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Novel quartz crystal microbalance immunodetection of aflatoxin B_1 coupling cargo-encapsulated liposome with indicator-triggered displacement assay

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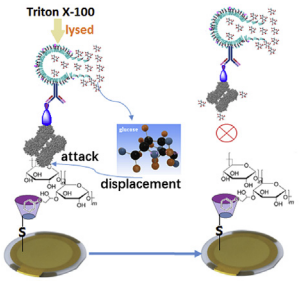
HIGHLIGHTS

- We designed an in-situ amplified quartz crystal microbalance immunoassay for aflatoxin B_1.
- Glucose-encapsulated nano liposome was used for the signal amplification.
- Indicator-triggered displacement reaction was employed for QCM measurement.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple and sensitive quartz crystal microbalance (QCM) immunosensing platform was designed for the high-efficient detection of aflatoxin B_1 (AFB_1) in foodstuff. Initially, phenoxy-derived dextran molecule was immobilized on the surface of QCM gold substrate by using thiolated β-cyclodextrin based on the supramolecular host-guest chemistry between phenoxy group and cyclodextrin. Then, AFB_1-bovine serum albumin (AFB_1-BSA)-conjugated concanavalin A (Con A) was assembled onto the QCM probe through the dextran-Con A interaction. Glucose-loaded nanoliposome, labeled with monocolonal anti-AFB_1 antibody, was used for the amplification of QCM signal. Upon target AFB_1 introduction, the analyte competed with the immobilized AFB_1-BSA on the probe for the labeled anti-AFB_1 antibody on the nanoliposome. Based on specific antigen-antibody reaction, the amount of the conjugated nanoliposomes on the QCM probe gradually decreased with the increment of target AFB_1 in the sample. Upon injection of Triton X-100 in the detection cell, the carried nanoliposome was lysed to release the encapsulated glucose molecules. Thanks to the stronger affinity of Con A toward glucose than that of dextran, AFB_1-BSA-labeled Con A was displaced from the QCM probe, resulting in the change of the local frequency. Under the optimum conditions, the shift of the functionalized QCM immunosensing interface in the frequency shift was proportional to the concentration of target AFB_1 within a dynamic range from 1.0 ng kg^{-1} to 10 μg kg^{-1} at a low detection limit of 0.83 ng kg^{-1}. In addition, the acceptable assayed

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results on precision, reproducibility, specificity and method accuracy for the analysis of real samples were also acquired. Importantly, our strategy can provide a signal-on competitive immunoassay for the detection of small molecules, *e.g.*, mycotoxins and biotoxins, thereby representing a versatile sensing schemes by controlling the corresponding antibody or hapten in the analysis of food safety.

1. Introduction

Mycotoxin (*e.g.*, AFB_1), a toxic secondary metabolite produced by organisms of the fungus kingdom, is capable of causing disease and death in both humans and animals [1]. Aflatoxins are poisonous carcinogens that are produced by certain molds (*e.g.*, *Aspergillus flavus* and *Aspergillus parasiticus*), which grow in soil, decaying vegetation, hay and grains [2,3]. The major aflatoxins of interest are designated B_1, B_2, G_1 and G_2. AFB_1 is a very potent carcinogen with a TD_{50} 3.2 μg/kg/day in rats (TD_{50}: median toxic dose) [4]. Aflatoxin B_1 is a common contaminant in a variety of foods including peanuts, cottonseed meal, corn, and other grains, as well as animal feeds [5,6]. AFB_1 is considered the most toxic aflatoxin and it is highly implicated in hepatocellular carcinoma in humans [7]. According to the Food and Agriculture Organization (FAO), the worldwide maximum tolerated levels of AFB_1 was reported to be in the range of 1.0–20 $\mu g\,kg^{-1}$ in food, and 5.0–50 $\mu g\,kg^{-1}$ in dietary cattle feed. This has prompted adoption of regulatory limits in several countries, which, in turn, requires the development of validated official analytical methods for rapid and cost-effective screening of AFB_1 on a large scale.

Different methods and strategies on the basis of various signal-generation principles have developed for the detection of AFB_1 [8,9]. Myndrul et al. designed a photoluminescence-based immunosensor for the determination of AFB_1 by using gold-coated porous silicon nanocomposite as a substrate [10]. Wang et al. established a simple, sensitive and cost-effective method for amplified optical detection of AFB_1 based on controlled growth of immunogold [11]. Machado et al. designed a multiplexed capillary microfluidic immunoassay with smartphone data acquisition for parallel mycotoxin detection [12]. Chen et al. developed a large Raman scattering cross-section molecular embedded surface-enhanced Raman scattering (SERS) aptasensor for ultrasensitive AFB_1 detection using chitosan-Fe_3O_4 [13]. Krittayavathananon and Sawanghruk successfully used impedimetric sensor of thiolated single-stranded DNA/reduced graphene oxide aerogel electrode toward AFB_1 detection [14]. Our group recently reported several preferable photoelectrochemical immunoassays for AFB_1 detection, *e.g.*, by using enzymatic product-etching MnO_2 nanosheets for dissociation of carbon dots [15], silver nanolabels-assisted ion-exchange reaction with CdTe quantum dots [16], dopamine-loaded liposomes [17] and enzyme-controlled dissolution of MnO_2 nanoflakes for colorimetric immunoassay [18]. Viter et al. investigated analytical, thermodynamical and kinetic characteristics of photoluminescence immunosensor for the screening of ochratoxin A [19]. Despite some advances in this field, there is still the request to explore new methods and protocols for the detection of AFB_1 in order to meet the requirements of small-molecular mycotoxins.

Quartz crystal microbalance (QCM) measures a mass variation per unit area by determining the change in frequency of a quartz crystal resonator [20]. The resonance is disturbed by the addition or removal of a small mass due to oxide growth/decay or film deposition at the surface of the acoustic resonator [21]. The QCM-based immunosensor with some advantages, *e.g.*, high sensitivity, low cost, real-time output, and label- or radiation-free entities, has been the active subject of investigating biomolecular interactions [22,23]. The Pohanka's group utilized magnetic bead-based piezoelectric biosensor for the detection of tumor necrosis factor alpha [24]. Pirich et al. used piezoelectric immunochip coated with thin films of bacterial cellulose nanocrystals for dengue detection [25]. Koutsoumpeli et al. explored antibody mimetics for the detection of small organic compounds using quartz crystal microbalance [26]. Although QCM-based immunosensors or immunoassays have been reported for the detection of AFB_1 [27–31], most methods were carried out based on the signal-off mode. In this regard, one major disadvantage of using the signal-off assay lies in the requirement of a relatively high background signal relative to the signal-on assay formation. To this end, our motivation in this work is to construct a competitive-type immunoassay for detection of small molecules with the signal-on mode.

To develop a high-efficient signal-on competitive immunoassay, design of the detection scheme is very important. Essentially, a classical competitive-type immunoassay usually exhibits a signal-off trend. From the dialectical point of view-'Two negatives make an affirmative', such a signal-on detection system can be executed by involving in another signal-off detection strategy. Displacement reaction mode, where part of one reactant is replaced by another reactant, is very usable for this purpose. Wu et al. developed a glucose-controlled cargo release system from concanavalin A (Con A)-gated mannose-functionalized mesoporous silica nanocontainers by using competitive-displacement reaction between glucose and mannose for Con A [32]. More et al. utilized an acac-boron-dipyrromethene (BODIPY) dye as a reversible 'ON-OFF-ON′ fluorescent sensor for Cu^{2+} and S^{2-} ions based on the displacement approach [33]. Nguyen et al. reported a signal-on electrochemical displacement heterogeneous immunosensor for diclofenac detection between the analyte and arylamine-coupled diclofenac for the corresponding antibody [34]. Con A, a tetrameric form under physiological conditions, is capable of specifically binding dextran and glucose epitopes in the presence of Mn^{2+} and Ca^{2+} ions but with higher affinity for higher affinity for glucose. Moreover, the glucose-Con A-dextran system has been utilized for the detection of glucose and biotoxins based on glucose/dextran displacement reaction [35–37]. Routine approaches consisted ligand-conjugated enzyme labels in these immunoassays. Unfavorably, most enzyme immunoassays are susceptible to interference and assay conditions during the signal-generation stage, *e.g.*, pH, temperature and instability caused by structural unfolding [38]. Therefore, another motivation in this study is to design an enzyme-free competitive-displacement immunoassay by coupling with the glucose-Con A-dextran reaction system.

Liposome with a spherical vesicle can be used as a vehicle for the administration of nutrients and pharmaceutical drugs [39]. Owing to the unique chemical structure, different cargoes and nutrients can be encapsulated into the liposome during the synthesis, *e.g.*, fullerenes [40], dopamine [17], enzyme [41], and glucose [42]. Significantly, cargo-encapsulated nanoliposome can be lysed by using surfactants, *e.g.*, Tween 20 and Triton X-100. Herein, we report on the proof-of-concept of simple and feasible competitive-type immunoassay for QCM sensing of AFB_1 based on cargo-

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