Analytica Chimica Acta xxx (2017) 1–7

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Highly sensitive colorimetric aptasensor for ochratoxin A detection based on enzyme-encapsulated liposome

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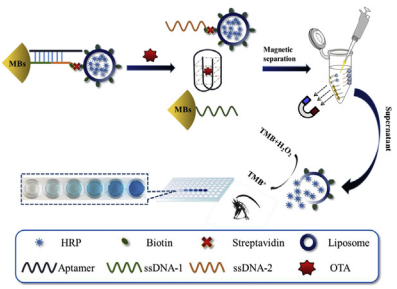
HIGHLIGHTS

- A simple, low-cost, and sensitive liposome-based colorimetric aptasensor has been developed to detect OTA.
- The strategy combines advantages of the signal amplification of liposome and the high specificity and low cost of aptamer.
- The signal amplification via HRP-encapsulated lipsomes can be achieved in one step without additional surfactant.
- The proposed colorimetric aptasensor has been applied to detect OTA concentration in corn samples with satisfied result.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 August 2017
Received in revised form
17 October 2017
Accepted 24 November 2017
Available online xxx

Keywords:
Liposomes
Aptamer
Colorimetric
Ochratoxin A

ABSTRACT

A simple, low-cost, and sensitive liposome-based colorimetric aptasensor has been developed to detect ochratoxin A (OTA). Specifically, a dumbbell-shaped probe was designed, including magnetic beads (MBs), double-stranded DNA (dsDNA), and enzyme-encapsulated liposome. The dsDNA formed by the hybridization between OTA aptamer and its complementary probe. And the dsDNA was used to contact the MBs and the enzyme-encapsulated liposome. In the presence of OTA, the aptamer preferred to combine with OTA to form G-quadruplex, resulting in the release of the detection probe and the enzyme-encapsulated liposome. Each liposome contained a large amount of HRP. Thus, when the liposome was lysed by adding the mixed solution of 3,3′,5,5′-tetramethylbenzidine (TMB) and H_2O_2, a large number of HRP were released. HRP could catalyze the H_2O_2-mediated oxidation of TMB and hence resulted in the color change from colorless to blue with the OTA concentration varying, and this variation can be observed by naked eyes easily. The result showed that the absorption intensity at 652 nm enhanced with the increase of OTA concentration ranging from 0.05 to 2.0 ng mL^{-1}, and the limit of detection was

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https://doi.org/10.1016/j.aca.2017.11.061

calculated to be 0.023 ng mL^{-1} (S/N = 3). The developed colorimetric aptasensor has been applied to detect OTA concentration in corn samples with satisfied results.

1. Introduction

Colorimetric assay is normally adopted to determine component content via the comparison or measurement of color change, and it has been paid much attention in different fields mainly because the detection of targets can be achieved by naked eyes without any sophisticated instruments [1–3]. Among the colorimetric method, enzyme-linked immunosorbent assay (ELISA) is a robust strategy that is available for the particular biomarker detection via the specific interaction between antigen and antibody and signal via color change [4]. However, its sensitivity is limited because only a small amount of enzyme that catalyzes chromogenic substrate can be modified on the immunocomplex. To address this issue, one way is to combine the immunocomplex with many enzymes.

Liposome is one kind of sphere-shaped vesicle, containing at least one lipid bilayer [5]. It has been widely investigated mainly because 1) it has hydrophobic and hydrophilic character, specifically, hydrophilic molecules can be encapsulated in the aqueous space and hydrophobic molecules can be embedded into the lipid bilayer [6]; 2) signal amplification is realized as a large quantity of molecules can be released from one liposome [7,8]. In this context, enzyme-encapsulated liposome is one of the best candidates for amplifying the signal and hence improves the sensitivity. Given the signal amplification of enzyme-encapsulated liposome, a highly sensitive chemiluminescence immunosensor has been designed for prostate-specific antigen detection because each immunocomplex contains a large quantity of enzymes [9]. However, the liposome is usually lysed by surfactants such as Triton X-100, making the procedure become complex; furthermore, the surfactants may have adverse effect on enzymes. At the same time, although immunoassay such as ELISA has advantages such as strong affinity, it still has some deficiencies. The stability of the antigen and antibody is poor, and the process of obtaining the antigen and antibody is relatively complex and hence the cost is slightly high. These disadvantages, to some extent, hinder the promising applications of liposome. Consequently, more improvement is necessary.

3,3′,5,5′-tetramethylbenzidine (TMB), a chromogenic substrate for colorimetric immunoassay, has little adverse influence on enzyme activity and it can lyse liposomes easily [7]. Aptamer has advantages of high stability, low cost, target versatility, and easy synthesis under a broad array of conditions [10]. So in this study, we report a simple, low-cost, and sensitive liposome-based colorimetric aptasensor for OTA detection in a TMB-H_2O_2 reaction system. It combines the advantages of the signal amplification of liposome and the high specificity and low cost of aptamer. Besides, the signal amplification can be achieved in one step without additional surfactant, so the assay time is short and there is little influence on enzymes. Ochratoxin A (OTA) is one of the most abundant food-contaminating mycotoxins and shows strong toxin effects in livers and kidneys, such as carcinogenicity and neurotoxicity [11], therefore it was selected as target in this study. Herein, to decrease the background, specifically, to release the HRP-encapsulated liposomes and remove the superfluous MBs-dsDNA-liposome dumbbell probe in the presence of OTA, magnetic beads were used. The proposed method was then applied for the real sample analysis.

2. Experimental section

2.1. Reagents and materials

L-α-phosphotidylcholine (PC), HRP, and Tween-20 were purchased from Sigma-Aldrich (USA). 2-(*N*-Morpholino)ethanesulfonic acid (MES), tris-buffered saline Tween (TBST, referring to 10 mM Tris buffer, 0.15 M sodium chloride, 1% Tween-20, pH 7.5), and sodium phosphate saline buffer (PBS, 10 mM phosphate buffer, 2 mM potassium phosphate, 137 mM sodium chloride, and 2.7 mM potassium chloride) were provided by Sangon Inc. (Shanghai, China). Ochratoxin A (OTA), ochratoxin B (OTB), ochratoxin C (OTC) were supplied by Pribolab (Qingdao, China). Aflatoxin B1 (AFB1) was provided by Huanan Magnech Bio-Tech Co., Ltd. (Beijing, China). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was supplied by TCI (Japan). Phosphoethanolamine-conjugated biotin (DSPE-PEG2000-biotin) and cholesterol were obtained from RiBio Co., Ltd. (Fuzhou, China). TMB and H_2O_2 were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). All reagents and solvents were analytical grade or better and used directly without further purification. Millipore purification system-based ultrapure water was used in this study (18.2 MΩ cm, Milli-Q, Millipore).

All oligonucleotides were synthesized and purified by Sangon Inc. (Shanghai, China) and the sequences were shown as follows:

OTA aptamer: 5′- GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-3′
Capture probe (ssDNA-1): 5′-NH_2- AAAAAAAAAAAATGTCCGATG-3′
Detect probe (ssDNA-2): 5′-CTCCCTTTAAAAAAAAAAAAA-biotin-3′

The underline part of ssDNA-1 and ssDNA-2 were complementary to underline part of OTA aptamer.

2.2. Preparation of the HRP-encapsulated liposomes

HRP-encapsulated liposomes were prepared using the enthanol injection method, as mentioned in previous work [7]. The hydrophobic compounds can be embedded into the spherical phospholipid bilayer liposomes and the stability of liposomes can be significantly improved after the addition of polyethylene glycol (PEG) [12], however PEG is not a hydrophobic compound. PEG was initially conjugated with hydrophobic phosphoethanolamine to make it easily insert into the membrane of liposome. The best way to combine liposomes with biotin successfully is to conjugate biotin with the end of PEG [13]. DSPE-PEG2000-biotin [14] can be embedded into the bilayers through the hydrophobic interaction. Briefly, PC (10 mg), cholesterol (2.04 mg), and DSPE-PEG2000-biotin (0.75 mg) (70:28:2 M ratio) were dissolved in chloroform solution (1 mL). To form liposomes, the chloroform was firstly removed under nitrogen to form a thin film, followed by being dissolved in ethanol (250 μL), the resulting ethanol solution was injected into phosphate buffer (10 mL, 10 mM, pH 7.4) containing HRP (0.2 mg mL^{-1}) for 1 h at room temperature under vigorous stirring. The liposomes were sonicated for 5–10 min by a probe-

Please cite this article in press as: C. Lin, et al., Highly sensitive colorimetric aptasensor for ochratoxin A detection based on enzyme-encapsulated liposome, Analytica Chimica Acta (2017), https://doi.org/10.1016/j.aca.2017.11.061