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A universal multi-wavelength fluorescence polarization immunoassay for multiplexed detection of mycotoxins in maize

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ABSTRACT

Multi-analyte immunoassays have attracted increasing attention due to their short assay times, low sample consumption, and reduced detection costs per assay. In this work, we describe a homologous and high-throughput multi-wavelength fluorescence polarization immunoassay (MWFPIA) for the multiplexed detection of mycotoxins. Three typical *Fusarium* mycotoxins, deoxynivalenol (DON), T-2 toxin and fumonisin B_1 (FB_1), were labeled with different dyes. Tracers and specific monoclonal antibodies (mAbs) were employed in the MWFPIA to simultaneously detect the three mycotoxins. Under optimal conditions, the limits of detection using MWFPIA were 242.0 μg kg^{-1} for DON, 17.8 μg kg^{-1} for T-2 toxin and 331.5 μg kg^{-1} for FB_1, providing sufficient sensitivity to meet the action levels of these three contaminants in maize as set by the European Union. The use of a methanol/water (2:3, v/v) mixture for sample pretreatment allowed recoveries ranging from 76.5–106.3%, with coefficients of variation less than 21.7%. The total time of analysis, including sample preparation, was less than 30 min. Twenty naturally contaminated maize samples were tested using MWFPIA and HPLC–MS/MS, with correlation coefficients (R^2) of 0.97 for DON and 0.99 for FB_1. By changing the targets of interest, homologous MWFPIA, a method with high sensitivity, a simple procedure and a short analysis time, can easily be extended to other chemical contaminants. Thus, MWFPIA represents a versatile strategy for food safety analysis.

1. Introduction

Mycotoxins are toxic, low-molecular-weight, secondary fungal metabolites that pose the greatest hazard among all food and feed contaminants in terms of chronic toxicity; therefore, mycotoxins are a major public health concern (De Saeger, 2011). Deoxynivalenol (DON), T-2 toxin and fumonisin B_1 (FB_1) (Fig. 1A) are three typical mycotoxins produced mainly by the *Fusarium* genus that are found globally in cereal crops (Joint FAO/WHO Expert Committee on Food Additives, 2002). Although their presence in food and feed are serious threats to human and animal health, it is also notable that these three mycotoxins always coexist in individual samples (Streit et al., 2012). Thus, the ability to monitor multiple mycotoxins is obviously preferable in order to gather more information about the sample through a single analysis.

Analytical methods such as liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) are well established for multi-mycotoxin analysis (Monbaliu et al., 2010; Ren et al., 2007; Sulyok et al., 2006). Recently, a multi-mycotoxin, LC–MS/MS-based method for simultaneously detecting 21 mycotoxins was reported (García-Moraleja et al., 2015). However, using LC–MS/MS as a confirmatory method is less suitable or practical for rapid, high-throughput screening of contaminants in large sample sets, because this approach requires sophisticated and expensive equipment, tedious sample pretreatment, and highly skilled technicians. Immunoassays, which have the advantage of being simple, rapid, sensitive, and cost effective, are being widely used to sift through targets in large sample sets. However, conventional immunoassays are designed to detect only one analyte per sample.

With the increasing desire to improve detection efficiency and

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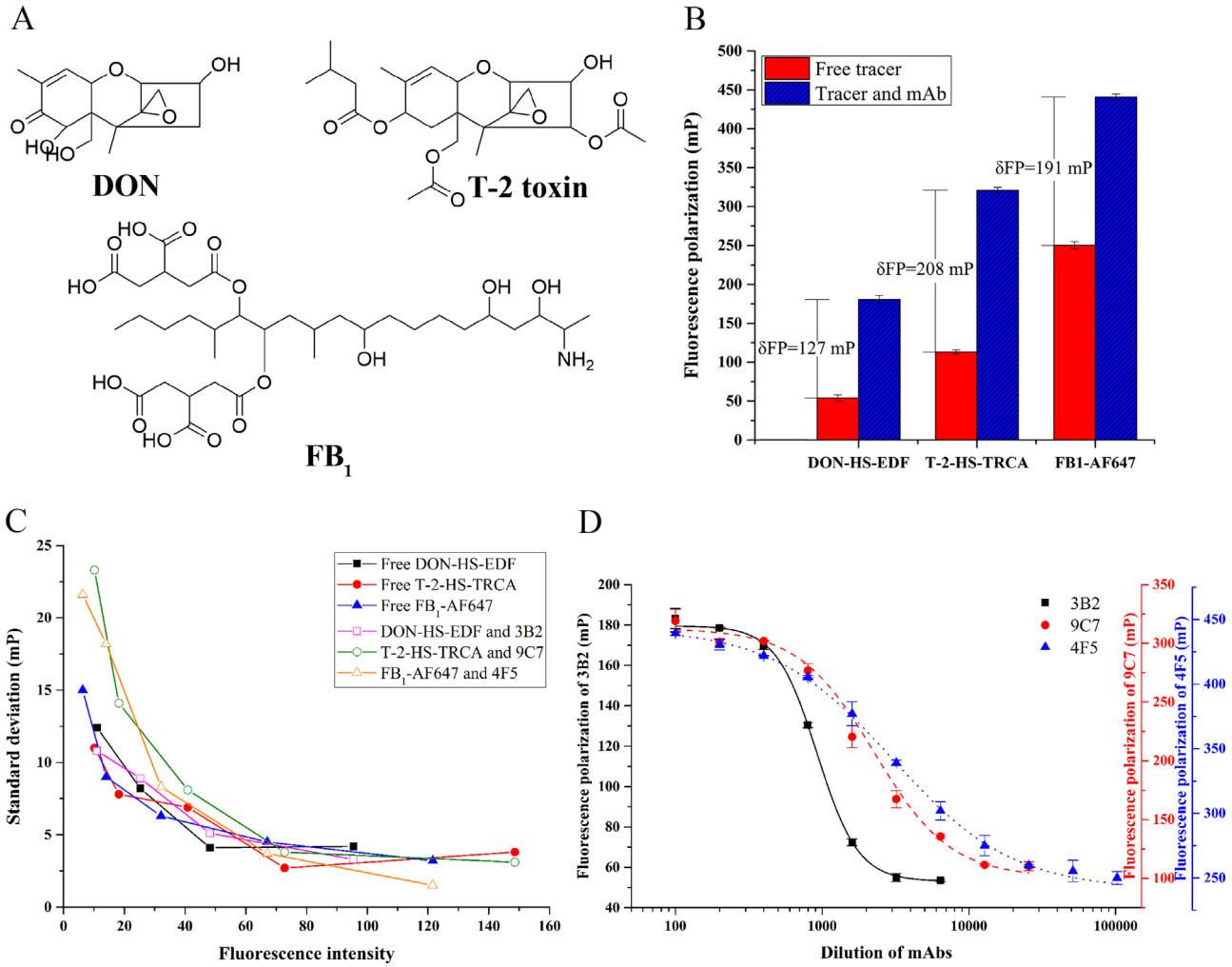

Fig. 1. (A) Chemical structures of the three mycotoxins (DON, T-2 toxin and FB_1). (B) Results of the three tracers binding with their corresponding 100-fold-diluted mAbs ($n=3$). (C) Effect of tracer concentration on the precision of the FP signal ($n=4$); the FI of the BB background was approximately 2.27 for 485 nm/530 nm ($\lambda_{ex}/\lambda_{em}$), 0.92 for 540 nm/580 nm and 0.77 for 640 nm/675 nm. (D) Antibody dilution curves of tracers with their corresponding mAbs ($n=3$).

reduce testing costs, major efforts have been dedicated to exploring alternatives methods of screening multiple analytes in a single run. Multiplexed immunoassays to simultaneously detect analytes without cross-reactivity generally employ one of two approaches. The first approach is based on spatial resolution in label-free or single-label platforms (Adrian et al., 2008; Basova et al., 2010; Dorokhin et al., 2011; Lattanzio et al., 2012; Oswald et al., 2013; Yu et al., 2015). The other approach uses multiple labels, including different radioactive markers, enzymes, metal ions, quantum dots, microbeads, microspheres, upconversion nanoparticles or fluorescent dyes (Geißler et al., 2013; Goldman et al., 2004; Huang et al., 2009; Jiang et al., 2013; Peters et al., 2013; Wang et al., 2013; Wu et al., 2015). Several of these multi-mycotoxin screening methods employing surface plasmon resonance (SPR), enzyme-linked immunosorbent assays (ELISA), lateral flow immunoassays (LFIA), flow-through immunoassays, and microarray-based immunoassays have been developed. However, these are heterologous, solid-phase immunoreactions, some of which require more than 2 h to achieve a plateau and have multiple incubations and washes to separate free and antibody-bound targets, and some primarily focus on qualitative “yes/no” testing.

The fluorescence polarization immunoassay (FPIA), which has a homologous format and the unique advantage of lacking separation and washing steps, has been primarily applied in pharmaceutical and medical research in order to shorten assay time, increase throughput, and simplify detection procedures (Nasir and Jolley, 1999). Recently, FPIAs based on FP changes in the solution phase, in which an analyte and a dye-labeled tracer compete for binding to a specific antibody, have been developed for detecting chemical contaminants such as aflatoxins, fumonisins, DON, ochratoxin A, and zearalenone in food and feed (Maragos, 2009). We previously developed an FPIA for detecting fumonisins in maize, showing that FPIA provided obvious advantages over conventional heterologous immunoassays (Li et al., 2015). The potential of using homologous FPIA to address “multiplexing” was easily realized. However, these methods have only recently been applied. These new applications include the use of a dual-wavelength FP to confirm a peptide (residues 733–767) from $p130^{CAS}$ can concurrently bind SH3 and SH2 domains of the Src protein and to simultaneously identify selective ligands of estrogen receptor α and/or progesterone receptor (Blommel et al., 2004; Lynch et al., 1999). To the best of our current knowledge, there are no publications that use multiple labels in an FPIA to simultaneously detect small molecules such as chemical contaminants in food. In this work, a homologous, high-throughput, and multi-wavelength fluorophore-based FPIA (MWFPIA) for the multiplexed detection of DON, T-2 toxin and FB_1 in maize was proposed. We combined multi-color tracers synthesized using different dyes and specific monoclonal antibodies (mAbs) to perform the MWFPIA. All the mycotoxins in the samples could be detected in just a single