Talanta 188 (2018) 644–650

Contents lists available at ScienceDirect

Talanta

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

Ultrasensitive determination of mercury ions (II) by analysis of the degree of quantum dots aggregation

Wenli Gao[a], Yifei Xu[a], Wei Wei[a], Da Wang[a], Xingbo Shi[a,b,*]

[a] Hunan Provincial Key Laboratory of Food Science and Biotechnology, College of Food Science and Technology, Hunan Agricultural University, Changsha, Hunan 410128, China

[b] State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, China

ARTICLE INFO

Keywords:
Mercury
Quantum dots
Degree of aggregation
Spectral imaging

ABSTRACT

In this work, an ultrasensitive fluorescence strategy on counting the degree of aggregation (DOA) of QDs aggregates was developed for Hg^{2+} detection, which is based on thymine-Hg^{2+}-thymine (T-Hg^{2+}-T) coordination chemistry. Oligonucleotides containing 12 thymines are modified on the surface of QDs which could protect QDs effectively from aggregation in the absence of Hg^{2+}. While in the presence of Hg^{2+}, QDs will be made to attach with each other by the structure of "T-Hg^{2+}-T" formed, and then aggregated in the sensing system. The DOA is acquired easily by recording the entire process of the blue shifting and photobleaching of QDs aggregates. First-order spectrum of each QD in the aggregates can be released from the original overlapped spectral image of the aggregates in sequence under continuous illumination because QDs exhibit the asynchronous spectra blue shifting. Employed 0.3 nM functional QDs in the sensing system, an ultralow detection limit of 4.6 pM was reached by the counting DOA-based sensing strategy. Besides, the sensor also shows high selectivity against other 12 metal ions even at high concentrations. At last, the application of the sensing strategy for river water shows that the real samples can be worked well using this method.

1. Introduction

Mercury is a widespread pollutant with high toxicity to human beings even at a very low concentration [1]. And it has been recognized that mercury accumulating in the human body can cause acute and permanent brain damage throughout the food chain [2]. Therefore, many current researchers have been paid considerable attention to develop efficient detection methods for Hg^{2+} with high sensitivity and selectivity. In 2004, the fact that Hg^{2+} specifically interacts with thymine forming the thymine-Hg^{2+}-thymine (T-Hg^{2+}-T) coordination has been proved by Ono and Togashi [3], and has stimulated many developments for DNA-based Hg^{2+} sensing strategies, including electrochemical [4–9], colorimetric [10–14] and fluorescent [15–23] strategies. However, these strategies still hold various deficiencies and limit the detection for Hg^{2+}.

Most of electrochemical sensors have to face difficulties that electrodes are required for complicated chemical modification [9] and a proper selectivity cannot be provided in the presence of some potential interferes like Cu^{2+} [24] and Cd^{2+} [25]. As for colorimetric sensors, how to improve the detection sensitivity is the primary considering problem for Hg^{2+} detection [10–14,26–30], because the limits of detection (LODs) of these methods are mostly either non-comparable to or slight lower than the allowable maximum levels (10 nM) in drinking water permitted by the U.S. Environmental Protection Agency (EPA). The fluorescence sensors, as a more promising method due to their better sensitivity compared with the colorimetric sensors, have been developed into many strategies by associating other techniques, such as normal S_N2 mechanism [21], hybridization chain reactions [16], fluorescence resonance energy transfer (FRET) [15,23] and nicking endonuclease [23].

However, there still remains great potential here for further improving the detection sensitivity. Au nanoparticles (Au NPs) and quantum dots (QDs), due to their excellent optical properties [31,32], are usually employed as colorimetric probe and fluorescence probe in biosensors, respectively. Most of their sensing strategies are based on the NPs aggregation/disaggregation induced by the detection target [13,14,33], and only utilize the relationship between the color, absorptivity, intensity or other optical signal changes to realize the sensitive for Hg^{2+}, without precisely counting the degree of aggregation (DOA) of NPs in the sensing systems. Therefore, the accuracy and sensitivity of these sensors are bound to be reduced greatly due to the loss of signal during the transformation process.

* Corresponding author at: Hunan Provincial Key Laboratory of Food Science and Biotechnology, College of Food Science and Technology, Hunan Agricultural University, Changsha, Hunan 410128, China.
E-mail address: shixingbo123@aliyun.com (X. Shi).

https://doi.org/10.1016/j.talanta.2018.06.039
Received 31 January 2018; Received in revised form 6 May 2018; Accepted 11 June 2018
Available online 12 June 2018
0039-9140/

Directly measuring the DOA of the NPs aggregation may avoid largely the loss of signal and further improve the detection accuracy and sensitivity. But it is difficult to measure the DOA of Au NPs aggregation without the help of the electron microscope analysis or optical signal fitting [34]. Fortunately, accurate measuring the DOA of QDs aggregates has been achieved in our previous work [35]. We found that photobleaching and blue shifting of individual QD among QDs assembly is asynchronous [35,36], and successfully utilized this unique properties to counting the number of QDs in an aggregate [35]. This achievement provides an opportunity to exploit novel sensors based on measuring the DOA of QDs aggregates.

In this paper, we developed a novel, ultrasensitive fluorescence method for Hg^{2+} detection, which is based on T-Hg^{2+}-T coordination inducing QDs aggregated and counting the DOA of QDs aggregates. Oligonucleotides containing 12 thymines were employed to modify the surface of QDs, and effectively protected QDs from aggregation in the absence of Hg^{2+}. While in the presence of Hg^{2+}, the QDs in the sensing system could aggregate due to the T-Hg^{2+}-T structure and which causes the increment of DOA gradually. By monitoring the DOA changes along with the increasing the Hg^{2+} concentration, we obtained an ultralow LOD of 4.6 pM, which largely lower than the U.S. EPA standards for drinkable water (10 nM). To the best of our knowledge, this work is the first time to analyse the DOA of QDs aggregates for Hg^{2+} detection.

2. Materials and methods

2.1. Materials and reagents

$Hg(NO_3)_2$ and other 12 metal salts ($FeCl_2{\cdot}4H_2O$, KBr, $ZnSO_4{\cdot}7H_2O$, $CaSO_4{\cdot}2H_2O$, $AlCl_3$, $MgCl_2{\cdot}6H_2O$, $Fe_2(SO_4)_3$, $CrCl_3{\cdot}6H_2O$, $NaSO_4$, $SnCl_2{\cdot}2H_2O$, $CrCl_2{\cdot}2.5H_2O$, $Pb(CH_3COOH)_2{\cdot}3H_2O$) were of analytical grade and were obtained from local reagent suppliers. QDs (carboxyl Qdots QD655 ITK) were purchased from Invitrogen/Molecular Probes (Eugene, OR). The polythymine oligonucleotide (DNA-T12: 5′NH_2-TTTTTTTTTTTT-3′) were synthesized in Sangon Biotechnology Co. Ltd. (Shanghai, China). N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich Co. Ltd. All of these metal salts were dissolved in ultrapure water prepared with a Millipore system and were filtrated with 0.45 μm membrane. The quantum dots were carried out in borate buffer solution (BBS buffer, 0.1 mM, pH 8.0).

2.2. Preparation of T12-functionalized QDs

Carboxyl-linked amino method was utilized to covalently conjugate between carboxyl QDs and polythymine oligonucleotides. A solution of carboxyl QDs (80 nM 20 μL) were mixed with excessive DNA-T12 (100 μM 6.4 μL), and then carbodiimide cross-linking agent EDC (40 μM 32 μL) and amine-reactive synergist NHS esters (10 mM 3 μL) were added into the mixture. The volume of this mixture was kept at 400 μL by adding BBS buffer (0.1 mM, pH 8.0). The cross-linking reaction was continued for 40 min at 45 ℃ under slight shaking. The DNA-T12-modified QDs complexes were separated from the free DNA-T12 by centrifugation using an ultra-filtration tube (UFC510096, Millipore, USA) at 2900 *g* for 5 min. Subsequently, the DNA-T12-modified QDs complexes were collected by centrifuging the reversing ultra-filtration tube at 100 *g* for 10 min. For sensing Hg^{2+} ions, the functionalized QDs was diluted to 3 nM, 0.6 nM and 0.3 nM using BBS buffer.

2.3. Formation of QDs aggregate

Two microlitres of functional QDs in BBS buffer (0.1 mM, pH 8.0) and 2 μL metal ions (such as Hg^{2+}, Fe^{2+}, K^+, Zn^{2+}, Ca^{2+}, Al^{3+}, Mg^{2+}, Fe^{3+}, Cr^{3+}, Na^+, Sn^{2+}, Cr^{2+} and Pb^{2+}) solution with different concentrations were mixed on the glass slide surface and immediately covered with a coverslip. To avoid water evaporation, bits of nail oil were utilized to seal the seam between the glass slide and coverslip. After the mixture on the glass slide surface reacted 5 min incubation at 45 ℃ water bath, the QDs aggregates were allowed to counting the DOA.

For the practical application, BBS buffer (0.1 mM, pH 8.0) was replaced by the river water. Other assay protocols were done as the same as the foregoing protocols. The river water was collected from Liuyang river near our university.

2.4. Spectral Imaging of QD aggregates

Observation of the QD aggregates on the glass slide were performed on an upright fluorescence microscope (Olympus BX 51, Japan) equipped with a 100× oil immersion objective (NA = 1.45, UPLSA-PO, Olympus, Tokyo, Japan). Fluorescence images of the QDs aggregates were captured by an electron-multiplied charge-coupled device (EMCCD, iXon3 DV897, Andor Technology, Northern Ireland). The camera's gain and temperature were set at 100 and − 75 ℃, respectively. The exposure time was set at 0.1–5 s. A transmission grating with 70 lines/mm (Edmund Scientific, Barrington, NJ) was placed in front of the EMCCD camera to obtain the zeroth-order spot and the first order streak of QD aggregate. To completely monitor the process of blue shift, we chose a long-pass filter (500–800 nm, Semrock, Rochester, USA) as the emission filter.

2.5. Transmission electron microscope (TEM)

TEM images were observed by using a high-resolution transmission electron microscope (JEOL JEM-2100).

2.6. Data analysis image processing

Data were processed using Image J software (National Institutes of Health, USA) and Origin software. For the SD of DOA, We chose five and more images to counting the DOA, and analysis of each image can provide a DOA. The five and more images contain one hundred aggregates at least. These values were based on to count the average DOA and SD.

3. Results and discussion

3.1. Basic principle of counting the DOA of QDs aggregates

The number of organic dyes in an assembly can be observed by single-step photobleaching. However, it cannot be used to count the number of QD in a QDs assembly. Because fluorescence intensity of each QD is heterogeneous, that hides the discrete steps in blinking. However, the blue shifting photo-property caused by photooxidation of the core [37,38] can help to resolve the challenge of counting QDs aggregates. Because photobleaching and blue shifting of QDs in the aggregate are asynchronous, that is due to the heterogeneity of QDs during synthesis and the different orientation of dipole moments in the aggregate,

In our previous works [39,40], we used the spectral imaging to monitor the spectra of single QDs and single Au NPs. Briefly, a transmission grating was placed in front of the EMCCD camera (Fig. 1**A**). The fluorescence image is divided into the zeroth-order spot and the first-order streak, and recorded on the same EMCCD chip. According to the equation $L = \lambda S/d$ (λ, fluorescence wavelength; S, distance between the grating and the EMCCD chip; d = 1/70 mm, grating constant), the distance between the orders (L) is related to the emission wavelength, so that monitoring the changes of L can distinguish the variation in QDs emission spectra. Movement of the first-order streak towards the zeroth-order spots indicates that the QDs are blue shifting. Fig. 1**B** is a typical fluorescence spectral image of QDs aggregates that was taken in the middle of the blue shifting process. In this image, we easily