The mixture of QDs and surfactant was dried under 80 °C for 10-15 minutes. After the mixture had been cooled down to room temperature, 6 mL water was added into the mortar to dissolve the product. The mixture was sonicated in an ultrasonic bath for 15 minutes, followed by centrifuged at 12000 rpm for 10 minutes. The precipitate was discarded and the upper clear aqueous supernatant was taken as the product, which were surface-charged water-soluble QDs. The product could be stored stably at room temperature for several months. In order to use these hydrophilic QDs in high efficiency, CdSe or CdSe/ZnS nanoparticles have to be the limiting reagent. Thus there was excess amount of surfactants in the product solution. The product can be further purified by dialysis with a MWCO 1000 dialyzer for 48 h or repetitive centrifugation at 16000 rpm/30 min to remove the excess surfactants completely. These hydrophilic QDs have good fluorescence visibility and monodispersity, and in the meantime keep the extraordinary spectral properties of original hydrophobic QDs (see Supporting Information).

3.3. Preparation of quantum dots/DNA bioconjugates

The purified surfactant-modified water-soluble product (10^{-7} mol/L) and Lambda DNA Marker (10^{-7} mol/L) were mixed in equal volume. After 10 minutes, a small amount of precipitated from solution was removed by

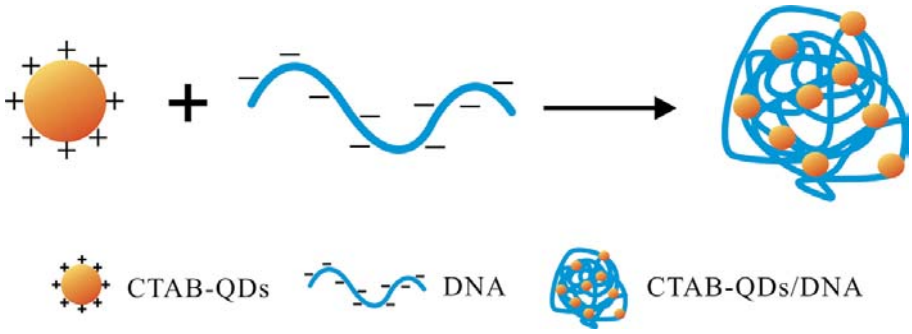

Figure 2. Formation of CTAB-modified QDs/DNA bioconjugates by electrostatic interaction.

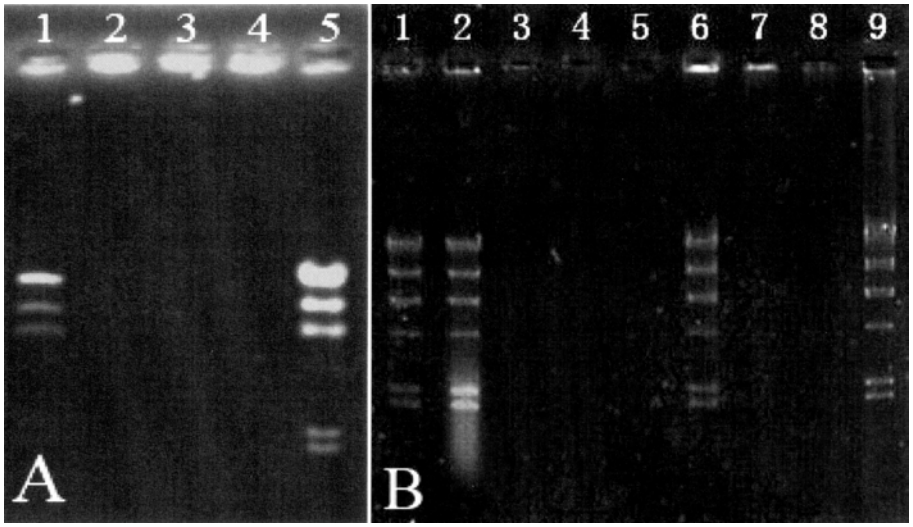

Figure 3. Fluorescence imaging of agarose gel electrophoresis of QDs/DNA bioconjugates. (A): (1) Pure DNA; (2) CTAB-QDs/DNA; (3) CTAB-QDs/DNA in 0.01 mol/L NaCl; (4) CTAB-QDs/DNA in 0.1 mol/L NaCl; (5) CTAB-QDs/DNA in 1 mol/L NaCl. (B): (1) Pure DNA; (2) SDS-QDs+DNA; (3) CTAB-QDs/DNA; (4) CTAB-QDs/DNA in 4.5×10^{-4} mol/L SDS; (5) CTAB-QDs/DNA in 7.5×10^{-4} mol/L SDS; (6) CTAB-QDs/DNA in 10^{-3} mol/L SDS; (7) CTAB-QDs/DNA in 5×10^{-4} mol/L Triton X-100; (8) CTAB-QDs/DNA in 10^{-3} mol/L Triton X-100; (9) CTAB-QDs/DNA in 1.5×10^{-3} mol/L Triton X-100. The conditions of electrophoresis: 0.7% agarose, and constant voltage mode at 100 V.

centrifugation at 16000 rpm for 30 min and re-dissolved in 0.5 mL of water.

3.4. Agarose gel electrophoresis

The controllably reversible forming process of QDs/DNA bioconjugates was characterized by agarose gel electrophoresis. 0.7% agarose gels were used through the whole characterization. The voltage for electrophoresis was set constantly at 100 V. We used 0.5×TBE (Tris/Borate/EDTA) as running buffer. The electrophoresis experiment was performed for 1.5 h and stained in Ethidium Bromide solution (0.5 μg/mL) for 0.5 h. The gels were illuminated and imaged by Bio-Print (Vilber Lourmat).

3.5. High resolution transmission electron microscopy

In order to investigate DNA condensation process induced by positively charged CTAB-modified quantum dots, the solution of QDs/DNA bioconjugates was observed by JEOL JEM 2010 FEF high resolution transmission electron microscopy (HR-TEM) with acceleration voltage at 200 kV.

4. RESULTS AND DISCUSSION

4.1. Reversible formation of quantum dots/DNA bioconjugates via electrostatic interaction

After surface modification by cationic surfactants, the positively surface-charged QDs can interact with the negatively charged DNA molecules to form bioconjugates via electrostatic force. Figure 2 is a cartoon showing the formation of CTAB-modified QDs/DNA bioconjugte. The reaction efficiency is quite high because electrostatic force is self-targeting. The combination ratio of CTAB-modified QDs to DNA molecules in the bioconjugates is determined by the concentration of QDs as well as the concentration and size of DNA sequences. The DNA molecules can be controllably released from the bioconjugates by deliberately changing the experimental conditions.

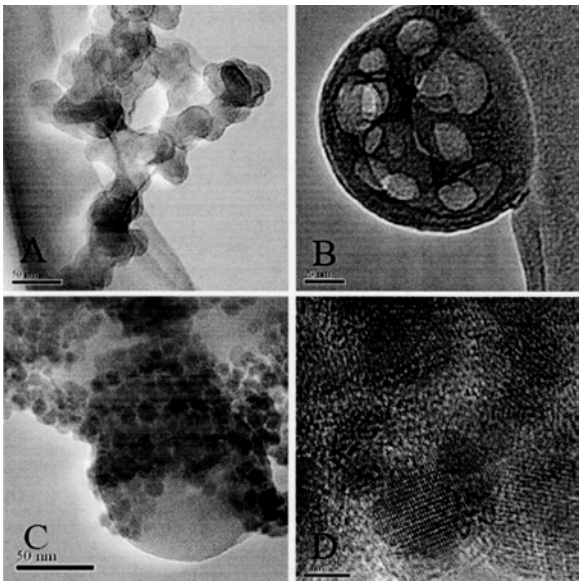

Figure 4. HR-TEM photos obtained at different stages of the formation of CTAB-QDs/lambda DNA conjugates. The scale bars in (A), (B), (C), and (D) are 50 nm, 20 nm, 50 nm, and 5 nm, respectively.

First, the stability of the CTAB-modified QDs/DNA bioconjugates was investigated by varying system ionic strength. Figure 3(A) is the fluorescent image for electrophoresis. The DNA sequences used in these experiments were Lambda DNA/Hind III Marker, which is composed of different segments of DNA sequences. The CTAB-modified QDs/DNA bioconjugates were either neutral or positively charged if DNA amount was limited, so the bioconjugates did not migrate to the anode in lane 2 in the absence of NaCl. In lanes 3 and 4, the bioconjugates were not destroyed by both 0.01 mol/L and 0.1 mol/L NaCl. When the concentration of NaCl was as high as 1 mol/L, as shown in lane 5, the DNA molecules were released from the bioconjugates. The "smile" strip in lane 5 was possibly due to the high salt concentration.

Anionic and neutral surfactants can also destroy CTAB-modified QDs/DNA bioconjugates, the amount of surfactants required is much less than that of NaCl. Figure 3(B) shows the influence of surfactants on the stability of CTAB-modified QDs/DNA bioconjugates. Because the negatively charged SDS-QDs did not interact with DNA molecules, lane 2 was the same as lane 1. The bright band in lane 2 was due to the drift of SDS-modified QDs. The threshold concentration of anionic and neutral surfactants which destroyed the bioconjugates of CTAB-modified QDs with the DNA Marker segments were 10^{-3} mol/L (for SDS) and 1.5×10^{-3} mol/L (for Triton X-100), respectively. When the concentration of surfactants in solution was lower than the threshold, the structure of the bioconjugate was preserved, which were shown in lanes 3, 4, 5, 7and 8. DNA molecules were successfully released in lanes 6 and 9 where concentrations of surfactants were higher than corresponding threshold values, and based on the fluorescence intensity of the respective DNA sequences, the release was almost complete. Comparing the position of the released DNA sequences with that of DNA Marker sequences indicated that the conformation of DNA molecules had little change during the reversible formation of these bioconjugates.

4.2. DNA condensation process induced by CTAB-modified QDs

In order to further prove the formation of CTAB-modified QDs/DNA bioconjugates and investigate their fine structures at molecular scale, HR-TEM was used to characterize the various stages in the process of bioconjugate formation. Lambda DNA was used in this experiment because its physical size is quite big (theoretical length is around 16 μm for a molecule of 48 kbp) and is very easily caught during HR-TEM measurements. The concentration of DNA in the reaction system was 1 μg/mL and the concentration of CTAB-QDs was 10^{-7} mol/L. Figure 4 shows the different stages during bioconjugate formation process in details. In Figure 4(A), the DNA long chain started to self-enwind and condense, induced by exposing to the CTAB-modified QDs, but there was no physical contact between CTAB-modified QDs and DNA since no nanoparticles could be found in this image. In Figure 4(B), DNA molecule continued to condense and became a globule with porous texture. No nanoparticles were also found in this DNA global structure. These two images prove that the CTAB-modified QDs play the key role for the early phase of DNA condensation but do not interact directly with DNA molecules during the condensation process. In Figure 4(C), the DNA molecule became a compact globule and many CTAB-modified QDs now stuck onto the surface of the DNA condensed globule by electrostatic force. There were no nanoparticles inside the DNA globule. This result further proves that CTAB-modified QDs do not bind to DNA during the DNA self-enwinding and condensing process. The binding of QDs to DNA only occurs on the surface of the DNA globule surface. Figure 4(D) is the feature image of some CTAB-modified QDs sticking on the surface of DNA globule in Figure 4(C). Based on above results, it can be concluded that the CTAB-modified QDs can induce the DNA condensation process, during which the DNA molecules are transformed from coiled long chains to compact globules. The binding of the positively charged QDs to DNA molecules does not occur at the early stage of the condensation process. After the DNA molecules are transformed into compact globules, the CTAB-QDs will stick onto the surface of DNA globules via electrostatic attraction.

The reversible formation of bioconjugates of CTAB-modified QDs and long chain lambda DNA were further confirmed by agarose gel electrophoresis. The concentration of SDS used to destroy bioconjugates of CTAB-modified QDs with lambda DNA was 0.01 mol/L. As shown in Figure 5, consistent with the result in Figure 3(B), SDS can also destabilize the bioconjugates of CTAB-modified QDs with long chain DNA such as lambda DNA. The very bright band in lane 3 resulted from QDs because excessive SDS in this system would reverse the charge on the QD surface. The positions of DNA bands in lanes 1 and

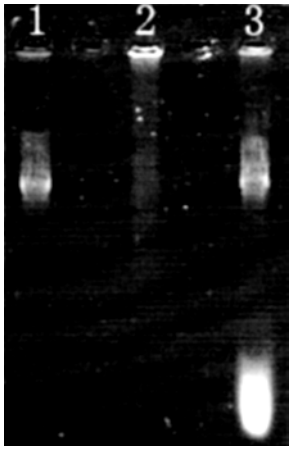

Figure 5. Fluorescence imaging of agarose gel electrophoresis of QDs/lambda DNA bioconjugates. (1) Lambda DNA; (2) CTAB-QDs/lambda DNA; (3) CTAB-QDs/lambda DNA in 0.01 mol/L SDS. The conditions of electrophoresis: 0.7% agarose, and constant voltage mode at 100 V.

3 were the same, indicating that the DNA molecules were restored from the globule structure to coiled chain conformation after they had been released from the bioconjugates.

In conclusion, high-quality surface-charged water-soluble QDs can be easily prepared in a high efficiency via surface modification with surfactants. Stable positively charged QDs/DNA bioconjugates can be formed via self-targeting electrostatic force. Our investigations on the controllably reversible formation of CTAB-modified QDs/DNA bioconjugates indicate that these positively charged QDs can induce the condensation of long chain DNA molecules, and the conformations of DNA molecules released from the bioconjugates keep intact. These easily prepared QDs/DNA bioconjugates have potential application for DNA delivery, because DNA structure has little change during formation process of QDs/DNA bioconjugates. Another advantage is that fluorescence of QDs can be utilized to track DNA during the transferring and releasing process.

5. ACKNOWLEDGEMENTS

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Abbreviations: QDs: quantum dots, DNA: deoxyribonucleic acid, FRET: fluorescent resonance energy transfer, TNT: trinitrotoluene, NLS: nuclear localization signal, FISH: fluorescent *in situ* hybridization, CTAB: cetyltrimethylammonium bromide, TOPO: trioctylphosphine oxide, SDS: sodium dodecyl sulfate, HR-TEM: high resolution transmission electron microscopy

Key Words: Quantum dots, Surface modification, DNA condensation, Bioconjugate

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