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Short communication

Chemiluminescence-based biosensor for fumonisins quantitative detection in maize samples

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ABSTRACT

A compact portable chemiluminescent biosensor for simple, rapid, and ultrasensitive on-site quantification of fumonisins (fumonisin B1 + fumonisin B2) in maize has been developed. The biosensor integrates a competitive lateral flow immunoassay based on enzyme-catalyzed chemiluminescence detection and a highly sensitive portable charge-coupled device (CCD) camera, employed in a contact imaging configuration. The use of chemiluminescence detection allowed accurate and objective analyte quantification, rather than qualitative or semi-quantitative information usually obtained employing conventional lateral flow immunoassays based on colloidal gold labeling. A limit of detection of 2.5 $\mu g\,L^{-1}$ for fumonisins was achieved, with an analytical working range of 2.5–500 $\mu g\,L^{-1}$ (corresponding to 25–5000 $\mu g\,kg^{-1}$ in maize flour samples, according to the extraction procedure). Total assay time was 25 min, including sample preparation. A simple and convenient extraction procedure, performed by suspending the sample in a buffered solution and rapidly heating to eliminate endogenous peroxidase enzyme activity was employed for maize flour samples analysis, obtaining recoveries in the range 90–115%, when compared with LC–MS/MS analysis. The chemiluminescence immunochromatography-based biosensor is a rapid, low cost portable test suitable for point-of-use applications.

1. Introduction

Portable devices and biosensors useful for "point-of-use" applications are gaining wide consideration for performing rapid and low-cost analyses directly where the sample is obtained, thus reducing assay costs and turnaround times with respect to traditional laboratory-based analytical systems. Devices for on-field sample analysis have been developed for a wide range of applications in the clinical (Kaittanis et al., 2010), environmental (Spier et al., 2011), forensic (Hoile et al., 2011) and agri-food (Fernandez et al., 2010) fields. In the context of food safety control, where the ability for early detection of contamination is of particular importance, cheap and rapid on-field tests are increasingly employed for first level screening purposes.

Fumonisins, mainly produced by *Fusarium* mould species commonly infecting corn and other agricultural products, represent a remarkable problem which appears to be more and more relevant in the context of global market. Due to their adverse effects in animals (Stockmann-Juvala and Savolainen, 2008) and humans (International Agency for Research on Cancer, IARC, 1993, pp. 301–366), maximum residue limits (MRLs) of fumonisins (as the sum of FmB1 + FmB2) in corn-derived foodstuff have been established by the European Union, ranging from 200 $\mu g\,kg^{-1}$ for baby food to 4000 $\mu g\,kg^{-1}$ for raw maize (EC No. 1126/2007).

Analytical methods for fumonisins have been recently reviewed (Krska et al., 2008; Maragos and Busman, 2010). Reference analytical methods are based on HPLC techniques (AOAC Official Methods 995.15 and 2001.04), while enzyme-linked immunosorbent assays are widely employed for screening purposes.

Since contamination by fumonisins can occur at any stage of the food chain (e.g., on field, at harvest, during storage and transportation) frequent analyses are required to promptly detect any contamination, thus reduce risks for the consumer. Various rapid and simple "point-of-use" analytical methods have been developed, including Lateral Flow Immunoassays (LFIAs) (Shiu et al., 2010; Wang et al., 2006) and biosensors (Sapsforda et al., 2006).

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In LFIAs a porous membrane with specific immunoreagents immobilized in definite areas is employed as a solid support, while other reagents are carried along the strip by the flow (driven by capillary forces) established upon sample addition. The immunological reactions occurring in correspondence of the functionalized strip areas and separation of antibody-bound and free fractions are expedited by the flow, thus reducing overall assay time. Both non-competitive sandwich-type and competitive LFIAs have been developed and a number of them are also commercially available (Posthuma-Trumpie et al., 2009). Conventionally LFIAs are based on the use of colloidal gold or latex particles labelling and the analyte is visually detected through color formation due to label accumulation in definite strip zones. This approach often suffers from poor and subjective quantitative discrimination and low assay sensitivity thus might be not suitable to fulfill the regulatory requirements (Reiter et al., 2009).

To obtain quantitative information, several hand-held devices capable of scanning the LFIA strip and estimating analyte content from the quantitative evaluation of the lines' color intensity have been proposed (Anfossi et al., 2010, 2011; Molinelli et al., 2009; Salter et al., 2006). In addition, detection systems based on enzyme amplification (Cho et al., 2006, 2009), fluorescent nanoparticles (Li et al., 2010; Xia et al., 2009; Zou et al., 2010) or electrochemical measurements (Fernandez-Sanchez et al., 2004; Muhammad-Tahir and Alocilja, 2003) have been developed to increase detectability.

Enzyme-catalyzed chemiluminescence (CL) relies in the use of an enzyme as a label, usually horseradish peroxidase (HRP) or alkaline phosphatase, which is detected with highly sensitive photomultiplier-based or CCD-based light measurement instruments upon addition of a suitable CL substrate. This approach has been widely demonstrated to provide superior analytical performance, yielding high detectability, amenability to miniaturization, short assay times and low sample and reagents consumption (Kricka, 2000; Magliulo et al., 2005; Roda et al., 2003). These features derive from both the amplification factor offered by the cyclic enzyme reaction and the intrinsic characteristics of CL. Indeed, since light emission is generated from dark by a chemical reaction, limitations typical of spectrophotometric or fluorescence detection techniques are circumvented (Kricka, 2000; Roda et al., 2003).

Recently, enzyme-catalyzed CL detection has been also proposed for LFIA, providing quantitative information and improved limits of detection with respect to color-formation-based LFIAs (Cho et al., 2009). Nevertheless, only a few examples have been reported in the literature, and in most cases the described systems rely on bulky instrumentation for photon emission measurements, thus reducing the on-field applicability of the methods.

In this work we describe the development a biosensor exploiting a CL competitive LFIA for fumonisins (FmB1 and FmB2). The competitive immunological reaction and the enzyme-catalyzed CL reaction were conducted sequentially in the LFIA strip and the HRP-labeled tracer antibody was revealed by CL to achieve objective quantitative information. To produce a compact and portable biosensor, the CL signal measurement was performed by contact imaging employing a compact light detection device equipped with an ultrasensitive cooled CCD sensor (Roda et al., 2011a, 2011b). In this set-up, the LFIA membrane was placed in contact with the CCD imaging sensor through a tapered fiber optic faceplate without the use of a lens-based optical system, thus allowing localization and quantification of the emitted photons with high light collection efficiency in a very compact device. The tapered configuration of the faceplate increased the useful analytical surface with respect to the CCD sensor area, making it compatible with the size of the LFIA membrane.

The developed LFIA with CL detection was able to provide sensitive and quantitative information on fumonisins content in corn flour samples, employing a simple pre-analytical sample clean-up, using a compact portable device and therefore enabling out-lab on-field application.

2. Materials and methods

2.1. Reagents

Bovine serum albumin (BSA), Tween-20, horseradish peroxidase (HRP), FmB1 (Oekanal standard solution), anti-HRP antibody, and HRP-labeled goat anti-rabbit immunoglobulin were purchased from Sigma Aldrich (St. Louis, MO, USA). Goat anti-rabbit antibody, the FmB1-BSA conjugate, and rabbit anti-fumonisin antibody were kindly supplied by Generon srl (Modena, Italy) and previously described (Anfossi et al., 2010). The Supersignal ELISA Femto CL substrate for HRP was purchased from Thermo Fisher Scientific Inc. (Rockford, IL).

The other reagents were of analytical grade and were employed as received.

Phosphate buffered saline (PBS) was prepared as follows: 10 mmol L^{-1} Na_2HPO_4, 2 mmol L^{-1} KH_2PO_4, 137 mmol L^{-1} NaCl, 2.7 mmol L^{-1} KCl, pH 7.4.

Assay strips for LFIA were prepared as previously described (Anfossi et al., 2010) by immobilizing the FmB1-BSA conjugate (T-line) and the goat anti-rabbit antibody (C-line) on nitrocellulose membranes, which were then assembled with an adsorbent pad and cut into sections (5 mm width). Details are available as Supplementary material.

2.2. Instrumentation

The biosensor, shown in Fig. 1, was assembled employing a previously described CCD-based contact imaging configuration (Roda et al., 2011b). In particular, the LFIA strip was positioned on the larger surface of a round fiber optic taper, which smaller surface was placed directly in contact with the CCD sensor. A mask was used to ensure reproducible strip positioning. This assembly was enclosed in a dark box to provide shielding from ambient light. During the acquisition the CCD sensor temperature was kept at −10 °C.

As a reference laboratory instrument, a Night OWL LB 981 luminograph (Berthold Technologies GmbH & Co KG, Bad Wildbad, Germany) equipped with a conventional lens-based optics and a highly sensitive, back-illuminated, double-Peltier-cooled CCD camera (Guardigli et al., 2005; Roda et al., 1996, 2002) was also used.

All CL images were processed and analyzed employing the WinLight software v. 1.2 (Berthold Technologies GmbH & Co KG, Bad Wildbad, Germany).

2.3. Assay procedure

The nitrocellulose strip was placed horizontally on the fiber optic taper surface, then the LFIA assay was started by depositing on the bottom of the strip a volume of 100 μL of solution, containing 40 μL of PBS with 3% BSA (w/v), 5 μL of HRP-labeled goat anti-rabbit antibody diluted 1:10,000 (v/v) in PBS, 5 μL of rabbit anti-fumonisin antibody diluted 1:500 (v/v) in PBS, and 50 μL of maize sample extract (or blank maize sample extract for the blank, or FmB1 standard solutions ranging from 1 to 500 μg L^{-1} prepared in blank maize sample extract to produce calibration curves). Upon complete migration of the solution (10 min), 80 μL of the CL substrate was added at the bottom of the strip and let flow through the membrane (4 min), which was kept at 25 °C. The CL signal was acquired with the contact CCD-based imaging device (10 s acquisition time) or with the Night OWL LB 981 luminograph (10 s acquisition time). Total analysis time was about 15 min. To obtain quantitative information, the mean photon emission was measured in the areas corresponding to C-line and T-line of each LFIA strips