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Influence of quantum dot's quantum yield to chemiluminescent resonance energy transfer

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ABSTRACT

The resonance energy transfer between chemiluminescence donor (luminol–H_2O_2 system) and quantum dots (QDs, emission at 593 nm) acceptors (CRET) was investigated. The resonance energy transfer efficiencies were compared while the oil soluble QDs, water soluble QDs (modified with thioglycolate) and QD–HRP conjugates were used as acceptor. The fluorescence of QD can be observed in the three cases, indicating that the CRET occurs while QD acceptor in different status was used. The highest CRET efficiency (10.7%) was obtained in the case of oil soluble QDs, and the lowest CRET efficiency (2.7%) was observed in the QD–HRP conjugates case. This result is coincident with the quantum yields of the acceptors (18.3% and 0.4%). The same result was observed in another similar set of experiment, in which the amphiphilic polymer modified QDs (emission at 675 nm) were used. It suggests that the quantum yield of the QD in different status is the crucial factor to the CRET efficiency. Furthermore, the multiplexed CRET between luminol donor and three different sizes QD acceptors was observed simultaneously. This work will offer useful support for improving the CRET studies based on quantum dots.

1. Introduction

Fluorescence resonance energy transfer (FRET) is an effective method to investigate the distance between donor and acceptor in nanometer scale. As its advantages, such as high sensitivity, wide applicability and good anti-jamming to environment, FRET has been an important tool to monitor the distance change in nanometer scale *in vivo* and *in vitro* researches. It offers a flexible and convenient strategy to explore the inner configuration, property, reaction mechanism and dynamic process of biomacromolecules, even quantitative analysis in bioresearches. And it has been widely used in determination of distance between donor and acceptor [1,2], explore of the interaction between single molecules [3], analysis of protein and nucleic acid conformational change [4,5], immunoassay [6], and signal transduction in cells [7].

The following primary conditions for fluorescence resonance energy transfer are essential. Donor and acceptor molecules must be in close proximity (typically 1–10 nm), and absorption spectrum of the acceptor must overlap fluorescence emission spectrum of the donor. It is also required that the excitation of the donor and the emission of the acceptor must be separated enough. In contrast to conventional fluorescent dyes, luminescent semiconductor nanocrystals (also called quantum dots, QDs) appeared in recent years, overcome some inherent limitations of conventional dyes,

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and are promising fluorescent material. As donor, QDs have broad excitation spectrum, so suitable excitation wavelength could be chosen flexibly to avoid the direct excitation of the acceptor. Narrow and tunable emission of QD will improve the FRET research while it is introduced in FRET. In recent years, QDs have been favorably adopted in the FRET-based studies, such as deriving QD–protein conjugate configuration [1], QD–protein sensing assemblies [8], QDs based DNA nanosensor [9], enzymatic activity and enzyme inhibitors [10], photochromic switching [11] and photodynamic medical therapy [12].

FRET has been shown to occur between quantum dot donors and organic dye acceptors [1,8], but it has been still argued whether quantum dots can be FRET acceptors for organic fluorophores because of the long exciton lifetime of the quantum dot acceptor compared with that of the dye donor and substantial direct excitation of quantum dots [13], which brings disadvantages in the QD based fluorescence resonance energy transfer in biochemical applications. As possessing broad excitation spectrum and high quantum yield, quantum dots are ideal acceptors in nature. In 2006, Ren and co-workers investigated the chemiluminescence resonance energy transfer (CRET) by using luminol–H_2O_2 system as energy donor and CdTe quantum dot as acceptor [14]. It is described in their report that the luminol chemiluminescence can excite the CdTe quantum dot acceptor efficiently, which means the accomplishment of the chemiluminescence resonance energy transfer between luminol donor and QD acceptor. Compared with FRET, CRET occurs by the oxidation of a luminescent substrate and does not need an exciting light source. Hence it will dramatically reduce the fluorescence bleaching and lessen the autofluorescence of system. And it will be a promising technique in biochemical application.

In the CRET system, luminol is adopted as the donor to excite the quantum dot acceptor. In our work, core-shell quantum dots (CdSe/ZnS) were chosen as acceptors. The influence of quantum dot acceptor's quantum yield to chemiluminescent resonance energy transfer efficiency was investigated. The results indicate that the quantum yield of the QD acceptor is the crucial factor to the CRET efficiency. This work will provide valuable support for QD-based CRET studies in biochemical filed.

2. Experimental

2.1. Materials and apparatus

Cadmium acetate, zinc acetate and selenium powder were from Acros Organics. Tri-*n*-octylphosphine oxide (TOPO), bis(trimethylsilyl)sulfide, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (EDC), hexadecylanmine (HDA) and 4-iodophenol were from Aldrich. Sodium thioglycolate and horseradish peroxidase (HRP) were obtained from Sigma. Luminol was purchased from Fluka. Hydrogen peroxide (H_2O_2) was from Shanghai Yuanda Peroxide Co. Ltd., China. All other chemicals and materials were of analytical grade.

The UV–vis absorption spectra of QDs were recorded on a UV-visible Spectrophotometer 2550 (Shimadzu, Japan). And the fluorescence spectra of QDs were achieved with a LS-55 spectrophotometer (PerkinElmer, USA) at room temperature. The chemiluminescence of luminol and the CRET spectrum of the Luminol–HRP–QD system were measured by using QE65000 fiber optic spectrometer (Ocean Optics, USA).

2.2. Preparation of oil soluble QD (OQD) and water soluble QD (WQD)

Core-shell quantum dots (ZnS/CdSe) were synthesized by using a two-step method according to literature procedures [15–17]. By controlling the temperature and the molar ratio of reactants in the reaction, different size quantum dot with different emission wavelength were synthesized.

Two different strategies were adopted to solubilize quantum dots (WQD). In the first strategy, 100 mg sodium thioglycolate powder was added to 500 μL 1.72×10^{-5} M CdSe/ZnS (em 593 nm) chloroform solution in centrifugal tube, stirred for 12 h, then 100 μL distilled water was added into the tube, stirred and lay for a while, the above layer of water solution was obtained, and was precipitated with acetone and dissolved three times, finally the precipitate was redissolved in deionized water. In the second strategy, an amphiphilic polymer was firstly prepared by using poly (acrylic acid-co-maleic acid) sodium salt according to literature procedure [18] reported previously, then the amphiphilic polymer and oil soluble quantum dots (em 675 nm) were mixed in the molar ratio of 10 to 1, stirred for 3 h, After vacuum drying, the encapsulated dots were soluble in a polar solvent (PBS and water). This solution was then centrifuged several times to remove the impurity, and at last clear water soluble quantum dots (WQD) solution was obtained.

2.3. Preparation of QD–HRP conjugate and column filtration analysis

Water soluble QDs were conjugated to horseradish peroxidase using EDC as a coupling reagent [19]. First, a reaction mixture containing WQD (1.7×10^{-5} M), HRP (4×10^{-5} M), and EDC (1 mg mL^{-1}) in PBS (0.01 M, pH 7.0) was prepared, stirred for 1 h at room temperature. Then, the mixture was purified using an ultra-filtration membrane (Micoron YM-50-50000 NMWL, Millipore, USA), the conjugates were obtained and stored at 4 °C.

Sephadex G-100 column (35 cm long and 1 cm diameter) was used to filter and analyse the conjugates, pre-equilibrated with PBS (0.01 M, pH 7.0), the wavelength of 280 nm of UV source was set to check the flowing specimen out from the column.

2.4. Detecting of CRET spectrum

CRET spectrum of the Luminol–HRP–QD system was recorded with a fiber optic spectrometer in a darkroom. In a 3 mL quartz cuvette (1 cm optical path), 10 μL H_2O_2 (9.8 M) and 400 μL mixture (containing luminol (10^{-4} M), 4-iodophenol (5×10^{-4} M), and $Na_2B_4O_7 \cdot 10H_2O$ (19 mg mL^{-1})) were mixed, then the QD–HRP conjugates or mixture of QDs and HRP (after sonicate) was quickly injected into the cuvette, synchronously,