Electrochemical Aptasensor for the Determination of Ochratoxin A at the Au Electrode Modified with Ag Nanoparticles Decorated with Macrocyclic Ligand

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Abstract
An electrochemical aptasensor for ochratoxin A (OTA) detection has been developed on the base of a gold electrode covered with electropolymerized neutral red and silver nanoparticles obtained by chemical reduction with macrocyclic ligands bearing catechol fragments. Thiolated aptamers against OTA were covalently attached to silver nanoparticles via Ag–S bonding. The interaction with OTA induced the conformational switch of the aptamer, which caused increase of the charge transfer resistance measured by EIS in the presence of ferricyanide ions. The *LOD* achieved (0.05 nM) was comparable to other electrochemical aptasensors employing sophisticated assembling technique and enzyme amplification of the signal. The aptasensor was validated in spiked beer samples. The recovery of the OTA determination was found to be 66.3 ± 14.1 % for light beer and 64.3 ± 1.8 % for dark beer.

Keywords: Aptasensor, Silver nanoparticles, Ochratoxin A, DNA aptamer, Electrochemical impedance spectroscopy

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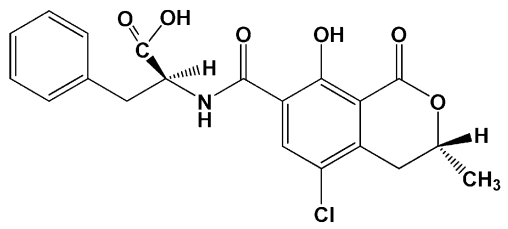
1 Introduction

Ochratoxin A (OTA) (Scheme 1) is a secondary metabolite produced by filamentous fungi of the genera *Aspergillus* and *Penicillium* present in a wide variety of foodstuffs, e.g., cereals, coffee, cocoa, grapes or spices. Due to the OTA stability, it is also frequently found in processed food, e.g., bread, beverages, and wine [1,2]. OTA exerts nephrotoxic, carcinogenic, teratogenic, immunotoxic and hepatotoxic effects and can probably cause nephropathies and urothelial tumors in humans [3,4]. There are evidences of the DNA adduct formation in the chronic exposure of OTA to rat and sub-acute exposure to pig [5].



Scheme 1. Structural formula of ochratoxin A.

The risks of OTA poisoning are related to high spread of the cereals contamination. The annual world crop of cereals exceeds 2000 million tones, and about 25–40 % of cereals are contaminated in the field or during the storage [6]. Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established for OTA provisional tolerable weekly intake (PTWI) of 112 ng/kg (0.28 nM) body weight per week [7]. The European Commission Regulation limits the OTA contamination in unprocessed cereals, including rice and buckwheat, up to 5 μg/kg (12.4 nM) [8].

The OTA contamination can be determined by HPLC with fluorescent detection after alkaline extraction of the mycotoxin residues from most matrices [9]. From other methods, ELISA [10–13] and competitive [14–16] immunoassay with fluorescent and SPR detection can be mentioned. Direct oxidation of OTA from alkaline solution at glassy carbon electrode was evaluated by measurement of the current using square-wave voltammetry [17]. Electrochemical immunosensors for OTA detection have been developed on the base of mono- and polyclonal antibodies attached to Au nanoparticles [18–21], magnetic beads [22,23] and ZnO [24] nanostructures. The signal was measured using cyclic voltammetry and electrochemical impe-

