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Adaption of Au Nanoparticles and CdTe Quantum Dots in DNA Detection*

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Abstract A DNA fluorescence probe system based on fluorescence resonance energy transfer (FRET) from CdTe quantum dot (QD) donors to Au nanoparticle (AuNP) acceptors is presented. CdTe QDs, 2.5nm in diameter, as energy donors, were prepared in water. Au nanoparticles, 16nm in diameter, as energy acceptors, were prepared from gold chloride by reduction. CdTe QDs were linked to 5′-NH_2-DNA through 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride (EDC) as a linker, and the 3′-SH-DNA was self-assembled onto the surface of AuNPs. The hybridization of complementary double stranded DNA (dsDNA) bound to the QDs and AuNPs (CdTe-dsDNA-Au) determined the FRET distance of CdTe QDs and Au nanoparticles. Compared to the fluorescence of CdTe-DNA, the fluorescence of CdTe-DNA-Au conjugates decreased extremely, which indicated that the FRET occurred between CdTe QDs and Au nanoparticles. The fluorescence change of this conjugate depended on the ratio of Au-DNA to CdTe-DNA. When the AuNPs-DNA to QD-DNA ratio was 10:1, the FRET efficiency reached a maximum. The probe system would have a certain degree of fluorescence recovery when a complementary single stranded DNA was introduced into this system, which showed that the distance between CdTe QDs and Au nanoparticles was increased.
Keywords CdTe, quantum dots, Au nanoparticle, fluorescence resonance energy transfer, DNA

1 INTRODUCTION

Colloidal semiconductor nanocrystals, also called quantum dot (QD), is a new class of fluorescent biological labels. Originating from the quantum confinement of electrons and holes within the nanocrystal material, the fluorescence from QDs is unique compared to that from traditional organic dyes and genetically engineered fluorescent proteins. Generally, organic dyes often have narrow absorption spectra, broad emission, and very low photobleaching thresholds, and they do not allow large Stoke shifts to be realized on account of the small spectral separation between the absorption and emission peaks[1]. QDs exhibit unique properties, such as high photobleaching threshold, chemical stability and their readily tunable spectral properties, have the potential to overcome many of the limitations in biological research encountered by conventional dye systems[2—5]. Since their introduction into biological imaging in 1998[2,3], many researches have emerged focusing on the synthesis, photophysical property characterization, and bioconjugation[6—9] of QDs. Fluorescence resonance energy transfer (FRET) occurs when the electronic excitation energy of a donor chromophore is transferred to an acceptor molecule nearby via a through-space dipole-dipole interaction between the donor-acceptor pair[10]. The FRET process is more efficient when there is an appreciable overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor. The strong distancedependence on the FRET efficiency has been widely exploited in studying the structure and dynamics of proteins and nucleic acids, in the detection and visualization of intermolecular association, and in the development of intermolecular binding assays[11]. Compared with conventional chemical analysis, FRET-based analytical method is higher in sensitivity and better in simplicity. As luminescent inorganic fluorophores, QDs are currently being widely used as biological probes, because QDs show a broad and continuous excitation spectrum, narrow size-tunable symmetric emission spectrum and high fluorescence quantum yield[12,13]. The size-dependent optical properties of QDs result from their quantum-confined electronic states[14]. The emission of the QDs is quenched when associated with gold nanoparticles that have opposite charges[15].

Recently, complex nanostructures formed by linking QDs and gold nanoparticles (AuNPs) through DNA hybridization or streptavidin-biotin interaction have also been realized[16—18] and applied in the sensing biomolecular concentration[17]. AuNPs have a high extinction coefficient and a broad absorption spectrum in visible light that is overlapped by the emission wavelength of the usual energy donors. The key challenge of the hybrid system QDs-dsDNA-AuNPs is to immobilize single stranded DNA (ssDNA) onto the surfaces of QDs and AuNPs, respectively.

The Gueroui group reported that carboxy-modified QDs, activated with 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC) and sulpho *N*-hydroxy-succinimide (NHS), could be covalently linked to ssDNA[18], and ssDNA modified AuNPs were achieved by ssDNA being covalently linked to

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AuNPs functionalized with a single NHS ester[18,19].

Previous studies confirmed that the synthetic oligonucleotides with —SH (HS-DNA) group could be easily self-assembled on the surface of Au[20]. Therefore, the objective of the present work was to utilize the self-assembly of HS-DNA on AuNPs to simplify the process of probe preparation, and prepare QDs-dsDNA-AuNPs (DNA probe) by the hybridization between QDs linked with a DNA sequence and AuNPs with a DNA strand. Because of that the emission spectrum of CdTe QDs and the absorption spectrum of AuNPs had an appreciable overlap, and the complex of QDs-dsDNA-AuNPs was expected to form donor-acceptor conjugate, as the emission of QDs was quenched by AuNPs in a distance-dependent manner (which is shown in Fig.1). When the complete complementary DNA, as target (Sequence 3), existed in the detection system of this complex, the double stranded DNA of the probe system was opened, and the distance between CdTe QDs and Au nanoparticles was increased. The target DNA could replace the DNA (Sequence 2) on the QDs surface and hybridize with the DNA (Sequence 1) on AuNPs, because the target DNA had more complementary base than the Sequence 2 DNA, which resulted in a stronger hydrogen bond between the target DNA and the Sequence 1 DNA. Thus, the fluorescent intention of the system would be recovered, and this complex could be used as a DNA probe for biosensors.

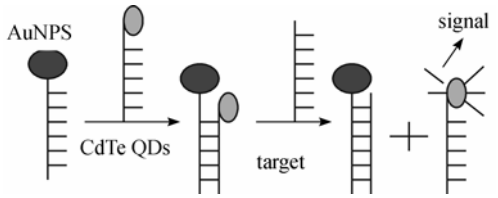


Figure 1 Schematic of the QD-DNA and AuNPs-DNA hybrid conjugates based on FRET

2 EXPERIMENTAL

2.1 Material

Gold chloride ($HAuCl_4 \cdot 3H_2O$), 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC), and trisodium citrate were purchased from Tianjin Delan Chemical Co. Other reagents were of analytical grade. All other reagents and solvents were of analytical grade and used as received without further purification. DNA oligonucleotides were purchased from Shanghai Invitrogen Biotechnique Co., and the sequences were listed as follows:

Sequence 1: 5′-AGT AAG CAA GAG AGA GCC GGG GGG-$(CH_2)_6$-SH-3′

Sequence 2: 5′-NH_2-$(CH_2)_6$-GGC TCT CTC TTG CTT ACT-3′

Sequence 3: 5′-CCC CCC GGC TCT CTC TTG CTT ACT-3′

Sequence 4: 5′-CCC CCC GGC TCT CTT TTG CTT ACT-3′

2.2 Preparation of AuNPs

AuNPs were prepared by the citrate reduction of $HAuCl_4$[21]. 2.5ml of 1% trisodium citrate solution was added to 1% $HAuCl_4$ quickly under stirring and reflux. After 15min, as the solution color changed from yellow to deep red, the reflux was stopped and the solution was stored at 4℃. The characterization of the particle size and morphology was determined by using Tecnai G2 20 S-TWN transmission electron microscope. The UV absorption spectra were obtained by Helios-γ thermo ultraviolet and visible spectrophotometers.

2.3 Preparation of CdTe QDs

According to Ref.[22], the preparation of NaHTe included two steps: First, Te powder and $NaBH_4$ was added into a 100ml flask with 50ml redistilled water. The reaction was in ambient temperature with N_2 protection. The reaction ended when the Te powder in the flask disappeared. Secondly, for the preparation of CdTe QDs, 86.6mg of $CdCl_2$ and 79.22μl of 3-mercaptopropionic acid (MPA) were dissolved in 297ml of redistilled water in a dry three-necked flask with N_2 protection. The pH of the mixture was adjusted to 9.1 with 1mol·L^{-1} NaOH solution under stirring. The NaHTe solution was added to the reaction mixture under N_2 protection under constant stirring for about 20min, and then the reaction mixture was heated from ambient temperature till it boiled at 100℃, and the reflux time to get the CdTe QDs was 1h.

2.4 Self-assembly of DNA on AuNPs surface

15ml of AuNPs solution and 33 μg of DNA (Sequence 1) were mixed in ambient temperature for about 16h to formulate the self-assembly program satisfactorily. Then, the solution was added into a phosphate buffer solution (PBS, pH=7.0). The reaction ended after 40h. The AuNPs-DNA was purified by repeating ultra-centrifugation (10000r·min^{-1} for 40min), decanting, and resuspension in PBS.

2.5 Preparation of DNA modified CdTe QDs

4ml of CdTe QDs, 33μg DNA oligonucleotide (Sequence 2), and 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC) amounting to 10 times the mole of DNA, were mixed in 0.05mol·L^{-1} Tris-HCl and 0.02mol·L^{-1} NaCl buffer (pH=7.2) under room temperature.

2.6 Hybridization of QDs-DNA and AuNPs-DNA and detection of target DNA

The hybridization of AuNPs-DNA and QDs-DNA occurred in a solution containing 0.02mol·L^{-1} Tris-HCl, 0.05mol·L^{-1} KCl, and 0.005mol·L^{-1} $MgCl_2$ (pH=8.0), at 37℃, for about 1h. Detection was as follows: target DNA (Sequence 3 and Sequence 4) was added into the QDs/AuNPs conjugate probe system. The fluorescence spectrum of the probe system was measured with a WGY-10 Luminescence Spectrometer.

According to Foster's theory, FRET efficiency, E, is given by

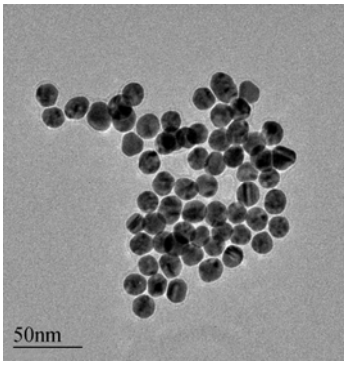
$$E = \left(1 - \frac{F_{\text{probe}}}{F_{\text{recovery}}}\right) \times 100\%$$

where F_{probe} is the fluorescence intensity of the QDs/AuNPs conjugates, and $F_{recovery}$ is the fluorescence intensity after addition of target DNA.

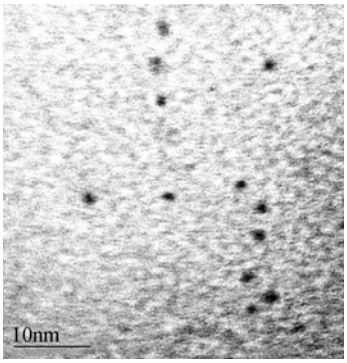
3 RESULTS AND DISCUSSION

3.1 Determination of the acceptor and donor

The TEM micrographs of AuNPs and QDs are illustrated in Fig.2. The particle sizes of AuNPs and QDs were (16±2)nm and (2±1)nm, respectively.



(a) AuNPs



(b) CdTe QDs

Figure 2 The TEM micrographs of AuNPs (a) and CdTe QDs (b)

According to the FRET process, when the distance between the donor and acceptor was 1—10nm and the emission spectrum of the donors could overlap the absorption spectrum of the acceptors, the energy could be transferred from the QDs to the AuNPs. Therefore, the absorption and emission spectra of CdTe QDs and Au nanoparticles were examined (Fig.3). From Fig.3, it can be seen that the emission spectrum of CdTe QDs and the absorption spectrum of Au nanoparticles had large overlap, 530nm, which indicated that the CdTe QDs and Au nanoparticles were a suitable donor-acceptor pair.

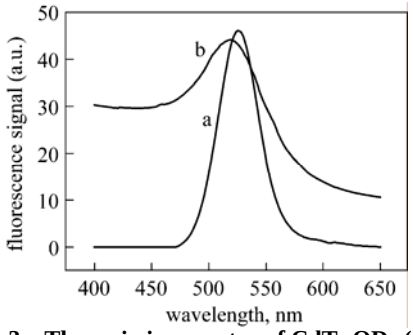


Figure 3 The emission spectra of CdTe QDs (a) and absorption spectra of Au nanoparticles (b)

3.2 The fluorescence of CdTe-dsDNA-AuNPs probe

After hybridization between CdTe-DNA and AuNPs-DNA, to form the fluorescence probe, the fluorescence intention of donors would decrease because of the energy transfer from the donors to acceptors. The emission fluorescence spectra of the CdTe-DNA, the CdTe-dsDNA-AuNPs probe, and the CdTe-dsDNA-AuNPs probe with a complementary single stranded DNA sequence (target DNA) are shown in Fig.4.

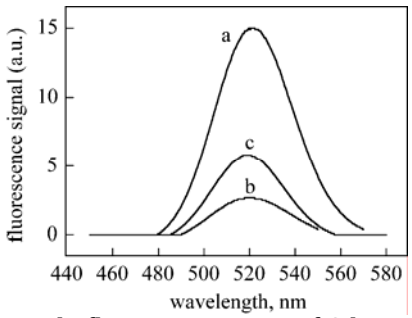


Figure 4 The fluorescence spectra of CdTe-DNA (a), CdTe-dsDNA-AuNPs (b) and CdTe-dsDNA-AuNPs probe with target DNA (c)

Compared with the fluorescence intensity of CdTe-DNA, the fluorescence intention of CdTe-dsDNA-AuNPs decreased tremendously, which indicated that the FRET process occurred in the CdTe-dsDNA-AuNPs conjugate system after the hybridization. The quenching efficiency was about 55%. After a complementary single stranded DNA (target DNA) was introduced into the probe system, the double stranded DNA of the probe system opened and the distance between CdTe QDs and Au nanoparticles was increased. Thus, the fluorescence intensity of the probe recovered partly, and the corresponding FRET efficiency is shown in Fig.5.

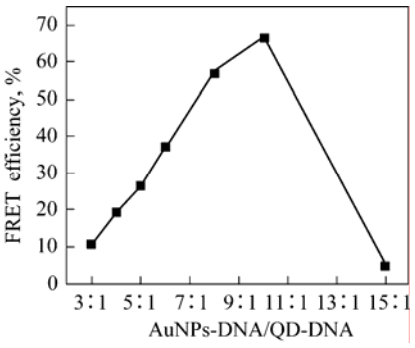


Figure 5 The effect of AuNPs-DNA/QD-DNA on the FRET efficiency

The FRET efficiency of this system was determined by the ratio of AuNPs-DNA to QDs-DNA.

With the ratio of AuNPs-DNA to QDs-DNA increasing, the FRET efficiency increased sharply and reached the maximum (about 67%) at 10∶1. After that, the FRET efficiency decreased enormously. It was considered that the unhybridized DNA on the AuNPs surface would remain when the amount of AuNPs in the CdTe-dsDNA-AuNPs probe was in excess. Moreover, the DNA (Sequence 1), which was self-assembled on the AuNPs surface, was complementary with target DNA (Sequence 3). Therefore, when the target DNA was added into the probe system, a competitive hybridization between unhybridized DNA on AuNPs and CdTe-dsDNA-AuNPs would exist. Consequently, this competitive hybridization would decrease the FRET efficiency.

3.3 The specificity of the CdTe-dsDNA-AuNPs probe

The specificity of the CdTe-dsDNA-AuNPs probe was detected by a mismatching base-pair with target DNA sequence. The results are shown in Fig.6.

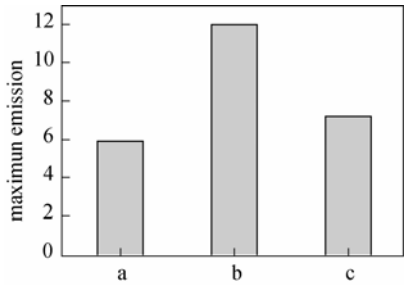


Figure 6 The specificity of the CdTe-dsDNA-AuNPs probe
a—probe; b—probe with complementary DNA;
c—probe with a mismatching base-pair DNA

Compared with the probe system, the fluorescence increased to about 100% when the target DNA was completely complementary with the DNA, however, when a mismatching base-pair DNA was the target DNA, the fluorescence increased only by about 20%. This difference in fluorescence intensity indicated that this CdTe-dsDNA-AuNPs probe had good specificity to distinguish a complementary DNA from a mutant.

4 CONCLUSIONS

A CdTe-dsDNA-AuNPs conjugate, as fluorescence DNA probe, based on the FRET process, was presented. CdTe QDs as donors and Au nanoparticles as acceptors linked with complementary DNA oligonucleotides prepared in different conditions. After the hybridization program, the FRET occurred because the donors and acceptors were in close proximity to each other. When a complete complementary DNA as target was added into this probe system, the fluorescence intention of this system was increased to about 100%. When the target was a mismatching base-pair DNA, the fluorescence intention was increased to only about 20%. These results demonstrated that this fluorescence probe system had an excellent specificity.

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