Sensors and Actuators B 259 (2018) 162–169

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Plasmonic ELISA for naked-eye detection of ochratoxin A based on the tyramine-H_2O_2 amplification system

Yi Liang[a,c,1], Xiaolin Huang[a,1], Xirui Chen[a], Wenjing Zhang[a], Guo Ping[b], Yonghua Xiong[a,c,*]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, PR China
[b] Jiangxi Food Inspection and Testing Research Institute, Nanchang, 330096, PR China
[c] Jiangxi-OAI Joint Research Institute, Nanchang University, Nanchang 330047, PR China

ARTICLE INFO

Article history:
Received 8 August 2017
Received in revised form
10 November 2017
Accepted 1 December 2017
Available online 12 December 2017

Keywords:
Immunoassay
Ochratoxin A
Hydrogen peroxide
Gold nanoparticle aggregation
Tyramine

ABSTRACT

A novel direct competitive plasmonic enzyme-linked immunosorbent assay (dc-pELISA) was applied to detect ochratoxin A (OTA) with naked eyes. In this assay, horseradish peroxidase (HRP) + hydrogen peroxide (H_2O_2) + tyramine (TYR)-induced gold nanoparticle (AuNP) aggregation was considered as a signal output; AuNP aggregation could be triggered through the phenol polymerization of TYR, which was induced by hydroxyl radicals from HRP-catalyzed H_2O_2; OTA-labeled catalase (CAT) was used as a competing antigen to consume H_2O_2. Owing to the combined advantages of ultrahigh CAT catalytic activity for H_2O_2 and dual-color responses (red and blue) generated through AuNP aggregation, the proposed method was highly sensitive and thus could be employed with naked eyes to detect OTA qualitatively with a cut-off limit of 150 pg/mL. Our method also demonstrated a good dynamic linear range (12.5–150 pg/mL) for quantitative OTA determination with a reliable correlation coefficient of $R^2 = 0.992$, a half-maximal inhibitory concentration of 84.75 pg/mL, and a detection limit of 17.8 pg/mL. In brief, this newly-designed technique is considerably suitable for high-throughput screening detection or point-of-care diagnostics in resource-constrained regions because of the easy readout of results by naked eyes without the use of advanced detection equipment.

1. Introduction

Enzyme-linked immunosorbent assay (ELISA) is a commonly used technique in clinical diagnosis, environmental monitoring, and food quality control because of its simplicity, low cost, and high throughput [1–6]. However, conventional ELISA involving horseradish peroxidase (HRP)-catalyzed tetramethylbenzidine (TMB) as a signal output is unsuitable for on-site detection in resource-constrained regions because yellow chromophores with different intensities are difficult to discriminate by naked eyes. Gold nanoparticles (AuNPs) are excellent signal indicators to fabricate biosensors because these indicators yield a high extinction coefficient and distinct localized surface plasmon resonance (LSPR) [7,8]. AuNPs can easily induce a distinct color change by slightly altering their compositions, shapes, sizes, and aggregation states [9]. With these advantages, AuNP-based nanosensors have been developed by combining them with a conventional ELISA platform called plasmonic ELISA (pELISA) to perform the colorimetric sensing of DNA [10], small molecules [11,12], bacteria [13,14], and other disease biomarkers [15–17].

In general, four strategies are used to modulate the LSPR of AuNPs: (i) controlled growth kinetics, (ii) metallization, (iii) etching, and (iv) AuNP aggregation [18]. Among these strategies, aggregation-induced changes in the color of AuNPs are the most easily recognized by the naked eyes because these changes can produce a remarkably contrasting color from red to blue. This strategy also yields a narrow dynamic linear range for target quantitative detection. Therefore, AuNP aggregation-based pELISA is more suitable for screening detection with visual interpretation in field scenarios than other format-based pELISAs. The color development of AuNP aggregation-based pELISA depends on interparticle distances among AuNPs. For example, a solu-

* Corresponding author at: State Key Laboratory of Food Science and Technology, and Jiangxi–OAI Joint Research Institute, Nanchang University, 235 Nanjing East Road, Nanchang 330047, PR China.
E-mail address: yhxiongchen@163.com (Y. Xiong).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.snb.2017.12.004
0925-4005/

tion appears red when AuNPs are monodispersed; otherwise, this solution turns purple or blue. Utilizing the enzyme-catalyzed substrate to trigger the AuNP aggregation remains a challenge in aggregation-based pELISA development. Few enzymes, such as alkaline phosphatase [15,19], acetylcholinesterase [20,21], and glucose oxidase [22], have been successfully combined with immunoassay platforms to trigger AuNP aggregation. Jiang et al. employed alkaline phosphatase-triggered click chemistry between azide/alkyne-functionalized AuNPs to induce AuNP aggregation for sensitive detection of IgG in a patient's serum [23]. Nie et al. conducted acetylcholinesterase-catalyzed hydrolysis of acetyl thiocholine to stimulate AuNP aggregation for the ultrasensitive detection of *Treponema pallidum* [21]. Yang et al. integrated glucose oxidase and benzene-1,4-diboronic acid (BDBA)-induced aggregation of AuNPs into conventional immunoassay to establish a novel pELISA involving glucose oxidase that hydrolyzes glucose to generate hydrogen peroxide (H_2O_2); H_2O_2 can also oxidize BDBA into hydroquinone to prohibit BDBA-induced AuNP aggregation [22].

HRP + H_2O_2 + tyramine (TYR) system has been widely used for signal amplification in electrochemical immunoassay because TYR can easily induce crosslinking through phenol polymerization by hydroxyl radicals; HRP can also catalyze H_2O_2 to produce hydroxyl radicals [24–26]. To the best of our knowledge, HRP+ H_2O_2 + TYR systems have not yet to be utilized to induce AuNP aggregation. In the present study, catalase (CAT) + H_2O_2 and HRP + H_2O_2 + TYR system were integrated with an ELISA platform to design a novel aggregation-based direct competitive pELISA (aggregation-based dc-pELISA). In the proposed system, citrate-capped AuNPs bind to TYR through strong electrostatic interactions, and TYR crosslinking further triggers AuNP aggregation. Subsequently, CAT consumes H_2O_2 into H_2O and O_2 [27]. One CAT molecule can convert approximately 4.0×10^7 molecules of H_2O_2 into H_2O and O_2 per second, which is about 2860-fold higher than that of alkaline phosphatase (1.4×10^4 molecules of substrate per second) [28].

Ochratoxin A (OTA) was selected as a model analyte to verify the sensitivity and robustness of dc-pELISA. OTA is a common mycotoxin mainly generated by several *Aspergillus* and *Penicillium* species; OTA has also been listed as a potential human group 2 B carcinogen by the International Agency for Research on Cancer [29]. Thus, many countries and regions have established the corresponding regulatory limits for OTA in different foodstuffs. For example, the European Commission has set up the maximum limits for OTA in raw and processed cereal products (5 and 3 μg/kg), wines (2 μg/kg), roasted coffee beans and soluble coffee (5 and 10 μg/kg), and baby foods (0.5 μg/kg) [30,31]. Our previous work demonstrated a highly sensitive pELISA for OTA quantification through target-induced the controlled growth kinetics of AuNPs. However, this method relied on the CAT-container based signal amplification that required a relative complex synthesis procedure [12]. Herein, we reported AuNP aggregation-based pELISA for naked-eye detection of OTA through introducing a simpler HRP + H_2O_2 + TYR signal amplification without any complex synthesis procedures. The proposed dc-pELISA was prepared by using an anti-OTA monoclonal antibody (anti-OTA mAb) and an OTA-labeled CAT conjugate (OTA–CAT) as a coating antibody and a competing antigen, respectively. Considering the combined advantages of the ultrahigh CAT-to-H_2O_2 catalytic activity and dual-color responses (red and blue) generated from AuNP aggregation, we observed that the proposed dc-pELISA was highly sensitive for OTA qualitative detection by naked eyes, with a cut-off limit at 150 pg/mL. This method also revealed a good linear range (12.5–150 pg/mL) for OTA quantitative detection. The accuracy and robustness of the proposed method were further evaluated by analyzing the OTA-spiked agricultural products. Our results demonstrated that the proposed dc-pELISA can be applied to detect OTA sensitively in actual corn samples by naked eyes or a plate reader.

2. Materials and methods

2.1. Materials

N-hydroxysuccinimide (NHS), *N*, *N*-dicyclohexylcarbodiimide (DCC), protein G, bovine liverCAT, $HAuCl_4$, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA). OTA, deoxynivalenol (DON), zearalenone (ZEN), fumonisin B_1 (FB_1), aflatoxin B_1 (AFB_1), and citrinin (CIT) were purchased from Huaan Magnech Bio-Tech Co., Ltd. (Beijing, China). Tetrahydrofuran (THF), H_2O_2, *N*,*N*-dimethylformamide (DMF), and trisodium citrate were obtained from Aladdin Chemisty Co., Ltd. (Shanghai, China). The anti-OTA mAb and commercial OTA ELISA kit were purchased from Wuxi Zodoboer Biotech. Co., Ltd. (Wuxi, China). The 96-well plates were obtained from Corning Inc. (New York, USA). Ultrapure water was prepared by Elix-3 and Milli-QA (Molsheim, France). All other chemicals reagents were of analytical grade and purchased from Sinopharm Chemical Corp. (Shanghai, China).

2.2. Synthesis of citrate-capped and cysteamine-capped AuNPs

A classical hydrothermal citrate-induced reduction method was used for the synthesis of citrate-capped AuNPs with the average diameter of 13 nm [32]. Briefly, an aqueous $HAuCl_4$ (1 mM, 250 mL) solution was heated to boil under vigorous stirring in the conical flask. Subsequently, 25 mL of 38.8 mM sodium citrate was rapidly added. During 15 min heating, the hue of the mixture solution changed from light yellow to a dark red. The solution was kept in an ice bath for 15 min to terminate the reaction and then filtered through a 0.22 μm filter membrane. The concentration of as-prepared AuNPs was approximately 17.5 nM according to Beer's law [33].

Cysteamine-capped AuNPs were prepared through the ligand exchange of cysteamine with citrate-capped AuNPs [34]. Briefly, the citrate-capped AuNPs were centrifuged at 13000*g* for 15 min to remove the excess sodium citrate. The precipitate was resuspended into 0.1% cysteamine solution with the mole ratio of AuNPs and cysteamine at 1:10000. Afterward, the mixture was allowed to react at room temperature for 8 h under continuous shaking. The resultant AuNP solution was centrifuged at 13000*g* for 15 min. The precipitate was further washed twice with ultrapure water. The as-prepared cysteamine-capped AuNPs were stored at 4 °C for further use.

2.3. Preparation of OTA–CAT conjugates

The OTA-CAT conjugates were synthesized by DCC/NHS-mediated formation of peptide bonds [35]. As described in previous work, the carboxyl group of OTA was activated by dissolving OTA (1 mg), NHS (0.55 mg), and DCC (2.05 mg) into 500 μL of anhydrous THF with a molar ratio of 1:2:4. The mixture was allowed to react in the dark at room temperature for 60 min under continuous shaking. Subsequently, the mixture was centrifuged at 10000*g* for 15 min to remove the white precipitate (byproduct). The supernatant was dried to remove the THF by nitrogen and then redissolved in 50 μL of DMF. The activated OTA was added in the CAT solution (0.1 M dicarbonate buffer, pH 7.4) with the mole ratio of 10:1. After reaction at 4 °C for 4 h under vigorous stirring, the mixture was dialyzed in 10 mM phosphate-buffered saline (PBS, pH 7.4) solution for 72 h. The OTA-CAT conjugate solution was stored at −20 °C for further use.