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ORIGINAL ARTICLE

Citrinin detection by intensified fluorescence signal of a FRET-based immunosensor using magnetic/silica core–shell

Behrooz Shojaee Sadi [a], Mansour Bayat [a,*], Parviz Tajik [b], Seyed Jamal Hashemi [c]

[a] *Department of Microbiology, Faculty of Veterinary Specialized Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[b] *Department of Clinical Sciences, School of Veterinary Medicine, University of Tehran, Tehran, Iran*
[c] *Department of Medical Parasitology and Mycology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

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Abstract The specific immune-reaction between the anti-citrinin antibody immobilized on the surface of magnetic/silica core–shell (MSCS) and the citrinin–Rho123–BSA conjugate brings the Rho123 fluorophore as an acceptor and the QDs as a donor in close spatial proximity and causes FRET for occurring upon photo-excitation of the QDs. The novelties of this study include: (1) immobilization of the MSCS; (2) large amount of the immobilized QDs, and (3) immobilization of a large amount of Rho123 on the BSA macromolecule. Cd/Te QDs were synthesized by the simultaneous reduction of cadmium chloride and tellurium in the presence of sodium borohydride. Magnetic nanoparticles were synthesized using $FeSO_4$ and $FeCl_3$. The prepared magnetic nanoparticles shelled by silica using tetraethoxysilane in the presence of ammonia. Transmission electron microscopy (TEM) analysis was used for investigating shape and monodispersity of the nanoparticles. EDC/NHS was used as a cross linking agent for immobilization of the QDs, conjugation of citrinin to amino groups of BSA, labeling of BSA with Rho123 and also for immobilization of the amino-functionalized MSCS on the immobilized QDs. Immobilization of the anti-citrinin antibody on the surface of the amino-functionalized MSCS was performed by Schiff-base mechanism. By using these three effective strategies, sensitivity of the designed nanobiosensor was incredibly enhanced as a very low limit of detection (up to 0.1 pM). The feasibility of this technique was tested by the detection of citrinin in the spiked human serum. Results showed that there was a linear correlation between the decreased fluorescence intensity of the Rho123 and increased fluorescence intensity of the QDs with increasing concentration of citrinin in the spiked samples in the range

* Corresponding author. Fax: +98 2177317716.
E-mail address: dr_Mansour_bayat@yahoo.com (M. Bayat).
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of 1–6 pM. According to obtained results, we conclude that this highly sensitive detection scheme is a easy, quick and impressive method that can be used in optical-based nanosensors.

1. Introduction

Mycotoxins are toxic secondary metabolites produced by species of filamentous fungi that may contaminate feed and food products including milk, oil seeds, dried fruits, nuts, spices and cereals (rice, corn, wheat, barley, etc). They are major problems for animal and human health. Several surveys reported that they are highly toxic, teratogenic, estrogenic, mutagenic, immunosuppressive and carcinogenic (Tavakoli et al., 2013; Mozaffari Nejad et al., 2013, 2014; Eslami et al., 2015; Heshmati and Mozaffari Nejad, 2015). Citrinin is produced by *Penicillium citrinum*, *Penicillium expansum* and *Penicillium verrucosum* and some species of *Monascus* and *Aspergillus* (Bragulat et al., 2008). In the previous studies, different kinds of nanoparticles such as gold (Kamelipour et al., 2014), QDs (Rad et al., 2012; Zekavati et al., 2013; Shamsipur et al., 2012; Shanehsaz et al., 2013) were used to develop nanobiosensors and to detect a variety of analytes. However, in the current study, we decided to use MSCS to intensify signals to make a highly sensitive nanobiosensor (Khaksarinejad et al., 2015). In this study, a highly sensitive competitive immunoassay for the determination of citrinin was designed based on intensified fluorescence signal using magnetic/silica core–shell. As briefly illustrated in Scheme 1, the QDs nanoparticles were immobilized on the micro-well plate based on self-assembly mono-layer technique. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were used as zero-valent cross-linker to bind carboxylic groups of the QDs and amino groups of the micro-well plate. By this strategy, a large amount of QDs could participate in the designed nanobiosensor. Then, the amino-functionalized magnetic/silica core–shells, as the second layer, were immobilized on the surface of the immobilized QDs. Because of the close proximity of the immobilized QDs and the immobilized magnetic/silica core–shell, the emission of the QDs passes through the silica layer of the MSCS and interestingly intensified. In fact, magnetic/silica core–shells act as nano-mirrors to multiply the emission light of the QDs. Anti-citrinin antibody, as a third layer, was then immobilized on the level of the immobilized amino-functionalized MSCS using glutaraldehyde, as a cross-linker, based on Schiff-base interaction. $NaBH_4$ was used as a reducing agent to reduce Schiff-base interaction. Bovine serum albumin (BSA) was used as a support to immobilize citrinin and Rho123. EDC/NHS activated carboxylic group of citrinin binds to amino groups of BSA and Rho123 molecules bind to carboxylic group of BSA using EDC and NHS. In this step, citrinin and Rho123 bind to BSA to make a Rho123-labeled citrinin. For this purpose, a large number of Rho123 are located in a close proximity to the immobilized QDs and MSCS when citrinin binds to the antigen binding site of anti-citrinin antibody. In fact, BSA was used just for conjugating a molecule of citrinin with a large number of Rho123 to enhance the obtained signal. Consequently, the signal of the designed nanobiosensor interestingly intensified by these three simple but effective factors: (a) a large number of the immobilized QDs on the bottom of micro-well plate (b) immobilized MSCS in a close proximity of the immobilized QDs and (c) a large number of Rho123 which are conjugated with carboxylic groups of BSA.

2. Materials and apparatus

2.1. Materials

Citrinin, anti-citrinin antibody, cadmium chloride ($CdCl_2$), sodium borohydride ($NaBH_4$), tellurium powder (Te), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) purchased from Sigma chemical company (St. Louis, Mo, http://www.sigmaaldrich.com). Thioglycolic acid (TGA), $FeSO_4$, $FeCl_3$, tetraethoxysilane (TEOS), (3-aminopropyl) tetraethoxysilane (APTES) and all materials were obtained from Merck chemical Co. (Germany). All materials were used as supplied without further purification. All solutions were prepared using double-distilled water. Shimadzu fluorescence spectrometer (Japan) was used for recording all fluorescence spectra. Moreover, all optical measurements were conducted under ambient conditions. A Malvern dynamic light scattering (DLS) apparatus (UK) was used for studding synthesized QDs size distribution. Experiments are done in triplicate.

2.2. Preparation and immobilization of TGA-capped Cd/Te QDs

The Cd/Te QDs was synthesized and characterized in accordance with previous relevant studies. (Rad et al., 2012; Zekavati et al., 2013). Briefly, Te powder (0.1 g) was reduced by $NaBH_4$ (0.280 g) in 10 mL of distilled water under continuous N_2 bubbling and vigorous stirring. Change in the color of the solution (from violet to white) was observed after 5 h. In order to remove the white precipitate of sodium tetraborate, the solution was filtered using an ultra-filter. Then, the fresh prepared oxygen-free NaHTe aqueous solution was added into a $CdCl_2$ 2.5 H_2O (0.358 g) in 200 mL nitrogen-saturated double-distilled water at pH 10 in the presence of TGA (200 μL). TGA was used as a stabilizing and capping agent. The mixture was refluxed under a nitrogen atmosphere while vigorously stirred. Different sizes of QDs could be achieved by prolonging the refluxing time. In order to remove excess substances, the prepared solution was washed three times with absolute ethanol using sigma high-speed centrifuge (15 min, 10,000×*g*). The produced precipitate was re-dispersed in 250 mL double-distilled water and kept in a dark place, oxygen-free condition. The QDs with maximum excitation of 375 nm and emission wavelength of 535 nm were used in this study. The QDs nanoparticles were immobilized on the amino-functionalized micro-well plate based on self-assembly mono-layer technique. EDC and NHS (1:1 molar ratio) were