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Rapid detection of foodborne contaminants using an Array Biosensor

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Abstract

Foodborne contaminants come in a variety of sizes ranging from simple chemical compounds to entire bacterial cells. Due to public health concerns, there is a current need in the food industry for a sensitive, specific and rapid method to monitor for the presence of these toxic species, either as a result of natural or deliberate contamination. The Array Biosensor developed at the NRL encompasses these qualities, including the ability to measure multiple analytes simultaneously on a single substrate. In this study, we demonstrate the Array Biosensor's ability to measure both large pathogens, such as the bacteria *Campylobacter jejuni* (*C. jejuni*), and small toxins, including the mycotoxins ochratoxin A, fumonisin B, aflatoxin B_1 and deoxynivalenol. Sandwich immunoassays were used to measure *C. jejuni* in buffer and a number of food matrices, while competitive immunoassays, taking only 15 min, were developed for the simultaneous detection of multiple mycotoxins. The combination of sandwich and competitive immunoassay formats on a single substrate was demonstrated, allowing the simultaneous detection of both large (*C. jejuni*) and small (aflatoxin B_1) food contaminants.

Keywords: Array Biosensor; Sandwich immunoassay; Competitive immunoassay; Bacteria; *Campylobacter jejuni*; Mycotoxins; Multi-analyte

1. Introduction

Foodborne contaminants can take a number of forms, ranging from whole bacterial cells or proteins to simple chemical compounds [1–4]. Food contamination, whether accidental or deliberate, can occur anywhere along the food processing line, from the source to the consumer, and is a major concern for the food industry. Traditional methods for the detection of food contaminants range from culture in selective media, followed by numerous biochemical and serological tests for bacterial or viral pathogens, to chromatography techniques for the smaller chemical compounds [1,3]. While these gold standard methods are typically very sensitive, they often require multiple time-consuming steps, such as extraction, sample clean-up or preconcentration for toxins or multiple cultures for pathogens, prior to measurement, resulting in analysis times which can run into days. Furthermore, they are designed to detect and measure only one particular target at a time. In an effort to reduce the analysis time while maintaining sensitivity, researchers have developed a variety of immunoassay-based measurements for pathogen and toxin detection, with the enzyme-linked immunosorbent assay (ELISA) format being the most common [1,3]. ELISAs have been used for a variety of food pathogens and analysis times range from 20 min to 2 days depending on the extent of sample pretreatment required.

The Array Biosensor developed at the Naval Research Laboratory (NRL) has successfully been used in the detection of a variety of protein toxins, organic molecules, physiological health markers, a virus and a number of bacteria, initially in buffer but increasingly in food, biological and

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environmental matrices [5–16]. The developed immunoassays are rapid (15–25 min), simple to perform and require little-to-no sample pretreatment prior to analysis, even for more complex sample matrices. In addition, the two-dimensional nature of the slide sensing surface facilitates simultaneous analysis of multiple samples for multiple analytes. In this study, the Array Biosensor measures both a large pathogen, the bacteria *Campylobacter jejuni* (*C. jejuni*), and small toxins, such as the mycotoxins ochratoxin A, fumonisin B, aflatoxin B_1 and deoxynivalenol. In an extension of a previous study [13], sandwich immunoassays were used to measure *C. jejuni* in an extended range of food matrices including milk, yogurt, turkey ham and turkey sausage. *Campylobacter*, which is found to inhabit the intestinal tracts of a variety of healthy mammals and birds, is the most common cause of intestinal and diarrheal disease in the US [1,2]. Infection typically results from the consumption of unpasteurized milk and milk products and undercooked poultry with symptoms including diarrhea, fever, abdominal and muscle pain, nausea and headache. In stark contrast to the large bacterial cells of *Campylobacter*, mycotoxins are small metabolites produced by fungi that grow on a number of agricultural products prior to harvest or during storage [3,4]. Mycotoxicosis results either from inhalation exposure or the ingestion of contaminated foodstuffs. The associated health problems, which range from vomiting to cancer, are dependent upon the specific mycotoxin to which an individual is exposed. In this study, competitive immunoassays taking only 15 min were developed for the simultaneous detection of multiple mycotoxins. Finally, the combination of sandwich and competitive immunoassay formats on a single substrate was demonstrated, which allows the simultaneous detection of both large (*C. jejuni*) and small (aflatoxin B_1) food contaminants.

2. Experimental

2.1. Materials

Unless otherwise specified, chemicals were of reagent grade and used as received. Borosilicate glass slides from Daigger & Co. Inc. (Vernon Hills, IL; www.daigger.com) were used as waveguides in all the assays described. Poly(dimethyl)siloxane (PDMS), used for making the assay flow cells, was obtained from Nusil Silicone Technology (Carpintera, CA; www.nusil.com). The 3-mercaptopropyltrimethoxy silane (MTS) and *N*-(γ-maleimidobutyryloxy) succinimide ester (GMBS) were purchased from Fluka Chemical Co. (St. Louis, MO; www.sigmaaldrich.com). EZ-link biotin-LC-NHS, biotin-LC-PEO-amine, biotin-PEO-amine, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS) and NeutrAvidin were obtained from Pierce (Rockford, IL; www.piercenet.com). The biotin-SP-conjugated rabbit anti-chicken IgY and Cy5-chicken IgY, used as positive controls, were purchased from Jackson ImmunoResearch (West Grove, PA; www.jacksonimmuno.com). The bacterial antigen *C. jejuni* (ATCC35918) used in the sandwich assay was grown as described, by Dr. Avraham Rasooly (FDA) and used under biosafety II conditions, requiring that the cells were killed prior to transportation to the NRL [13]. The polyclonal antibody against *C. jejuni*, rabbit anti-*C. jejuni*, was obtained from Biodesign International (Saco, ME; www.biodesign.com). The mycotoxins, ochratoxin A (OTA), fumonisin B (FB), aflatoxin B_1 (AFB_1) and deoxynivalenol (DON), used in the competitive assays were purchased from Sigma (St. Louis, MO; www.sigmaaldrich.com). Rabbit anti-OTA was purchased from ImmuneChem Pharmaceuticals Inc. (Burnaby, BC, Canada; www.immunechem.com). Monoclonal mouse antibodies against FB, AFB_1 and DON were kindly supplied by Dr. C. Maragos of the USDA (Peoria, IL). Fluorescent labeling of the antibodies was achieved using Cy5 bisfunctional dye from Amersham Biosciences (Arlington Heights, IL; www.amershambiosciences.com). Bovine serum albumin (BSA), gelatin, potassium hydroxide (KOH), sodium chloride (NaCl), sodium phosphate mono- and di-basic, polyoxyethylenesorbitan monolaurate (Tween 20), dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF) and toluene were supplied by Sigma–Aldrich (www.sigmaaldrich.com). Absolute ethanol was obtained from Warner-Graham Co. (Cockeysville, MD). All food was purchased from local grocery stores. A Waring two-speed commercial blender, equipped with a mini-sample container (37–110 ml), used for some of the food preparation, and isopropyl alcohol were purchased from Fisher Scientific (Pittsburgh, PA; www.fisherscientific.com).

2.2. Preparation of biotin-labeled mycotoxins

2.2.1. Ochratoxin A-biotin

To a test tube containing a small stir bar, 6.3 mg NHS and 10.3 mg EDC were added and the tube closed with a septum. Next, a solution of 10 mg OTA in 0.4 ml DMF was added to the test tube. After stirring for 1 h, a solution of 22.6 mg biotin-LC-PEO-amine in 1 ml 0.05 M carbonate–bicarbonate buffer (pH 9.5) was added and then stirred for 24 h at 4 °C. The reaction mixture was then transferred to a 500 MWCO dialysis bag and dialyzed against several changes of 1 l phosphate buffered saline (PBS). The biotin-OTA product was then stored in a vial at 4 °C [15].

2.2.2. Deoxynivalenol-biotin

In a 20 ml vial, 10 mg DON was dissolved in 2 ml of acetone. Next, 110 mg of 1,1′-carbonyldiimidazole was added and the reaction stirred for 1 h at room temperature. Water, 40–50 μl, was then added followed, by a solution of 28.3 mg biotin-PEO-amine in 1 ml of 0.1 M sodium bicarbonate buffer (pH 8.5) and stirred for 24 h at 4 °C. The reaction mixture was then transferred to a 500 MWCO dialysis bag and dialyzed several times against 1 l PBS. The biotin-DON product was then stored in a vial at 4 °C.