Biosensors and Bioelectronics 36 (2012) 174–178

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Oligonucleotide-based fluorogenic sensor for simultaneous detection of heavy metal ions

Changlong Hao [a], Liguang Xua [a], Changrui Xing [a], Hua Kuang [a,*], Libing Wang [b,*], Chuanlai Xu [a]

[a] School of Food Science & Technology, State Key Laboratory of Food Science & Technology, Jiangnan University, Wuxi 214122, China
[b] Reaearch Center of Hunan Entry-Exit Inspection and Quarantine Bureau, Changsha, 410001, China

ARTICLE INFO

Article history:
Received 6 February 2012
Received in revised form
26 March 2012
Accepted 9 April 2012
Available online 23 April 2012

Keywords:
Fluorogenic sensor
Silver ion
Mercury (II) ion
Simultaneous detection

ABSTRACT

In this study, we report a new fluorogenic sensor based on fluorescence resonance energy transfer (FRET) for detection of heavy metal ions in aqueous solution. The method showed the advantage of being simple, highly sensitive and selective, and rapid. The donor (CdTe QDs) and acceptor (TAMRA or Cy5) are brought into close proximity to one another due to Hg^{2+} and Ag^{+} form strong and stable T-Hg^{2+}-T complexes and C-Ag^{+}-C complexes, which quenches the fluorescent intensity of CdTe QDs and enables the energy transfer from donor to acceptor. This sensor showed high sensitivity and selectivity when only one kind of ion (Ag^{+} or Hg^{2+}) exists. Furthermore, the assay can also simultaneously detect Ag^{+} and Hg^{2+} in water media with the limit of detection (LOD) of 2.5 and 1.8 nM, separately, which satisfactorily meets the sensitivity demands of Environmental Protection Agency (EPA) and World Health Organization (WHO). This assay also exhibits excellent selectivity toward Ag^{+} and Hg^{2+}. Therefore, this method is of great practical and theoretical importance for detecting heavy metal ions in aqueous solution.

1. Introduction

Heavy metal ions, such as silver ion (Ag^{+}) and mercury ion (Hg^{2+}), are severe environmental pollutants. They contaminate water and soil, pose severe risks to human health and induce toxic effects in animals and plants (Aragay et al., 2011). Mercury ion has accumulative and toxic properties in the environment and long-term exposure to high levels of Hg^{2+} causes perpetual and severe damage of the central nervous system (Kim et al., 2012). Silver ion has been proven to be highly toxic to aquatic organisms. Thus, the development of more sensitive and selective analytical methods for determining these two ions is of great importance.

Traditional detection methods such as cold-vapor atomic fluorescence spectrometry (CV-AFS), cold-vapor atomic absorption spectrometry (CV-AAS), and inductively coupled plasma-mass (ICPMS) are associated with many drawbacks including being labor-intensive and high-cost and involving complex processes. Therefore, much effort has been directed towards developing new sensors to overcome these problems. FRET is a physical phenomenon first proposed by Theodor Förster over 50 years ago, which has many applications such as FRET imaging (Jares-Erijman and Jovin, 2003), single-molecular FRET (smFRET) (Roy et al., 2008). In the recent years, FRET has been used to many fluorescent detections such as detecting tartrazine (Huang et al., 2012), lysozyme (Wang and Liu, 2009), DNA (Li et al., 2011a), and histamine (Gustiananda et al., 2012).

As has been known to all, Hg^{2+} and Ag^{+} can form stable complexes by bridging specific nucleotide bases (Freeman et al., 2009; Miyake and Ono, 2005). Hg^{2+} ions specifically bridge thymine bases and form strong and stable T-Hg^{2+}-T complexes, while Ag^{+} ions form cytosine-Ag^{+}-cytosine(C-Ag^{+}-C) complexes. Numerous oligonucleotide-based sensors including electrochemiluminescence (ECL) sensors (Li et al., 2010; Zhou et al., 2012), electrochemical sensors (Zhu et al., 2009) and optical sensors (Li et al., 2009) have been developed for the selective and sensitive analysis of Hg^{2+} or Ag^{+} ions. More recently, our group has used an oligonucleotide functional magnetic nanoparticles sensor based on magnetic resonance imaging for highly selective and sensitive detection of Hg^{2+} (Ma et al., 2011). However, the water-soluble magnetic nanoparticles are sort of unstable and tend to aggregate quite easily, and magnetic resonance imaging (MRI) is expensive and requires professional assistance.

The sensors based on fluorescence resonance energy transfer for Hg^{2+} detection have attracted considerable attention. Li's group (Li et al., 2011b) developed a fluorescent sensor based on the quantum dot/DNA/gold nanoparticle ensemble to detect Hg^{2+}. Ono's group (Ono et al., 2008), Tseng's group (Lin and Tseng, 2009) and Luo's group (Xie et al., 2012) all developed

* Corresponding authors. Tel.: +86 510 85329076.
E-mail addresses: khecho@163.com (H. Kuang), wanglb1@126.com (L. Wang).

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.04.008

oligonucleotide-based Ag^+ sensors that contained adequate C–C pairs to measure Ag^+. However, all these assays have been focused on single metal ion analysis though they are effective and efficient. Commonly, Ag^+ and Hg^{2+} are simultaneously present in water. Therefore, we need to use only one sensor to monitor them at trace levels in the aqueous solution. To date, only few sensors have been designed for the simultaneous detection of Ag^+ and Hg^{2+}. In some ways, these sensors are useful, for example, the sensor developed by Lin's group (Lin et al., 2011) can simultaneously detect Pb^{2+}, Ag^+, and Hg^{2+} with good sensitivity. However, they have some limitations including poor selectivity, insufficient sensitivity to Hg^{2+} (Lin et al., 2010) or both (Freeman et al., 2009), or the need to use a masking agent (Lin et al., 2011).

Compared with routine methods, herein we present a simple sensing platform for highly sensitive and selective detection of Ag^+ and Hg^{2+} based on the FRET between luminescent CdTe quantum dots (QDs) and dye-labeled single-strand DNA (DNA-dye) probes.

2. Experimental

2.1. Materials and instrumentation

Three-mercaptopropionic acid (MPA), cadmium chloride ($CdCl_2$) and Tellurium (Te) powder were bought from Sigma to Aldrich. $Mg(NO_3)_2$, $Cd(NO_3)_2$, $Ca(NO_3)_2$, $Zn(NO_3)_2$, $Pb(NO_3)_2$, $Fe(NO_3)_2$, $Fe(NO_3)_3$, $Hg(NO_3)_2$ and $AgNO_3$ stock solutions were purchased from Sigma to Aldrich. All the other chemicals used were of the highest quality commercially available. The oligonucleotide probes were obtained from Sangon Biotech (Shanghai) Co., Ltd. Their sequences were as follows:

DNA1: 5'-NH_2-GTACA AGATG-3′; DNA2: 5′-GAGCT TTTCA GACGC ATCTT GTACG ACTCG CTCCC CATAC-3′; TAMRA-labeled ssDNA (TAMRA-DNA): 5′-TAMRA-TTTT GCTC-3′; Cy5-labeled ssDNA (Cy5-DNA): 5′-Cy5-GTAT CCCC-3 ′.

The morphology of QDs was characterized using a JEOL JEM-2100 transmission electron microscope (TEM), and all the fluorescent spectra were determined by an F-7000 fluorescence spectrophotometer.

2.2. Synthesis of CdTe quantum dots (QDs)

Water-soluble CdTe QDs were synthesized in aqueous solution by using 3-mercaptopropionic acid (MPA) as the capping ligand according to our published procedure (Chen et al., 2009; Zhao et al., 2011). Briefly, Te powder, $NaBH_4$ and ultrapure water were mixed together to obtain a solution of NaHTe, and then a crude orange and yellow CdTe solution without photoluminescence was prepared by the reaction between $CdCl_2$ and NaHTe solution. The molar ratio of Cd^{2+}:Te^{2-}: MPA was 1:0.47:2.45. Finally, the CdTe precursor was transferred to a Teflon-lined stainless steel autoclave and heated at 160 °C for 20 min. Before use, the CdTe QDs had to be dialyzed for three days with PBS buffer (pH 7.0). It is evident from Fig. 1(B) that the QDs were well dispersed.

2.3. Fabrication of oligonucleotide-based fluorogenic probe

The QD-DNA1 conjugates were prepared by reaction of the carboxylate groups of the QD and amino groups of DNA1 according to our published method (Wang et al., 2011; Xu et al., 2011). Briefly, 320 μL 0.1 mM EDC, 200 μL 0.0125 mM NHS and 1280 μL QD solution were mixed together. After activation under gentle shaking for 15 min in the dark, 100 μL DNA1 aqueous solution at 10 μM was added and reacted at 25 °C for 4 h. The QD-DNA1 conjugates were then ultrafiltered by using Millipore's Amicon Ultra centrifugal filter units with 14,000 molecular weight cutoff (5000 rpm for 15 min) to remove the excess DNA1, and washed three times with water. According to the fluorescence method (Sun and Gang, 2011; Xu et al., 2011), the average number of the DNA1 on every single QD is about 1.

Next, the 900 μL QD-DNA1 conjugates at 1 μM were then hybridized with 100 μL 20 μM DNA2 at room temperature for 6 h. The similar ultrafiltration process was carried out to remove the excess DNA2. Finally, 100 μL 20 μM Cy5-DNA and 100 μL 20 μM TAMRA-DNA were added to the QD-DNA1/DNA2 conjugates to obtain DNA-based fluorogenic probe.

2.4. Ag^+ and Hg^{2+} detection

The test was conducted in the MOPS buffer system (100 nM DNA-based fluorogenic probe, 10 mM NaH_2PO_4/Na_2HPO_4, 20 mM 3-morpholinopropanesulfonicacid (MOPS), and 50 mM $NaNO_3$) due to the weak binding between Ag^+ and MOPS (Lin and Tseng, 2009). In order to determine the hybridization kinetics, time-dependent fluorescent emission intensity (503 nm) of the QD/dye solution was monitored after addition of 0.1 μM Ag^+ and Hg^{2+}.

For Ag^+ sensing, the Ag^+ sample solutions (50 μL) of different concentrations were added to the test solution (450 μL) prepared

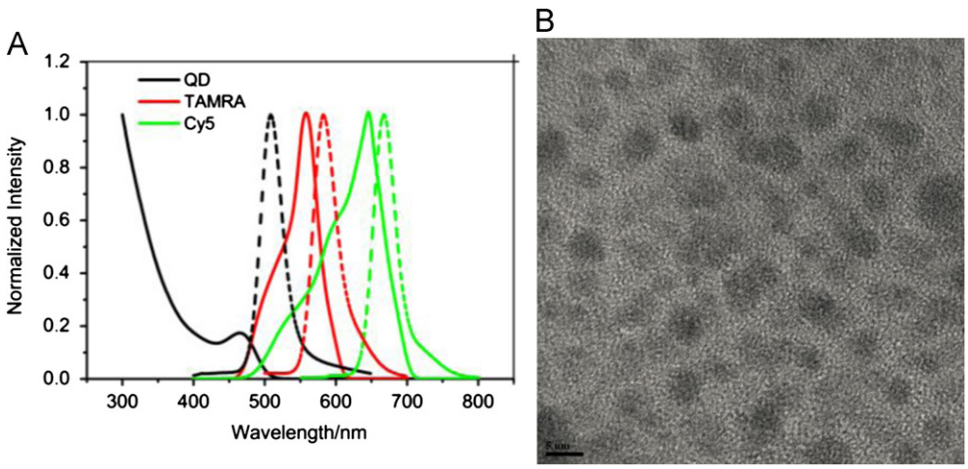

Fig. 1. Normalized absorption (solid lines) and emission (dashed lines) spectra of CdTe QD, TAMRA-DNA, and Cy5-DNA (A); TEM picture of CdTe quantum dots (B). Scale bar = 5 nm.