Food Chemistry 220 (2017) 527–532

Contents lists available at ScienceDirect

Food Chemistry

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

FRET-based aptamer biosensor for selective and sensitive detection of aflatoxin B1 in peanut and rice

Fereshte Sadat Sabet [a], Morteza Hosseini [a,b,*], Hossein Khabbaz [a], Mehdi Dadmehr [c], Mohammad Reza Ganjali [d,e]

[a] Department of Life Science Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran, Iran
[b] Medical Biomaterials Research Center, Tehran University of Medical Sciences, Tehran, Iran
[c] Department of Biotechnology, Payame Noor University, Tehran, Iran
[d] Center of Excellence in Electrochemistry, Faculty of Chemistry, University of Tehran, Tehran, Iran
[e] Biosensor Research Center, Endocrinology & Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 8 June 2016
Received in revised form 26 September 2016
Accepted 1 October 2016
Available online 4 October 2016

Keywords:
Aflatoxin B1
Aptasensor
FRET
Sensitive detection
Fluorescence
Quantum dots
Nanobiosensor
Food
Au nanoparticle

ABSTRACT

Aflatoxins are potential food pollutants produced by fungi. Among them, Aflatoxin B1 (AFB1) is the most toxic. Therefore, a great deal of concern is associated with AFB1 toxicity. In this work, utilizing a FRET-based method, we have developed a nanobiosensor for detection of AFB1 in agricultural foods. Aptamer-conjugated Quantum dots (QDs) are adsorbed to Au nanoparticles (AuNPs) due to interaction of aptamers with AuNPs leading to quenching effect on QDs fluorescence. Upon the addition of AFB1, the specific aptamers are attracted to AFB1, getting distance from AuNPs which result in fluorescence recovery. Under optimized conditions the detection limit of proposed nanobiosensor was 3.4 nM with linear range of 10–400 nM. Selectivity test demonstrates that the nanobiosensor could be a promising tool for specific evaluation of food stuff. This method was successfully applied for the analysis of AFB1 in rice and peanut samples.

1. Introduction

Aflatoxins (AFs) are toxic compounds produced as secondary metabolites by the fungi namely *Aspergillus flavus* and *Aspergillus parasiticus* (Yabe, Ando, & Hamasaki, 1988). Among more than 20 identified aflatoxins which contaminate several agriculture crops, Aflatoxin B1 (AFB1) is considered the most toxic (Nonaka, Saito, Hanioka, Narimatsu, & Kataoka, 2009). AFB1 has been classified as a Group I carcinogen in humans while having mutagenic, hepatotoxic and teratogenic effects in animal species (Bacaloni et al., 2008). There has been several reports on AFB1 outbreaks, in which the most shocking was reported in 2004 with 125 deaths in Kenya (Khayoon et al., 2010; Nonaka et al., 2009).

Considering its potential threat, many analytical methods have been developed for determination of AFB1 in various matrices including liquid chromatography (LC), thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) immunoaffinity chromatography (IAC), enzyme-linked immunosorbent assay (ELISA) and electrochemical immunosensor (Amaya-González, de-los-Santos-Álvarez, Miranda-Ordieres, & Lobo-Castañón, 2013; Bacaloni et al., 2008; Khayoon et al., 2010; Nonaka et al., 2009; Piermarini, Micheli, Ammida, Palleschi, & Moscone, 2007). However due to limitations associated with these methods, including extensive sample preparation, expensive procedure and unavailability for on-site screening, increasing demand has been emerged especially in developing countries for more simple and cost-effective methods.

Semiconductor quantum dots (QDs) as a new type of fluorescent probes, have unique optical characteristics such as photostability and high quantum yield originated from "quantum size" effect, and have been proven to be of many use in biosensing application (Alivisatos, 1996; Zhang, Xu, Zhang, Ji, & He, 2012).

Aptamers are artificially-synthesized single-stranded DNA or RNA sequences which can take secondary and tertiary structures allowing them to bind to specific targets with high affinity (Iliuk, Hu, & Tao, 2011). Since their report in early 1990s (Ellington &

* Corresponding author at: Department of Life Science Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran, Iran.
E-mail address: smhosseini@khayam.ut.ac.ir (M. Hosseini).

http://dx.doi.org/10.1016/j.foodchem.2016.10.004
0308-8146/

Szostak, 1990; Robertson & Joyce, 1990; Tuerk & Gold, 1990), aptamers have gained increasing interest due to their advantages including no limitation for their target and ease of reproduction (Kim, Raston, & Gu, 2016). In this work, utilizing a QD-aptamer-AuNP FRET system we have developed a sensitive nanobiosensor for detection of AFB1 in rice and peanut samples.

2. Experimental

2.1. Reagents

Hydrogen tetrachloroaurate (III) tetrahydrate, cadmium chloride hydrate, tellurium powder, N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and sodium citrate were purchased from Merck and all other commercially available substances were purchased from Aldrich and Acros, and used without further purification. The 50-mer 5′-amino-modified AFB1 aptamer oligonucleotide (5′-GTTGGGCACGTGTTGTCTCTCTG TGTCTCGTGCCCTTCGCTAGGCCCACA-3′) was synthesized by Shanghai Generay Biotech Co (Shanghai, China) and was based on a patent obtained by NeoVentures Biotechnology Inc. The aflatoxin B1 (AFB1) standard sample was purchased from Sigma–Aldrich, Co. All other reagents were of analytical reagent grade and ultra-pure water (Milli-Q plus, Millipore Inc., Bedford, MA) was used throughout the reactions.

2.2. Apparatus

Absorption spectra were studied on a Perkin-Elmer lambda 25 spectrometer and all the fluorescence measurements were carried out on a Perkin-Elmer LS55 luminescence spectrometer.

2.3. Synthesis of CdTe quantum dots

The experimental procedure was based on (Silva et al., 2012). In summary, Cd solution (0.4 mmol) and thioglycolic acid (TGA) (1.4 mmol) were solvated in 80 mL distilled water with pH adjusted to 10.0 using NaOH solution. Next, sodium borohydrate (0.8 mmol) and Te powder were diluted in 10 ml distilled water in a flask, with vigorous stirring under argon flow. The mixture was heated to 80 °C to get a clear red NaHTe solution. Cd-TGA solution was heated at 100 °C under argon flow in a 250 mL three-neck flask. Then the freshly prepared NaHTe solution (4.0 mL) was added to the flask, and the resulting solution was refluxed at 100 °C for 2 h. The characterization of CdTe quantum dots was carried out through transmission electron microscopy and spectrofluorometer. (Fig. S1).

2.4. Conjugation of ssDNA aptamers to CdTe QDs

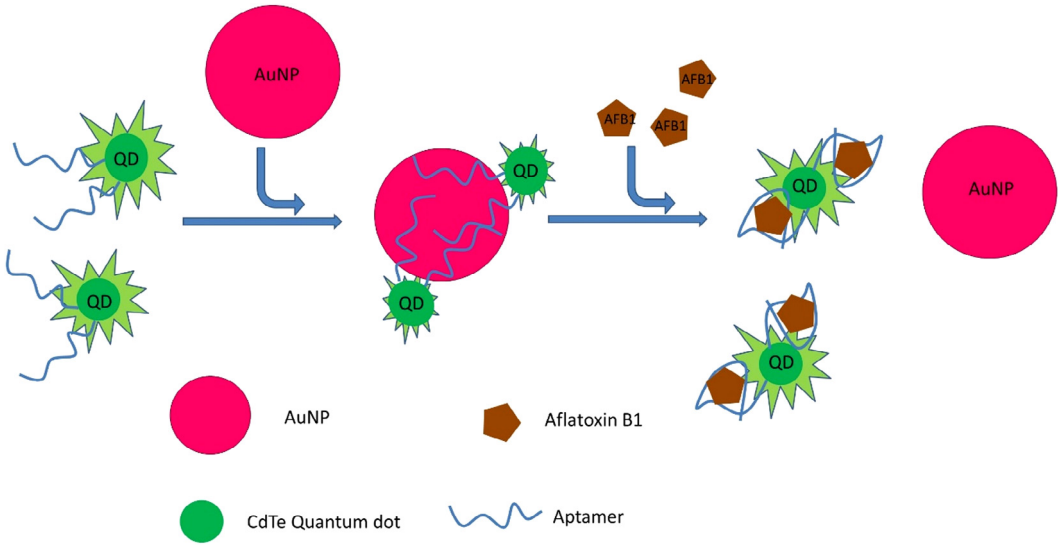
Amine-modified aflatoxin aptamers were dissolved in TE Buffer (1X). Immobilization was carried out similar to Kim and Jurng (2011). Firstly, the activation of carboxyl-functionalized QDs was conducted using EDC (1 mM) and NHS (1 mM) for 30 min. Freshly activated QDs were incubated with amine modified aptamers for 2 h. Non-reacted COOH groups on the QDs were blocked using ethanolamine solution (10 mM). Eventually, purification of aptamer/QDs conjugates was conducted by centrifugation and resuspension in phosphate buffer.

2.5. The synthesis of citrate-protected AuNP solution

The synthesis was carried out as follows: 50 ml of $HAuCl_4{\cdot}4H_2O$ aqueous solution (1 mM) was heated in a flask with until reaching boiling state. Then 10 mL trisodium citrate (38.8 mM) was added to boiling solution while stirring. The mixture was boiled for another 10 min. The yellow color of mixture changed to wine red. The heating was stopped and the solution was cooled down in room temperature while being stirred. (Borghei et al., 2016; Hosseini, Khabbaz, Dadmehr, Ganjali, & Mohamadnejad, 2015). After that, the resulting AuNPs solution was stored at 4 °C. The characterization of synthesized AuNPs was conducted by TEM. The concentration of AuNPs was calculated using the extinction coefficient ($2.7 \times 10^8\ M^{-1}\ cm^{-1}$) at 520 nm and Beer's law to be about 10 nM. As shown in Fig. S2 the diameter of AuNPs was about 13 nm.

2.6. Assay procedure

Unmodified AuNPs were added to aptamer conjugated QDs solution followed by a 15 min mild shaking. Then, sample solutions containing different concentrations of AFB1 were added into the mixture and incubated for 30 min at room temperature. The fluorescent intensity of the solution was measured by a Luminescence Spectrometer using excitation wavelength of 350 nm.



Scheme 1. Schematic illustration of detection procedure by FRET-based nanobiosensor.