Sensors and Actuators B 266 (2018) 115–123

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Microarray surface enhanced Raman scattering based immunosensor for multiplexing detection of mycotoxin in foodstuff

Yu Li[a], Qian Chen[b], Xuefang Xu[c], Yongpeng Jin[a], Yuan Wang[a], Liying Zhang[a], Wenjun Yang[a], Lidong He[d], Xiaoyu Feng[e], Yiqiang Chen[a,*]

[a] State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China
[b] Department of Cancer Genetics and Epigenetics, City of Hope National Medical Center, 1500 East Duarte Road, Duarte, CA 91010, USA
[c] State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Diseases Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China
[d] Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL, USA
[e] Beijing Centers for Disease Control and Prevention, Beijing 100112, China

ARTICLE INFO

Article history:
Received 23 December 2017
Received in revised form 5 March 2018
Accepted 11 March 2018
Available online 14 March 2018

Keywords:
Surface enhanced Raman scattering
Immunoassay
Sensor
Mycotoxin
Multiplex
Foodstuff

ABSTRACT

A surface enhanced Raman scattering (SERS) based immunosensor was developed for the detection of three mycotoxins (aflatoxin B_1, AFB_1; zearalenone, ZEA; ochratoxin A, OTA) in foodstuff. Gold nanoparticles (GNPs) were labeled with 5,5-dithiobis(succinimidyl-2-nitrobenzoate) (DSNB) as Raman reporter and covalently linked with anti-mycotoxin antibodies as SERS nanoprobes, while AFB_1-BSA, ZEA-BSA, and OTA-BSA conjugates were covalently linked onto micro-array gold surface as corresponding capture addresses. This design allows three independent immunoreactions multiplexed on a single gold chip. After optimization, the limits of detection of the developed assay are 0.061–0.066 μg/kg for AFB_1, 0.53–0.57 μg/kg for ZEA, and 0.26–0.29 μg/kg for OTA in foodstuff. The spiked experiments presented an acceptable assay accuracy with recovery of 83.8%–108.1% and a reasonable assay precision with variation of coefficient less than 15%. Furthermore, the analysis of actual samples by our method demonstrated consistent results by comparison with conventional instrumental analysis. These results indicate that the developed SERS immunosensor can be a promising tool for simultaneously and rapidly monitoring multiple-mycotoxin levels in foodstuff.

1. Introduction

Mycotoxin is a type of toxic secondary metabolite produced by fungi on agricultural product in the field and during storage [1,2]. There are several hundreds of mycotoxins identified at present, which exhibit great structural diversity and demonstrate a variety of chemical and physicochemical properties [1]. Mycotoxins are potent toxins and can cause many adverse effects on humans and animals, such as cytotoxicity, nephrotoxicity, neurotoxicity, carcinogenicity, mutagenicity, immunosuppressive and estrogenic effects [1–3]. Therefore, their contamination in foodstuff has been considered as a potential threat to human health. To ensure consumer's health, maximum tolerable levels of major mycotoxins such as aflatoxin (AFB_1), zearalenone (ZEA), and ochratoxin A (OTA) in foodstuff have been established by countries of the world [4,5].

A number of analytical methods have been developed for the detection of mycotoxins in various samples [6,7]. These methods can be divided into instrumental analysis and immunoassay. The former is often performed in laboratories with high accuracy and precision, while the latter is generally used for rapid detection in a non-laboratory environment. Instrumental methods are typically represented by liquid chromatography (LC) [8] and LC coupled with tandem mass spectrometry (LC–MS/MS) [9,10]. However, these methods require sophisticated equipment, highly skilled personnel, and generally take hours or days to obtain results, which limits their applications in common laboratories. The requirement for timely monitoring mycotoxin contamination in food industry has demanded more rapid and cost-effective methods, therefore, immunoassay including enzyme immunoassay [11–13], lateral flow immunoassay [14–16], together with some novel immunosensors [17–19] have been developed for mycotoxin detection. Nevertheless, most of these immunoassays can only detect a single mycotoxin at one time. Because mycotoxins often co-occur in crops [20], simultaneous detection of multiple

* Corresponding author.
E-mail address: yqchen@cau.edu.cn (Y. Chen).

https://doi.org/10.1016/j.snb.2018.03.040
0925-4005/

mycotoxins is preferred to monitor mycotoxin contamination in foodstuff more effectively.

Surface enhanced Raman scattering (SERS) based immunosensor acts as a good alternative for multiplexing, rapid, and highly sensitive detection of mycotoxin. It is a novel immunosensing platform which combines SERS labeling technique and antigen-antibody interaction. The origin of SERS arises from the electromagnetic enhancement and chemical enhancement of Raman label adsorbing onto the surface of roughened coinage metals, e.g. gold or silver nanoparticle [21]. SERS labeling has several advantages: First, Raman spectrum has strong molecular characteristics (1/10 to 1/100 narrower than fluorescence), and therefore it is very suitable for multiplex labeled immunoassay. Second, single wavelength light can excite multiple Raman label, which can beneficial for the multiplexing SERS readout. In addition, Raman label is not prone to self-quenching, and thus increasing the amount of Raman label leads to higher Raman signal and better assay sensitivities [22]. In recent years, SERS-based immunosensor has been introduced into the field of biomedicine (e.g., for diagnosis of cancer biomarker [23] and virus antigens [24]) and the field of food safety. Zhu et al. [25] developed a SERS immunoassay for highly sensitive detection of clenbuterol, which demonstrates that SERS-based immunosensor owns great potential in the detection of small molecules based on competitive immunoassay format. Subsequently, many researchers have separately developed several SERS-based immunosensors for the detection of zearalenone (ZEA) [26], chloramphenicol [27], ochratoxin A (OTA) [28], and salbutamol [29] in different sample matrixes. However, there was no study on developing a SERS-based immunosensor for the multiplexing detection of small molecules. To our knowledge, this is the first microarray-based SERS immunosensor for the simultaneous detection of three major mycotoxins in foodstuff. The method validation of this immunosensor was also presented.

2. Experimental

2.1. Chemicals

Chemical standards of AFB_1, ZEA and OTA, bovine serum albumin (BSA), chloroauric acid were purchased from Sigma-Aldrich (St. Louis, USA). (3,3′-dithiobis[sulfosuccinimidylpropionate]) (DTSSP) was purchased from Pierce Biotechnology (Rockford, IL, USA). Goat anti-mouse IgG was obtained from Beijing Dingguo Changsheng Biotechnology Co. Ltd. (Beijing, China). De-ionized water was prepared using a water purification system (Millipore, Bedford, USA). Other chemical reagents were bought from Beijing Regent Corporation (Beijing, China). 5,5-Dithiobis(succinimidyl-2-nitrobenzoate) (DSNB) was synthesized according to a previously reported procedure [30]. The synthesis of AFB_1-BSA, ZEA-BSA and OTA-BSA conjugates and the preparation of respective monoclonal antibodies (mAbs) were described in supplementary material.

2.2. Synthesis of gold nanoparticles (GNPs)

GNPs were synthesized according to the method of Frens [31] with some modifications. Briefly, 100 mL of 0.01% (m/v) chloroauric acid in water was heated to boiling, then 1.2 mL of 1.0% trisodium citrate (w/v) was added into the solution. After reaction under constant stirring for 15 min, the solution was cooled at room temperature, and de-ionized water was complemented to the initial volume of 100 mL. The obtained GNPs solution is stable at 4 °C for at least three months. The particle size of GNPs was then determined by transmission electron microscopy (TEM, JEOL USA Inc., Peabody, USA).

2.3. Preparation of SERS nanoprobes

SERS nanoprobes were prepared according to the procedure of Granger et al. [32] with some modifications. Briefly, 10 mL of 36 nm GNPs as prepared above was mixed with 400 μL of 50 mM borate buffer (pH 8.5), followed by the addition of 200 μL 1 mM DSNB in acetonitrile. After 0.5 h reaction at ambient temperature, appropriate volumes of 1.0 mg/mL mAbs (anti-AFB_1, anti-ZEA or anti-OTA) were added and reacted for 1 h with mild agitation. Finally, 1 mL of 10% BSA in 2.0 mM borate buffer was used for blocking any uncoated sites on GNP surface. To remove excessive reagent, these suspensions were centrifuged at 8000*g* for 10 min. The resuspension and centrifugation steps were then repeated twice and finally the precipitate was re-suspended in 10.0 mM phosphate buffer (pH 7.4) containing 1% BSA. The three SERS nanoprobes were then mixed at a volume ratio of 2:2:1 (anti-AFB_1: anti-ZEA: anti-OTA) and ready for use.

2.4. Hydrodynamic size determination

Nano *ZS* Zetasizer (Malvern Instruments Ltd., Worcestershire, UK) was used to determine the hydrodynamic sizes of GNPs and SERS nanoprobes. Three repeats for each measurement were

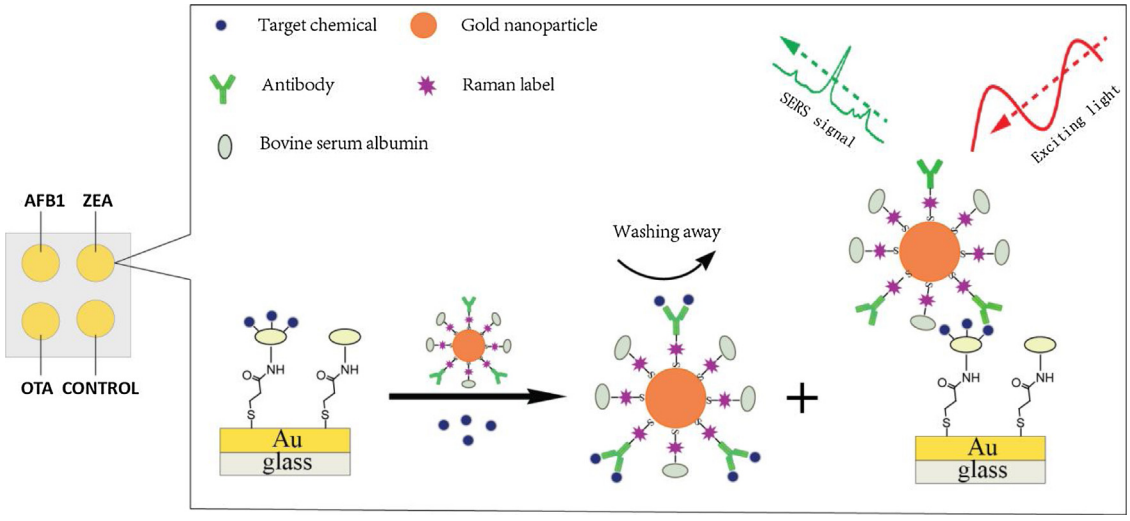

Fig. 1. Schematic illustration of multiplex SERS-based immunosensor.