Biosensors and Bioelectronics 36 (2012) 168–173

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A new nanoprobe based on FRET between functional quantum dots and gold nanoparticles for fluoride anion and its applications for biological imaging

Mei Xue [1], Xu Wang [1], Lili Duan, Wen Gao, Lifei Ji, Bo Tang *

College of Chemistry, Chemical Engineering and Materials Science, Engineering Research Center of Pesticide and Medicine Intermediate Clean Production, Ministry of Education, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Normal University, Jinan 250014, PR China

ARTICLE INFO

Article history:
Received 14 February 2012
Received in revised form
5 April 2012
Accepted 9 April 2012
Available online 26 April 2012

Keywords:
CdTe quantum dots
Gold nanoparticles
FRET
Fluoride anion
Living cell

ABSTRACT

A new nanoprobe was designed for the fluorescence imaging of fluoride anion (F^-) in living cells with high sensitivity and selectivity. The design is based on the fluorescence resonance energy transfer (FRET) between CdTe quantum dots (CdTe QDs) and gold nanoparticles (AuNPs) through the formation of cyclic esters between phenylborinic acid and diol. In the presence of F^-, the boronate ester, a "hard acid", strongly reacts with F^-, a "hard base". Therefore, the boronate ester is converted to trifluoro borate, which causes the breakage of the linkage and disassembles CdTe QDs from AuNPs, resulting in the fluorescence recovery of the quenched CdTe QDs. The interaction mechanism was investigated by ^{19}FNMR on a model that was constructed by a small molecule and F^-. Quantum chemical calculations also testify the reactivity of boronate ester to F^- and the sensing mechanism. Experimental results show that the increase in fluorescence intensity is proportional to the concentration of F^- in the range of 5.0–45 μM. The detection limit and the relative standard deviation were 50 nM and 2.6%, respectively. Fluorescence imaging of F^- in macrophages cells indicates good cell membrane penetration ability and low cytotoxicity of the nanoprobe, providing a viable alternative to detection of F^- in biological or environmental samples.

1. Introduction

Among the range of biologically important anions, fluoride ion (F^-) is of particular interest owing to its duplicitous nature. Fluoride is used for prevention of dental caries, enamel demineralization while wearing orthodontic appliances, and treatment for osteoporosis (Horowitz, 2003; Farley et al., 1983). However, pathological studies show that excessive intake of fluoride will result in gastric and kidney disorders, dental and skeletal fluorosis (Riggs, 1984; Kleerekoper, 1998), and even cancers (Arhima et al., 2004; Matsui et al., 2007). The cellular studies show that excessive exposure to fluoride can inhibit cell growth (Berry and Trillwood, 1965), induce inflammatory reactions(Gutowska et al., 2011), and even cell apoptosis (Hirano and Ando, 1996). Therefore, the quantitative detection of fluoride is of growing importance in chemical and biological systems.

Ion-selective electrode and ion chromatography have been extensively investigated in the analysis of fluoride in various samples. However, the relatively complicated equipment and extending operating time limit their application in real-time analysis. The optical sensors, which are highly rapid and selective, have been designed for F^- analysis (Cho et al., 2005; Lee et al., 2007; Lin et al., 2006). Among these, the fluorescent sensors are proved to be a powerful tool for fluoride analysis because of their high sensitivity, easy operation, and noninvasion character (Lee et al., 2007; Bao et al., 2011; Zhu et al., 2011; Zhang et al., 2011; Cao et al., 2011; Kim et al., 2009; Gong et al., 2011). However, as to the nano-based optical probes for fluoride, to the best of our knowledge, there are only two literatures that have been reported for fluoride in water (Watanabe et al., 2005; Xue et al., 2011). One is a colorimetric probe based on the fluoride-induced aggregation of thioglucose-capped gold nanoparticles (AuNPs) (Watanabe et al., 2005). The other is a "turned on" type of fluorescence resonance energy transfer (FRET) nanoprobe initiated by the breakage of hydrogen bond by fluoride that is proposed by our group (Xue et al., 2011). Considering the bond energy, hydrogen bonding interactions belong to the range of Van der Waals' forces, a weak interaction. As we know, the intermolecular forces are much weaker than covalent bond. Therefore, in the endocytic processes, hydrogen bond is susceptible to be broken, resulting in the disassembly of the nanoprobe. Considering this, a nanoprobe fabricated through covalent bond for F^- imaging in living cell is attractive and promising.

Compare to organic fluorescence molecules, quantum dots (QDs) have unique optical and electronic properties, including

* Corresponding author. Tel.: +86 531 86180010; fax: +86 531 86180017.
E-mail address: tangb@sdnu.edu.cn (B. Tang).
[1] These authors contributed equally to this work.

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.04.007

broad excitation spectra, narrow and tunable emission spectra, high quantum yield, and high resistance to photobleaching (Alivisatos, 1996; Chan et al., 2002). Meanwhile, FRET from the excited state of the QDs to acceptors has been regarded widely as an extremely useful tool for the sensitive determination of molecules (Peng et al., 2007; Shi et al., 2007; Zhang et al., 2005). As an excellent fluorescent quencher, AuNPs open new perspectives to detect molecules with high sensitivity in FRET systems owing to their high extinction coefficients as well as broad absorption spectrum within the visible light range that overlaps with the emission wavelengths of common energy donors (Chen and Chang, 2004; Wargnier et al., 2004; Tang et al., 2008a,b). The superiority of QDs–AuNPs donor–acceptor pairs designed as a nanoprobe for analysis with high sensitivity and selectivity has attracted increasing attention (Oh et al., 2005; Tang et al., 2008a,b; Dyadyusha et al., 2005).

Up to now, studies on nanoprobes in bioassays and fluorescence bioimaging have been reported (Huang, et al., 2011; Li, et al., 2011; Napp, et al., 2011; Paquet, et al., 2012; Yeh, et al., 2010). According to the literatures, the nano-based platform is easy to be assembled and have shown some distinguished features. Therefore, in the present work, we designed a new type of covalent bond-based FRET nanoprobe for the direct determination of F^- in water and living cells. This design is based on the FRET between CdTe QDs and AuNPs through the formation of cyclic esters between phenylborinic acid and diol. In the presence of F^-, the boronate ester is converted to trifluoro borate, which causes the breakage of the linkage and disassembles QDs from AuNPs, resulting in the fluorescence recovery of the quenched QDs. Unlike our previous work, the new nanoprobe was assembled by covalent bond. This allows quantitative analysis of F^- in water with high selectivity and sensitivity. Importantly, as our expectation, the nanoprobe was successfully applied to the fluorescence imaging of F^- in living cells.

2. Experimental

2.1. Preparation of 3-amino-1,2-propanediol conjugated CdTe QDs (QDs-diol)

2.1.1. Preparation of NaHTe

In a two-necked flask (25 mL), 15.0 mg of tellurium powder and 10 mL of water were deaerated with argon for 10 min. Then slightly excessive $NaBH_4$ was added and the resulting suspension was stirred under argon until $NaBH_4$ was completely dissolved. After that, the resulting suspension was heated to 35 °C under vigorous stirring and argon bubbling. During 15 min, the colored suspension changed its characteristic color from violet to colorless. Fresh NaHTe was obtained and was cooled to room temperature.

2.1.2. Preparation and purification of TGA/CdTe QDs

In a three-necked flask (250 mL) equipped with a reflux condenser, cadmium acetate dihydrate (2 mmol) was dissolved in 100 mL of water. After thioglycolic acid (TGA, 1.0 mmol) was added, the solution was adjusted to pH 10 with aqueous NaOH (1.0 M) and stirred under argon at room temperature for 30 min. Then 2 mL of NaHTe solution was injected under argon and the mixture was refluxed. The color of the precursor's mixture turned from colorless to orange, supporting the growth of the nanocrystals. After refluxing for an hour, stable water-soluble TGA-capped CdTe QDs were obtained. Then, the QDs were precipitated with an equivalent amount of 2-propanol, followed by resuspension in a minimal amount of ultrapure water. Excess salts were removed by repeating this procedure three times, and the purified QDs were dried overnight at room temperature in vacuum.

2.1.3. 3-Amino-1,2-propanediol conjugation

TGA/CdTe QDs (5 mg) was dissolved in 10 mL of 10 mM of phosphate buffer saline (PBS) (pH=7.4) solutions. 10 mg of NHS and 20 mg of EDC were added to the QDs solution and incubated for 30 min at room temperature with continuous gentle mixing to activate the carboxylate groups on QDs. After that, 1.20 mg of 3-amino-1,2-propanediol was added to the activated QDs solution. After reacted overnight, the diol-conjugated QDs were separated from the mixture through ultrafiltration under centrifugation at 12,000 rpm for 5 min. The final concentration of QDs-diol is about 7.9×10^{-7} mol/L. The concentration is denoted to be 1x.

2.2. Preparation of mercaptophenyl-boronic acid modified AuNPs (AuNPs-MPBA)

20 mg of mercaptophenyl-boronic acid (MPBA) was added to 10 mL of the citrate modified AuNPs solutions under stirring in the dark for 24 h. After that, unbound MPBA was removed by repeated centrifugation (12,000 rpm, 7 min), followed by dispersing the red precipitation in 10 mL of pure water to get MPBA-modified AuNPs. At last, the concentration of AuNPs-MPBA was calculated to be approximately 2.0 nM. The concentration is denoted to be 2x.

2.3. Fluorescence detection

2 mL of AuNPs-MPBA solution was dropped into 1 mL of QDs-diol for 1 h to obtain the nanoprobe. The concentration of the assembled nanoprobe QDs-diol–MPBA-AuNPs is denoted to be 3x. 100 μL of 0.10 M PBS (pH=7.4) buffer solution was added to 300 μL of the probe solutions. Afterwards, different concentrations of F^- were added respectively. Subsequently, each sample solution was diluted with ultrapure water to a final volume of 1.00 mL. After 30 min reaction, the fluorescence spectra were obtained.

2.4. Cytotoxicity of the assembled nanoprobe

RAW264.7 macrophage cells were seeded on 96-well microtiter plates to a total volume of 100 μL/well. Plates were maintained at 37 °C in a 5% CO_2 incubator for 24 h. The nanoprobe (3x) of different volume was loaded into each well, with six duplicates for each probe concentration. No probe was added to the control cells. After 24 h of incubation, the supernatant was removed, and the cells were washed with PBS three times. To evaluate cell viability, 100 μL of MTT solution (0.5 mg/mL in PBS) was added to each well and the mixture was incubated at 37 °C for 4 h. After incubation, the remaining MTT solution was removed, and 100 μL of dimethyl-sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The optical absorbance was measured at 490 nm on a microplate reader.

2.5. Cell imaging

RAW264.7 macrophages were added to glass coverslips in the culture plates. After 2 h of incubation at 37 °C in a 5% CO_2 incubator, the nonadherent cells were removed by vigorous washing (3 times) with warm serum-free medium, and the adherent cells were incubated overnight in complete medium to form macrophage monolayers. A set of cells were supplemented with KF (50 μM in serum-free DMEM medium, 1.0 mL) at 37 °C for 1 h. After the cells were washed with DMEM for three times,