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Single-walled carbon nanotubes based quenching of free FAM-aptamer for selective determination of ochratoxin A

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ABSTRACT

Ochratoxin A, a toxin produced by *Aspergillus ochraceus* and *Penicillium verrucosum*, is one of the most abundant food-contaminating mycotoxins in the world. It has been classified by the International Agency for Research on Cancer (IARC) as a possible human carcinogen. In this paper, a sensitive and selective fluorescent aptasensor for ochratoxin A (OTA) detection was constructed, utilizing single-walled carbon nanotubes (SWNTs) as quencher which can quench the fluorescence of free unfolded toxin-specific aptamer attached with FAM (carboxyfluorescein). Without any coating materials as compared to graphene-oxide based sensor, we obtained the detection limit of our sensing platform based on SWNTs to be 24.1 nM with a linear detection range from 25 nM to 200 nM. This technique responded specifically to OTA without interference from other analogues (N-acetyl-L-phenylalanine, warfarin and OTB). It has also been verified for real sample application by testing 1% beer containing buffer solution spiked with a series of concentration of OTA.

1. Introduction

Nowadays, a variety of nanomaterials such as nanoparticles, nanotubes and nanowires have been used to create new types of analytical tools for life science and biotechnology [1]. Carbon nanotubes have emerged as one of the most extensively studied nanomaterials due to their unique chemical, electrical, and mechanical properties [2,3]. The potential for application of carbon nanotubes ranges from molecular electronics to ultrasensitive biosensors. Compared to graphene-oxide which has flat surface, single-walled carbon nanotube has curved surface which may lower down the unspecific adsorption. In previous study [4], we have shown that uncoated graphene oxide has very poor sensitivity. Although coated graphene-oxide has significantly improved the detection limit, uncoated material which provides a simple approach and comparable detection limit is highly desirable.

The superpower of the above-mentioned carbon nanomaterials lies in that they can quench the fluorescence of the adsorbed dyes, which offers great opportunities for building a variety of biosensors especially when in combination with aptamer technology [5]. In recent years, aptamers as newly emerging molecular recognition agents have been extensively studied [6]. Aptamers are single strand DNA that can bind target molecule with high specificity and strong binding affinity [7,8]. As compared to antibody, aptamer possesses several advantages which attract extensive research in recent years [5,9,10]: easily synthesized, commercially available, flexible modification with a variety of groups and good stability [11]. Until now, there are several reports which have demonstrated the powerful potential of biosensors based on combination of single-walled carbon nanotube and dye-modified DNA (or aptamer) [12–16]. Analytes such as ATP, cocaine, Hg^{2+} and DNA have been systematically investigated by using the above-mentioned approach. Among these experiments, the target analyte induced the DNA probes to be detached from single-walled carbon nanotube due to the strong binding between target and DNA probes. In our study, we will show that this sensing strategy can also be applied to detect target which has much weaker interaction with aptamer probes.

Recently, aptamer selected for specific binding with OTA has been used to construct different versions of biosensors. As compared to those methods based on toxin-specific antibody [17–20], biosensors based on aptamer specific for OTA are highly flexible [4,21–32]. Although in recent two years more and more papers come out about the detection of OTA by utilizing aptamer, those procedures and modification steps are still not simple and straightforward enough, which always need more than one DNA probe and more expensive labeling.

OTA, as one of the most abundant food-contaminating mycotoxins in the world [33,34], was chosen as the prototypic analyte in this paper. Human exposure to OTA occurs mainly through consumption of improperly stored food products, particularly contaminated

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grain and pork products, as well as coffee and wine grapes [35]. This toxin has also been found in the body of animals, including human blood and breast milk [36]. OTA can cause cancer in human beings [37]. It has been shown to be weakly mutagenic and can cause immunosuppression as well as immunotoxicity [34,38,39]. With the recognition of severe toxic effect of this fungi toxin, a vast amount of study have been devoted to develop simple sensing platforms to detect this fungi toxin [40].

In this study, in combination with single-walled carbon nanotube, an aptamer specific for OTA was utilized, which can fold to form antiparallel G-quadruplex structure upon exposure to OTA [21,41]. The formation of antiparallel G-quadruplex structure will prevent the adsorption onto the SWNTs. Coexistence of unfolded single-stranded DNA (ssDNA) and SWNTs will favor the spontaneous self-assembly in aqueous solution via hydrophobic driving force between DNA bases and SWNTs sidewall [42]. By employment of dye-labeled DNA as the recognition component, the strong interaction between ssDNA and SWNTs can bring the dye close to SWNTs, which, in turn, results in complete quenching [13]. Therefore we designed a sensing strategy for detection of OTA: FAM (carboxyfluorescein) modified aptamer after binding to OTA cannot be quenched by SWNTs, however, FAM modified aptamer without binding to OTA could be significantly quenched. Indeed, the results in our experiment also confirmed that our design strategy was very successful. By combining SWNTs with aptamer modified in single end, the major advantage is that it averts expensive dual labeling of aptamer in comparison to conventional molecular beacon. Furthermore, the unspecific adsorption of target molecules onto the single-walled carbon nanotubes based sensor is extremely low compared to the case for graphene oxide [4], which needed polymer coating.

2. Experimental

2.1. Materials and reagents

OTA's aptamer (5′-GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA-FAM-3′) were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Single-walled carbon nanotubes were purchased from Chengdu Organic Chemicals Co. Ltd. (Chengdu, China). DNA stock solution was prepared by dissolving oligonucleotides in 10 mM Tris buffer (pH 8.5) containing 120 mM NaCl and 5 mM KCl and 20 mM $CaCl_2$ and was stored at 4 °C before use. The concentration of oligonucleotide was quantified using the absorbance at 260 nm. Tris ($C_4H_{11}NO_3$) was purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). OTA, *n*-acetyl-L-phenylalanine (NAP) and warfarin were obtained from Sigma–Aldrich (St. Louis, MO, USA). Ochratoxin B (OTB) was obtained from BioAustralis Pty Ltd (NSW, Australia). The stock solution of OTA (1 mM) was prepared by dissolving ochratoxin in dimethyl sulfoxide (DMSO) and stored at −20 °C. Sodium chloride (NaCl), potassium chloride (KCl) and anhydrous calcium chloride ($CaCl_2$) were purchased from Beijing Chemical Reagent Company (Beijing, China). Ginsber Beer was produced by SiPing Ginsber Beer Co. Ltd. (SiPing, China). All other chemicals were of analytical reagent grade and were used as received without further purification. Solutions were prepared with deionized (DI) water processed with a Milli-Q ultra-high purity water system (Millipore, Bedford, MA, USA).

2.2. Instrumentation

Cary 500 Scan UV/Vis Spectrophotometer (Varian, USA) was used to quantify the oligonucleotides. A JASCO J-810 spectropolarimeter (Tokyo, Japan) was utilized to collect the circular dichroism (CD) spectra in the 10 mM Tris buffer (pH 8.5). Fluorescence intensities were recorded on a LS-55 luminescence spectrophotometer (Perkin-Elmer, USA). The emission spectra were recorded in the wavelengths of 500–630 nm upon excitation at 492 nm with slit widths for the excitation and emission set at 2.5 and 15 nm, respectively.

2.3. Analysis of aptamer conformation with CD

CD spectropolarimeter using solutions of DNA aptamer (1 μM) in a optical chamber (1 cm path length, 1 mL volume) which was deoxygenated with dry purified nitrogen (99.99%) before use and kept in the nitrogen atmosphere during experiments. Each CD spectrum was the accumulation of three scans at 200 nm/min with a 1 nm band width and a time constant of 1 s. Data was collected from 230 nm to 340 nm at 0.1 nm intervals. The background of the buffer solution was subtracted from the CD data.

2.4. Fluorescent detection of OTA

For quantitative measurement of OTA, 200 μL solution containing 400 nM concentration of FAM-modified aptamer was mixed with 400 μL solution of different concentrations of OTA, and allowed to settle for 15 min. Then, 200 μL of solution with 100 μg/mL SWNTs was added and the total final volume was 800 μL. After the solution was equilibrated for 30 min, fluorescence measurement was carried out.

3. Results and discussion

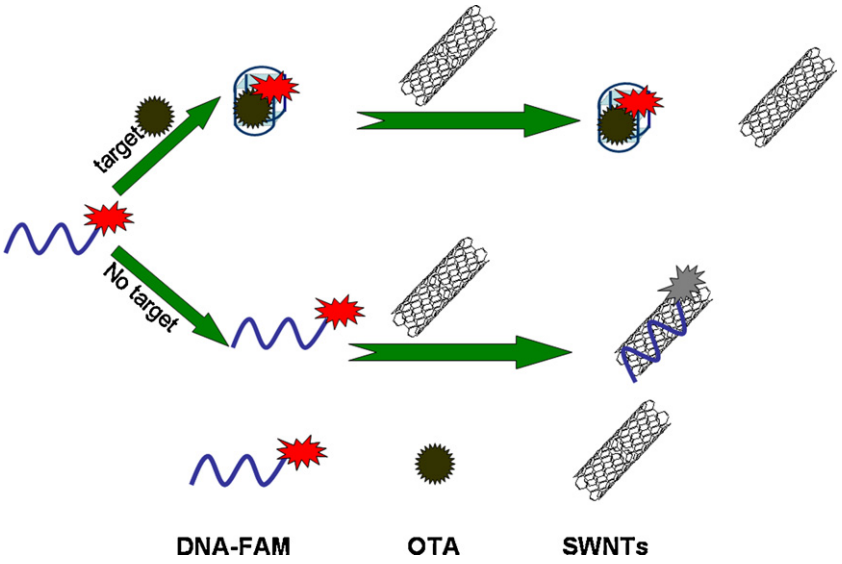
3.1. Design strategy for ochratoxin detection

Scheme 1 illustrates the sensing strategy for detection of OTA. In the absence of target molecule (OTA), FAM-modified aptamer is wrapped around SWNT through π–π stacking interaction between the nucleotide bases and the SWNT sidewall to form stable complex [12,43]. Consequently, the fluorescence of FAM is quenched readily via energy transfer from dye to carbon nanotube [13]. In the presence of target molecules (OTA), the conformation of the aptamer specific for OTA is switched from a random coil to an antiparallel G-quadruplex which is resistant to being wrapped onto the carbon nanotube [21,41]. Therefore, the fluorescence of FAM cannot be quenched and the fluorescent intensity as a function of OTA concentration was measured correspondingly.

CD measurement was utilized to monitor the conformation change of FAM-modified aptamer in different cases (Fig. 1). Before addition of OTA into the solution containing aptamer, the CD spectrum of aptamer showed the characteristic of a random coil DNA (curve a in Fig. 1). Upon adding OTA of 1 μM, the CD spectrum of aptamer had a greatly enhanced positive band at 290 nm and a greatly enhanced negative band at about 265 nm (curve b in Fig. 1). This is the typical characteristic of antiparallel G-quadruplex structures [44]. This change of CD spectra should be attributed to the interaction of aptamer with OTA, leading to the increase in its ellipticity at 290 nm and 265 nm. It has to be noted that there was only small change of ellipicity at 245 nm upon addition of OTA.

3.2. Optimization of the concentration ratio of SWNTs to FAM-modified aptamer

The correct concentration ratio of SWNTs to FAM-modified aptamer is highly important. As shown in Fig. 2, the maximum peak intensity of fluorescence spectra decreased along with increasing the concentration of SWNTs at the fixed concentration of 100 nM FAM-modified aptamer. When the concentration of SWNTs



Scheme 1. Schematic illustration of SWNTs based sensing platform for detection of OTA.

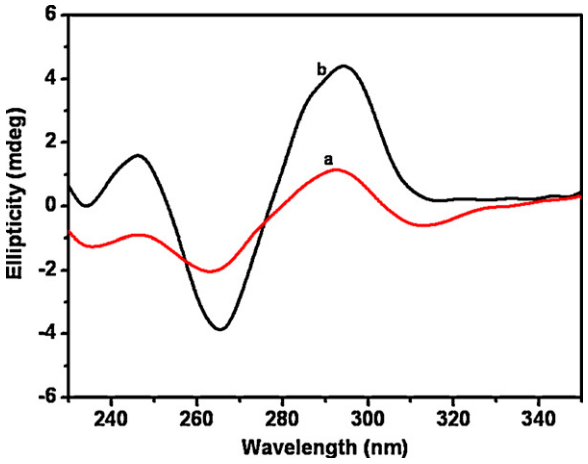


Fig. 1. CD spectra of aptamer (1 μM) at different conditions: (a) aptamer (1 μM) in 10 mM Tris buffer (pH 8.5); (b) aptamer (1 μM) + OTA (1 μM) in 10 mM Tris buffer (pH 8.5).

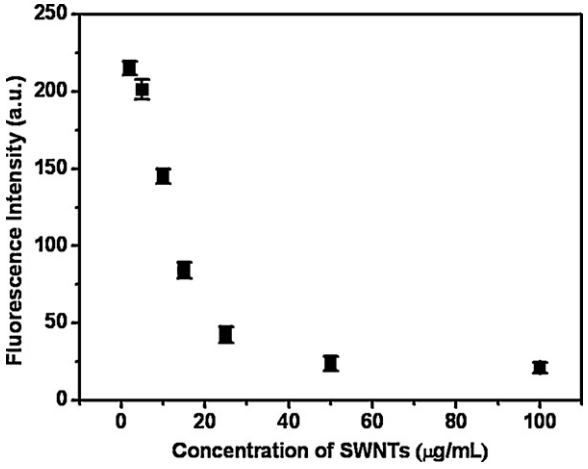


Fig. 2. Effect of SWNTs concentration on the quenching efficiency toward FAM-modified aptamer. The aptamer concentration is fixed at 100 nM, the optimum concentration of SWNTs is 25 μg/mL. Error bars were obtained from three experiments.

reached 25 μg/mL, 90% of the fluorescence intensity of FAM-modified aptamer was quenched. Since higher concentration of SWNTs cannot quench the fluorescence intensity more efficiently, higher concentration than 25 μg/mL was not necessary. According to this result, we utilized the SWNTs with concentration of 25 μg/mL in the throughout experiment.

3.3. Quantitative measurement of OTA

The premixing of aptamer/OTA is important in this study [45], allowing sufficient freedom of the interaction between aptamer and OTA. As illustrated in Fig. 3A, fluorescence measurement showed that the fluorescence intensity in the solution increased along with increasing the concentration of OTA. Calibration curve of fluorescence intensity as a function of concentration from 25 nM to 5 μM was plotted (Fig. 3B). The limit of detection (LOD) was defined as the concentration corresponding to the fluorescence signal at 3 times standard deviation of blank without OTA. Then the limit of detection (LOD) was calculated to be 24.1 nM. With the existence of 100 nM aptamer and 25 μg/mL SWNTs in the binding buffer solution, the linearity of the fluorescence response to concentration of OTA was located between 25 nM and 200 nM (inset in Fig. 3B). Compared to the sensor based on graphene oxide, this new approach eliminated the coating process and the detection limit was even better than that obtained in the previous study [4].

3.4. Selectivity of SWNTs based sensing platform

In order to determine the specificity of this method, we tested the sensing platform against various structure analogues. N-acetyl-L-phenylalanine (NAP) whose molecular structure represents one part of OTA was used. Warfarin and OTA share the same similarity in the fact that they occupy the same binding site in human serum albumin [46]. Ochratoxin B that lacks the chlorine atom in the isocoumarin ring is a structural analogue of OTA. As shown in Fig. 4 (column 6), addition of target molecule (OTA) with concentration of 1 μM induced dramatic fluorescence increase; however, other analogues with concentration of 1 μM (columns 2– 4) did not induce apparent fluorescence increase. Therefore, this aptamer was highly specific to OTA, which was further confirmed by the

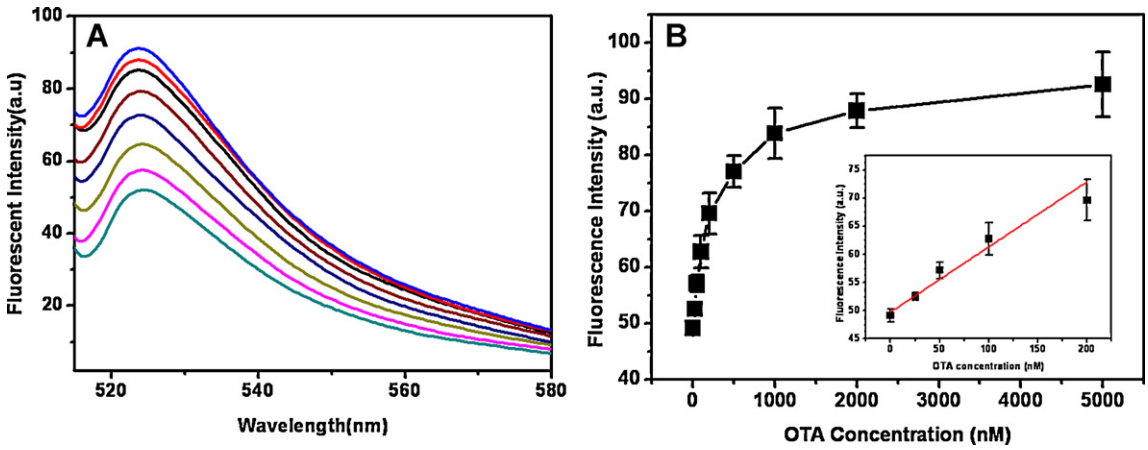


Fig. 3. (A) Fluorescence spectra of solutions corresponding to various concentrations of OTA under the condition of premixing of aptamer/ochratoxin before addition of SWNTs. (B) The maximum peak of fluorescence spectra in (A) were plotted as a function of concentration of OTA. Inset in B: linear part of the plot in B. Error bars were obtained from three experiments.

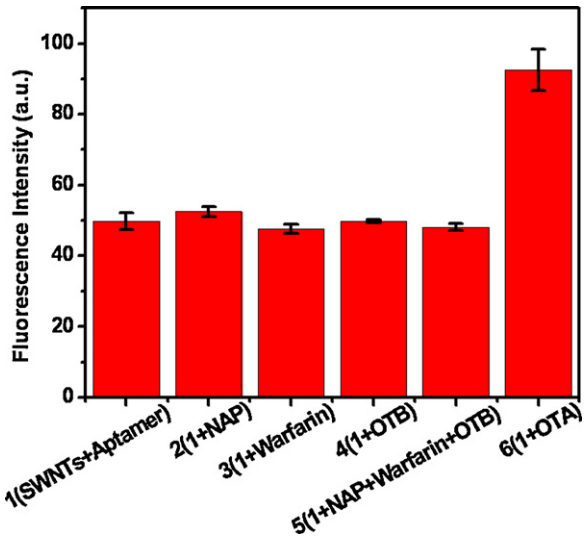


Fig. 4. Selectivity of the sensor toward OTA (1 μM) and against other structure analogues (1 μM). SWNTs (single-walled carbon nanotubes), OTA (OTA), OTB (ochratoxin B), NAP (N-acetyl-L-phenylanaline) and warfarin. Error bars were obtained from three experiments.

slight fluorescence response to the coexistence (column 5) of N-acetyl-L-phenylalanine (1 μM), warfarin (1 μM) and ochratoxin B (1 μM).

3.5. Practicability of SWNTs based sensing platform

In order to conform to the practicability for real sample analysis, we challenged our system with beer, which can be contaminated by ochratoxin A. Buffer solutions containing 1% beer were spiked with various concentration of OTA. Fig. 5A illustrates that the fluorescence intensity depends upon the concentration of OTA. Calibration curve of fluorescence intensity versus concentration of OTA was plotted (Fig. 5B). Concentration of OTA down to 25 nM was tested. With the existence of 100 nM aptamer, 25 μg/mL SWNTs in the binding buffer solution, the linearity of the fluorescence response to the concentration of OTA was from 25 nM to 200 nM. The sensing capability of this platform was much better than previously reported study with using graphene-oxide as quencher [44].

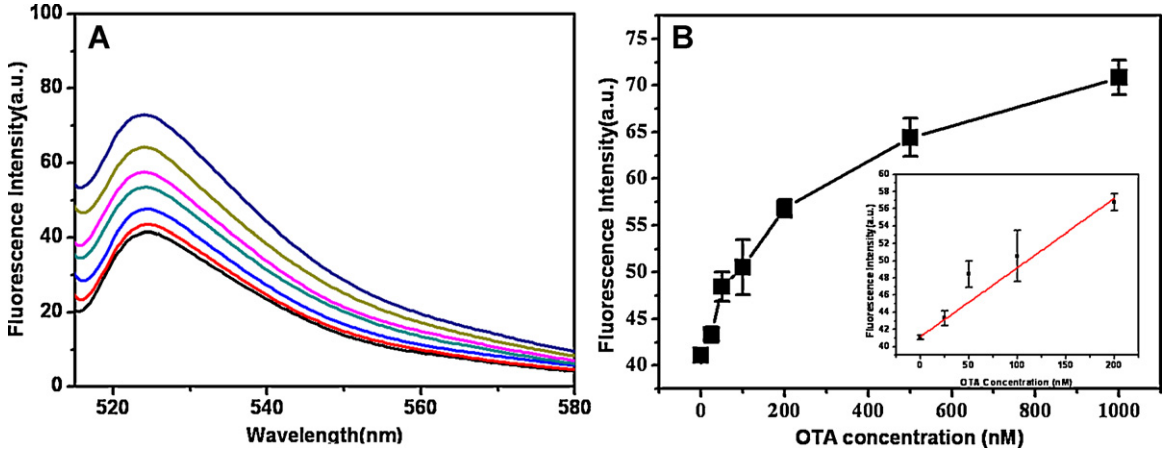


Fig. 5. (A) Fluorescence spectra of buffer solutions containing 1% beer spiked with various concentration of OTA under the condition of premixing of aptamer/ochratoxin before addition of SWNTs. (B) The maximum peak of fluorescence spectra in (A) were plotted as a function of concentration of OTA. Inset in B: linear part of the plot in B. Error bars were obtained from three experiments.

4. Conclusions

To summarize, we have developed a simple, selective and sensitive fluorescent aptasensor for OTA detection by using a dye-labeled aptamer and SWNTs. A limit of detection of 24.1 nM was achieved for detection of OTA. The advantages of this analytical method rely on its simple measurement performance, allowing measurements to be performed almost in a real-time manner even at room temperature. The integration of nanostructures such as SWNTs with the use of specific recognition molecules such as nucleic acids to recognize and bind target analytes in the sample matrix has shown a promising future for the detection of toxins and pathogens in food.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.08.015.

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