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Electrochemiluminescence resonance energy transfer between quantum dots (QDs) as the donor and Cy5 dye molecules as the acceptor in QD-Cy5 conjugates with biomolecules as the linker

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ABSTRACT

Electrochemiluminescence resonance energy transfer (ECRET) between CdSe/Zns quantum dots (QDs) as the donor and cyanine dye (Cy5) molecules as the acceptor in QD-Cy5 conjugates with DNA or protein as the linker was reported. When a negative potential was applied, the excited-state CdSe/ZnS* was produced in 0.1 mol/L phosphate buffer (pH 7.4) containing 0.1 mol/L $K_2S_2O_8$ and 0.1 mol/L KNO_3 (PB-$K_2S_2O_8$). The CdSe/ZnS* went back to the ground-state CdSe/ZnS to emit light at 590 nm or to transfer energy to proximal ground-state Cy5 molecules. The resultant excited-state Cy5 molecules relaxed to their ground state by emitting a light at 675 nm. The ECRET between QDs and Cy5 was used to evaluate interactions between DNAs and to measure conformational changes of DNAs and proteins.

1. Introduction

Quantum dot (QD) electrochemiluminescence (ECL)-based ECL resonance energy quenching (ECREQ) has been reported [1–3]. In these ECREQ methods, only ECL emission of QD donors was detected. Recently, Wu et al. developed a ECL resonance energy transfer (ECRET) system between QD as the donner and $Ru(bpy)_3^{2+}$ as the acceptor [4]. They used a series of optical filters with different wavelengths to obtain inaccurate ECL quasi-spectra consisting of several ECL intensity points measured by a photomultiplier tube. Since no emission of acceptors or no accurate ECRET spectra was obtained, applications of all the methods mentioned above in monitoring molecule interactions and measuring conformational change of biomolecules were limited. More recently, we reported a new ECRET technique between luminol as the donor and QD as the acceptor based on accurate ECRET spectra and its application in the measurement of interactions and conformational changes of proteins [5]. In this work, we developed another new ECRET system that could be used to study the interactions and conformational changes of biomolecules. In the system, ECRET took place between ECL CdSe/Zns QDs as the donor and Cy5 as the acceptor in the QD-Cy5 conjugates with DNA or protein as the linker.

2. Experimental

2.1. Chemicals

Octadecylamine-capped CdSe/ZnS QDs from NN-Labs (Fayetteville, AR, USA), single-stranded DNAs from Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. (Shanghai, China) and human plasma fibronectin (Fn) from Millipore Trading Co., Ltd (Billerica, MA, USA) were used in this work. Other chemicals were obtained from standard reagent suppliers. The QDs were rendered water soluble by replacing the organic capping shell with mercaptopropionic acid (MPA) using a similar procedure reported by Smith et al. [6]. To prepare QD-DNA1-DNA2-Cy5 conjugates, 10 μL of 1×10^{-6} mol/L MPA-coated QD was incubated for 1 h with 5 μL of 1×10^{-6} mol/L 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), 5 μL of 1×10^{-6} mol/L *N*-hydroxysuccinimide (NHS) and 100 μL of 0.2 mol/L borate buffer (pH 7.4). The solution was incubated for 2 h with 10 μL of 1.0×10^{-4} mol/L NH_2-DNA1. The resultant QD-DNA1 was washed with 0.2 mol/L borate buffer by ultrafiltering. After diluting to 100 μL with 0.2 mol/L borate buffer, the solution was incubated for 4 h with 100 μL of 1.0×10^{-5} mol/L Cy5-DNA2 and 100 μL of 1 mol/L NaCl at room temperature. After washing with 0.2 mol/L borate buffer by ultrafiltering, the resultant QD-DNA1-DNA2-Cy5 conjugates were diluted to 100 μL with 0.2 mol/L borate buffer. A similar approach in our previous work [5] was used to prepare QD-Fn-Cy5 conjugates.

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2.2. Apparatus and measurements of ECRET spectra

An electrochemical analyzer coupled with an imaging spectrograph (Shamrock SR-303i-A, Andor Tech. Ltd., UK.) was used to record ECRET spectra of the conjugates consisting of QDs and DNA or Fn on a Au electrode holding at −2.0 V for 5 s in PB-$K_2S_2O_8$ according to our previous work [5]. To immobilize the conjugates on the electrode, 1 μL of the solution containing the conjugates was applied onto the electrode and dried at 40 °C for 20 min in an oven.

3. Results and discussion

3.1. ECRET of the QD-Cy5 conjugates with DNA as the linker

We found that ECL of CdSe/ZnS QDs was strongly dependent on the ZnS shell and that the carboxyl group-capped CdSe/ZnS QDs with a Zn mole amount of 13% could emit ECL ~2 orders of magnitude stronger than that of the CdSe/ZnS QDs with a Zn mole amount of >20%, which exhibited very weak ECL due to poor conductivity [7]. The CdSe/ZnS QDs with a Zn mole amount of 13% were used in this work. The ECL emission spectra of QDs and absorption spectrum of Cy5 (Fig. 1B) indicated that ECRET between the QDs with emission maximum (λ_m) between 590 and 620 nm and Cy5 can occur based on spectral overlap. The maximum number of Cy5 molecules on each QD (N) can be estimated from Eqs. (1)–(3).

$$c_2 = c_1 A_2 / A_1 \tag{1}$$

$$M_{Cy5} = (c_1 - c_2)V \tag{2}$$

$$N = M_{Cy5} / M_{QD} \tag{3}$$

where c_1 and c_2 as well as A_1 and A_2 are the concentration and absorption of Cy5-DNA2 in the solutions before and after reaction with QD-DNA1, respectively. V is the solution volume that is used to measure A_1 and A_2. M_{Cy5} and M_{QD} are the amounts of Cy5 and QD to form QD-DNA1-DNA2-Cy5 conjugates. In the experiment, V, c_1 and M_{QD} were 500 μL, 4.00×10^{-6} mol/L and 1.0×10^{-10} mol, respectively. The detected A_1 and A_2 were 0.446 and 0.263. From the data, the N value was calculated to be 8.2.

The ECRET principle of the QD-DNA1-DNA2-Cy5 conjugates for the QDs with a λ_m of 590 nm in PB-$K_2S_2O_8$ is shown in Fig. 1C. Referencing the ECL mechanism of CdSe QDs [8], CdSe/ZnS QDs immobilized on an Au electrode and $S_2O_8^{2-}$ are electrochemically reduced to negatively charged radicals CdSe/ZnS$^{-\bullet}$ QDs and anion sulfate radicals $SO_4^{-\bullet}$, respectively. The $SO_4^{-\bullet}$ reacts with CdSe/ZnS$^{-\bullet}$ to produce the excited-state CdSe/ZnS*. The excited-state CdSe/ZnS* emits a light at λ_m = 590 nm or transfers energy to proximal Cy5. The resultant excited-state Cy5 may relax to their ground state by emitting a light at 675 nm. For the system, Förster distance (R_0) was calculated to be 3.6 nm according to the theoretical equation [9]. In this system, the R_0 was the distance between the surface of the QDs and the Cy5 molecules. The ECRET mode could be used to evaluate the interactions between DNAs (here DNA1 and DNA2) as shown in Fig. 2A. One of a pair of DNAs was bound to QDs to form QD-DNA1, followed by incubating with the Cy5-labeled other of the pair of DNAs (Cy5-DNA2). If DNA2 was complementary to DNA1 and the QD-Cy5 distance was small enough, the photoluminescence (PL) of Cy5 could be observed. The spectra of the QD-DNA1 before and after reaction with the complimentary Cy5-DNA2 are shown in Fig. 2A.

The ECRET mode could also be used to measure conformational changes of DNAs. A hairpin DNA was selected as a model target DNA (tDNA). The tDNA was bound to QDs and labelled with Cy5 to form QD-tDNA-Cy5 conjugates (Fig. 2B). The stem-loop structure of the tDNA brought both the Cy5 and the QD into close proximity. For the conjugates, two peaks corresponding to QD ECL at 590 nm and Cy5 PL at 675 nm via ECRET on its spectrum were observed. When another DNA (DNA3) was hybridized with the tDNA, the stem portion of the tDNA was opened. In this case, the Cy5 PL peak decreased or disappeared and the QD ECL peak enhances, indicating conformation change of the tDNA. The spectra before and after incubating QD-tDNA-Cy5 conjugates with pDNA at different concentrations are

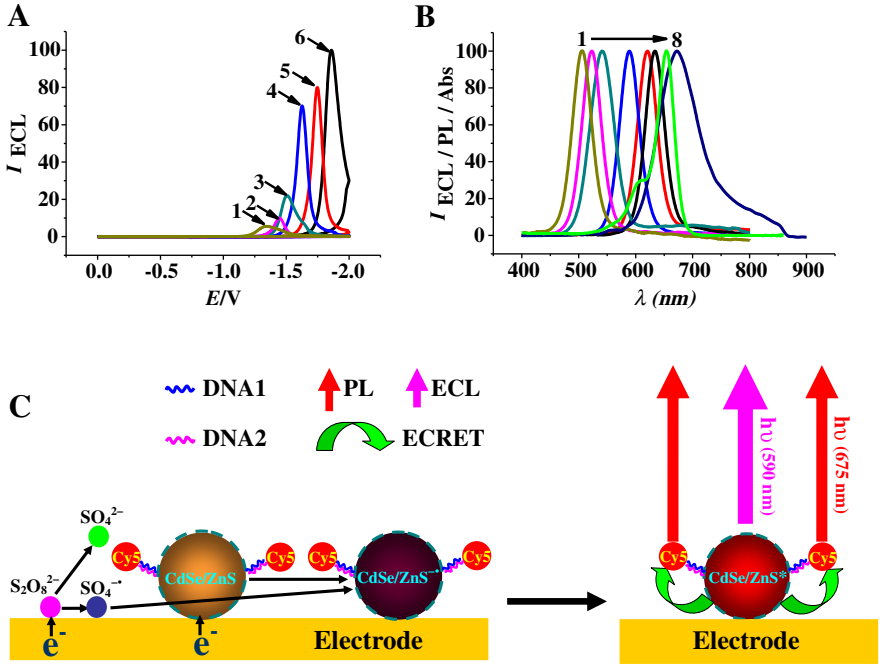

Fig. 1. (A) ECL intensity (I_{ECL}) versus applied potential (E) curves and ECL emission spectra of six carboxyl group-capped CdSe/ZnS QDs (1–6) for λ_m at (1) 505, (2) 520, (3) 540, (4) 590, (5) 620 and (6) 630 nm, as well as absorption (7) and photoemission spectrum of Cy5 dye (8), and (C) basic principle of ECRET for QD-DNA1-DNA2-Cy5 conjugates in the presence of $S_2O_8^{2-}$.