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A label free aptasensor for Ochratoxin A detection in cocoa beans: An application to chocolate industries

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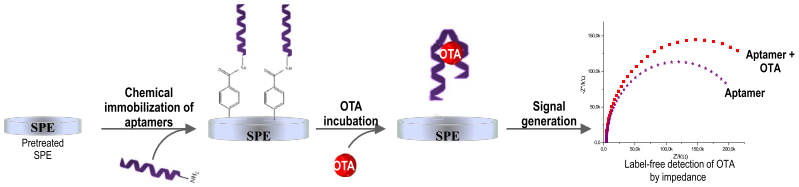
HIGHLIGHTS

- Simple and facile method to detect OTA.
- The aptasensor exhibited a very good limit of detection (LOD) as low as 0.15 ng/mL.
- The first report on OTA detection in cocoa beans using impedimetric aptasensor.

GRAPHICAL ABSTRACT



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ABSTRACT

Contamination of food by mycotoxin occurs in minute/trace quantities. Nearly 92.5% of the cocoa samples present Ochratoxin A (OTA) levels at trace quantity. Hence, there is a necessity for a highly sensitive and selective device that can detect and quantify these organic toxins in various matrices such as cocoa beans. This work reports for the first time, a facile and label-free electrochemical impedimetric aptasensor for rapid detection and quantitation of OTA in cocoa beans. The developed aptasensor was constructed based on the diazonium-coupling reaction mechanism for the immobilization of anti-OTA-aptamer on screen printed carbon electrodes (SPCEs). The aptasensor exhibited a very good limit of detection (LOD) as low as 0.15 ng/mL, with added advantages of good selectivity and reproducibility. The increase in electron transfer resistance was linearly proportional to the OTA concentration in the range 0.15–2.5 ng/mL, with an acceptable recovery percentage (91–95%, RSD = 4.8%) obtained in cocoa samples. This work can facilitate a general model for the detection of OTA in cocoa beans based on the impedimetric aptasensor. The analysis can be performed onsite with pre-constructed and aptamer modified electrodes employing a portable EIS set up.

1. Introduction

Food contamination is one of the most important worldwide issues and has been paid much attention with the fast development of economics and improvement of human life. Among all, contamination by toxins is one important branch. Ochratoxin A (OTA) is frequently presents in various agricultural commodities during storage [1]. OTA is the most toxic and known to be hepatotoxic, nephrotoxic, teratogenic and mutagenic to a wide variety of mammalian species [2,3]. Thus, it is characterized as possibly carcinogenic to humans (Group 2B, according to the IARC classification) [4]. It occurs naturally in a wide variety of raw commodities and finished food products of vegetal origin such as cereals, coffee beans, pulses, and dried fruits as well as cocoa derived products. Cocoa is a cash crop produced in many countries of the world with

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about 71% of the world production from West African countries especially from the Ivory Coast (30% of the world's production), but also from Ghana and Nigeria. Cocoa is also produced in Asia (15.8%) and Latin America (12.4%) [5]. Cocoa beans after post-harvest treatments are mainly exported to Europe and North America to be turned into liquor, butter and cocoa powder [6]. Cocoa and various derivatives contamination (International Cocoa Organization [ICCO]) with OTA has been revealed by numerous studies using conventional techniques [5,7,8].

The health risks associated with the consumption of OTA-contaminated foods necessitates drastic measures to protect worldwide consumer health. Accordingly, in the European market, food products are subject to maximum allowable threshold concentrations of OTA. The EU 1881/2006 regulation sets maximum levels of OTA in foodstuffs: 5 μg/kg in cereals, 3 μg/kg in cereal-processed products, and 2 μg/kg in cocoa, wine and spices [9]. The Superior Council for Public Hygiene of France (Conseil Supérieur de l'Hygiène Publique de France [CSHPF], 1999) and the Scientific Committee on Food (Scientific Committee on Food [SCF], 1998) established a tolerable daily intake (TDI) of 5 ng/kg body weight/day based upon data from "in vivo" experiments accounting for nephrotoxicity in rats.

However, with the maximum admissible value of OTA established in 2 mg/kg, about 40% of the cocoa which arrives in Europe may be rejected at the ports [10]. Despite the fact that cocoa beans are not ingested raw, and most of OTA (80%) are in the shells, cocoa processing does not always completely eliminate the toxin [6,11]. Thus, beans and cocoa derivatives can only be exported if there are well-established quality criteria, including the analysis of OTA occurrence.

The clearly undesirable presence of trace amounts of OTA in foodstuffs requires suitable sampling procedures and highly sensitive techniques to detect and control OTA concentrations at trace level [12]. Apart from precision and accuracy, the wide varieties of matrices where OTA can be found pose a great challenge to analytical scientists. Furthermore, the presence of several mycotoxins in a same sample may produce synergistic effects, what firmly encourages researchers to search for analytical techniques able to perform highly selective measurements [13–15]. High performance liquid chromatography (HPLC) with fluorescence detectors have been widely adopted as the standard methods for OTA detection [16,17]. In addition, gas chromatography-mass spectrometry, thin layer chromatography (TLC) and enzyme linked immunosorbent assay (ELISA) also showed good performance for OTA detection. However, new methods that are more direct, highly sensitive have attracted attentions on defining strategies suitable for real time onsite analysis and adaptable to different complex matrices.

In the current state of the art, ample of OTA detection methods are available by exploiting the properties of aptamers such as folding mechanism upon target recognition [18], coupling of aptamers by click chemistry and also by azido modified aptamers which was recently demonstrated by our group [19,20]. Immobilization of azido aptamers on SPE is difficult using click chemistry as it requires the involvement of toxic organic solvents such as acetonitrile. Moreover, azido aptamers have stability issues and the obtained yields are is less compare to the amino modified aptamers which subsequently affects the sensitivity and increases the cost of the assay or biosensors. Apart from it, recently, numerous aptamer based biosensors based on enzyme-labels, immuno based, nano particles and label free detection methods have been exploited for OTA detection [21–23] but none of them have attempted to detect OTA in cocoa beans [24–27]. Alternative to antibody, the aptamer selected for OTA exhibits a high level of binding affinity specificity, showing a100-fold less affinity to Ochratoxin B (OTB) [28].

Based on these virtues, the aptamer functionalized biosensor is developed for OTA detection in cocoa samples. This proposed aptasensor for OTA has advantages of simplicity, high sensitivity, acceptable selectivity good reproducibility and stability. Therefore, this work lays a potential foundation for chocolate industries to pre-asses the quality of cocoa beans and cocoa powder.

To the best of our knowledge, this is the first report on OTA detection in cocoa beans using impedimetric aptasensor at low level (2 μg/kg) thus, meeting the EU standards. The transducer substrate used in the development of sensor are highly stable, and could be directly used onsite, thus can be extended for onsite monitoring of OTA at the origin of cocoa beans.

2. Experimental

2.1. Chemicals and materials

The amino modified aptamer was purchased from Eurogentec (France). The binding site of the aptamer is shown below:

5′-GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-3′),
5′-NH_2.

All other chemicals, sodium phosphate dibasic Na_2HPO_4, potassium phosphate monobasic KH_2PO_4, Magnesium chloride ($MgCl_2$), potassium chloride (KCl) sulfuric acid (98%), sodium chloride (NaCl) ethanol (98%), sodium nitrite ($NaNO_2$), N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyle)-N-ethyle-carbodiimide hydrochloride (EDC), potassium ferrocyanide ($K_4[Fe(CN)_6]$), potassium ferricyanide ($K_3[Fe(CN)_6]$), 4-aminobenzoic acid, sodium nitrate, ethanolamine and bovine serum albumin were purchased from Sigma (France). Aptamer solutions prepared in binding buffer (BB pH 7.4) containing 1 mM $MgCl_2$, 140 mM NaCl, 2.7 mM KCl, 0.1 mM Na_2HPO_4 and 1.8 mM, KH_2PO_4 were used. All solutions were prepared in deionized Milli-Q water (Millipore, Bedford, MA, USA). Ochratoxin A was purchased from Trilogy (France), was firstly dissolved in methanol (5 mg/mL) and then diluted in BB.

2.2. Apparatus

The electrochemical measurements were performed on an Autolab PGSTAT100 potentiostat/galvanostat equipped with a frequency response analyzer system (Eco Chimie, Netherlands) controlled by two Autolab softwares; Frequency Response analyzer (4.9) for impedance and General purpose Electrochemical system (4.9) for voltammetry. Screen printed carbon electrodes (SPCEs) were fabricated using a DEK 248 screen-printing system as reported for 2-electrode systems [29]. The SPCE consists of conventional three electrode configuration with graphite as working (4-mm diameter disk) and counter (16 mm × 1.5 mm curved line) electrode, and Ag/AgCl (16 mm × 1.5 mm straight line) as pseudo reference electrode. The impedance spectra were recorded using a sinusoidal ac potential perturbation of 5 mV (rms), in the frequency range 10^4–0.5 Hz, superimposed on a dc potential of 0.1 V. All measurements were performed in a solution of 1.0 mM ferri/ferrocyanide couple $[Fe(CN)_6]^{4-/3-}$ in BB, pH 7.4, as a background electrolyte.

2.3. SPCE surface modification with film of diazonium salts

Prior to modification, SPCE was subjected to electrochemical pretreatment by applying several potential cycles between 1 and −1.5 V/pseudo Ag reference electrode with 100 mVs^{-1} scan rate in mixture of 0.5 M sulfuric acid and 0.1 M KCl until the CV characteristic for a clean SPCE surface is obtained. The clean SPCE surface modification was performed in the diazotation mixture