Letter to the Editor

The Development of A Fluorescence Polarization Immunoassay for Aflatoxin Detection*

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A fluorescence polarization immunoassay (FPIA) was developed for the analysis ofaflatoxins (AFs) using an anti-aflatoxin B_1 (AFB_1) monoclonal antibody and a novel fluorescein-labeled AFB_1 tracer. The FPIA showed an IC_{50} value of 23.33 ng/mL with a limit of detection of 13.12 ng/mL for AFB_1. The cross-reactivities of AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, and AFM_2 with the antibody were 100%, 65.7%, 143%, 23.5%, 111.4%, and 2%, respectively. The group-specificity of anti-AFB_1mAb indicated that the FPIA could potentially be used in a screening method for the detection of total AFs, albeit not AFG_2 and AFM_2. The total time required for analyzing 96 samples in one microplate was less than 5 min. This study demonstrates the potential usefulness of the FPIA as a rapid and simple technique for monitoring AFs.

Aflatoxins (AFs) are a group of toxic secondary metabolites that are mainly produced by *Aspergillusflavus*and *A. parasiticus*[1]. The four most important are AFB_1, AFB_2, AFG_1, and AFG_2 (Figure 1), which are often found in different types of matrices, such as maize, peanuts, cottonseed, fruit, and meat[2]. Of those compounds, AFB_1 and AFB_2 are the most commonly occurring. The hydroxylated forms of AFB_1 and AFB_2 are AFM_1 and AFM_2, respectively, which can sometimes be found in milk and dairy products. These compounds are generated when AFB_1 and AFB_2 are ingested from contaminated feed and metabolized into AFM_1 and AFM_2. Because of the widespread occurrence of AF-producing fungi and the occurrence of the AFs in a number of agricultural commodities, robust efforts have been made to develop new methods for AF detection[3-4]. The fluorescence polarization immunoassay (FPIA) is a homogeneous method that reaches equilibrium in minutes or even seconds, and involves no separation or washing steps. The principles and applications of FPIA to the detection of chemical contaminants in food have been reviewed previously[5]. FPIA is more suitable for use in the high-throughput screening of large numbers of samples than the conventional enzyme-linked immunosorbent assay (ELISA) method. To date, many FPIAs have been developed for the detection of food contaminants, the majority of which are pesticides[6-7]. However, few applications of FPIA for detecting mycotoxins have been reported, partly because of the unavailability of fluorescein-labeled mycotoxin tracers. Only Nasir and Jolley have previously reported on the use of FPIA for detecting AFs by a method that used of a tracer derived from AFB_1 and fluoresceinamine (isomers I and II)[8]. In this study, we report the synthesis of a novel AFB_1 tracer and describe our preliminary efforts towards developing a FPIA for the detection of AFB_1.

We developed a novel synthesis of the AFB_1 derivative (Figure 2). We added 20 mg (60 μmol) Carboxymethoxylamine (CMO,) 10 mg AFB_1 (30 μmol), and 5 mL pyridine to a round-bottom flask and maintained it for 24 h at room temperature. The pyridine was evaporated in a rotary evaporator at 50 °C, and the remaining 2 mL yellow-brown oil product was dissolved in 10 mL pure water. We added 0.1 mol/L NaOH (about 5 mL) drop-wise to adjust the solution to pH=8.0, completely dissolving the product. To this solution, 2×5 mL benzene was added to separate the organic and aqueous phases. The aqueous phase was acidified with 5 mol/L HCl to pH=2, anda white-brown precipitate formed. Then, the compound was extracted with ethyl acetate (3×20 mL) and then dried with 2 g anhydrous Na_2SO_4. The organic fraction was filtered through Whatman No. 1 filter paper. The solvent was evaporated and

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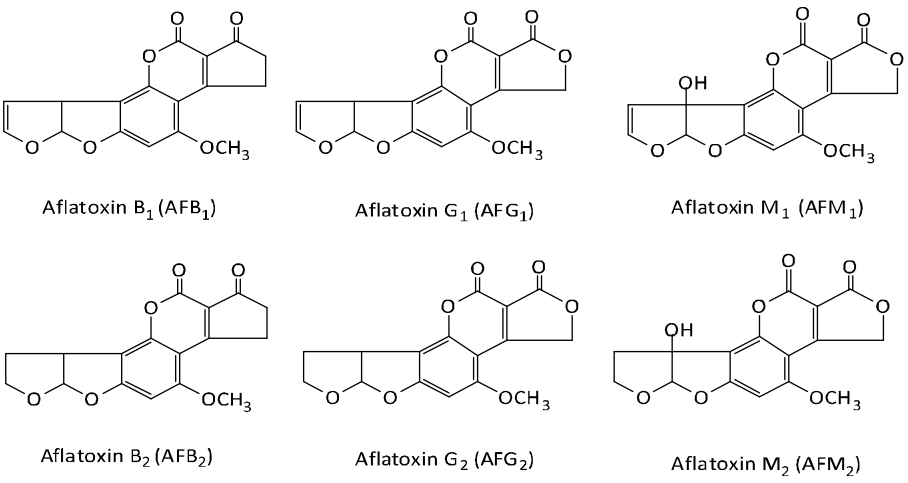

Figure 1. The chemical structures of the aflatoxins: AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, and AFM_2.

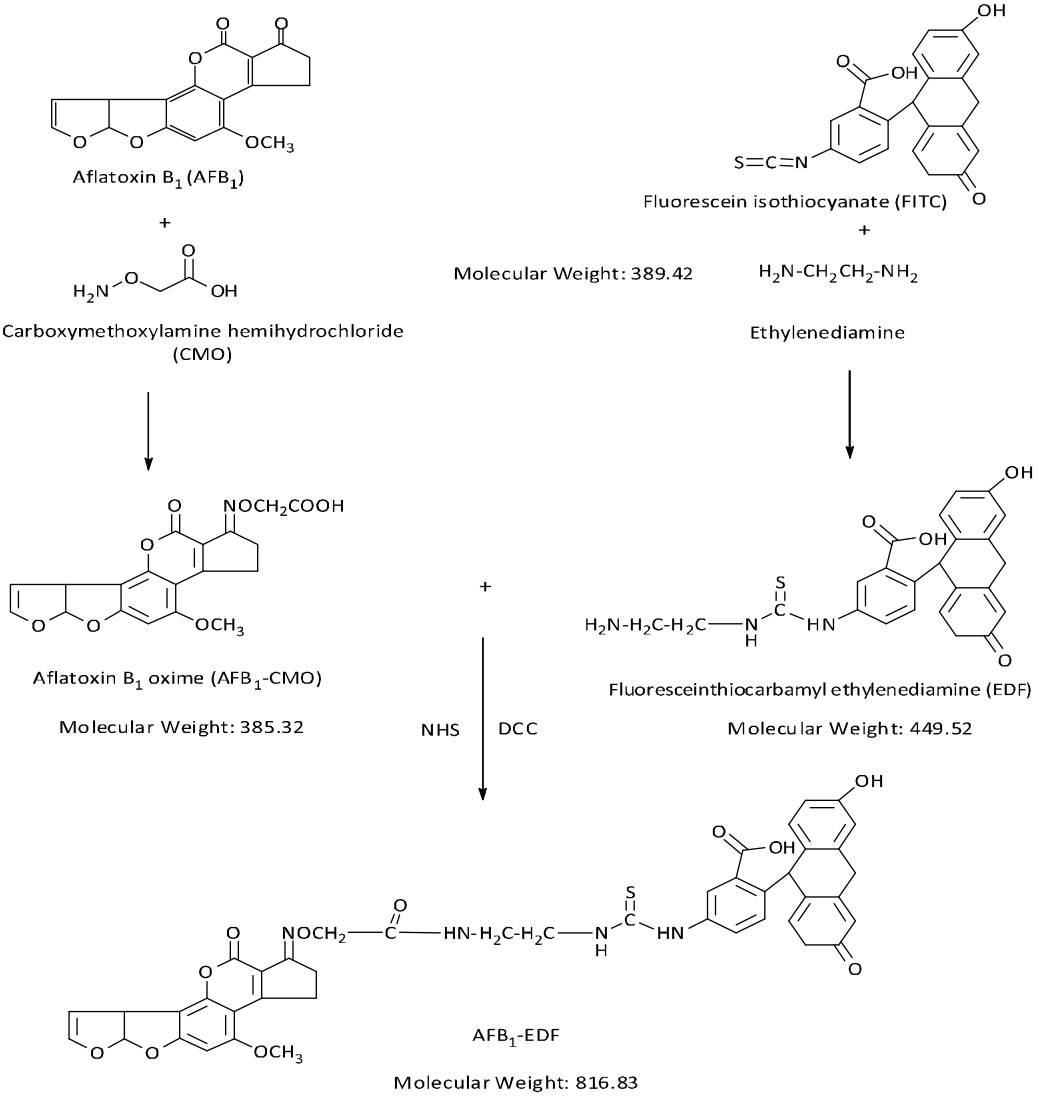

Figure 2. The chemical synthesis of AFB_1-EDF from AFB_1 and EDF.