Biotechnology Reports 8 (2015) 144–151

Contents lists available at ScienceDirect

Biotechnology Reports

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

Screening of self-assembled monolayer for aflatoxin B1 detection using immune-capacitive sensor

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ARTICLE INFO

Article history:
Received 16 July 2015
Received in revised form 23 September 2015
Accepted 4 October 2015
Available online 27 October 2015

Keywords:
Capacitive
Sensor
Aflatoxin
Self-assembled monolayer
Capacitance

ABSTRACT

A capacitive biosensor was used for detection of aflatoxin B1. Two different methods for cleaning gold electrodes were evaluated using cyclic voltammetry in the presence of ferricyanide as redox couple. The methods involve use of a sequence of cleaning steps avoiding the use of Piranha solution and plasma cleaner. Anti-aflatoxin B1 was immobilized on self-assembled monolayers (SAM). The immune-capacitive biosensor is able to detect aflatoxin B1 concentrations in a linear range of 3.2×10^{-12} M to 3.2×10^{-9} M when thiourea was used to form the SAM; 3.2×10^{-9} M to 3.2×10^{-7} M when thioctic acid was used. When the gold surface was isolated with tyramine-electropolymerization linear ranges of 3.2×10^{-13} M to 3.2×10^{-7} M and 3.2×10^{-9} M to 3.2×10^{-7} M where obtained, respectively. The results obtained show the difference in linear range, limit of detection, and limit of quantification when different self-assembled monolayers are used for aflatoxin B1 detection.

1. Introduction

Aflatoxins represent a major class of mycotoxins having deleterious impact in human and animal health. Aflatoxins are produced by *Aspergillus* fungi, section *flavi*, which includes *Aspergillus flavus* and *Aspergillus parasiticus* [1–3]. Studies of aflatoxins have shown mutagenic, teratogenic, and highly hepatotoxic and hepatocarcinogenic effects. Regulatory limits have been established in terms of concentration, which differs from country to country [4,5]. The fungi are contaminating various crops and produce aflatoxins. Contamination of oil-rich crops such as corn, peanuts, cottonseed, and tree nuts [6] are common. Aflatoxin B1, which is a small hydrophobic molecule with a molecular weight of 312.3 Da is the major aflatoxin produced by toxigenic strains [7,2].

The primary methods for aflatoxin detection are thin layer chromatography (TLC) [8], high performance liquid chromatography (HPLC) [9], and enzyme-linked immunosorbent assay (ELISA) [10]. TLC analysis is relatively economic, but is tedious and time consuming. HPLC analyses require extensive time for cleanup but similar as for TLC, the detection sensitivity is rather low. ELISA is the most commonly used method since the analytes can be detected relatively fast and quantified even at low concentrations among a multitude of other substances [10]. One new technique for detection of analytes at low concentration is the capacitive biosensor. This biosensor is highly sensitive, selective, requires low sample volumes and the samples do not need to be purified [11–13]. The principle of capacitive biosensor is based on measuring the change in capacitance caused by the change of dielectric properties when the target analyte binds to the immobilized biorecognition element (antibodies, receptors, etc.) attached to the sensor chip. The gold surface is isolated by a self-assembled monolayer and the biorecognition element is immobilized to that. When the analyte binds a resulting decrease of the capacitance is registered [14]. Capacitive biosensors have been used for the monitoring of a broad range of compounds/particles from heavy metal ions via glucose, to soluble proteins as well as larger aggregates such as virus particles and even microbial cells.

The capacitive biosensor is composed of three electrodes: a working electrode, a reference electrode and an auxiliary/counter electrode. The working electrode (i.e. transducer) is constructed as a gold surface onto which the sensing element, the ligand, is immobilized. Immobilization techniques based on different self-assembled monolayers (SAMs) [15–18] have been adapted to the capacitive sensor surface in combination with various biorecognition elements [19]. The self-assembled monolayers of sulfur-containing compounds are used for insulation of the gold electrode. SAMs are formed from e.g. thioctic acid, thiourea or mercaptopropionic acid [17], An alternative technique is to use

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http://dx.doi.org/10.1016/j.btre.2015.10.001

electropolymerization of e.g. tyramine [20] thereby creating an insulating layer and concomitantly introducing amino groups on the electrode surface. The amplitude of the signal registered from the sensor is a function of the surface area of the working electrode. Surface expansion might be achieved by adding gold nanoparticles (AuNPs). Proteins are easily adsorbed to the nanoparticles, and that constitutes a convenient way of immobilizing antibodies. Such electrode surface has a good biocompatibility [21].

The effect of SAMs and electropolymerized tyramine as insulators of the electrodes was studied. Depending on the kind of insulator used when constructing the aflatoxin B1 sensitive electrode, the limit of detection and limit of quantification varied. Additionally, one simple cleaning method for flat gold surfaces was introduced and compared with the commonly used cleaning method for flat gold electrodes.

2. Materials and methods

2.1. Materials

Anti-Aflatoxin B1, aflatoxin B1 (Fig. 1), thiourea, thioctic acid, tyramine, *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) were obtained from Sigma–Aldrich (Steinheim,Germany), 1-dodecanethiol was obtained from Aldrich (Milwaukee, USA), . All other chemicals used were of analytical grade. All buffers were prepared from water treated with a MilliQ system from Millipore (Bedford MA, USA). This treated water is called MilliQ water in the rest of this paper. The buffers were filtered and degassed before use.

Samples from contaminated Brazilian nuts containing contaminated and non-contaminated nuts were kindly provided by Tahuamanu S.A. Company (Pando,Bolivia).

2.2. Methods

2.2.1. Cleaning surface methods for flat gold electrodes

Disposable flat electrodes with a gold thickness 4000 Å and diameter of 3 mm were prepared as described by Teeparuksapun et al. [38] and cleaned using two different methods: (A) The gold electrodes were immersed first in acetone and then in ethanol under sonication for 1 min each, rinsed with water and dried with nitrogen gas before they were immersed in Piranha solution (sulphuric acid:hydrogen peroxide 3:1) under sonication for 1 min. Each step was followed by rinsing the electrode with MilliQ water (18 ΩM cm) and drying with pure nitrogen gas, finally the electrodes were placed into a plasma cleaner (Mod. PDC-3XG, Harrich, NY) for 15 min. (B) In the second method flat gold electrodes were immersed in acetone and ethanol under sonication for 5 min in each solvent, the two steps were followed by rinsing the electrode with MilliQ water (18 ΩM cm) and drying with pure nitrogen gas.

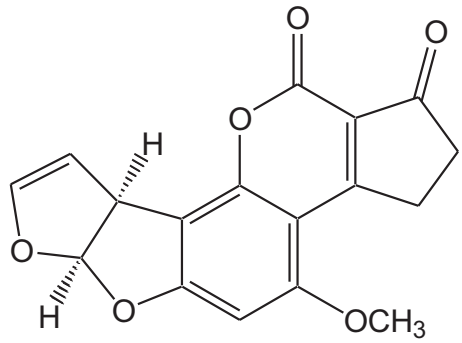

Fig. 1. Aflatoxin B1 structure.

2.2.2. Self-assembled monolayer and electropolymerization

The cleaned flat gold electrodes recently rinsed with MilliQ water(18 ΩM cm) and dried with pure nitrogen gas were immediately immersed in thiol solutions (thiourea or thioctic acid 250 mM dissolved in ethanol, Fig. 2A and 2B respectively), at room temperature for 12–18 h, rinsed with MilliQ water (18 ΩM cm) and dried with pure nitrogen gas [17].

Tyramine electropolymerization (Fig. 2C) was performed using two different solutions separately. The first solution was NaOH (300 mM) dissolved in pure methanol, and the second solution was potassium phosphate buffer (10 mM):ethanol in a rate of 3:1. Tyramine (100 mM) was prepared in each solution and electropolymerized on the gold surface electrode. Electrodes were rinsed with MilliQ water (18 ΩM cm) and dried with pure nitrogen gas. The tyramine electropolymerization was carried out in a range of 0–1.5 V vs Ag/AgCl reference electrode for 15 scans using (8 PGSTAT12, Eco Chemie, The Netherlands), before the electrode was immersed in gold nanoparticles solution (Ø 2.6 nm) [22] for 18–24 h.

2.2.3. Immobilization of anti-Aflatoxin B1

Anti-Aflatoxin B1, used as the biorecognition element, was immobilized covalently on SAMs built from thiourea and thioctic acid as follow: Flat gold thiourea electrode was immersed in a solution of glutaraldehyde 5% v/v in potassium phosphate buffer 10 mM for 20 min, rinsed with the same potassium phosphate buffer and dried with pure nitrogen gas. This chemical reaction is used for introduction of aldehyde groups that can be used to bind the anti-aflatoxin B1. A volume of 20 μL of anti-aflatoxin B1 (100 mg/mL) were added on the modified part of the flat gold electrode and incubated at 4 °C overnight. In the case of thioctic acid the carboxylic group was activated with 1% w/v of EDC in acetonitrile for 5 h, rinsed with MilliQ water (18 ΩM cm) and dried with pure nitrogen. A volume of 20 μL of anti-aflatoxin B1 in a concentration of 100 μg/mL was pipetted over the modified part of the flat gold electrode and incubated at 4 °C overnight. For the electrode modified with tyramine-gold nanoparticles, the electrode was rinsed with MilliQ water (18 ΩM cm) and dried with pure nitrogen, a volume of 20 μL of anti-aflatoxin B1 in a concentration of 100 μg/mL were pipetted over the reactive part of the gold electrode and incubated at 4 °C overnight. As a final step, the electrodes were treated with a solution of 1-dodecanethiol (10 mM) in ethanol for 20 min. This step is used to block pinholes in the insulation of the electrode surface.

2.2.4. Capacitive measurement

The capacitive measurement was carried out using the newly developed technology [37] where current pulses are used instead of the potential pulses that most often are used. The advantage of using current pulses is that one can follow a linear change in potential as a result of the current pulse. In the older procedure, the potential pulse gave rise to a current signal that decayed logarithmically over time. The new concept give a far better stability of both baseline and of readings, and it contributed to an even higher sensitivity of the assay than what was achieved with the older procedure. The sensor chip is placed in a flow cell that is an integrated unit in a continuous flow system. The electrochemical flow-cell involves three electrodes, the working electrode that is the gold electrode which has been insulated and modified by immobilizing affinity binder structures on its surface [37]. The reference and an auxillary electrode are both made of platinum wire [12,13,37,38].