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Direct detection of OTA by impedimetric aptasensor based on modified polypyrrole-dendrimers

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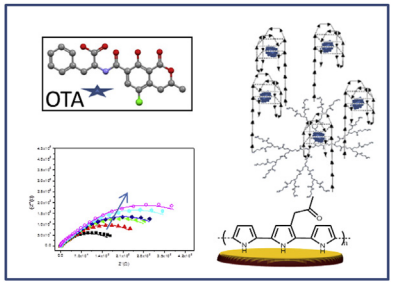
HIGHLIGHTS

- Development of innovative platform for direct and ultra-sensitive toxins detection.
- Aptasensor based on modified conductive polypyrrole layer.
- We demonstrate the conformation change of aptamer upon toxin binding.
- We highlight that detection was obtained by modification of charge of polypyrrole.
- Detection of OTA in wine was demonstrated.

GRAPHICAL ABSTRACT



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ABSTRACT

Ochratoxin A (OTA) is a carcinogenic mycotoxin that contaminates food such as cereals, wine and beer; therefore it represents a risk for human health. Consequently, the allowed concentration of OTA in food is regulated by governmental organizations and its detection is of major agronomical interest. In the current study we report the development of an electrochemical aptasensor able to directly detect trace OTA without any amplification procedure. This aptasensor was constructed by coating the surface of a gold electrode with a film layer of modified polypyrrole (PPy), which was thereafter covalently bound to polyamidoamine dendrimers of the fourth generation (PAMAM G4). Finally, DNA aptamers that specifically binds OTA were covalently bound to the PAMAM G4 providing the aptasensor, which was characterized by using both Atomic Force Microscopy (AFM) and Surface Plasmon Resonance (SPR) techniques. The study of OTA detection by the constructed electrochemical aptasensor was performed using Electrochemical Impedance Spectroscopy (EIS) and revealed that the presence of OTA led to the modification of the electrical properties of the PPy layer. These modifications could be assigned to conformational changes in the folding of the aptamers upon specific binding of OTA. The aptasensor had a dynamic range of up to 5 μg L^{-1} of OTA and a detection limit of 2 ng L^{-1} of OTA, which is below the OTA concentration allowed in food by the European regulations. The efficient detection of OTA by this

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electrochemical aptasensor provides an unforeseen platform that could be used for the detection of various small molecules through specific aptamer association.

1. Introduction

OTA is a low molecular weight mycotoxin naturally produced by *Aspergillus ochraceus*, *Aspergillus carbonarius* and *Penicillium verrucosum* and its toxicity is greatly enhanced by the coproduction of penicillic acid by *A. ochraceus* or of citrinin by *Aspergillus* spp. and *Penicillium* spp [1]. More importantly, this toxin is one of the predominant mycotoxins contaminant in a wide variety of improperly stored food and feed [2]. It is known to contaminate cereals, dried fruits, nuts, spices, coffee beans, cacao, beer and wine [3], additionally it is suspected of being the main etiological agent responsible for the Balkan endemic nephropathy [4]. Due to its genotoxic effect *via* oxidative DNA damage, as well as to its teratogenic and immunotoxic effects [5], OTA was classified by the International Agency for Research on Cancer as a human carcinogen (group 2B) [6]. Furthermore, the European Commission has set OTA consumption limits for a large range of agricultural commodities, for example the limit is 5 μg kg^{-1} of raw cereal grains, 10 μg kg^{-1} of soluble coffee, 2 μg L^{-1} of grape juice and wines [7].

In order to further ensure food safety and considering the high risks of contamination by OTA and subsequently its consumption, it is essential to develop rapid and sensitive tools to detect its presence in food and feed. The development of biosensors for OTA detection have been reported using different signal transducers based on immuno-detection processes [8–10]. Meanwhile, due to their distinctive selectivity and affinity, aptamers have been involved in several bioassays for OTA detection using different detection methods such as Surface Plasmon Resonance (SPR) [11,12], colorimetric [13–15] and electrochemical techniques [16,17]. The low sensitivity of the previously described specific OTA aptasensors was mainly caused by the low molecular weight of OTA and the aptasensors responses had to be further enhanced by either a competitive assay or the application of an amplification method after the OTA/aptamer interactions. Direct OTA detection and alternative ways to develop biosensing systems with simple manufacturing techniques are accordingly of major interest. Electrochemical impedance spectroscopy (EIS) is a powerful technique to follow the affinity interaction between OTA and aptamer. EIS was employed for OTA detection based on label-free impedimetric biosensors using various transducers for example gold electrode [18] or iridium oxide nanoparticle [19]. For this purpose, we developed an optimized OTA electrochemical biosensor based on aptamers as bioreceptor with poly (amidoamine) dendrimers of the fourth generation (PAMAM G4) covalently bound to conductive modified polypyrrole (PPy) coating gold electrode as transducer. The detection of OTA was achieved by following the modification of the electrical properties of the conducting PPy layer upon the interaction of OTA with the aptamer. This conducting polymer could be easily formed by electrochemical oxidation of a mixture of pyrrole and functionalized pyrrole monomers and has been shown to possess redox properties and high electrical conductivity [20]. Furthermore, functionalized PPy has been used to provide a suitable interface for the grafting of bioreceptors and has allowed the conversion of the probe/target biological interactions into electrochemical signals [21]. PPy is known to have p-doping process and its ability to interact with negatively charged DNA has been previously demonstrated [22]. These properties have been exploited for monitoring DNA hybridization using the subsequent changes in the electrical properties of PPy [23]. In this work, we took advantage of these unique properties of PPy to develop an aptasensor for the sensitive detection of OTA. The association of PPy with other nanomaterials such as PAMAM G4 [24,25] has been shown to improve the surface to volume ratio and to bring numerous primary amine groups useful for the coupling of bioreceptors. The association of dendrimers in the construction of biosensors has been previously proven including the case of DNA biosensors [26–28]. We also previously demonstrated that the high surface coverage of PPy with PAMAM G4 improves aptamers attachment and sensitivity and provides a larger dynamic range of detection during prion protein sensing [24,29].

In this work we highlight that the G-quadruplex formation due to aptamers/OTA interaction lead to changes in the electrical properties of the modified PPy. These changes were followed by Electrochemical Impedance Spectroscopy (EIS) measurements allowing the assessment of the biosensor in detecting OTA in buffer and in wine.

2. Materials and methods

2.1. Reagents

The amine modified aptamer was formed by 36 nucleotides with the sequence NH_2-$(CH_2)_6$-5′-GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA-3′, it was provided by Eurogentec (France). Ochratoxin A *N*-{[(3*R*)-(5-chloro-8-hydroxy-3-methyl-1-oxo-7-isochromanyl) carbonyl]-L-phenylalanine} (OTA, from *A. ochraceus*) and ochratoxin B (OTB, from *A. ochraceus*) were purchased from Santa Cruz Biotechnology (Germany). Pyrrole (Py) was purchased from Sigma–Aldrich and distilled under argon before use. The pyrrole-3-acetic acid (PyCOOH) was synthesized according literature procedure [30]. Poly(amidoamine) dendrimers of the fourth generation (PAMAM) (G4), 1-Ethyl-(3-dimethylaminopropyl)-carbodiimide and glutaraldehyde were purchased from Sigma–Aldrich. Phosphate-Buffered Saline (PBS) was prepared from 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, and 137 mM NaCl at pH 7.4. All solutions were prepared with deionized (DI) Milli-Q water (Milipore, El Paso, USA) and were filtered with 0.22 μm sterile membranes before use.

2.2. Biolayer formation and OTA detection

A cleaning step of the gold working electrode was performed as following before surface functionalization. The gold disk for SPR measurement was dipped for 3 min into a freshly prepared H_2SO_4/H_2O_2 (piranha, 3:1 (v:v)) solution followed by rinsing with deionized water, ethanol and dried with argon. The electrodes for CV and EIS measurements were polished with diamonds powder with (6 μm, 3 μm and 1 μm particle diameter) and were washed vigorously with water.

The various steps of the aptasensor construction are described in Scheme 1. The first step required the electrochemical polymerization of a mixture of Py and PyCOOH monomers on the electrode surface to form the film layer. In the second step, PAMAM G4 was covalently grafted on the copolymer film. Finally, the aptamers