Biosensors and Bioelectronics 26 (2010) 333–337

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Ultrasensitive mycotoxin detection by STING sensors

Paolo Actis [a], Olufisayo Jejelowo [b], Nader Pourmand [a,*]

[a] Department of Biomolecular Engineering, University of California Santa Cruz, 1156 High Street, Santa Cruz, CA 95064, USA
[b] Department of Biology, Texas Southern University, 3100 Cleburne Street, Houston, TX 77004, USA

ARTICLE INFO

Article history:
Received 25 May 2010
Received in revised form 16 July 2010
Accepted 12 August 2010
Available online 19 August 2010

Keywords:
Nanopipette
Label-free
Single-molecule detection
Biosensor
Nanopore
STING

ABSTRACT

Signal transduction by ion nano-gating (STING) technology is a label-free biosensor capable of identifying DNA and proteins. Based on a functionalized quartz nanopipette, the STING sensor includes specific recognition elements for analyte discrimination based on size, shape and charge density. A key feature of this technology is that it does not require any nanofabrication facility; each nanopipette can be easily, reproducibly, and inexpensively fabricated and tailored at the bench, thus reducing the cost and the turnaround time. Here, we show that STING sensors are capable of the ultrasensitive detection of HT-2 toxin with a detection limit of 100 fg/ml and compare the STING capabilities with respect to conventional sandwich assay techniques.

1. Introduction

Mycotoxins are small (MW ~700 g/mol), toxic chemical products formed as secondary metabolites by a few fungal species that readily contaminate crops in the field or after harvest. These compounds pose a potential threat to human and animal health through the ingestion of food products prepared from contaminated commodities.

At this time, the great diversity of toxins represents a challenge; detection methods are currently specific for individual toxins or groups of similar toxins. Because each toxin requires a different method, standardization of techniques to detect all mycotoxins remains elusive. Likewise, practical requirements for high-sensitivity detection and the need for a specialized laboratory setting create challenges for routine analysis. Therefore, depending on the physical and chemical properties of the toxins, procedures have been developed around existing analytical techniques, which offer flexible and broad-based methods of detecting compounds (Turner et al., 2009).

Traditionally, thin-layer chromatography (TLC) and high-pressure liquid chromatography (HPLC) have been employed for toxin detection. However, the tedious sample preparation and cleanup often lead to inconsistent results and poor sensitivity (Daly et al., 2000). Various research groups have employed surface plasmon resonance (SPR) - based sensors for applications such as inhibition immunoassays (Stubenrauch et al., 2009) and antibody affinity analysis (Reid et al., 2007). SPR analysis changes in the interfacial optical properties of modified electrodes induced by the binding of biomolecules on the surface. Although the SPR platform is capable of label-free, real time monitoring of molecules as small as 200 Da, this requires highly sophisticated and expensive equipment (Skottrup et al., 2008).

In their 2002 study, Schnerr et al. (2002) developed an inhibition immunoassay for the rapid quantification of the trichothecene mycotoxin deoxynivalenol using the SPR-based Biacore system. Despite its versatility, the complexity and the cost of the Biacore instrumentation remain very high (Mullett et al., 1998). The low molecular weight of mycotoxins is often not enough to induce significant change upon binding to the sensor surface. Consequently, an alternative assay strategy is required for mycotoxin detection using SPR. One of the most established laboratory-based biochemical assays for pathogen detection is ELISA, which is based on the detection of pathogen-specific surface epitopes using antibodies (Cunningham, 2000). With its very high specificity and exceptional sensitivity, ELISA is often referred to as the gold standard of toxin detection. Nevertheless, current assays typically involve reporter molecules or labels conjugated to enzymes or fluorescent markers, which makes ELISA restricted to advanced laboratory settings with specialized read-out equipment (Skottrup et al., 2008). Accurate and rapid read-out on site would provide vital efficiency in toxin detection, reducing potential risks of further unnecessary food-borne pathogen contamination. However, implementing ELISA into a point-of-use test remains challenging due to the sheer complexity of the instrumentation involved. In 2009, Valdés et al. (2009)

* Corresponding author. Tel.: +1 650 812 2002; fax: +1 650 812 1975.
E-mail address: pourmand@soe.ucsc.edu (N. Pourmand).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.08.016

reviewed the application of nanotechnology-based platforms for the detection of mycotoxins.

More recently, our lab employed a magnetic nanotag (MNT) detection platform for multiplexed mycotoxin detection (Mak et al., 2010). Real-time measurements were conducted upon the addition of MNTs onto the spin-valve sensor surface immobilized with capture antibodies for mycotoxins (aflatoxin-B1, zearalenone and HT-2). The MNT technology demonstrated detection limits for mycotoxins in the pg/ml level.

Here we describe a new technique, signal transduction by ion nano-gating (STING), which uses a functionalized quartz nanopipette as an electrochemical biosensor. A key feature of this technology is that it does not require any nanofabrication facility; each nanopipette can be easily, reproducibly, and inexpensively fabricated and tailored at the bench, thus reducing the cost and the turnaround time. The electrochemical sensitivity of the device is maximized at the nanopipette tip, essentially an elongated cone, making the dimension and geometry of the tip orifice crucial for biosensor performance (Umehara et al., 2009). Permanent blockade, or gating, from binding events at the nanoscale-sized tip opening cause distinctive changes to the nanopipette electrical signature. The electrical changes are then detected with simple electrochemical measurements in real time without any need for labeling. For a more detailed explanation of the ion nano gating mechanism and the electrochemical characteristic of nanopipette electrodes, see our recent review, (Actis et al., 2010). The selectivity of the nanopipette sensor can be customized for many different targets by introducing highly specific bio-recognition agents such as antibodies (Umehara et al., 2009), DNA (Fu et al., 2009), and aptamers (Ding et al., 2009). The quartz pipettes also provide an ideal interface to append such bio-receptors using established surface-modification chemistry. Nanopipette-based platforms have been used to investigate single-molecule biophysics (Clarke et al., 2005), for the controlled delivery of molecules inside a single cell (Laforge et al., 2007), and to image cells at the nanoscale (Klenerman and Korchev, 2006). We have recently demonstrated that STING technology can selectively detect interactions such as biotin–streptavidin and protein–protein binding (Umehara et al., 2009).

In this paper, we discuss application of the STING platform for the ultrasensitive detection of a mycotoxin belonging to the species *Fusarium*, HT-2 toxin. The detection of HT-2 toxins presents unique challenges due to their low molecular weight (<500 Da) and their insolubility. We examine the sensor's limit of detection and linear range and compare the STING capabilities with respect to conventional sandwich assay techniques.

2. Experimental

2.1. Reagents

Poly-L-lysine (PLL; 19320-A) was purchased from Electron Microscopy Sciences (Hatfield, PA). Sulfo-SMCC was purchased from G biosciences (Maryland Heights, MO). Monoclonal antibody for HT-2 (anti-HT-2, clone C6B4) was purchased from Advanced Immuno-Chemical Inc. (Long Beach, CA). HT-2 toxin was acquired from Sigma–Aldrich (T4138). Polyclonal antibody HPV16 E6 (C-19) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and used as a control. PBS solutions at pH 7.4 were prepared using standard method. Ridascreen® ELISA kits for zearalenone was purchased from RBiopharm AG (Darmstadt, Germany). *N*,*N*-Dimethylformamide (99% purity) was purchased from Sigma–Aldrich and used without further purification. Aqueous reagents were prepared using nanopure water with >18 MΩ cm^{-1} resistance.

2.2. STING sensors fabrication

Nanopipettes were fabricated from quartz capillaries with filaments, with an outer diameter of 1.0 mm and an inner diameter of 0.70 mm (QF100-70-5; Sutter Instrument Co.). Prior to pulling, glass capillaries were cleaned with sulfuric acid/hydrogen peroxide (piranha solution) (Tabard-Cossa et al., 2007).

Caution: The piranha solution is a highly energetic oxidizer. It reacts very violently with organic materials.

The capillary was then pulled using a P-2000 laser puller (Sutter Instrument Co.) preprogrammed to fabricate nanopipettes with an inner diameter of 50 nm. Parameters used were: Heat 700, Fil 4, Vel 60, Del 150, and Pul 192. The resulting nanopipette tips had inner diameters ranging from 37 to 82 nm, with the mean diameter of 56 nm (Karhanek et al., 2005).

2.3. Surface functionalization: antibody immobilization

Antibodies were immobilized through the following steps. First, nanopipettes were internally coated by filling with a 0.01% solution of poly-L-lysine in water, followed by centrifugation at 4600 rpm for 3 min. The centrifugation step helps to get the solution to the very tip of the nanopipette. After the removal of excess PLL solution, the nanopipettes were baked at 120 °C for 1 h to stabilize the PLL coating (Umehara et al., 2006). The nanopipette was then filled with a sulfo-SMCC solution (2 mg/ml, 10 mM EDTA and 50 mM PBS), centrifuged at 4600 rpm for 3 min and then incubated at room temperature for 1 h. Nanopipettes were then filled with 0.01 M PBS and centrifugated for at least 3 times to remove any unreacted sulfo-SMCC molecules. Sulfo-SMCC contains an amine-reactive *N*-hydroxysuccinimide (NHS ester) that reacts with the PLL amino groups, leaving a maleimide group available for the antibodies cross link through a thioether bond. The nanopipettes were then incubated with antibody solution (10 μg/ml IgG, PBS, 1 h, 37 °C). Antibody-functionalized nanopipettes were then filled at least 3 times with PBS and centrifuged, rinsed two more times with a PBS (0.01 M)/DMF (80/20) solution to remove any unbound antibody and to provide a smooth electrolyte filling throughout the tip.

2.4. Measurement setup

Since the current flowing through the nanopipette is too small to polarize a reference electrode (Wei et al., 1997), a two electrode setup was used. A typical setup is shown in Fig. 1. The STING sensor, acting as the working electrode, is backfilled with the working buffer, and a Ag/AgCl electrode is inserted. Another Ag/AgCl electrode is placed in bulk solution acting as auxiliary/reference electrode. Both electrodes are connected to the Axopatch 700B amplifier with the DigiData 1322A digitizer (Molecular Devices), and a PC equipped with pClamp 10 software (Molecular Devices). Since nanobubbles are the dominant source of noise in solid state nanopores (Smeets et al., 2006), every solution was degassed prior use. The system remained unstirred for the duration of the measurement, which was conducted at room temperature.

3. Results and discussion

Fig. 1 schematically illustrates the operation of the STING platform. The electrochemical sensitivity of the device is maximized at the nanopipette tip, essentially an elongated cone, making the dimension and geometry of the tip orifice crucial for biosensor performance (Umehara et al., 2009). The STING sensor is based on nanopipettes fabricated using a P-2000 laser puller where the protocol was optimized to get reproducible pore opening within the range 30–70 nm. We extensively tested the reproducibility of the STING sensor fabrication as well as the surface chemistry and these