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RESEARCH NOTE

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Detection of Fumonisin B_1 by a Batch Type Surface Plasmon Resonance Biosensor

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Abstract Levels of fumonisins, mycotoxins produced by fungal species, must be accurately and rapidly monitored to ensure food safety. In this study, using surface plasmon resonance sensor, a batch-type biosensor was fabricated to detect fumonisin B_1. By applying this biosensor to fumonisin B_1 solutions of 0 to 6 ppm, a significant calibration model was developed for measurement. Coefficient of determination in regression analysis for the model was 0.920. Results indicate that detection of fumonisin B_1 by surface plasmon resonance biosensor was highly feasible.

Keywords: fumonisin B_1, surface plasmon resonance, biosensor, batch type

Introduction

Fumonisins are mycotoxins produced by several fungal species, including *Fusarium moniliforme* and are subgrouped into B_1, B_2, B_3, B_4, A_1 and A_2 depending on formation of substituents. Among the subgroups, fumonisin B_1 (FB_1) has been shown to induce a wide range of adverse biological effects, including fatal leukoencephalomalacia in horses, pulmonary edema in pigs, and nephrotoxicity and liver cancer in rats, thus requiring accurate and rapid monitoring of fumonisin level to ensure safe food. However, analytical methods for detection of fumonisins, such as high-performance liquid chromatography, gas chromatography, and capillary electrophoresis, have several disadvantages in that they require lengthy sample preparation time and sophisticated equipments. Therefore, to overcome this problem, alternatives such as immunoassay have been studied (1-3).

The potential of characterizing biological and chemical quantities by surface plasmon resonance (SPR) has been recognized during the late 1970s. Since the first application of the SPR phenomenon to sensing biological and chemical quantities, this method has made great strides both in terms of instrumentation development and applications. SPR sensor technology has been commercialized, and SPR biosensors have become central tools for characterizing and quantifying biomolecular interactions (4, 5). In the food-related field, the applications of SPR sensor to the measurements of *E. coli* concentration, alcohol content of alcoholic beverages, and polar compound content in frying oils have been reported (6-8).

The principle of SPR is as follows. When incident light is coupled into the surface plasmons, which are packets of free electrons on a thin metal film of about 20 nm thickness, evanescent waves propagate through the metal and excite the surface plasmons on the other side of the film immersed in liquid. To obtain this surface palsmon resonance phenomenon, surface configurations of metals such as gold and silver are necessary. Three general configurations of SPR sensors enable coupling of the light into the surface plasmons: prism known as Kretschmann geometry, grating, and wave-guide types (4).

Here, we report on the detection of FB_1 by a SPR biosensor, which has a batch-type sensing device.

Materials and Methods

Chemicals and antibodies Standard solutions containing FB_1 and anti-fumonisin B_1 antibody were obtained using the Beacon Fumonisin Plate Kit, (Beacon Analytical Systems Inc., Portland, ME, USA). Other chemicals including Tween 20, bovine serum albumin (BSA), and phosphate-buffered saline (PBS) with 20 mM NaH_2PO_4 and 140 mM NaCl were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Apparatus To detect FB_1 using a SPR biosensor, a batch-type SPR system was fabricated (Fig. 1). This system consisted of a Spreeta® SPR sensor (Texas Instruments, Dallas, TX, USA), an analog-to-digital converter, a serial communicator of RS-232C, and a lap-top computer. Spreeta®, a compact type SPR sensor integrated into a small container, was composed of 840 nm NIR light emitting diode with a polarizer, thin gold film, reflecting mirror, and a photodiode array with 128 pixels.

The SPR curve obtained was converted into a refractive index by calculating the 1st moment of points below the

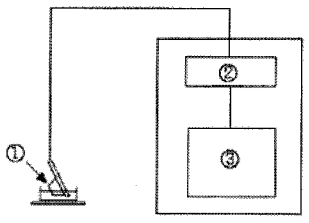

Fig. 1. A batch type SPR biosensor. 1. SPR sensor immobilized with fumonisin B_1 antibodies, 2. Analog-to-digital converter, and 3. Lap-top computer.

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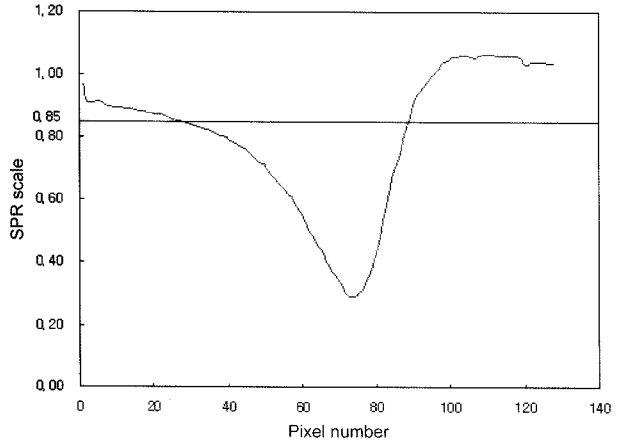

Fig. 2. Calculation of the 1st moment of points below the baseline of 0.85 in the SPR curve.

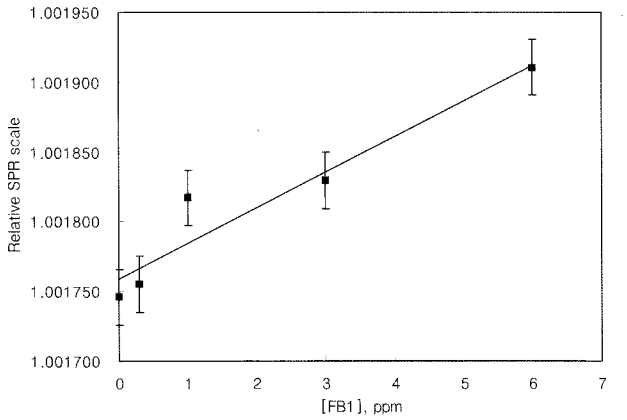

Fig. 4. Calibration line of relative SPR scale for measurement of fumonisin B_1 concentrations using a SPR biosensor.

baseline of 0.85 in the SPR curve (Fig. 2).

Biosensing procedure Biosensing measurement for the detection of FB_1 was performed as follows. After the sensor surface was cleaned with 10 mM NaOH and methanol, the sensor was initialized by measuring the SPR value of deionized water. The surface of the SPR sensor was soaked in the FB_1 antiserum for 1 hr, during which time the antibodies were immobilized on the surface. Subsequently, the antibodies unbound on the sensor surface were rinsed with deionized water. Finally, after incubating the immobilized FB_1 antibodies in the FB_1 solution with PBS for 10 min, the SPR value was measured.

Results and Discussion

Figure 3 shows the sensogram of the SPR biosensor during FB_1 detection, with stages 1, 2, 3, and 4 representing the sensor surface cleaning, antibody immobilization on the gold film of SPR sensor, wash-out of unbound antibodies, and ligand-analyte binding, respectively. In this sensogram, the SPR scales indicate the refractive indices based on pure water. The relative SPR scale to represent FB_1 concentration was determined as the ratio of refractive index at stage 4 to that at stage 3. This newly defined SPR scale can minimize the possible error caused by antibody

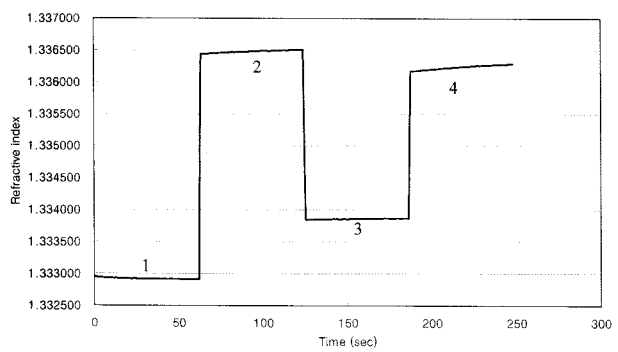

Fig. 3. A sensogram of a SPR biosensor during detection of fumonisin B_1. 1. Cleaning, 2. Antibody immobilization, 3. Wash-out of unbound antibodies, and 4. Ligand-analyte binding.

immobilized on the sensor surface during the replication. The measured and predicted values for the FB_1 solutions were in the range of 0 to 6 ppm (Fig. 4). Using the following linear regression equation developed in this study for the measurement of FB_1 concentration, the coefficient of determination and F value were determined to be 0.920 and 34.6, respectively, at the statistical significance level of 0.01.

$$[FB_1] = -36119.5 + 36056.3\ SPR_{RS} \quad (1)$$

where $[FB_1]$ and SPR_{RS} are the concentration of FB_1 (ppm) and relative-scaled SPR value, respectively.

Our results show the high feasibility of using the SPR biosensor to detect FB_1 (Fig. 4). Furthermore, near real-time measurement of FB_1 concentration will be possible with the preparation of a pre-calibrated SPR biosensor, which has proceeded up to stage 3 (Fig. 3). This type of SPR biosensor will allow an on-site or portable instrument for the rapid detection of FB_1. Further study should follow using lower detection limits at the level of ppb.

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