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A novel β-Cyclodextrin-QDs optical biosensor for the determination of amantadine and its application in cell imaging

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ABSTRACT

In this paper, a novel optical biosensor for amantadine (AD) determination has been constructed successfully based on the fluorescence resonance energy transfer (FRET) between water-soluble β-Cyclodextrin (β-CD)-functionalized CdTe quantum dots (QDs) and Rhodamine B (RB). RB could enter the cavity of β-CD by hydrophobic interaction, and the process of FRET between QDs and RB occurred. However, the process of FRET was switched off with the addition of AD, due to its larger hydrophobic association constant with β-CD than that of RB. The fluorescence intensity of CdTe QDs (donor) would increase gradually with the increasing concentration of AD, which shown a good linear relationship in the range of 1×10^{-5}–1.6×10^{-4} mol/L with a correlation coefficient $R^2=0.998$. We also obtained a satisfactory result using this spectrophotometric method for the determination of AD in pharmaceutical formulation. Furthermore, β-CD-functionalized CdTe QDs with AD in the cavity were incubated with target HepG2 cells and could be observed in the cytoplasm of cells. The β-CD-functionalized CdTe QDs could act as a visible biomarker for AD in cancer cells fluorescence imaging, which presents a potential application in biomedical field.

1. Introduction

As a new kind of fluorescent and semiconductor material, quantum dots (QDs) have got more and more attention in nanomaterial and bioanalytical fields in recent years, including solar cell [1], cell imaging [2], drug delivery [3], protein tracking [4] and cancer diagnoses [5]. Compared with traditional organic dyes, QDs have many unique properties that make them widely used in the development of optical biosensor. Firstly, they have enormous absorption extinction coefficients and high fluorescent quantum yields. They have broad excitation spectrum, narrow and size-tunable emission spectrum and large Stokes shift. QDs with different emission wavelengths can be excited at a single wavelength, enabling them applied in multiplexed experiments. Secondly, the resistance to photobleaching and high stability of QDs in biological environment enable their applications extend to biomedical field. With these outstanding properties, QDs have been widely applied as an excellent optical biomarker in biological fields. Ishihama et al. successfully observed the movement of individual mRNAs labeled by QDs in interchromatin regions of Cos7 cells [6]. Singh utilized biocompatible Fe_3O_4–ZnO core–shell magnetic quantum dots (M-QDs) for cancer imaging and therapy [7].

For the application of QDs in complex physiological environment, the methods for QDs modification was important [8–10]. Most molecules can link with QDs by covalent bond [11], hydrophobic interaction [12], electrostatic interaction [13] or chelation [14]. Different QDs modification methods not only have effect on the combination of QDs and biomolecule, but also lead distinct distribution of QDs in various organs in vivo [15–17]. Ballou et al. studied the effects of four kinds of amphiphilic polymer-coated QDs on mice after injection into their tail veins [15]. Deposition of four kinds of QDs was seen in the liver, spleen, born marrow, lymph nodes and other organs, depending on the various coatings used.

Cyclodextrins (CDs), as a well-known molecular host, have been used as an ideal functional molecule to modify the surface of QDs [18–20]. CDs are cyclic oligosaccharides that consist of six, seven, or eight glucopyranose units in α, β and γ forms, respectively [21]. The β-CD-functionalized QDs have been applied in biomedical field for their biocompatibility and chiral selectivity [22,23]. Zhao et al. synthesized QDs coated with β-CD and used them to deliver siRNA in live cells [23]. The results showed that the β-CD coupled to amino acids outlayers greatly improved the biocompatibility of QDs and endowed it with lower cytotoxicity even at very high concentration.

Amantadine (AD) is an antiviral agent used against infection with influenza A virus and Parkinsons disease [24,25]. Several analytical methods, such as high performance liquid chromatography (HPLC) [26–29], gas chromatography (GC) [30], capillary electrophoresis (CE)

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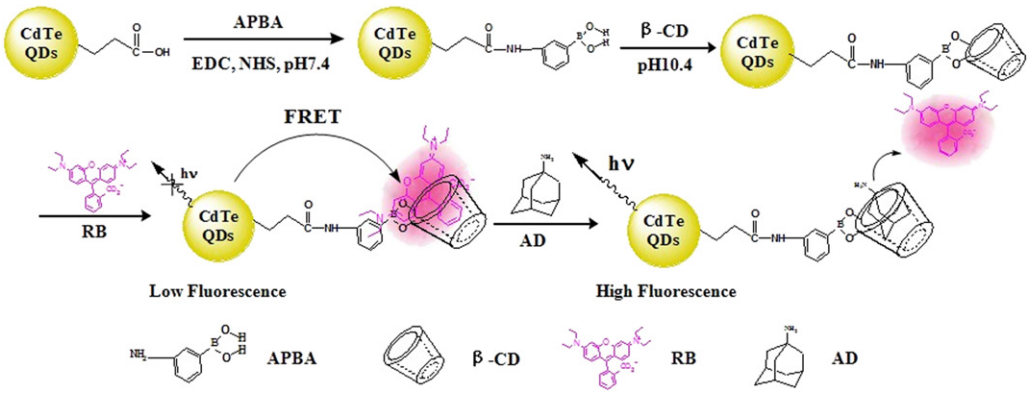

Scheme 1. Illustration of the optical biosensor for AD determination via FRET mechanism.

[31], have been reported for the determination of AD. Although some chromatographic methods have been the primary methods applied for the determination of the antiviral drugs in pharmaceutical formulations [27–29], however, the procedures are tedious, difficult to perform, and require more expensive apparatus, which could not be available in many laboratories. Spectrophotometric analysis is considered more convenient alternative technique because of its inherent simplicity and high sensitivity. However, few spectrophotometric methods have been reported for the determination of AD in pharmaceutical formulations, mainly due to that AD does not possess any chromophore in its molecule, which is essential for spectrophotometric methods. Therefore, derivatization [32] and formation complex with AD [33] were common ways before determination of AD by spectrophotometric methods. However, this process was laborious, time consuming, and not real time measurement. Therefore, it is significant to develop a simple, rapid, sensitive and non-derivative spectrophotometric method for AD determination. FRET as a fast, sensitive and non-destructive method has been widely used to develop various biosensors for the determination of bioactive molecules [34–37]. Freeman et al. reported a competitive optical sensing and chiral selective sensing for different substrates by using β-CD-functionalized CdSe/ZnS QDs, which was based on the fluorescence resonance energy transfer (FRET) or electron transfer (ET) mechanism [37]. This system combining QDs and β-CD not only has good selectivity and sensitivity, but also has excellent biocompatibility in vivo.

In this paper, we described a novel optical biosensor for amantadine determination based on FRET mechanism. We designed a FRET system by using water-soluble β-CD-functionalized CdTe QDs and Rhodamine B (RB). As shown in Scheme 1, RB could enter the cavity of β-CD by hydrophobic interaction and the process of FRET between QDs (donor) and RB (acceptor) occurred. When AD replaced RB in the cavity based on its larger hydrophobic association constant with β-CD, the process of FRET was switched off. We also obtained a satisfactory result using this method for the determination of AD in pharmaceutical formulations. Furthermore, β-CD-functionalized CdTe QDs with AD in the cavity were incubated with HepG2 cells and can be observed in cytoplasm by using fluorescence microscopy. The labeled QDs could reveal the location of AD in cells and this visible approach might offer a new visible biomarker for AD in cancer cells fluorescence imaging.

2. Materials and methods

2.1. Materials and reagents

All chemicals used were of analytical reagent grade without further purification. 3-mercaptopropyl acid (MPA), N-Hydroxysulfosuccinimide (sulfo-NHS) and 3-aminophenyl boronic acid (APBA) were purchased from J&K Chemical Co. Ltd. Tellurium powder, $CdCl_2$, $NaBH_4$ and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Aldrich Chemical Co. Ltd. β-CD and Rhodamine B (RB) were purchased from Beijing DingGuo Biotechnology Co. Ltd. and AD was obtained from Dalian Meilun biotechnology Co. Ltd. respectively. Glucose, starch, vitamin C, sucrose, sodium citrate, cellulose and dextrin were purchased from Tianjin Guangfu Chemical Technology Co., Ltd. The pharmaceutical capsules were purchased from local drugstore. Dulbecco's modified Eagle's medium (DMEM) with high glucose was obtained from Invitrogen Co. and Fetal bovine serum (FBS) was purchased from Hyclone. 6-well plates were purchased from Corning Incorporated Co. The water used in all experiments had a resistivity higher than 18 MΩ/cm.

2.2. Instruments

Fluorescence experiments were performed on an RF-5301 PC spectrofluorophotometer (Shimadzu Co., Japan) and a 1 cm path-length quartz cuvette was used to measure the fluorescence spectrum. UV–vis absorption spectra were obtained using a GBC Cintra 10 e UV–vis spectrometer. The FT-IR spectrum was recorded on a Nicolet AVATAR 360 Fourier transform infrared spectrometer. Inverted fluorescence microscope (Olympus FV1000 IX71) equipped with a multispectral imaging system (Nuance, CRI, Woburn, MA, USA) was used to observe the location of β-CD-functionalized CdTe QDs in the cells.

2.3. Preparation of β-CD-functionalized CdTe QDs

CdTe QDs were synthesized by refluxing routes as described in detail in Ref. [38]. Briefly, the precursor solution of CdTe QDs was formed in water by adding fresh NaHTe solution to 1.25×10^{-3} mol/L N_2 saturated $CdCl_2$ solution at pH 11.4 in the presence of MPA as stabilizing agent. The molar ratio of Cd^{2+}/MPA/HTe^- was 1:2.4:0.5. The CdTe precursor solution was subjected to reflux at 100 °C under open-air conditions with condenser attached and different sizes of CdTe QDs were obtained at different refluxing times. The photoluminescence (PL) wavelength of CdTe QDs used in this study was 546 nm and the concentration of QDs was 1×10^{-6} mol/L.

β-CD-functionalized CdTe QDs were prepared by covalent link with APBA as a bridge. Briefly, 0.3 mL 2.5×10^{-6} mol/L EDC and 0.2 mL 1.5×10^{-6} mol/L sulfo-NHS were added into as-prepared QDs solution to activate carboxyl of QDs with stirring for 0.5 h. Then 0.5 mL 2.5×10^{-6} mol/L APBA solution was added and stirred for 3 h to form an amide bond between QDs and APBA. All the reactants above were prepared in 0.1 mol/L phosphate buffer solution (PBS, pH7.4). After adjusting the pH value from