Sensors and Actuators B 247 (2017) 400–407

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Development of highly selective and sensitive fluorimetric label-free mercury aptasensor based on cysteamine@CdTe/ZnS quantum dots, experimental and theoretical investigation

B. Rezaei*, M. Shahshahanipour, Ali A. Ensafi, H. Farrokhpour

Department of Chemistry, Isfahan University of Technology, Isfahan, 84156-83111, Iran

ARTICLE INFO

Article history:
Received 24 August 2016
Received in revised form 13 March 2017
Accepted 16 March 2017
Available online 18 March 2017

Keywords:
Mercury ions
CdTe/ZnS core/shell quantum dots
Folding of the aptasensor
Fluorescence method

ABSTRACT

In this paper, an ultra-sensitive and highly selective label-free fluorescent aptasensor was developed for the rapid detection of Hg(II), using water soluble cysteamine-capped CdTe/ZnS quantum dots (cysteamine@CdTe/ZnS QDs) as an luminescent probe and a single strain DNA aptamer designed to specifically bind to Hg(II) ions. Negative charge aptamers could aggregate the cationic cysteamine@CdTe/ZnS core/shell quantum dots, so the fluorescence quenching occurred. When Hg(II) ions, as a target, were added to the quantum dots solution, the aptamers with thymine (T)-rich sequences were selectively bound to the mercury ions. This is due to the powerful affinity of Hg(II) ions to the T bases of the DNA aptamer. It leads to the formation of an Hg (II)-bridged T base pair and the aptamers rearrangement into a hairpin-like structure. In the presence of Hg(II), de-aggregation of the quantum dots was occurs, so the fluorescence intensity was gradually increased with enhancing its concentration. Hg(II) could be measured in the range of 5.0×10^{-10} to 1.0×10^{-6} mol L^{-1} with a low limit of detection, 8.0×10^{-11} mol L^{-1}. The fabricated fluorescent aptasensor also demonstrated excellent selectivity for Hg(II) detection, this principal was investigated by theoretical and experimental methods so it was applied lucratively for the determination of Hg(II) in real water and waste water samples.

1. Introduction

Mercury pollution is due to nature and human activities [1]. This ion is one of the most stable and toxic heavy metals existing in water, soil and food [2]. As well, it is not biodegradable; thus it remains in the environment [3]. It can simply pass through skin, breathing and tummy tissues; also, it tends to accumulate in many organs and tissues persistently, leading to mitosis damage and perpetual damage to the central nervous system [4,5]. According to its public health peril, many organizations have imposed severe constraints on mercury concentrations in drinking water; for example, the World Health Organization mandated 29.9 nM for maximum mercury level [6].

Due to the anthropogenic activities, the whole concentration of mercury in environment has been increased [7]. So creation of extremely sensitive and selective techniques for trace determination of Hg(II) is essential [8]. Based on the literature, many analytical methods have been developed for trace levels of Hg(II) detection, such as atomic absorption/emission spectrometry [9], fluorescence assay [10], electrochemical methods [11] and inductively coupled plasma mass spectrometry [12].

Most of these methods may have practical limitations; for example, they may be time-consuming, expensive, and inappropriate for field measurements and real-time analysis [13]. To overcome these weaknesses and plan highly selective and sensitive mercury sensors, in recent years many inventive measures using inorganic molecules [14], polymers [15], oligonucleotides [16], and nanoparticles [17].

Recently, single-stranded nucleic acid molecules namely aptamers, selected through the systematic evolution of ligand via an exponential enrichment (SELEX) process. The aptamers have been widely used as the ideal recognition elements for detecting metal ions [18]. Aptamers have some important advantages such as stability and resistance against denaturation, high solubility in aqueous solutions, rather easy synthesis, and ready structural adjustment. Furthermore aptamers have high affinity into metal ions through hydrogen bonding, electrostatic and Van der Waals forces, or a mixture of these interactions [19]. Also they have a specific linkage of certain metal ions like Hg(II) [20]. These artifi-

* Corresponding author.
E-mail addresses: rezaei@cc.iut.ac.ir, rezaeimeister@gmail.com (B. Rezaei).
URL: http://rezaei@cc.iut.ac.ir%20 (B. Rezaei).

http://dx.doi.org/10.1016/j.snb.2017.03.082
0925-4005/

cial nucleic acids can specifically recognize various targets through tuning the base sequences [21].

Quantum-dots (QDs) are nanomaterial crystalline clusters (1–10 nm), which have received great attention as fluorescence bioprobes. The desirable optical properties of QDs are large absorption spectrum, symmetric and narrow emission bands (full width at the half maximum of 25–35 nm), size-tunable photo luminescence (PL), high quantum yield, and usually long PL decay times [22]. Typically, core/shell QDs with an inorganic structures capping layer of ZnS around the core are synthesized to improve the optical properties [23].

In this work, a special sequence aptamer was used as an aggregator agent for positive charged QDs, leading to decreasing its fluorescence signal. In the presence of Hg(II), the aptamer aggregating quantum dots was released to form a strong hairpin complex with Hg(II). High selectivity and sensitivity, very low detection limit, and a wide dynamic range are the advantages of this aptasensor.

2. Experimental

2.1. Materials and reagents

Cysteamine hydrochloride, Na_2TeO_3, $CdCl_2$, $ZnSO_4$. $7H_2O$, $Hg(NO_3)_2$, $NaBH_4$ and $Na_2S{\cdot}9H_2O$ were all purchased from Aldrich (London, UK). The specific aptamer sequence for Hg(II) (5′-TTCTTTCTTCCCCTTGTTTGTT −3′) [18] was from Sinaclon bioscience Co, Ltd (Karaj, Iran). Deionized water was used during all experiments and all chemicals were of the highest degree of purity.

A 10.0 mmol L^{-1} stock solution of Hg(II) was prepared by dissolving an suitable amount of $Hg(NO_3)_2$ into 0.4 mL of 0.1 mol L^{-1} nitric acid solution and then diluted to 100.0 mL with deionised water in a volumetric flask. Lower Hg(II) concentrations were obtained by stepwise dilution of the Hg(II) stock solution.

2.2. Apparatus

Photoluminescence spectra were registered by a spectrofluorometer (Jasco FP- 750; Tokyo, Japan). UV.Vis absorption spectra were registered by a spectrophotometer (Jasco V-570 UV/Vis/NIR; Tokyo, Japan). The size and morphology of the QDs were analyzed using transmission electron microscopy (TEM) method. Tests were performed by Philips CM30 300 kV TEM. The diameter and the particle dimension distribution of cysteamine-stabilized CdTe/ZnS QDs in aqueous solution were acquired using a Malvern ZEN3600 dynamic light scattering (DLS) instrument (UK). Metrohm pH meter (Model 827) with a glass electrode (Corning) was used to read the solution pH. For recording FT-IR spectra, a Jasco 680-plus FT-IR spectrophotometer (Tokyo, Japan) in the range of 400–4000 cm^{-1} was used, using KBr powder-pressed pellets.

2.3. Synthesis of cysteamine@CdTe/ZnS core/shell QDs

Water soluble cysteamine@CdTe/ZnS QDs core/shell was synthesized according to the published protocol [24]. In brief, at first, for the synthesis of CdTe core, 545.0 mg of cysteamine hydrochloride and 365.0 mg of $CdCl_2{\cdot}2.5H_2O$ were dissolved into 80.0 mL of deionized water. Then, the pH was regulated at 5.7, using NaOH solution. Afterwards, 17.0 mg of Na_2TeO_3 and 80.0 mg $NaBH_4$ were added and the solution was heated to 100 °C under stirring and refluxing for 1.5 h. Also, 272.0 mg of cysteamine hydrochloride and 29.0 mg of $ZnSO_4{\cdot}7H_2O$ were added to 40.0 mL of deionized water and its pH was adjusted at 5.7 using NaOH solution. For the synthesis of cysteamine@CdTe/ZnS core/shell QDs, this solution was mixed with 40.0 mL of freshly synthesized cysteamine-stabilized CdTe QDs solution and then 2.0 mg of $Na_2S{\cdot}9H_2O$ was added in the solution. The mixture was refluxed again under 100 °C for 2.0 h. The obtained QDs solution was cooled under room temperature and after centrifuging, it was stored in a dark container at 4.0 °C for further use. According to the absorption spectrum, the amount of cysteamine@CdTeZnS-QDs was calculated as 4.22 μmol L^{-1} [25].

For XRD and IR spectroscopic studies, the obtained QDs were precipitated by adding 5 mL acetone. After centrifuging for 10 min at 9000 rpm, the sedimented QDs were separated and dried in nitrogen atmosphere and the analyses were carried out.

2.4. Measurement procedure

For the determination of Hg(II), 100 μL of the aptamer solution (10.0 nmol L^{-1}), representing an appropriate volume of sample or standard Hg(II), and 20 μL of 4.22 μmol L^{-1} cysteamine@CdTe/ZnS-QD solution were, respectively, mixed into the vial and the final volume was reached to 3.0 mL with phosphate buffer solution (pH 7.0). Then, it was incubated for 5.0 min at room temperature. After that, the solution was poured into the fluorimetric quartz cuvette then its fluorescence spectra were registered at the excitation wavelength of 450 nm and in the wavelength limited area of 510–550 nm (excitation slit of 5.0 nm and emission slit of 10.0 nm). In this work the analytical signal was defined by ($\Delta F = F - F_0$) values of the solution, where F_0 and F relegate to the fluorescence intensity at 529 nm in the absence and presence of Hg(II), respectively. For the measurement of Hg(II) in real samples, the standard addition technique was used.

2.5. Sample preparation

All water samples (drinking water, Zayande rood river water and waste water from Esfahan's Mobarekeh Steel Co.) were gathered and filtered through a 0.22 μm pore-sized membrane immediately to eliminate physical impurities and afterward three drops of concentrated HNO_3 were added and kept in polyethylene containers at 4 °C.

3. Results and discussion

3.1. Characterization of QDs

The obtained QDs were characterized by florescent emission spectra, UV–vis absorption spectra, TEM and DLS. As shown in Fig. 1(A), the absorbance spectrum of the QDs was wide and the florescent spectrum was very narrow, with full width at half maximum being about 20 nm, which resulted from narrow size distribution of QDs. DLS diagram (Fig. 1 (B)) and TEM image (Fig. 2(A)) demonstrated that the average size of the QDs was 7.0 nm. As can be seen in Fig. 2(A), the particles were round.

The X-ray pattern of cysteamine@CdTe/ZnS displayed the specification zinc blend planes of 311, 220, and 111 at 48.76°, 41.72°, and 24.94° for CdTe/ZnS, respectively [26]

The FT-IR spectra of free cysteamine (Fig. 2(D)), showed a stretching vibration peak of its S—H group at ~2500 cm^{-1} and the stretching vibration of N—H (—NH_2) and O—H (H_2O molecules) in the limited area of 3000–3500 cm^{-1}. The peak at 1600 cm^{-1} could be obviously allotted to the stretching type of the NH_2 group. In the FT-IR spectra of the cysteamine@CdTe/ZnS QDs (Fig. 2(E)), the S—H stretching vibration peak was not observed at about 2500–2600 cm^{-1}. Other cysteamine peaks were observed at 1600 and 3500, so cysteamine was covalently bounded by —SH on the surface of QDs.