

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

Abstract. A novel kind of fluorescent DNA Probe based on fluorescence resonance energy transfer (FRET) was presented in this paper when CdTe/CdS/SiO₂ core/multishell fluorescent nanoparticles were as energy donors and Au nanoparticles (AuNPs) were as energy acceptors. The DNA probes were prepared when energy donors and acceptors were conjugated with two single-stranded complementary oligonucleotides respectively and hybridized with each other. Compared with the fluorescent intensity of single-stranded DNA (ssDNA) conjugated CdTe/CdS/SiO₂ nanoparticles (CdTe/CdS/SiO₂-DNA), the fluorescent intensity of DNA probe was significantly increased and the FRET efficiency was about 71%. The detection results indicated that this novel DNA probe had excellent detection and specificity of completely complementary target ssDNA.

Streszczenie W pracy zaprezentowano nowy rodzaj fluoryzującej próbki DNA opartej o rezonansowe przenoszenie energii fluoryzacji (FRET), gdzie donorami energii są nanocząstki CdTe/CdS/SiO₂ z jądrową fluorescencją powłokową a akceptorami energii nanocząstki Au (AuNPs). W przygotowanych próbkach DNA energia donora i akceptora są odpowiednio sprzężone z dwoma pojedynczo skręconymi komplementarnymi oligonukleotydami i hybrydizowane ze sobą. Intensywność fluorescencji próbki DNA ze sprzężonymi nanocząstkami CdTe/CdS/SiO₂ (CdTe/CdS/SiO₂-DNA) w porównaniu z próbką pojedynczo-skręconego DNA (ssDNA) znacząco wzrasta. Wydajność FRET wynosi około 71%. Wyniki wykrywalności wykazują, że nowa próbka DNA ma doskonałą wykrywalność i swoistość do całkowicie komplementarnego docelowego ssDNA. **Fluorescencyjna próbka DNA oparta o nanocząstki kompozytowe CdTe/CdS/SiO₂ z fluorescencją jądrową powłokową**

Keywords: CdTe/CdS/SiO₂, AuNPs, Fluorescence resonance energy transfer, DNA probe

Słowa kluczowe: CdTe/CdS/SiO₂, AuNPs, Rezonansowe przenoszenie energii fluoryzacji (FRET), Próbkę DNA

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 widely used in gene delivery and cell labelling because they are bioinert, nontoxic and readily synthesized and functionalized [5, 6]. By virtue of the high extinction coefficient and broad absorption spectrum in visible light, AuNPs are usual 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 exists. Therefore, the coating of QDs with bioinert 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'-TTATAAAGCTCGTTGGATGCAGGTACG-SH-3'

DNA₃ (target DNA):

5'-CGTACCTGCATCCAACGAGTTTATAA-3'

DNA₄ (target DNA):

5'-CGTACCTGCATCTAACGAGTTTATAA-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 [9]. 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 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 HS-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 Förster's theory, The FRET efficiency (quenching efficiency) could be measured experimentally and was commonly defined as [15]:

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

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.

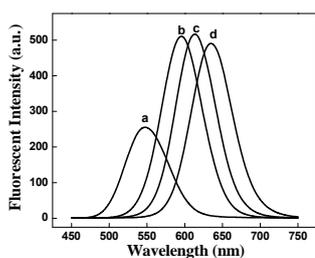


Fig.1. Fluorescence spectra of CdTe/CdS QDs with different thiourea/Cd²⁺ (mol/mol) when the reaction time was 1 h a) 0:1; b) 1:1; c) 2:1; d) 4:1

Compared with pure CdTe QDs (thiourea:Cd=0:1) in the same conditions, CdTe/CdS QDs exhibited stronger fluorescent 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 Cd²⁺ 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/Cd²⁺ 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/SiO₂ 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/SiO₂ 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 acceptor. Therefore, the emission spectrum of CdTe/CdS/SiO₂ fluorescent nanoparticles and the UV absorption spectrum of AuNPs were compared in Fig.3, which indicated that the emission spectrum of CdTe/CdS/SiO₂ fluorescent nanoparticles and the absorption spectrum of AuNPs had a large overlap, which indicated that they were a suitable donor-acceptor pair of FRET.

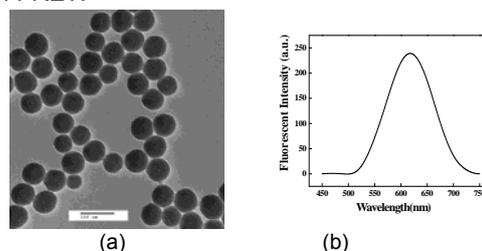


Fig.2. TEM micrograph (a) and fluorescent spectrum (b) of CdTe/CdS/SiO₂ nanoparticles

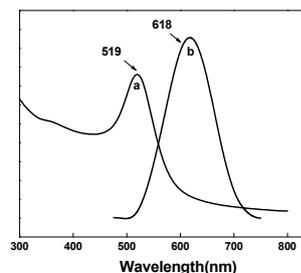


Fig.3. The UV Absorption spectrum of AuNPs (a) and emission spectrum of CdTe/CdS/SiO₂ (b)

When CdTe/CdS/SiO₂ fluorescent nanoparticles and AuNPs were conjugated with complementary ssDNA respectively and hybridized each other, the fluorescent DNA probes into double-stranded DNA conjugated CdTe/CdS/SiO₂ nanoparticles and AuNPs (CdTe/CdS/SiO₂-dsDNA-AuNPs) structure were obtained and the fluorescent emission intensity of DNA probes was decreased because the distance between CdTe/CdS/SiO₂ donors and AuNPs acceptors in a probe was close enough and the FRET occurred from the donors to acceptors (Fig.4(b)) [14]. In this work, the quenching efficiency of probe was about 71%.the partial maximum terms space will be given.

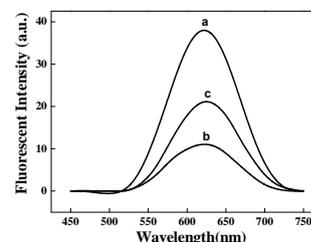


Fig.4. Fluorescence spectra of (a) CdTe/CdS/SiO₂-DNA₁, (b) CdTe/CdS/SiO₂-dsDNA-AuNPs probe and (c) CdTe/CdS/SiO₂-dsDNA-AuNPs probe with target ssDNA

When the target ssDNA (DNA₃, completely complementary with DNA₂) was introduced into the probe system, the target ssDNA could replace the DNA₁ on the CdTe/CdS/SiO₂ surfaces and hybridize with DNA₂ on

AuNPs surfaces because more complementary bases of DNA₃ and stronger hydrogen-bonding hybridization was between DNA₃ and DNA₂. Consequently, the CdTe/CdS/SiO₂-DNA₁ 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).

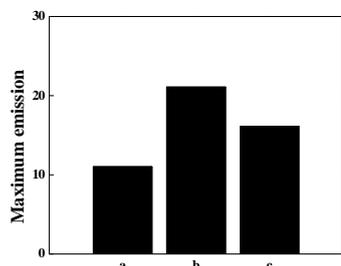


Fig.5. The specificity of the CdTe/CdS/SiO₂-dsDNA-AuNPs probe (a) probe; (b) probe with complementary ssDNA; (c) probe with a mismatching base-pair ssDNA

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 multshell 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 fluorescence probe system had excellent detection ability and specificity.

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REFERENCES

- [1] Förster T., Zwischenmolekulare Energiewanderung und Fluoreszenz, *Annalen der Physik*, 437 (1948), No. 1, 55–57
- [2] Oh E., Hong M. Y., Lee D., Nam S. H., Yoon H. C., Kim H. S., Inhibition Assay of Biomolecules Based on Fluorescence Resonance Energy Transfer (FRET) between Quantum Dots and Gold Nanoparticles, *J. Am. Chem. Soc.*, 12 (2005), No. 10, 3270-3271
- [3] Zhang C. Y., Yeh H. C., Kuroki M. T., Wang T. H., Single-Quantum-Dot-Based DNA Nanosensor, *Nat. Mater.*, 4 (2005), No. 11, 826-831
- [4] Cady N. C., Strickland A. D., Batt C. A., Optimized Linkage and Quenching Strategies for Quantum Dot Molecular Beacons, *Mol. Cell. Probe.*, 21 (2007), No. 2, 116-124
- [5] Daniel M. C., Astruc D., Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications Toward Biology, Catalysis, and Nanotechnology, *Chem. Rev.*, 104 (2004), No.1, 293-346
- [6] Connor E. E., Mwamuka J., Gole A., Murphy C. J., Wyatt M. D., Gold Nanoparticles are Taken up by Human Cells but do not Cause Acute Cytotoxicity, *Small*, 1 (2005), No. 3, 325-327
- [7] Dyadyusha L., Yin H., Jaiswal S., Brown T., Baumberg J. J., Booy F. P., Melvin T., Quenching of CdSe Quantum Dot Emission, a New Approach for Biosensing, *Chem. Commun.*, (2005), No. 25, 3201-3203
- [8] Derfus A. M., Chan W. C. W., Bhatia S. N., Probing the Cytotoxicity of Semiconductor Quantum Dots, *Nano Lett.*, 4 (2004), No. 1, 11-18
- [9] Gu Z. Y., Zou L., Fang Z., Zhu W. H., Zhong X. H., One-pot Synthesis of Highly Luminescent CdTe/Cds Core/Shell Nanocrystals in Aqueous Phase, *Nanotechnology*, 19 (2008), No. 13, 135604
- [10] You X. G., He R., Gao F., Shao J., Pan B. F., Cui D. X., Preparation and Characterization of CdTe@SiO₂ Core/Shell Luminescent Composite Nanoparticles, *Acta Chim. Sinica*, 65 (2007), No. 6, 561-565
- [11] Zhang H., Zhou Z., Yang B., Gao M. Y., The Influence of Carboxyl Groups on the Photoluminescence of Mercaptopropionic Acid-Stabilized CdTe Nanoparticles, *J. Phys. Chem. B*, 107 (2003), No. 1, 8-13
- [12] Yang Y. H., Jing L. H., Yu X. L., Yan D. D., Gao M. Y., Coating Aqueous QDs with Silica Via Reverse Microemulsion Method: Toward Size-Controllable and Robust Fluorescent Nanoparticles, *Chem. Mater.*, 19 (2007), No. 17, 4123-4128
- [13] Zhao C. T., Feng X. X., Wu F., Chen J. Y., Preparation and Carboxyl Modification of Single Scattered Nano Silica Microspheres, *J. Funct. Mater.*, 40 (2009), No. 11, 1942-1945
- [14] Dai Z., Zhang J. M., Dong Q. X., Guo N., Xu S. C., Sun B., Bu Y. H., Adaption of Au nanoparticles and CdTe QDs in DNA Detection, *Chinese J. Chem. Eng.*, 15 (2007), No. 6, 791-794
- [15] Pons T., Medintz I. L., Sapsford K. E., Higashiya S., Grimes A. F., English D. S., Mattoussi H., On the Quenching of Semiconductor Quantum Dot Photoluminescence by Proximal Gold Nanoparticles, *Nano Lett.*, 7 (2007), No.10, 3157-3164
- [16] Aldana J., Wang Y. A., Peng X. G., Photochemical Instability of CdSe Nanocrystals Coated by Hydrophilic Thiols, *J. Am. Chem. Soc.*, 123 (2001), No.36, 8844-8850

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