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Detection of ochratoxin A in aptamer assay using total internal reflection ellipsometry

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ABSTRACT

The current work is a continuation of our research targeted the development of novel optical sensing technologies for detection of mycotoxins. The method of (TIRE) was developed in the last decade as a combination of spectroscopic ellisometry and SPR and was proved to be a highly sensitive analytical tool in bio-sensing particularly attractive for detection of low molecular weight analytes, such as mycotoxins. The use of aptamers as highly specific artificial molecular receptors to ochratoxin A (OTA) in conjunction with the method Total Internal Reflection Ellipsometry (TIRE) is reported here for the first time. Our results showed a possibility of label-free optical detection of OTA down to 0.01 ppb in concentration in direct assay with specific aptamer. The kinetics of aptamer/OTA binding was studied with dynamic TIRE spectral measurements and allowed evaluating the affinity constant $K_D = 1.8\ 10^{-8}$ Mol which is characteristic for highly specific aptamer/OTA binding.

1. Introduction

The main goal of this work is the development of novel optical sensing technologies for detection of mycotoxins. One of the most attractive optical biosensing technology developed in the last decade was the method of total internal reflection ellipsometry (TIRE) which is a combination of spectroscopic ellipsometry (SE) and surface plasmon resonance (SPR) [1].

This method appeared to be more sensitive than SPR [28], and thus suitable for detection of small molecular analytes, including pesticides (atrazine and simazine) [2] mycotoxins T2 [2,3], zearalenone [4], and aflatoxin B1 [5], alkyl-phenols [6], and microcystine [7].

On the biochemistry side, the specific bio-receptors for the above mentioned toxins were IgG-based antibodies (Ab). In majority of cases, the direct immunoassay sensing format was used with the antibodies immobilized electrostatically on the surface of gold via the layers of polycations (PAH or PEI) and proteins A or G [7,28]. Such immobilization procedure was relatively simple, universal (in respect of using different substrates, i.e. gold, glass, silicon), and was providing quite strong binding of Ab to the substrate (second strongest after covalent binding). However the stability of immobilized antibodies was always in question as well as a possibility of non-specific binding which resulted in a number of negative control tests to perform. Also, a multi-stage process of Ab immobilization prolongs the time of analysis.

Aptamers were developed recently as a synthetic alternative to antibodies in bio-sensing applications. Aptamers are linear biopolymers with specifically designed sequences of RNA or DNA oligonucleotides which bind to target molecules of both organic and inorganic origins [8,9]. The technology of aptamers synthesis improved dramatically in the last few years, so they became and commercially available for a wide variety of analytes, and in many cases less expensive than antibodies. Aptamers have a number of advantages over traditional antibodies, mainly in their robustness and simple immobilization chemistry. Aptamers being relatively small size receptors seem to be particularly suitable for optical detection of small toxin molecules because of a large relative increment of thickness (or refractive index) when binding analytes. Aptamers were recently used successfully in detection mycotoxins, such as ochratoxin A [10].

Ochratoxin A (OTA), an object our present study, is a mycotoxin produced by some of *Aspergillus* or *Penicillium* fungi species. It is one of the most-abundant food contaminant known by its carcino-

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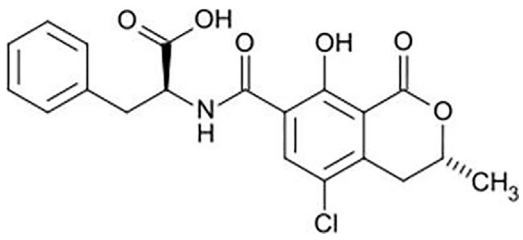

Fig. 1. Chemical structure of ochratoxin A.

genic, genotoxic and mutagenic actions on human [11–13]. OTA is a relatively small molecule (see chemical structure in Fig. 1) with the molecular weight of 403.8 Da.

Traditional biosensing technique such as ELISA is capable of detecting OTA in the concentration range down to of 5–100 ng/ml [14]. More recent work [15] achieved the detection limit for OTA of 1 ng/ml using ELISA aptamer assay. The detection limit for OTA detection in aptamer assay was lowered substantially down to single ng/ml using other transducing techniques such as electrochemical, optical and piezo-electrical methods [16–19]. Aptamers can be immobilized on the surface of gold via functional thiol group on one end [20], and may contain either fluorescent groups or redox labels such as methylene blue on the other end as described by [21,22] and therefore can be used in optical or electrochemical biosensors. The chromatographic strip assay method [23] utilising labled aptamer for rapid toxin detection. High sensitivity of toxin detection can be achived in a sandwich assay format, for example using a pair of aptamer and antibody specific to toxins [24–27]. The sensitivity of electrochemical detection can be boosted using competitive aptasensor assay test for OTA coupled with paramagnetic beads. In this work we report for the first time the label-free optical detection of ochratoxin A in the direct assay with highly specific aptamers using the method of TIRE.

2. Experimental methodology

2.1. Samples' preparation and immobilization of aptamers

Standard microscopic glass slides were cleaned in hot piranha solution (3:1 mixture of H_2SO_4 and H_2O_2) for 1 h followed by rinsing with di-ionized Milli-Q water and drying under a stream of nitrogen gas. Gold layers of about 25 nm in thickness were evaporated on glass slides using Edwards E306A metal evaporator unit. A thin (2–3 nm) layer of chromium was evaporated first to improve the adhesion of a gold layer to glass. Such two-stage evaporation was carried out without breaking the vacuum of no less than 10^{-6} Torr.

DNA-based aptamers specific to OTA acquired from M/sMicrosynth (Schutzenstrasse, Balgach, Switzerland) have a following oligonucleotides sequence: 5′-SH-GATCGGGTGTGGGTGGC GTAAAGGGAGCATCGGACA-3′. The aptamer was functionalized with thiol group (C3-SH) on the 5′ terminal position to obtain a strong and oriented binding to gold. The immobilization of aptamers on gold surface was carried out by mixing the original 100 μM aptamer solution with 2 mM of 1,4-Dithiothreitol (DTT) diluted in 100 mM HEPES buffer (pH 7.4) supplemented with 2 mM of $MgCl_2$ in order to remove the protecting group and subsequently release aptamers with the SH end-groups which then bind to gold [10,16,27]. Before immobilization, the liquid aptamer samples were activated by quick (5 min) heating up to 90 °C followed by 5 min cooling to 4 °C using thermo-cycling PCR unit (Master cycler personal Eppendorf VWR, Leuven, Belgium). Immobilization was carried out by casting aptamer solution onto gold coated slides; the immobilization time was 10–12 h at room temperature in moist atmosphere. The unreacted oligonucleotide was removed from the gold slides by several rinsing stages with HEPES/$MgCl_2$ buffer. Then, gold coated glass slides with immobilized aptamers were kept in HEPES/$MgCl_2$ to prevent aptamers from coiling. Interestingly, the samples prepared were quite stable and keep their functionality for few weeks.

2.2. TIRE measurements

The method of total internal reflection ellipsometry (TIRE) and its application for detection of mycotoxins has been described previously in detail [5,28]. In the TIRE method being a combination of spectroscopic ellipsometry and SPR, the spectra of two ellipsometric parameters Ψ and Δ were recorded, where Ψ and Δ represent, respectively, the amplitude ratio and phase shift between *p*- and s- components of polarized light. The spectrum of Ψ resembles the traditional SPR graph, while Δ-spectrum exhibits a phase drop near the plasmon resonance, position of which is much more sensitive (as compared to Ψ) to molecular adsorption. That is why, Δ-spectra are typically recorded in TIRE biosensing [28] and thus used in this work.

The TIRE experimental set-up (shown schematically as inset in Fig. 2) is based on J.A. Woollam M2000 spectroscopic ellipsometer with the addition of a 68° glass prism (providing the light coupling at total internal reflection conditions) optically connected via index matching fluid with the gold coated glass slide. The reaction cell with the inlet and outlet tubes were attached underneath to the gold surface and allowed the injection of the required chemicals to perform binding reactions.

In our case of gold coated glass slides with immobilized aptamers, the injected solution was ochratoxin A (OTA) acquired from Microsynth (Switzerland), the original stock solution (10 μg/ml) of OTA in acetonitrile was multiply diluted with PBS buffer to obtain the required concentrations of 0.01, 0.1, 1, 10, 100, and 1000 ng/ml.

Two types of ellipsometric measurements were carried out: (i) dynamic measurements, e.g. a large number of spectroscopic scans taken during the binding of analytes to receptors which give the information on the reactions kinetics, and (ii) single spectroscopic scans carried out in a standard buffer solution after completion of the adsorption (or binding) stage. The latter measurements are actually used for sensing. Typically, the shift of spectra of Δ (a phase related ellipsometric parameter), constitutes the TIRE sensor response.

3. Results and discussion

3.1. TIRE detection of OTA in direct assay with specific aptamer

A typical series of TIRE Δ-spectra recorded after injecting OTA of different concentrations is shown in Fig. 2. As one can see a progressive blue (e.g. to the shorter wavelengths) spectral shift

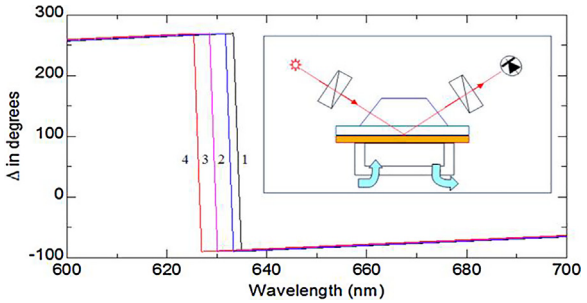

Fig. 2. Total Internal Reflection Ellipsometry (TIRE) spectra recorded on aptamer layer (1) and after binding Ochratoxin A (OTA) of 0.01 ng/ml (2), 1 ng/ml (3) and 10 ng/ml (4).