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SHORT COMMUNICATION

Detection of *Bifidobacterium* Species-specific 16S rDNA Based on QD FRET Bioprobe

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Abstract Fluorescence resonance energy transfer (FRET) that consists of quantum dot as donors and organic fluorophore dyes as acceptors has been a very important method to detect biomolecules such as nucleic acids. In this work, we established a new FRET detection system of *Bifidobacterium* species-specific 16S rDNA using QD—ROX FRET bioprobe, in which 525 nm QD-DNA conjugation consisted of the carboxyl-modified QD and the amino-modified DNA in the presence of EDC. Both ROX-DNA and the conjugation above could hybridize with the target DNA after forming the QD—ROX bioprobe. When the hybridization made the distance between the QD and ROX to meet FRET effect needed, 525 nm QD fluorescence intensity decreased and ROX fluorescence intensity increased. In the control, there was no notable change of fluorescence intensities without target DNA. It is very clear that the change of the QD and ROX fluorescence intensities provide the good base and guaranty for this rapid and simple detection system.

Keywords Quantum dots · Sequence alignment · Bioprobe · FRET · 16S rDNA detection

Introduction

Quantum dots (QD), Luminescent colloidal semiconductor nanocrystals, have been attracted more and more attention in diverse research in the past few years [1–4]. Because of the unique photophysical properties, especially the unique Size-dependent, narrow tunable emission, symmetric, bright, longevity, stable fluorescence, resistance to both photobleaching and chemical degradation, QD have been used as a powerful tools for studying biological and biomedical [5] problem from biomolecule fluorescent imaging to cell tracking in vivo and vitro [6, 7]. Benefit from the advantage of broad absorption spectrum, the narrow and symmetrical emission spectrum, QD that can be allowed to select a proper excitation wavelengths among a wide range to reduce background signal by direct excitation of dye (acceptor), has become the excellent donors in fluorescence resonance energy transfer (FRET)-based sensor. Of all the recently technique development of QD synthesis [8, 9], surface modification, biomacromolecule connection [10], and increasingly improvement of the quantum yield, QD FRET-based sensor have been widespreadly used for probe biologyical phenomena such as enzymatic reactions or used for detect biomolecule such as enzymes [11, 12], proteins [13, 14], nucleic acids [15, 16], pathogens [17, 18] and ATPs [19].

As a kind of beneficial bacteria living in human intestinals, Bifidobacterium bifidum can not only inhibit the growth of the harmful bacteria, but also reduce toxin excretion from harmful bacteria. It also plays a very important role in intestinal flora balance maintenance, anti-aging, anti-tumor, hypertension prevention, digestion improvement, and so on. The survival number of Bifidobacterium bifidum is one of the criterions indicating whether a man is healthy or not. The traditional detection of the Bifidobacterium bifidum are often time-consuming, with a low specificity and complex operations or exhibit other ones, such as lab-contamination and stain-carcinogenesis [20–23].

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rDNA that include conservative fragment and variable sequence in its' primary structure exists widespreadly in organism. Conservative fragment of rDNA indicates the genetic relationship among the creature species, but high variable sequence indicates the difference. This characteristic makes the rDNA become the molecular basis for identification of different categorization level organisms. The probe based on 16S rDNA has been used for strain detection or identification. At present, there are enormous 16S rDNA sequences of bacteria available in the Genbank. The species-specific sequence of 16S rDNA should be easily obtained based on the sequence alignment. FRET can cater to the complicated system as it has the advantage of high resolution and high sensibility. In order to detect the Bifidobacterium bifidum rapidly, we established FRET-based sensor consisting of the QD as an energy donor and the x-rhodamine (ROX) as an energy acceptor, to detect the 16S rDNA of the Bifidobacterium bifidum (Fig.1). The QD-DNA conjugation consisted of the carboxyl-modified QD and the amino-modified DNA (amino-DNA) by the dehydration of EDC. Both ROX-DNA and the QD-DNA conjugation above could hybridize with the target DNA as soon as the QD—ROX bioprobe was formed. When the hybridization narrowed the distance between the QD and ROX into FRET effect required, QD was excited at 340 nm and the energy would transmit from the QD to the ROX, resulting in ROX fluorescence emission at 605 nm because of acception of energy from QD. By contrast, there would be no notable fluorescence of ROX emission at all without target DNA.

Experimental

Material

525 nm carboxyl-modified CdSe/ZnS quantum dot (QD) and 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC) were obtained from Invitrogen company (America). Each QD was modified on the surface by about 80 to 100 carboxyl groups which could connect to amino groups by dehydration of EDC (Product information). As the speciesspecific-16S rDNAs were obtained through sequence alignment of the 16S rDNA (16S rRNA) sequences of 32 species of Bifidobacterial [22], their complemental sequences could be used to design the detecting probe. All of DNA (Table 1) (ROX-DNA, amino-DNA and other unmodified DNA, purified by PAGE) used in this experiment were synthesized by the Sangon Biotechnology company (shanghai, China). Other reagents were purchased from Tian Heng Company (Changsha, China). Conjugation buffer (10 mM Borate buffer pH 7.4) and hybridization buffer (50 mM borate buffer pH8.0) were prepared with ultrapure MilliQ water (resistance >18 MΩ cm). All the water used in this study was purified by the Millipore-Q system (Millipore Inc. America).

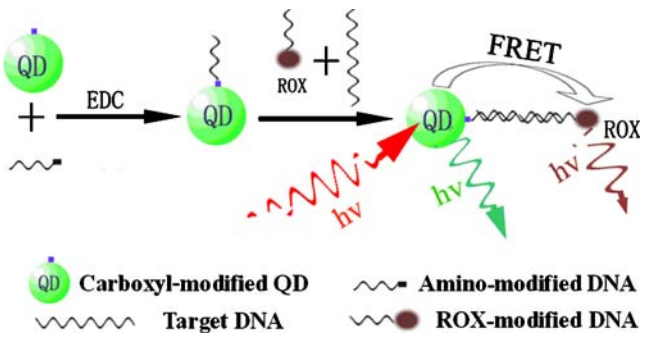

Fig. 1 The mechanism of detecting the 16S rDNA of the Bifidobacterium bifidum based on the QD-ROX FRET bioprobe. To simplify the process, one carboxyl group was given on the surface of the QD

Preparation of QD-DNA conjugation

To prepare QD-DNA conjugation, the carboxyl-modified QD (6 pmol) was conjugated to the amino-modified DNA (0.48 nmol) in the presence of EDC (9 nmol) and appropriate volume of borate buffer (10 mM, pH=7.4), and reacted for 2 h at room temperature in a eppendorf tube [24]. As reported before [25], excess EDC resulted in QDs aggregation during the coupling reaction. The solution was filtered through a 0.2 um PES syringe to remove any large aggregates. Unreacted DNA and free EDC were removed by spin filtration in Millipore Microcon 50,000 molecular weight cut-off spin filters [10]. The flow-through was discarded and the residue was collected by spining at 1,000×g for 3 min and then resuspended in 300 ul borate buffer. The final retentate (QD-DNA) was resuspended in 200 ul hybridization borate buffer after two repeats. In this work, at 2 pmol QD, six different ratios of amino group modified DNA to carboxyl group modified QD (1:1. 20:1. 40:1. 60:1. 80:1. 100:1) were studied at the same conditions, respectively. All of the ROX-DNA and target DNAs were kept 0.8 nmol in these reactions and the final concentrations were 2 uM.

Table 1 DNA Sequence used in this work

DNA code	Sequence(5′→3′)
ROX-DNA	GGTAGCACCCG-ROX
Amino-DNA	NH_2-CATGAAAGTG
Target DNA	CGGGTGCTACCCACTTTCATG
Two bases mismatch DNA	CGGATGCTACTCACTTTCATG
Eight bases mismatch DNA	CGCATGCAACTCACGCTCTCG

Detection of 16S rDNA with match DNA and mismatch DNA

As soon as the QD-DNA conjugate formed, ROX-DNA and target DNA were added and mixed thoroughly and the total volume of the hybridization solution was kept 400 ul. The fluorescence spectra were recorded after 4 h at room temperature in dark. Five different concentrations of the target DNAs from 0.2 uM to 1 uM were tested. And to detect the mismatch DNA, two different mismatched oligonucleotides (two and eight bases mismatch DNA shown in Table 1) were thoroughly tested. Control experiments were carried out under the same conditions. The concentration of ROX-DNA and the mismatched DNA were kept constant of 1.2 uM, respectively.

Instrumentation

All fluorescence spectra were obtained from a LS50B luminescence spectrometer (PerkinElmer, Inc.) at 28 °C. The fluorescence signals were recorded from 360 nm to 660 nm when the solutions were excited at 340 nm. Excitation and emission slits were set to 15 nm and 20 nm, respectively. The scan rate was 1,500 nm/s.

Results and discussion

It is noticed that the ratio of acceptor to donor is a very important factor for FRET efficiency. In this work, the ratio of acceptor to donor was determined on the ratio of amino-modified DNA to carboxyl-modified QD when the ROX-DNA concentration kept a constant. The high ratio of amino-DNA to carboxyl-modified QD was coupling with a high concentration of the QD-DNA conjugation which benefit to a high ratio of acceptor to donor. Since we could only obtain information on the average number of carboxyl groups attached to the QD from the supplier, five different ratios of amino group modified DNA to carboxyl group modified QD (1:1. 20:1. 40:1. 60:1. 80:1. 100:1) were measured. QD was kept 2 pmol for QD-DNA conjugation preparation at all of these different ratios. After the QD-DNA conjugation was prepared and resuspended in 200 ul hybridization borate buffer, 0.8 nmol ROX-DNA and 0.8 nmol target DNA were added in. Then, the total volume of the mixture in every tube was kept 400 ul with hybridization solution. All of these ratios tests (corresponding amino-DNA were 2 pmol, 40 pmol, 80 pmol, 120 pmol, 160 pmol and 200 pmol) were taken under the same conditions and at the same time. The fluorescence spectra were recorded and analyzed after the mixture reacted for four hours at room temperature in dark. Fluorescence ratio is defined as Fa/Fd, where Fa is the fluorescence intensity of ROX at 605 nm and Fd is the fluorescence intensity of QD at 525 nm. As shown in Fig. 2, the value of Fa/Fd became bigger with the increase of the ratio and tended to be constant near the ratio of 80:1, indicating that the reaction reached equilibrium. This phenomenon suggested that the more DNAs attached to QDs, the stronger change the Fa/Fd did. And it also indicated that there should be energy change between QD and ROX. Theoretically based on the information above, DNA modified by about 80–100 amino groups can be conjugated to one QD as 80–100 carboxyl groups were modified on the surface of each QD (Product information). The reaction reached equilibrium just at 80:1 of the amino-DNA/QD, but not at 100:1. It might be explained that the EDC had an insufficient effectiveness between the amino-DNA and QD conjugation and the sterical resistance prevented more DNAs from attaching to QD. Therefore, the ratio of 80:1 was used in latter work.

The hybridization of target DNA with the QD-DNA conjugation and ROX-DNA was carried out in hybridization buffer. In this section, five different concentrations of target DNAs in 400 ul (0.2 uM, 0.4 uM, 0.6 uM, 0.8 uM and 1.0 uM) were studied at the same conditions, respectively. 6 pmol of QD was used for QD-DNA conjugation preparation according to the method above, then 0.08 nmol target DNA and 0.48 nmol ROX-DNA were added in the hybridization. After the total volume of every tube was keep 400 ul, the reaction lasted for four hours at the room temperature in dark. The corresponding fluorescence spectra of QD and ROX were recorded and shown in Fig. 3. It's clear that there was the lowest fluorescence signal (background signal) of ROX and the highest fluorescence signal of QD simultaneously when target DNA were absent. But as soon as a certain target DNA was added in, the fluorescence signal of ROX was stronger

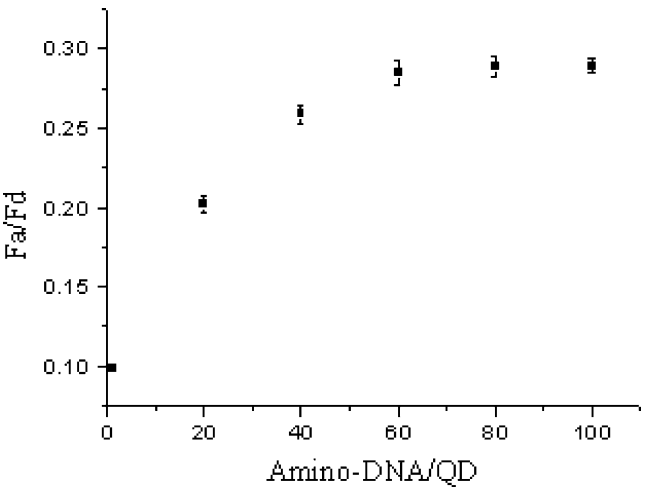

Fig. 2 The influence of the ratio of DNA to QD on the FRET. *Error bars* SD (n=3)

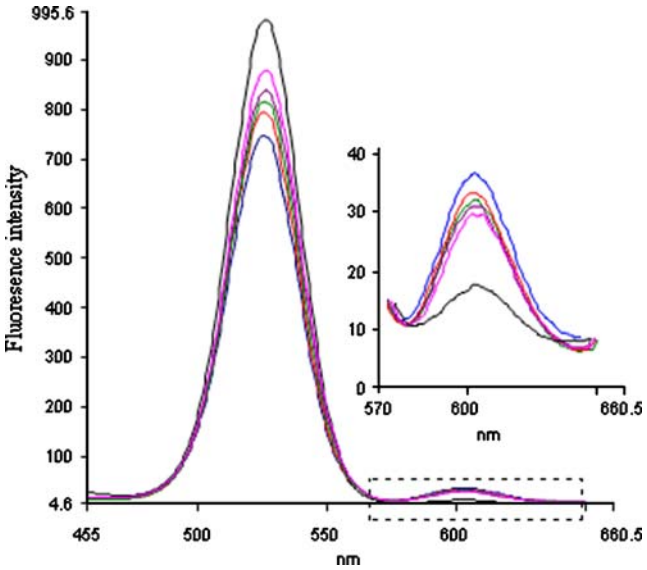

Fig. 3 Fluorescence spectra of QD and ROX conjugate after adding five different concentrations of the target DNA. From up to down, the *pink*, *violet*, *green*, *red* and *blue curves* represent the fluorescence spectra of QD and ROX with the concentrations of target DNA are 0.2 uM, 0.4 uM, 0.6 uM, 0.8 uM, 1.0 uM, respectively. The *black* represent the fluorescence spectra of QD and ROX in the control

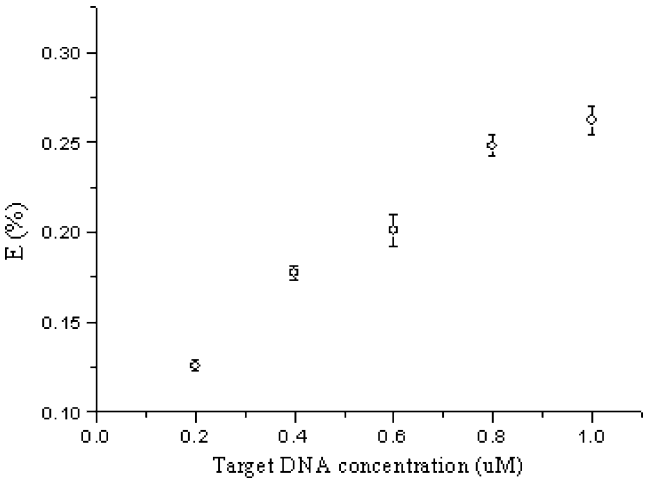

Fig. 4 The FRET efficiency under different concentrations of the target DNA. Error bars SD (n=3)

and QD's was weaker simultaneously. It could be explained that the hybridization of target DNA with QD-DNA conjugates and ROX-DNA decreased QD fluorescence intensity emission at 525 nM, while enhanced ROX fluorescence intensity emission at 605 nM via FRET. And the fluorescence intensity was shown that a 200 nM concentration of the target DNA could become detectable. Fig. 4 is the corresponding FRET efficiency from the Fig. 3. The FRET efficiency (E) is defined according to Eq. (1) [26],

$$E = 1 - F_{DA}/F_D \quad (1)$$

where F_{DA} is the QD fluorescence intensity in the presence of ROX acceptors and F_D is the QD fluorescence intensity in the absence of ROX acceptors.. As it displayed, the FRET efficiency tends upwards coupling with increasing the concentration of target DNA. It indicated that the results of target DNA detection should be significant when target DNA concentration was higher. It also suggested that the reaction between target DNAs and QD-DNA-ROX tended to be unsaturated under the 1 uM target DNA because the FRET efficiency has an increasing tendency when the concentration is 1 uM. Although the change of E is the same as our anticipation, it should be stressed that the E value is lower in this experiment. A typical Forster distance R_0, that produces 50% FRET efficiency, for the QD (donor)—dye (acceptor) FRET system is between 4–7 nm [26, 27]. The distance between QD and ROX is the most important factor for FRET efficiency because FRET is a distance-dependent phenomenon. Since reported from the supplier and some paper [10], the average hydrodynamic radius of unmodified carboxyl-QDs should be increased from 7±2.2 nm to between 13.5±3.2 and 18±6 nm after modification with various DNA molecules. Carboxyl-modified QD with the 21 bases was longer than R0, and result in a low FRET efficiency. Additionally, low efficiency of amino DNAs conjugate to carboxyl groups during the EDC coupling reaction should also influence QD fluorescence efficiency.

Mismatch test were carried out with two bases mismatch DNA and eight bases mismatch DNA. Control experiment was carried out with perfect match DNA under the same conditions. At the present of QD-DNA and ROX-DNA, two bases mismatch DNA was failed to markedly changed the fluorescence intensity of QD. However, there should be

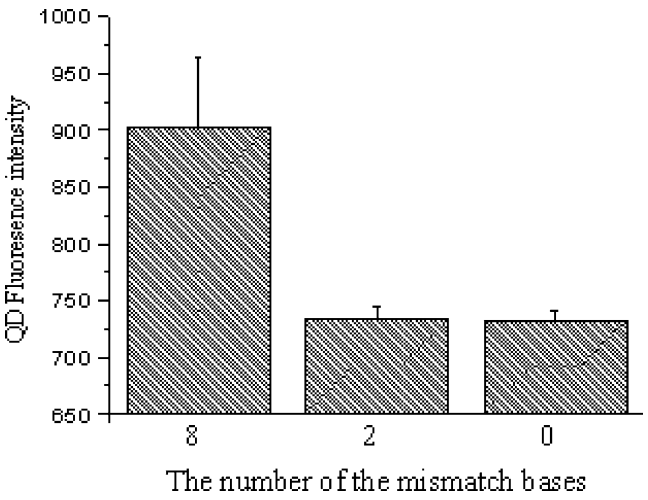

Fig. 5 QD fluorescence intensity at different numbers of the mismatch base. Error bars SD (n=3)

apparent QD fluorescence intensity increased when the eight bases mismatch DNA added in (Fig. 5). It indicated that although had a similar fluorescence intensity of two bases mismatch DNA and perfect match DNA, this method has the discriminability with mismatch DNA still.

Conclusion

In this work, we have successfully constructed a covalently coupled functional QD-DNA conjugation and made it with the ROX-DNA as a FERT sensor to detect speciesspecific 16srDNA of the Bifidobacterium based on taking the advantage of fluorescent properties of QD. This method can be used to detect 200 nM target DNA in solution. Clearly, it is simple, convenient, and rapid. Besides its bacteria classification and identification, this method can be also used to detect biomolecules with lower concentration such as RNA, protein or virus. To improve greatly its detection sensitivity, we further work on reducing the radius of QD and mofied group and exchanging the different groups modified on the surface of QDs.

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