Talanta 176 (2018) 277–283

Contents lists available at ScienceDirect

Talanta

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

Facilely self-assembled magnetic nanoparticles/aptamer/carbon dots nanocomposites for highly sensitive up-conversion fluorescence turn-on detection of tetrodotoxin

Hui Jin[a], Rijun Gui[a,*], Jie Sun[b], Yanfeng Wang[b]

[a] College of Chemistry and Chemical Engineering, Laboratory of Fiber Materials and Modern Textile, The Growing Base for State Key Laboratory, Qingdao University, Qingdao 266071, PR China

[b] Institute of Materia Medica,Medica, Shandong Academy of Medical Sciences, Jinan 250062, PR China

ARTICLE INFO

Keywords:
Aptamer
Carbon dots
Up-conversion fluorescence
Tetrodotoxin
Detection

ABSTRACT

In this work, magnetic Fe_3O_4 nanoparticles (NPs) were modified with aptamer of tetrodotoxin (TTX) to form Fe_3O_4/aptamer complex. The self-assembling of carbon dots (CDs) and the aptamer generated Fe_3O_4/aptamer/CDs nanocomposites. The nanocomposites exhibited down-conversion fluorescence and up-conversion fluorescence (UCF) emissions simultaneously. When excited at a wavelength of 780 nm, the UCF (peaked at 475 nm) of nanocomposites increased regularly with the increase of TTX concentration [TTX]. In the [TTX] range from 0.1 ng mL^{-1} to 0.1 mg mL^{-1}, UCF peak intensities almost linearly increased with the increase of Log [TTX], with a high linear coefficient (R^2) of 0.9975 and a low detection limit of TTX (0.06 ng mL^{-1}). The nanocomposites were developed as a novel UCF turn-on probe of TTX. Experimental results confirmed the highly selective and sensitive UCF responses of the probe on TTX, over potential interferences. In real samples, the probe of TTX showed superior analysis performance with high detection recoveries. Based upon the combined advantages from spectrofluorimetric methods, CDs and UCF, the novel probe of TTX would be superior especially in real biological sample analysis, when compared to previous methods for the detection of TTX.

1. Introduction

Tetrodotoxin (TTX) belongs to one of potent nonpeptidic neurotoxins and extensively exists in different species, including vertebrates (puffer fish, goby, newt, frog, etc) and invertebrates (crab, starfish, blue-ringed octopus, flatworm, gastropod, etc) [1]. TTX mainly exists in different species of puffer fishes, especially concentrated in the liver and ovary [2–4]. TTX is frequently involved in the fatal food poisoning of humans, which most often results from the ingestion of delicious puffer fishes. Generally, TTX in puffer fishes cannot be destroyed only under cooking conditions. TTX can selectively block Na^+ channels on nerve cell membranes and further leads to nerve paralysis, respiratory failure and eventual death [2,5,6]. As a highly toxic neurotoxin, TTX at a low dose of 0.5–3 mg can make an adult die of poisoning. Through intraperitoneal injection, the median lethal dose (LD_{50}) of TTX in mammals is 2–10 μg kg^{-1}. Moreover, TTX can be used in neurophysiology because it enables the relieving of pain in clinic [4,5]. Hence, it is significant to develop a simple, low-cost, highly selective and sensitive method for TTX detection, which has attracted increasing attention in recent years.

Currently, some traditional methods based on different principles have been developed for TTX detection, including mouse bioassay, surface plasmon resonance (SPR) assay, high performance liquid chromatography (HPLC) assay, liquid chromatography-mass spectrometer (LC-MS) assay, liquid chromatography-mass spectrometer-electrospray ionization (LC-MS-ESI) assay, enzyme- linked immunosorbent assay (ELISA), immunoaffinity chromatography assay, genotoxicity assay, chemoprobe selective assay, and so forth [4,7–14]. These traditional methods are often restricted due to low efficiency and lacking practicality. For instance, the mouse bioassay is time-consuming and labor-intensive. The SPR, genotoxicity and chemoprobes selective assays are often limited in laboratory scientific researches. HPLC, LC-MS and LC-MS-ESI assays usually require expensive equipments, tedious sample preparation, as well as the requirement of skilled analysts. ELISA and immunoaffinity chromatography assays commonly need complicated steps and high cost. Because these traditional analysis methods possess a variety of drawbacks, currently the achievement of a simple, economic and highly efficient method serving as an alternative method for TTX detection has become a challenging research work.

* Corresponding author.
E-mail addresses: guirijun@163.com, guirijun@qdu.edu.cn (R. Gui).

http://dx.doi.org/10.1016/j.talanta.2017.08.043
Received 19 April 2017; Received in revised form 4 August 2017; Accepted 11 August 2017
Available online 14 August 2017

Notably, the electrochemical probe (ECS) methods of TTX were reported firstly [15]. A glassy carbon electrode was modified with a selective aptamer immobilized on the electroactive polymer platforms. The aptamer served as a biorecognition probe and was used for the amperometric and impedimetric determination of TTX. Most recently, an electrochemiluminescence (ECL) probe was developed at a Nafion-graphene-Ru(bpy)$_3^{2+}$ composite-modified electrode for TTX detection [5]. By contrast, spectrofluorimetric methods are of great interest due to their unique advantages, such as simplicity, rapidity, low cost and high sensitivity. However, to date there is still no report that refers to the spectrofluorimetric method for TTX detection.

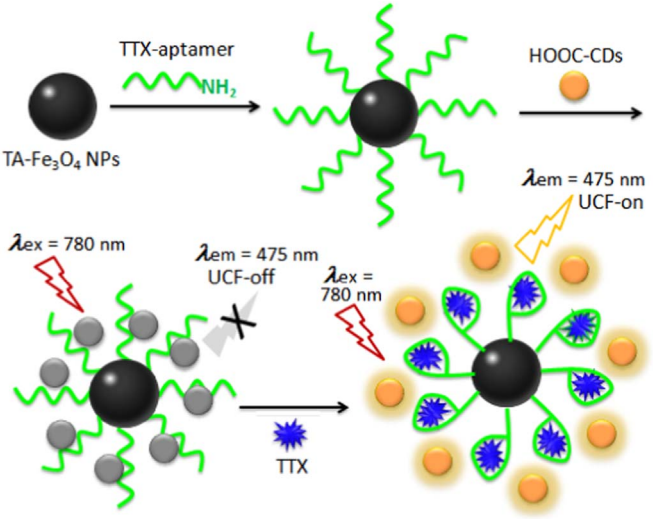
During the past decade, carbon dots (CDs) have served as one of the ideal alternatives of other fluorescent nanomaterials, originating from ultralow toxicity, biocompatibility, low cost and high stability of CDs [16]. In addition to the traditional down-conversion fluorescence (DCF), CDs also possess unique up-conversion fluorescence (UCF) [17,18]. UCF emission is an interesting optical property wherein the emission wavelength is shorter than its corresponding excitation wavelength, whereas DCF (usually so-called fluorescence) has the opposite situations [19,20]. Especially, the excitation wavelength in the near-infrared (NIR, 700–1000 nm) region is attractive, which helps to UCF applications in biomedical systems, owing to improved photon-penetration, reduced photon- damage, minimized background fluorescence and autofluorescence. Thus, the utilization of UCF allows for sensitive bio-sensing and high resolution bio-imaging [21]. In view of these combined advantages from spectrofluorimetric methods, CDs and UCF, herein we put forward a novel CDs- based UCF probe of TTX. In previous reports, CDs-based UCF sensing was mainly used for cell imaging [22–25]. Only two papers referred to CDs-based UCF detection of ClO^- and H_2S [18,26]. So far, there is still no report relative to CDs-based UCF sensing of TTX.

As artificial oligonucleotides (DNA/RNA), aptamers can specifically bind to target molecules, with high selectivity, specificity and affinity. Compared with other natural receptors (antibody or enzyme), aptamers have remarkable advantages, such as easy production by chemical synthesis, commercial acquisition and satisfactory stability. The structural flexibility of aptamers allows for adaptation of significant conformational changes, once the binding of aptamers with their targets. Thus, aptamers as ideal bio-sensing molecules have been exploited in biorecognition applications of toxins, proteins, cancer cells, drugs and other organic or inorganic molecules [27,28]. In this article, thiodiglycolic acid (TA)-stabilized Fe_3O_4 nanoparticles (NPs) were combined with -NH_2 terminated TTX-aptamer to generate Fe_3O_4/aptamer complex *via* carbodiimide-activated coupling (Scheme 1). CDs with ample -COOH on the surface were prepared from one-step hydrothermal treatment of fresh juice of black fungus, and then were added in Fe_3O_4/aptamer complex to form Fe_3O_4/aptamer/CDs nanocomposites through π-π stacking self-assembling between aptamer and CDs [29]. Upon excitation at 780 nm, the obvious decreased UCF emission (peaked at 475 nm) of Fe_3O_4/aptamer/CDs nanocomposites was observed when compared with that of CDs, attributed to photo-induced electron transfer (PET) from CDs to aptamer [30,31]. With the addition of TTX, the strong binding affinity between TTX and its aptamer could induce the unwinding of CDs from TTX-aptamer, together with the UCF recovery of CDs. Based on the competitive assembling and the unwinding between TTX-aptamer and CDs, Fe_3O_4/aptamer/CDs nanocomposites-based UCF probe enabled the highly sensitive and selective detection of TTX. The probe would be especially suitable for TTX detection in biological samples.

2. Experimental section

2.1. Materials

TTX-aptamer (*5′-NH_2-AAAAATTTCACACGGGTGCCTCGGCTGTCC-3′*) was synthesized and purified by Shanghai Sangon Biotech Co., Ltd., China. Aflatoxin B_1-B_2 (AFB_1, AFB_2), botulism neurotoxins A-B (BoNTA, BoNTB), Staphylococcus aureus enterotoxins A-B (SEA, SEB) and TTX were bought from Shanghai Sangon Biotech Co., Ltd., China. Histidine, cysteine, uric acid, ascorbic acid, glucose, thiohydracrylic acid, glutathione, *N*-hydroxysuccinimide sodium (NHS) and 1-ethyl-(3-(3-dimethylamino)propyl) carboxylate hydrochloride (EDC) were purchased from Sigma-Aldrich. Other chemicals with analytical reagent were obtained from Shanghai Sinopharm Chemical Reagent Co., Ltd., China. All chemicals could be directly used as received without any further purification.



Scheme 1. Schematic illustration of the fabrication procedures of Fe_3O_4/aptamer/CDs nanocomposites-based UCF turn-on probe of TTX.

2.2. Preparation of Fe_3O_4 NPs and CDs

TA-stabilized Fe_3O_4 NPs were prepared based on a modified version of reported methods [32]. Detailed preparation and purification steps are available in Supplementary material (Part S1). CDs were prepared by directly hydrothermal treatment of fresh black fungus. Typically, 10 g of fresh black fungus was broken into black fungus juice in distilled water. Then, 10 mL of black fungus juice was transferred into 50 mL Teflon-lined autoclave, which was heated at 180 °C for 2 h. At room temperature, the products were filtered with a 0.22 μm membrane, and washed with ethanol and dichloromethane to move unreacted components. The resulting upper aqueous suspension was collected, and treated by evaporating water and precipitated with acetone. Crude precipitates were collected by centrifugation and washed with ethanol repeatedly. Final precipitates were collected and dried to achieve the dry samples of CDs, or dispersed in distilled water to achieve the aqueous suspension of CDs for subsequent experiments.

2.3. Preparation of Fe_3O_4/aptamer and Fe_3O_4/aptamer/CDs

Fe_3O_4/aptamer complex was prepared through carbodiimide-activated coupling of TA-stabilized Fe_3O_4 NPs with TTX-aptamer. Briefly, 15 mg of EDC and 9 mg of NHS were added in 5 mL of phosphate buffer saline (PBS, 1 mM, pH7.4) containing 10 mg Fe_3O_4 NPs to form homogeneous mixture, which was treated by sonication for 30 min in the dark. Under magnetic stirring, TTX- aptamer (50 μL, 50 μM) dispersed 5 mL of PBS was added in the mixture, followed by continuous reaction for 6 h. The reaction mixture was precipitated by adding acetone, and precipitates were collected by centrifugation and washed with ethanol repeatedly. The following purification steps were similar to that of CDs. In the preparation of Fe_3O_4/aptamer/CDs nanocomposites, 10 mL of PBS containing 5 μg mL^{-1} of CDs was drop-by-drop added into 10 mL of PBS containing the Fe_3O_4/aptamer