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Aptamer-based microcantilever array biosensor for detection of fumonisin B-1†

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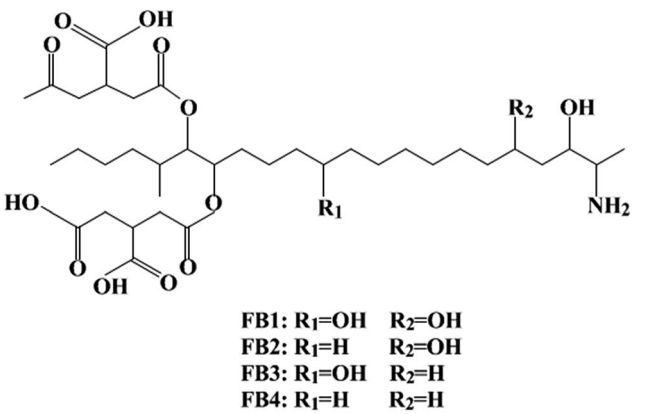
An aptamer-based microcantilever array sensor for detection of fumonisin B-1 (FB1) was developed. The sensing cantilevers in the array were functionalized with self-assembled monolayers (SAMs) of thiolated FB1-specific aptamer, while the reference cantilevers were modified with 6-mercapto-1-hexanol SAMs to eliminate interference from the environment by detecting the deflection induced by nonspecific interactions. This differential deflection amplitude between sensing and reference cantilevers shows a linearity relation with the concentration of FB1 in the range from 0.1 to 40 μg mL^{-1}, with a limit of detection of 33 ng mL^{-1} ($S/N = 3$). The sensor exhibits good specificity over ochratoxin A (OTA) and deoxynivalenol (DON). This biosensor provides a promising approach to detect FB1 in food and agricultural products.

1 Introduction

Mycotoxins are secondary metabolites,[1] produced by molds and fungi, which always occur in grain, forage and silage.[2] Most mycotoxins have toxic effects on animals, as they can be teratogenic, carcinogenic to organic systems and detrimental to reproductive functions of animals.[3–5] Fumonisins are a group of most common mycotoxins in food and feedstuff. Fumonisins, mainly produced by *Fusarium moniliforme Sheld*, are a group of water-soluble diester compounds, composed of different types of polyhydric alcohol and tricarballylic acid. In 1988, Gelderblon first isolated fumonisins from *Fusarium moniliforme Sheld* culture medium.[6] The fumonisin analogs are divided into four types, fumonisin A, B, C, P series.[7] Among the 28 fumonisin analogs, fumonisin B (Scheme 1) are most important and naturally widespread in the contaminated maize, with fumonisin B-1 (FB1) accounting for 70%.[8] FB1 can cause damage to brain, liver, lung and kidney, and especially affect horses, resulting in equine leukoencephalomalacia,[9] which is related to esophageal cancer.[10] International Agency for Research on Cancer (2002) has classified FB1 into group 2B.[11] The levels for total fumonisin (combined FB1, FB2, FB3) should be less than 2 mg kg^{-1} in degermed dry-milled corn products according to the draft guidance that has been published by United States Food and Drug Administration (FDA).[12] Numerous researches have been done to determine concentration of the FB1 in the food and feed.[13] Cawood used thin-layer chromatography (TLC) for the preparative-scale isolation of the fumonisin B from corn cultures of *Fusarium moniliforme*.[14] Compared to other chromatographic approaches, this method is easy to operate and low cost but limited by its poor precision. Gas chromatography tandem mass spectrometry (GC-MS)[15] can be applied to determine FB1 in maize with good sensitivity and specificity by detecting tricarballylic acid. In the sample preparation, tricarballylic acid must be removed from FB1 molecule by hydrolysis and derivatizing agent is required to derivatize the amino to increase its volatility. Due to the complex sample preparation, it was displaced by other methods. High performance liquid chromatography (HPLC) is widely used to determine FB1 with low detection limit, high precision, good repeatability and high sensitivity. Martins *et al.*[16] detected



Scheme 1 Molecular structure of fumonisin B.

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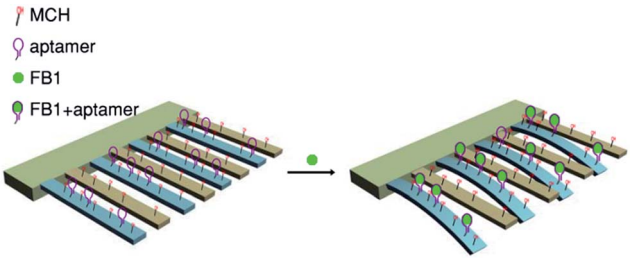
FB1 from swine feed and horse feed with a detection limit of 50 ng kg^{-1}. However, the sample must be treated with proper derivatizing agent, for there is no UV-absorbing side group in FB1 molecule. Liquid chromatography tandem mass spectrometry (LC-MS)[17] has been applied to detect FB1 with a detection limit of 0.1 μg kg^{-1}. It also has the same disadvantage with other chromatographic approaches that require complex pretreatment procedures and trained personnel. Enzyme-linked immunosorbent assay (ELISA) has advantage in rapid detection, high specificity and sensitivity, and has been conducted to detect FB1.[18] But antibody is expensive to produce and vulnerable to environmental factors. Thus, new methods of determination of FB1 are desirable in the food industry.

In the last decade, aptamers, selected *in vitro* through a progress referred to as systematic evolution of ligands by exponential enrichment (SELEX),[19,20] have emerged as promising recognition ligands for the specific binding of mycotoxin molecules.[1] As an alternative to antibodies, aptamers have gained more attention as recognition receptors with low cost, small size, high affinity, good selectivity and thermodynamic stability.[21] In 2010, McKeague *et al.* selected a DNA aptamer that can bind with FB1 molecule specifically.[22] Wu *et al.* presented an aptasensor based on multiplexed fluorescence resonance energy transfer.[23] However, their experiments require complex sample preparation.

Since the advantage of microcantilever in fast response, high sensitivity, small size and their compatible integration into "lab-on-a-chip" devices,[24] they have become an emerging and promising biosensors for chemicals and biooganisms.[25] In our previous works, aptamer-based cantilever sensor has been used for the detection of a series of antibiotics, such as oxytetracycline,[26] kanamycin,[27] and vancomucin.[28] Antibiotics are a type of antimicrobial used specifically against bacteria, and are often used in medical treatment of bacterial infections. Here we reported the detection of mycotoxins, FB1. Mycotoxins are secondary metabolites, produced by molds and fungi, most of them have toxic effects on animals. FB1 can cause damage to brain, liver, lung and kidney of animals. The working principle of microcantilevers is based on the translation of the biochemical reaction between target molecule and probe molecule covalently anchored to the cantilever surface into a mechanical motion. The biochemical reaction translates into deflection of cantilevers in the static mode, while the changes of resonance frequency of cantilevers in the dynamic mode. The microcantilever arrays can screen reference coatings in parallel and under identical experimental conditions[29] to eliminate the influence of environmental disturbances. They are amenable for parallelization for high-throughput screening of different biomolecule interactions between target molecule and different kinds of probe molecules modified on individual cantilevers in an array. Herein, we report a new simple aptasensor based on microcantilevers to detect FB1 specifically and sensitively. This method is easy to operate and label-free. Scheme 2 illustrates the basic principle of using microcantilevers to detect FB1 in static mode. FB1 aptamers were immobilized on the sensing cantilevers (blue) to bind with FB1. Meanwhile, to eliminate the interferences from the environment, the reference ones (yellow) were functionalized with 6-mercapto-1-hexanol (MCH) self-assembled monolayers (SAMs) to detect the deflection induced by nonspecific interactions, system thermo or mechanical shifts. The specific binding of FB1 with aptamers induced differential surface stress between the functionalized gold side and silicon backside, driving cantilevers to deflect. FB1 can be detected by the microcantilever biosensor effectively and sensitively.



Scheme 2 Illustration of microcantilever modification and detection of FB1. The sensing cantilevers were modified with aptamer SAMs to bind with FB1. Meanwhile, the reference cantilevers were coated with MCH SAMs. The binding of FB1 induced the sensing cantilevers to bend downward compared to reference ones.

2 Experimental section

2.1 Chemicals

MCH was bought from Sigma-Aldrich (St. Louis, MO., USA). Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from Alfa Aesar(USA). FB1 was purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). Ochratoxin A (OTA) and deoxynivalenol (DON) were purchased from Pribolab Pte. Ltd. (Singapore). Ethanol was guaranteed reagent and other chemicals were of analytical reagent grade and used as received with no further purification. Deionized water (18.2 MΩ cm^{-1}) from a Milli-Q-water purification system (Millipore) was used in all the experiments. FB1 aptamer was 5′-SH-$(CH)_6$-ATA CCA GCT TAT TCA ATT AAT CGC ATT ACC TTA TAC CAG CTT ATT CAA TTA CGT CTG CAC ATA CCA GCT TAT TCA ATT AGA TAG TAA GTG CAA TCT-3′,[22] synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Aptamers were dissolved in immobilization buffer (10 mmol L^{-1} Tris–HCl, 100 mmol L^{-1} NaCl, 100 μmol L^{-1} TCEP, pH 7.4) and stored at −20 °C. Binding buffer contained 10 mmol L^{-1} Tris–HCl, 100 mmol L^{-1} NaCl at pH 7.4.

2.2 Functionalization of microcantilever array

Arrays of eight identical silicon cantilever beams (500 μm long, 90 μm wide, and 1 μm thick) with a 20 nm gold layer coated on the top side were bought from Micromotive GmbH (Mainz, German). The array was cleaned using piranha solution (98% H_2SO_4 : 30% H_2O_2, 7 : 3) (Caution: piranha solution is a very strong oxidant and reacts violently with many organic materials, it must be handled with extreme care) for 30 seconds and then rinsed three times with deionized water. After being cleaned in a UV-ozone cleaner for 30 minutes, cantilevers were rinsed three times with ethanol and deionized water respectively, and then

dried by infrared light. Microcantilevers were functionalized with aptamers after these cleaning procedures as above. As shown in Fig. S1,† four of the cantilevers on the array were modified by immersing in 25 μL 1 μmol L^{-1} DNA solution in a group of capillaries for 3 hours. And then the whole cantilevers were immersed in 2 mmol L^{-1} MCH ethanol solution for 1 hour to prevent non-specific adsorptions on the sensing cantilevers and to block the active sites on the reference ones.

2.3 Measurement of deflection curves

The deflection measurements were conducted on the commercial Cantisens sensor platform (Concentris GmbH, Switzerland). The microcantilever array was mounted in an array holder which was inserted into the measurement cell filled with binding buffer. As shown in Fig. S2,† a constant flow vertical to the cantilevers at a small rate of 0.42 μL s^{-1} was maintained during the experiment to keep the enough time for the interaction for aptamers and FB1. The temperature in the experiment was constantly controlled in an automatic calibration system. The arrays were equilibrated in binding buffer in the measurement cell at 25.00 °C until a stable differential signal (deflection of sensing cantilevers minus deflection of reference ones) was obtained. To measure the deflection of every cantilever *in situ*, a laser beam (8 semiconductor class 1 M laser sources, max. 1.21 mW each, 850 nm) reflected off the tip of each cantilever (Fig. S2†), and the differential signal can be extracted simultaneously. As the differential signal was stable, 250 μL of FB1 sample was injected into the measurement cell at the desired time, and the measurement curves of deflection induced by reaction between FB1 and aptamers can be achieved. The cantilevers bending toward the gold side or bending toward silicon side is defined as bending up (positive) or bending down (negative), respectively.

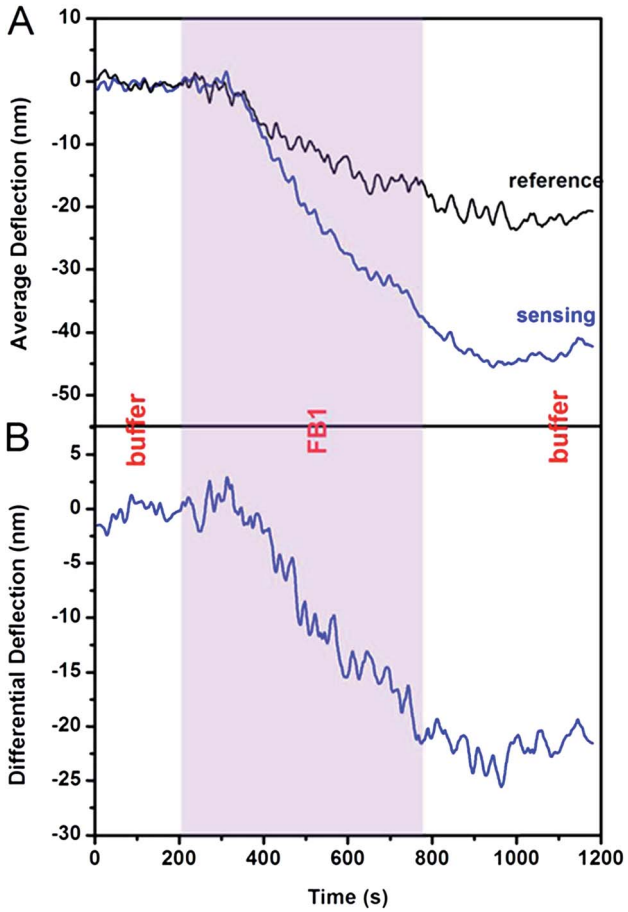


Fig. 1 Deflections of aptamers functionalized microcantilever in a flowing cell with injection of FB1 (100 ng mL^{-1}). (A) Average deflection of sensing cantilevers (blue) and average deflection of reference cantilevers (black). (B) Differential deflection of cantilevers. The light violet region shows the period of FB1 sample flowing through the cell.

3 Results and discussion

3.1 Deflection of microcantilever induced by FB1

The cantilever array functionalized with aptamer SAMs on the gold side was applied to investigate the interaction between FB1 and aptamers. Fig. 1A shows the average deflection of cantilevers as injecting FB1 sample at the concentration of 100 ng mL^{-1} and the corresponding differential signal is shown in Fig. 1B. After recording a baseline for 200 seconds, FB1 reacted with aptamers (the light violet region). The binding of FB1 induced all the sensing cantilevers to bend downward. The signals induced by non-specific adsorption, thermo or mechanical shifts, can be eliminated by extracting the differential signal from sensing cantilevers and reference ones. Consequently, the differential signal is only related to the combination of FB1 and aptamers.

The specific binding of FB1 and aptamers *via* surface stress changes was transduced to nanomechanical response of microcantilevers. As shown in Scheme 2, FB1 bound to the loop region of aptamers, resulting in sterically crowded, which induced steric repulsive force between the adjacent aptamers. Besides, electrostatic repulsive force between the neighboring FB1-aptamer complexes was another primary cause of the bending of cantilevers, as FB1 is negatively charged.[30] The two major elements both induced compress stress of the cantilevers, which impelled them to bend downward. As an additional evidence, the morphological change of aptamer SAMs on glod resulted from the binding of FB1 has been discerned by atomic force microscope (AFM) images as shown in Fig. S3.†

3.2 Relationship between deflection amplitudes and concentrations of FB1

As shown in Fig. 2A, the differential deflection increases as a result of increasing the concentration of FB1 in the range of 0.1 μg mL^{-1} to 40 μg mL^{-1}. To assess the reproducibility of the experiment measurement, at least three experiments were repeated for each concentration. Fig. 2B shows the calibration curve of average differential deflection of cantilevers *versus* FB1 concentration. There is a linearity in the concentration range of FB1 from 0.1 μg mL^{-1} to 40 μg mL^{-1}. While a steady state was reached when the concentration of FB1 exceeded 40 μg mL^{-1}. The linear equation is $Y = 27.15 + 4.65X$ ($R = 0.9905$), with limit of detection of 33 ng mL^{-1} ($S/N = 3$), where Y is the differential deflection (nm), and X is the concentration of FB1 (μg mL^{-1}).

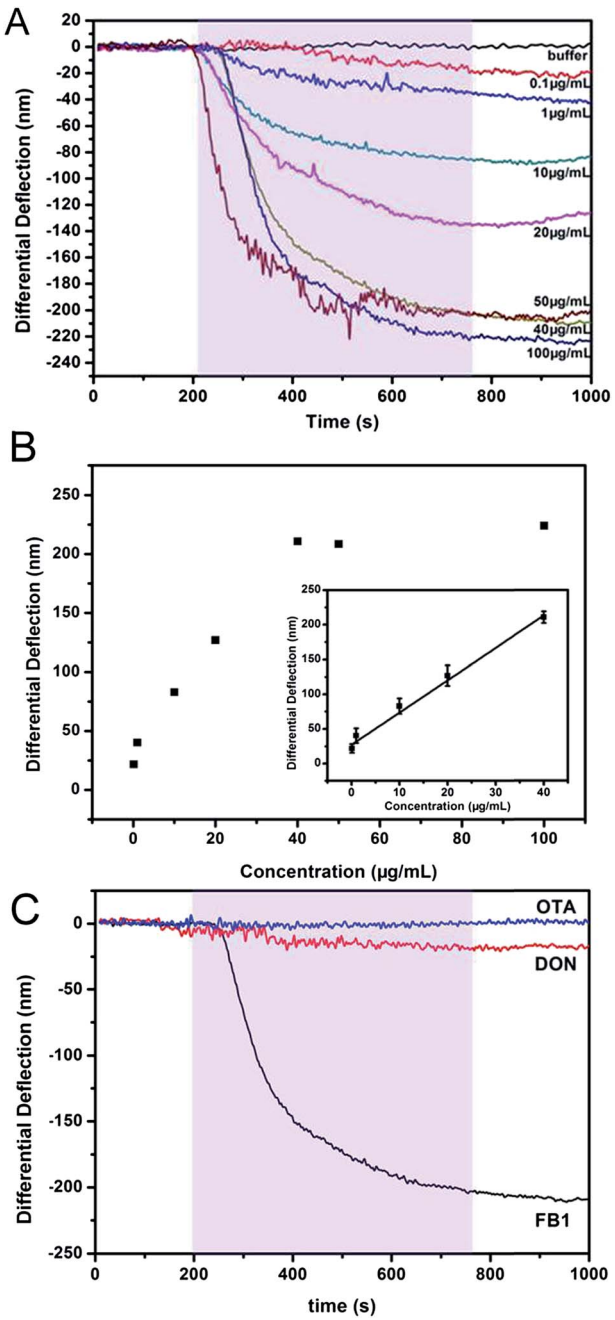


Fig. 2 (A) Differential deflection curves of cantilevers as a function of time with injection of different concentration of FB1. The light violet region shows the period of FB1 sample flowing through the cell. (B) Calibration curve of cantilever sensor for FB1. The error bars represent the standard deviation of data. Inset illustrates the linear response of differential deflection *versus* FB1 concentration. (C) The specificity test of the assay for FB1 on the aptamer modified cantilevers. The light violet region shows the period of OTA sample or DON sample flowing through the cell.

3.3 Specificity evaluation of microcantilever array sensor

Control experiments were performed to verify specificity of the biosensor. Cantilevers functionalized with FB1 aptamers was exposed to ochratoxin (OTA) and deoxynivalenol (DON), other structurally similar mycotoxins found in foods, at the same concentration with FB1 of 40 μg mL^{-1}, respectively. As shown in Fig. 2C, FB1 aptamers does not selectively bind with OTA and DON, which indicates that microcantilever array biosensor can detect FB1 with high selectivity and specificity.

4 Conclusion

In summary, a simple and label-free FB1 aptasensor based on microcantilever array sensor has been developed by functionalized aptamers on the gold side *via* forming Au–S bonds. The developed aptasensor exhibits high selectivity and stability for detecting FB1 with a linear working range of 0.1 μg mL^{-1} to 40 μg mL^{-1} and a limit detection of 33 ng mL^{-1} ($S/N = 3$). The promising microcantilever array sensor based on aptamers would have great potential applications in food analysis.

Acknowledgements

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