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An Electrochemical Immunosensor for Rapid and Sensitive Detection of Mycotoxins Fumonisin B1 and Deoxynivalenol

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ABSTRACT

We report an electrochemical immunosensing method for rapid and sensitive detection of two mycotoxins, fumonisin B1 (FB1) and deoxynivalenol (DON). A disposable screen-printed carbon electrode (SPE) was used as sensing platform. The working electrode part of SPE was modified by gold nanoparticles (AuNPs) and polypyrrole (PPy)-electrochemically reduced graphene oxide (ErGO) nanocomposite film for effective anti-toxin antibody immobilization, enhanced electrical conductivity, and biocompatibility. Under optimized test conditions, the limit of detection and linear range achieved for FB1 were 4.2 ppb and 0.2 to 4.5 ppm (%RSD = 4.9%); and the corresponding values for DON were 8.6 ppb and 0.05 to 1 ppm (%RSD = 5.7%). The immunosensor can specifically detect the target toxin in co-existing toxins environment. The sensor exhibited high sensitivity and low matrix interference when tested using extracts obtained from spiked corn samples. Hence, our electrochemical immunosensing scheme can be adopted for highly sensitive and rapid detection of multiple co-contaminant mycotoxins in food and feed products.

1. INTRODUCTION

Mycotoxins such as aflatoxin (AF), deoxynivalenol (DON), fumonisin B1 (FB1), ochratoxin A (OTA) and zearalenone are a group of toxic secondary metabolites produced by certain fungi. They naturally contaminate foods and feeds [1], which lower the product quality, pose severe health risk to humans and animals, and cause profound economic losses worldwide [2]. FB1 is the most common and economically important form of fumonisin (FUM), given its hepatotoxic and nephrotoxic effects in all animal species tested [2,3]. DON occurs predominantly in grains such as wheat and barley infected by *Fusarium* head blight or scab [4,5]. The US Food and Drug Administration has suggested action levels of 2 to 4 parts per million (ppm) for FUM and 1 ppm for DON in foods intended for human consumption [6]. In addition, the co-occurrence of mycotoxins in nature has increased their likelihood of consumption and may cause additive and/or synergistic effects [7,8]. For example, AF and FUM are co-contaminants often found in corn [9–11] and milled corn fractions [5,9]; DON and OTA are common co-contaminants in wheat [4,12,13].

Due to the widespread prevalence of multiple mycotoxins in foods and feeds, research has focused on developing effective methods for highly sensitive and selective detection of mycotoxins to screen foods and feeds. Traditional analytical methods include liquid chromatography–mass spectrometry (LC-MS), high performance liquid chromatography (HPLC), and thin-layer chromatography (TLC), which can detect toxins in the range of ~0.01 to 0.1 parts per billion (ppb) [14–18]. However, these laboratory methods are not suitable for rapid, on-site testing because they are labor- and time-intensive as well as expensive [19]. Other screening methods used for mycotoxins detection are enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR), lateral flow immunoarray (LFI), immunochip, electronic nose, etc. [18]. Though ELISA is widely used, its shortcomings include cross-reactivity and possible false-positive or false-negative results [18]; also, additional reagents are necessary to ensure the stability of stored antibody (Ab) coating on the microwell plates [20]. SPR sensor is sensitive to temperature, and LFI and electronic nose have low sensitivity, while immunochip involves complex labeling process and requires professional technicians to run the test [18].

Electrochemical biosensors are attractive due to their advantages of operational simplicity, high sensitivity, low cost, and portable on-field use. Kadir and Tothill developed the ELISA-based chronoamperometric immunosensor using screen-printed gold

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electrode for detection of FUM with a limit of detection (LOD) of 5 μg/L (5 ppb) [21]. However, the use of secondary Ab in this work increases the cost and complexity. Ezquerra et al. reported an electrochemical immunoassay with the help of dispersed paramagnetic particles for FB1 detection with LOD of 0.58 ± 0.05 μg/L [22]. But this method requires additional tracer as well as an external magnetic field, also involves a step to transfer modified magnetic beads to the electrode surface, which is somewhat complicated.

Novel nanomaterials are widely used for effective Ab immobilization and highly sensitive biosensor development. Srivastava et al. synthesized and deposited chemically active reduced graphene oxide (rGO) onto an indium tin oxide-coated glass substrate and this platform has been utilized for covalent attachment of the AFB1 antibodies for AFB1 detection (LOD = 0.12 ng/mL) [23]. Romanazzo et al. developed a recombinant fragment antigen-binding fragment based electrochemical immunosensor for DON detection [24]. Zhilei et al. reported a sensor for using fullerene C-60, an ionic liquid and ferrocene on a chitosan film at a glassy carbon electrode to detect DON [25]. Some literature report that incorporating nanomaterials and conducting polymers together in sensor platform afford large surface area, biocompatibility, ease of functionalization, and significantly enhances the sensitivity, reproducibility, and stability [26,27]. Among nanomaterials, graphene oxide (GO)/polypyrrole (PPy) nanocomposite, electrochemically reduced graphene oxide (ErGO) and gold nanoparticles (AuNPs) are particularly suitable for biosensor applications due to their high electrical conductivity, good biocompatibility and chemical stability [28–33]. PPy is one of the most studied conducting polymers due to its great electrical conductivity, ease of preparation and low cost. However, it has some drawbacks such as poor mechanical strength and instability in conductivity [33]. Researchers have discovered that GO demonstrates a synergistic effect in PPy/GO composites that could improve both mechanical properties and electrical conductivity compared to PPy [33]. Therefore, combining such advantages of conducting polymers and nanomaterials [26,34], we developed a label-free electrochemical immunosensing method using disposable screen-printed carbon electrode (SPE) modified by AuNPs and PPy/ErGO film for effective Ab immobilization and improved electrical conductivity to enable highly sensitive and selective detection of FB1 and DON.

2. EXPERIMENTAL

2.1. Materials and chemicals

SPEs were purchased from CH Instruments, Inc. (TE100, Bee Cave, TX, USA). Graphite flakes (~150 μm), FB1, DON and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$), nitric acid (63%), pyrrole (99% extra pure) and 3-mercaptopropionic acid (MPA, 99+ %) were supplied by ACROS Organics (Morris Plains, NJ, USA). Monoclonal anti-DON mouse Ab (1 mg/mL) and monoclonal anti-FB1 mouse Ab (1 mg/mL) were purchased from Antibodies-online.com (Atlanta, GA, USA). Immobilization of antibodies onto the electrode surface was performed using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) chemistry with a minimum purity of 98% or higher (Bioworld, Dublin, OH, USA and Acros Organics). Phosphate buffered saline (PBS) 10X, methanol (99.8%), sulfuric acid (96%), hydrogen peroxide (50%), and hydrochloric acid (36.5–38%) were from Fisher Scientific (Rockford, IL, USA). Potassium ferrocyanide trihydrate (reagent grade) and potassium chloride were acquired from Fisher Science Education (Hanover Park, IL, USA). Potassium ferricyanide, sodium phosphate monobasic (NaH_2PO_4) and sodium phosphate dibasic (Na_2HPO_4) were certified A.C.S. reagents from Thermo Fisher Scientific (Fair Lawn, NJ, USA). All chemicals were used as received without any purification, and deionized (DI) water of resistivity ≥18.2 MΩ·cm (Ultrapure water system, Millipore, Billerica, MA, USA) used for solution preparation and all experiments.

Methods for all solution preparation, synthesis of GO and AuNPs, and extraction of spiked toxins in corn samples are presented in Supplementary Material.

2.2. Immunosensor fabrication

The immunosensor fabrication steps in preparing the working electrode surface of SPE is illustrated in Scheme 1A. The SPE is first rinsed with DI water to remove any existing impurities. PPy/GO nanocomposite film, grown using our modified procedure [27], is electrochemically deposited over the bare SPE placed in nitrogen-purged solution containing 0.025 M pyrrole, 0.33 mg/mL GO and 0.1 M KCl under gentle stirring. Cyclic voltammetry (CV) scans are then applied between −0.2 V and +0.9 V for 15 complete cycles at 50 mV/s scan rate to electro-polymerized pyrrole into PPy and deposit PPy/GO nanocomposite film on the SPE, with GO being partially reduced during the process. GO is fully reduced and PPy/ErGO film is obtained by CV from 0.0 to −1.4 V at 50 mV/s scan rate in nitrogen-purged 0.05 M phosphate buffer (Na_2HPO_4/NaH_2PO_4) solution (pH 7.0) for 25 complete cycles [35]; it is then rinsed with DI water and dried at room temperature. The ErGO/PPy film surface is drop-coated with 20 μL AuNPs solution and allowed to dry under ambient conditions. The AuNPs-PPy/ErGO-modified SPE is immersed in 2 mL of 10 mM MPA solution and incubated for 6 h at room temperature to form Au-S bond [36]. After rinsing with DI water, the MPA-functionalized electrode is transferred to a mixture of EDC/NHS as crosslinker and incubated for one hour at room temperature to activate the carboxyl groups for effective immobilization of antibodies on the electrode surface [37]. The electrode is washed three times with DI water to remove any excess EDC/NHS and by-products followed by incubating with 40 μL of anti-FB1 or anti-DON Ab solution (pH 9.0) at 4 °C for 12 h to allow sufficient Ab immobilization. Finally, the electrode is incubated with 1% BSA for 30 min to block unoccupied sites and washed with 1X PBS [38]. The fabricated Ab-AuNPs-PPy/ErGO-SPE is stored in 1X PBS (pH 7.4) at 4 °C when not in use.

2.3. Instruments and measurements

Fourier transform infrared spectrometer (FT-IR, Spectrum-100, PerkinElmer) was used for characterization of synthesized GO. UV/Vis spectrophotometer (Lambda-25, PerkinElmer) was also used to characterize the synthesized GO and AuNPs. All electrochemical experiments were performed in CHI-660D electrochemical workstation (CH Instruments, Inc. USA) with a conventional three-electrode system. CV and differential pulse voltammetry (DPV) of redox couple 5 mM $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ containing 1 M KCl as supporting electrolyte was employed to investigate the electron transfer behavior of SPE after each modification step and for target toxins (FB1/DON) detection. Scanning electron microscopy (SEM) images were obtained using a LEO 1530 scanning electron microscope.

3. RESULTS AND DISCUSSION

The sensing scheme employed the target mycotoxin detection system is illustrated in Scheme 1B. With the target mycotoxin-specific Ab attached on the working surface of SPE, a blank sample is tested and the DPV peak current is recorded as the signal baseline. When samples containing mycotoxins are tested, after