Fluorescent DNA probe based on CdTe/CdS/SiO₂ core/multishell composite nanoparticles

Introduction

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 [1]. Fluorescent DNA probe is one kind of optical DNA biosensor based on FRET mechanism and has been widely studied [2, 3]. A promising improvement of fluorescent DNA probes is the application of inorganic nanoparticles, such as quantum dots (QDs) and gold nanoparticles (AuNPs), as the FRET energy donors and acceptors, respectively [4]. AuNPs are usually used in gene delivery and cell labelling because they are biocompatible, nontoxic and readily synthesized and functionalized [5, 6]. By virtue of the high extinction coefficient and broad absorption spectrum in visible light, AuNPs are utilized as energy acceptors in fluorescent DNA probes [7]. On the other hand, QDs have an excellent luminous property, which makes QDs become a kind of novel luminescent fluorophores and have wide applications in biosensors, DNA detection, cell labelling and imaging.

However, researchers are still facing some important problems when QDs are used in biological fields, such as, cytotoxicity as a result of the release of heavy metal ions upon photo-oxidation, as well as chemical and colloidal instabilities of the QDs in harsh environments [8]. Core/shell QDs have been investigated to improve the fluorescent intensity, stability and quantum yield of QDs greatly, such as CdTe/CdS QDs [9]. However, the toxicity of Cd²⁺ and fluorescent ultra-sensitivity to the surface states of CdTe/CdS QDs still exist. Therefore, the coating of QDs with biocompatible material is a promising method to utilize the excellent fluorescence of QDs in biological fields. In this paper, a novel kind of fluorescent material, CdTe/CdS/SiO₂ core/multishell nanoparticles, was prepared and used as the energy donors of fluorescent DNA probe. The CdTe/CdS had excellent fluorescent properties than CdTe QDs and the silica layer was non-toxic, biocompatible and easily modified [10]. Therefore, this novel kind of fluorescent DNA probe would have great potentials in DNA detection, labelling and imaging of living cells.

Experiments

The sequences of DNA were listed as follows:
DNA₁ (NH₂-DNA):
5'-NH₂-GCATCCAACGAGTTTATAA-3'
DNA₂ (HS-DNA):
5'-TTATAAACTCGTTGGATGCAGGTACG-SH-3'
DNA₃ (target DNA):
5'-CGTACCTGATCCAAAGAGTTTATAA-3'
DNA₄ (target DNA):
5'-CGTACCTGATCTACAGAGTTTATAA-3'

CdTe QDs cores were synthesized according to the reference work in aqueous solution when 3-mercaptopropionic acid (MPA) was as stabilizer [11]. The obtained CdTe solution was divided equally into four 100mL three-necked flasks. Thiourea solution (thiourea:Cd (mol/mol) = 0.1:1.1:2.1:4.1) was added into the reaction system respectively and refluxed at 100 °C for 1 h under no nitrogen protection [8]. CdTe/CdS/SiO₂ fluorescent nanoparticles were synthesized according to the reverse microemulsion method [12]. 500 µL aqueous solution of CdTe/CdS QDs and 250 µL of ammonia aqueous solution (25 wt%) were introduced into a liquid system containing 7.5mL of cyclohexane, 1.8mL of n-hexanol, and 1.77 mL of polyethylene glycol tert-octylphenyl ether (Triton X-100) under stirring for 30 min. Then, 100 µL tetraethyl orthosilicate (TEOS) was added into the reaction system under vigorous stirring. The silica growth was completed in the dark at room temperature for 24 h. The core/shell nanoparticles were isolated from the microemulsion by ultracentrifuge.

The CdTe/CdS/SiO₂ fluorescent nanoparticles were modified by using silane coupling agent (3-Aminopropyl) diethoxymethylsilane (APDEMS) and succinic anhydride to get carboxyl groups on the surface (CdTe/CdS/SiO₂-COOH nanoparticles) [13]. Then, 25 mg of CdTe/CdS/SiO₂-COOH nanoparticles, 33 µg of NH₂-DNA (DNA₁) and 4.4 µL of EDC were dispersed in 1 mL ultrapure water. The mixture was diluted with 4 mL of Tris-HCl buffer (0.05 M, pH=8.0) and incubated for 24 h at 25°C.

AuNPs were prepared by reducing HAuCl₄ with trisodium citrate in aqueous solution [14]. Then, 1.5 mL of AuNPs aqueous solution, 33 µg of H₂-DNA (DNA₂) and 2 mL 0.1M NaCl-10mM PBS (pH=7) were mixed under shake.
at 37°C for 40 h to get the DNA2 conjugated AuNPs (Au-DNA2). The Au-DNA2 was purified by ultra-centrifugation (10,000 r/min) for 40 min, dispersed in PBS and kept in the dark at 4°C.

200 μL of CdTe/CdS/SiO2-DNA1 solution and 1000 μL of Au-DNA2 solution were hybridized about 12 h at 37°C to obtain DNA probe. The detection process was as following: 10 μg/mL of target DNA was added into the probe system about 12 h, then fluorescence spectrum of the probe system was measured with a F380 Luminescence Spectrometer. According to Föster's theory, the FRET efficiency (quenching efficiency) could be measured experimentally and was commonly defined as [15]:

\[
E = \left(1 - \frac{F_{DA}}{F_D}\right) \times 100\%
\]

where \(F_{DA}\) is the integrated fluorescence intensity of the donor in the presence of the acceptor(s) and \(F_D\) is the integrated fluorescence intensity of the donor alone (no acceptors present).

**Results and Discussion**

CdTe/CdS QDs were synthesized in aqueous solution when MPA was as stabilizer. In alkaline conditions, mercapto group of MPA was linked with quantum dots and the negatively carboxylic group of MPA stabilized QDs in aqueous solution.

**Fig.1.** Fluorescence spectra of CdTe/CdS QDs with different thiourea/Cd$^{2+}$ (mol/mol) when the reaction time was 1 h
a) 0:1; b) 1:1; c) 2:1; d) 4:1

Compared with pure CdTe QDs (thiourea:Cd=0:1) in the same conditions, CdTe/CdS QDs exhibited stronger fluorescence intensities (Fig.1) because the thiourea could be decomposed at high temperature and provide a steady sulfur source which would be combined with the unreacted Cd$^{2+}$ onto the surface CdTe and formed a CdS shell around CdTe core seeds [11]. The amount of thiourea influenced the fluorescent emission spectra of CdTe/CdS QDs, extremely (Fig.1). When the amount of thiourea was increased, fluorescent emission spectra of QDs shifted from 547.6 nm to 634.8 nm and the fluorescent intensity reached the maximum when the thiourea/Cd$^{2+}$ was 2:1 (Fig.1(c)). By virtue of the existence of the surface defects and the proceeding of photooxidation on the QDs surfaces, the coating of stable material on the QDs surfaces with core/shell structure could improve the fluorescent ability of QDs[16]. However, when the amount of thiourea was increased extremely, the particle size of CdTe/CdS QDs could be too large and the crystal structure of QDs was partly faulty, which could result in the decrease of fluorescence intensity of CdTe/CdS QDs.

CdTe/CdS/SiO$_2$ fluorescent nanoparticles were prepared by reverse microemulsion method [13] and the TEM micrograph and fluorescent emission spectrum of these nanoparticles were shown in Fig.2. The results indicated that the CdTe/CdS/SiO$_2$ nanoparticles were generally spherical shape and their size about 62 nm with core/shell structures.

According to the FRET process, when the distance between the donor and acceptor was 1-10 nm and the emission spectrum of the donors could overlap the absorption spectrum of the acceptors, the energy could be transferred from the donor to the acceptor1. Therefore, the emission spectra of CdTe/CdS/SiO$_2$ fluorescent nanoparticles and the UV absorption spectrum of AuNPs were compared in Fig.3, which indicated that the emission spectrum of CdTe/CdS/SiO$_2$ fluorescent nanoparticles and the absorption spectrum of AuNPs had a large overlap, which indicated that they were a suitable donor-acceptor pair of FRET.
AuNPs surfaces because more complementary bases of DNA3 and stronger hydrogen-bonding hybridization was between DNA3 and DNA4. Consequently, the CdTe/CdS/SiO2-DNA4 would depart from the probe, which resulted that the fluorescent intensity of the system was recovered about 1.92 times than that of probe system (Fig. 4).

The specificity of fluorescent DNA probe is a key factor. In this work, when a mismatching base-pair ssDNA (DNA4) was as the target DNA, the fluorescent emission intensity of detection system was increased only about 28%, while fluorescent intensity of detection system could be increased about 192% when the completely complementary ssDNA (DNA3) was as target DNA. This difference of fluorescence intensity indicated that this DNA probe had a good specificity to distinguish the target DNA was mutant or not.

**Conclusions**

In this work, a novel kind of fluorescent nanoparticles, CdTe/CdS/SiO2 with multishell structure was prepared and introduced into fluorescent DNA probe as energy acceptors. The coating of CdS on CdTe QDs could result in a higher fluorescent emission intensity of QDs than that of CdTe QDs and the coating of SiO2 on CdTe/CdS QDs could decrease the ultra-sensitivity, cytotoxicity as well as the chemical and colloidal instabilities of QDs. The quenching efficiency of this novel kind probe was about 71%. When completely complementary ssDNA was as the target DNA, the fluorescent intensity of detection system was increased about 192% than that of probe. When a mismatching base-pair ssDNA was as the target DNA, the fluorescent emission intensity of detection system was increased only about 28%. These results demonstrated that this fluorescent probe system had excellent detection ability and specificity.

**Acknowledges**

This work was supported by the National Natural Science Foundation of China (No.21172171 and No.21106101) and Natural Science Foundation of Tianjin (No. 11JCYBDJC22300).

**REFERENCES**


**Authors:** Dr. Zhao Dai, Key Laboratory of Hollow Fiber Membrane Materials and Processes, School of Environmental and Chemical Engineering, Tianjin Polytechnic University, Tianjin 300387, China E-mail: daizhao@gmail.com; Master student Jiaojiao Song, Tianjin Polytechnic University, Tianjin 300387, China E-mail: songjiaojiao139@139.com; Dr. Wenjuan Guo, Tianjin Polytechnic University, Tianjin 300387, China E-mail: guowenjuan@yahoo.cn; Dr. Ying Li, Tianjin Polytechnic University, Tianjin 300387, China E-mail: liying_791190@163.com.