Microchemical Journal 142 (2018) 335–342

Contents lists available at ScienceDirect

Microchemical Journal

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

Label-free optical detection of aflatoxin by using a liquid crystal-based immunosensor

Zongfu An, Chang-Hyun Jang*

Department of Chemistry, Gachon University, Seongnam-daero 1342, Sujeong-gu, Seongnam-si, Gyeonggi-do 13120, Republic of Korea

ARTICLE INFO

Keywords:
Aflatoxin B1
Immune competition
Liquid crystals (LCs)
4-Cyano-4′-pentylbiphenyl (5CB)

ABSTRACT

Aflatoxin is one of the most toxic mycotoxins and a severe threat to human health. Here, we report a novel liquid crystal (LC) cell system that can be used to visualize the immune competition reaction between an antibody and the target antigen aflatoxin. The sensing system involves co-immobilization of the antibody and *N*, *N*-dimethyl-*N*-octadecyl (3-aminopropyl) trimethoxysilyl chloride (DMOAP) on the surface of a glass slide, resulting in a random orientation of the LCs in the film on these surfaces. In this system, the LC molecules are disordered, producing bright optical signals upon binding of the antibody to the surface-immobilized antigen. In the presence of aflatoxin, the interaction between the target antigen and antibody results in an immune competition reaction, which disturbs the orientation of LCs. This change in orientation can be observed by the naked eye through a crossed polarizer. The results demonstrated that this method possesses high specificity with an antigen detection limit as low as 100 pg/mL. This method does not require laboratory-based instruments, and the test results can be observed with naked eyes through a polarized microscope.

1. Introduction

Mycotoxins are toxic metabolites produced by fungi mainly via growth on various kinds of foodstuff, including animal feeds by many plant pathogens [1]. Among these, aflatoxin (AFT) is a natural, toxic secondary metabolite that is produced by several fungal species [2]. AFT is a fungal metabolite that can interfere with the development in children [3,4], and can cause immune suppression [5,6], cancer [7–9], and in severe acute exposure, even death [10]. AFT has been widely detected in cereal-derived products, dried fruits, spices, beer, and milk [11–16], and is one of the most toxic mycotoxins [17]. Since the mycelium of the *Aspergillus* spp. is usually colorless and thermally stable, it is difficult to know whether the food is contaminated by aflatoxins. Therefore, the development of efficient methods for rapid detection of aflatoxins is of great importance for human and animal health.

Various analytical methods have been established for the determination of AFT. The most widely used methods are thin-layer chromatography (TLC) [18], high performance liquid chromatography (HPLC) with fluorescence [19] and mass spectrometry (HPLC-MS) detection [20], and gas-chromatography with mass spectrometry detection (GC–MS) [21]. However, these methods generally require multiple steps, including extraction, extensive sample cleaning, pre-concentration, and analyte derivatization prior to detection. Such sample treatments not only make the analysis time-consuming and expensive, but also require trained personnel. Enzyme-linked immunosorbent assays (ELISAs) are also used for routine detection of mycotoxins [22]. While these immunoassays provide a simple and economical alternative to instrumental methods for AFT analysis, ELISA is a heterogeneous method and involves multiple wash steps, high labor, time, and reagent consumption, all of which compromise its cost-effectiveness. In addition, the storage and application conditions of the antibody, such as temperature, pH, and ionic strength, are strictly defined. Thus, owing to the limitations associated with these methods, including extensive sample preparation, expensive procedure, and lack of availability of on-site screening, there is an increasing demand, especially in developing countries, for more simple and cost-effective methods.

Lately, assays based on a liquid crystal (LC)-polarized light system have emerged as alternatives to more conventional sensors, offering advantages of low costs and simple experimental processes (for instance, monitoring with the naked eye instead of by complex instruments). LC technologies are currently being developed for use as biosensors in a variety of systems such as for the observation of lipids [23,24], DNA molecules [25–27], proteins [28,29], surfactants [30,31], and viruses [32,33]. Optical detection of biological materials has been achieved through the observation of local changes in the orientation of the nematic director of the aligned LC in contact with the desired target.

* Corresponding author.
E-mail address: chjang4u@gachon.ac.kr (C.-H. Jang).

https://doi.org/10.1016/j.microc.2018.07.013
Received 17 May 2018; Received in revised form 12 July 2018; Accepted 12 July 2018

The nature and extent of these changes are influenced by the structure of the modifying material at the LC interface (e.g., surfactant tail length or headgroup structure) [34,35], and the types of processes at the LC interface that disrupt or perturb the LC orientation (e.g., local pH change by an enzymatic reaction) [36–46]. The biological target induces a local perturbation in the nematic director field, which is amplified through the long-range orientational order of the LC. This LC-based detection via a change in orientation offers potential advantages over conventional techniques as it does not require complex instrumentation or labeling. Most previous studies have focused on using nematic LCs to sense biomaterials at LC interfaces.

In the present study, we developed a new LC sensing system based on immune competition and LC orientation that is extremely sensitive to the physical and chemical properties of the surfaces. Minute differences on the surfaces (e.g., structure and chemical composition) caused by the attachment of different substances to the surfaces lead to different orientations of LCs on these surfaces. Based on these observations, we hypothesize that changes in surface roughness can change the orientation of the LCs, thereby enabling determination of the presence or absence of the antibody-bound sample. To test this hypothesis, we immobilized various concentrations of BSA-aflatoxin suspensions with or without antibody on glass slides modified with DMOAP/APTES, and compared the orientations of LCs on these surfaces. Indeed, a planar orientation of LCs was induced when the antibody was immobilized along with BSA-aflatoxin composites. When the antibody association was disrupted with complementary aflatoxin targets, the planar orientation of LCs was disrupted. This method provides a label-free aflatoxin detection scheme that is simple to perform.

2. Experimental section

2.1. Materials

Unless otherwise stated, all materials used were obtained from commercial sources. Aflatoxin, anti-aflatoxin, glutaraldehyde solution (GA), (3-Aminopropyl) triethoxysilane (APTES), dimethyloctadecyl [3-(trimethoxysilyl)propyl] ammonium chloride (DMOAP), bovine serum albumin (BSA), *N*,*N*′-dicyclohexylcarbodiimide (DCC), *N*,*N*-dimethylformide (DMF), ochratoxin, fumonisins, zearalenone, and sodium acetate buffer solution (3 M, pH 5.2) were purchased from Sigma-Aldrich (St. Louis, USA). *N*-heptane (anhydrous), methanol, ethanol (anhydrous), methylene chloride, acetone, ethylene glycol, and sulfuric acid were purchased from Daejung Chemicals & Metals Co. Ltd. (Siheung-si, South Korea). Ethyl alcohol was purchased from Duksan Pure Chemicals Co. Ltd. (Ansan, South Korea). The 4-cyano-4′-pentylbiphenyl (nematic LC, 5CB) was purchased from Tokyo Chemical Industry Co. Ltd. (TCI, Tokyo, Japan). Hydrogen peroxide (30% w/v) was purchased from Junsei Chemical Co. Ltd. (Tokyo, Japan). Glass microscope slides were purchased from Matsunami Glass Ind. Ltd. (Osaka, Japan). All aqueous solutions were prepared with deionized (DI) water (18.2 MΩ/cm) obtained from a Milli-Q water purification system (Millipore; Bedford, MA).

2.2. Preparation of BSA-aflatoxin conjugates

The chemical structures of AFB1 and its derivatives are illustrated in Fig. 1. In the first step, the oxime groups were added to aflatoxin, using the method described earlier [47]. Briefly, 5 mg (20 μmol) of aflatoxin and 12 mg (60 μmol) of carboxymethoxylamine hemihydrochloride were dissolved in 7 mL pyridine and incubated overnight at 37 °C. Carboxymethoxylamine hemihydrochloride (12 mg, 60 μmol) was then added to the mixture and incubated again at 37 °C for 6 h. Pyridine was evaporated from the mixture, and the product, a yellowish-brown oil, was dissolved in 10 mL of 0.1 mol/L NaOH. The mixture was extracted twice with 5 mL dichloromethane. The aqueous phase was acidified to a pH of 2 with 5 mol/L HCl, resulting in the formation of a white residue.

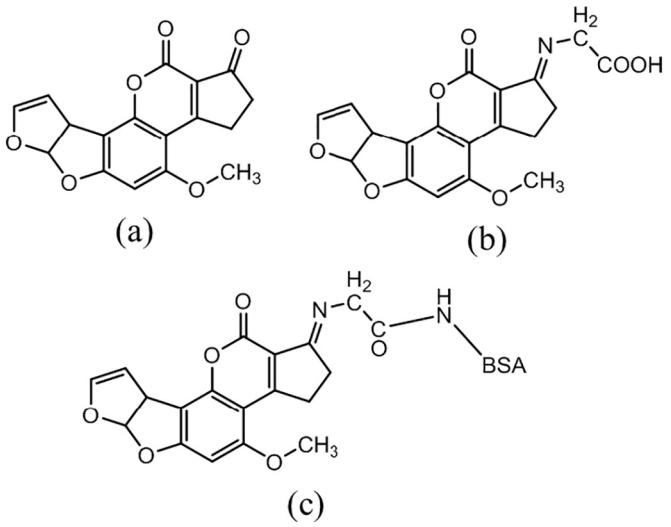

Fig. 1. Chemical structures of (a) aflatoxin, (b) aflatoxin-oxime, and (c) aflatoxin-BSA conjugates.

The crude compound was extracted thrice with 10 mL ethyl acetate. The combined organic phases were dried (2 g anhydrous sodium sulfate), filtered, and the solvent was evaporated. The product was separated by TLC using dichloromethane/methanol (4:1, v/v) as a mobile phase. About 3.3 mg (7 μmol) of aflatoxin-oxime was eventually recovered.

The BSA-aflatoxin conjugate was synthesized using the activated ester method [48]. Next, 11.5 mg (100 μmol) of NHS and 20.5 mg (100 μmol) of DCC were dissolved in 2 mL of DMF, and then 1 mL of this solution (containing 50 μmol NHS and 50 μmol DCC) was added to 3.3 mg (7 μmol) of aflatoxin-oxime, and the reaction mixture was incubated overnight at 25 °C. BSA (15 mg, 0.25 μmol) was dissolved in 2 mL of 0.05 mol/L carbonate buffer (pH 9.6) and cooled at 4 °C for 2 h. The solution of the active ester (300 μL) was slowly added (drop by drop, with shaking) to the protein solution and kept at room temperature for 2 h, followed by overnight incubation at 4 °C. The mixture was then centrifuged at 3000 rpm for 10 min. The mixture was finally dialyzed against 2 L of PBS for 3 days, with the buffer being changed thrice daily.

2.3. Preparation of glass slides for LC assembly

Glass microscope slides were treated with piranha solution (70% H_2SO_4, 30% H_2O_2) at 80 °C for 30 min under a stream of N_2 gas (0.2 MPa) (warning: piranha solution reacts strongly with organic compounds and should be handled with extreme caution; do not store in closed container) as described previously [49–51]. The slides were then rinsed with DI water, ethanol, and methanol, and then dried under a stream of ultrapure N_2 gas (0.2 MPa) followed by heating at 120 °C overnight.

LC assembly was performed on the cleaned slides by the classic LC optical cell method (Fig. 2). Briefly, the glass slides were immersed in an ethanol solution containing 0.5% (v/v) DMOAP for 20 min, rinsed with ethanol and DI water, and then dried under gaseous N_2 (0.2 MPa). These DMOAP-coated glass slides acted as top slides in the LC cells and were used immediately.

Another set of glass slides were immersed in an ethanol solution containing 1% (v/v) APTES and 1% (v/v) DMOAP at 50 °C for 1.5 h, rinsed with ethanol and DI water, and dried under nitrogen. Subsequently, the glass slides were immersed in GA water solution containing 2% (v/v) GA at °C for 1 h, rinsed with deionized water, and dried under N_2. These GA plus DMOAP-coated glass slides acted as bottom slides in the LC cells and were also used immediately.