Aptamer-based microcantilever-array biosensor for ultra-sensitive and rapid detection of okadaic acid

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ABSTRACT

Okadaic acid (OA) is widely present in seafood and consuming OA contaminated seafood can cause carcinogenic or teratogenic effects. Therefore, developing ultra-sensitive and rapid detection methods for OA is crucial. Here, an aptamer-based microcantilever array sensor was developed for ultra-sensitive and rapid detection of OA. OA aptamers were immobilized on a microcantilever through a thiol group in one step, thus greatly simplifying cumbersome preprocessing procedures. The detection limit of this assay for OA is 1 pg mL\(^{-1}\), the lowest of reported aptamer-based OA detection method. More importantly, compared with reported methods, our method based on micro-cantilever array sensor has the advantages of being rapid, label-free, economical and more applicable for food sample detection. We proposed a method of using enzyme-catalyzed amplification on microcantilever arrays and then used this method to verify the successful modification of aptamers. The sensor also showed the applicability in food sample detection and specificity to OA in shellfish toxin. Therefore, our results prove that this highly sensitive assay based on microcantilever sensor has practical application prospect in assessment in the quality and toxicity monitoring of seafood.

1. Introduction

Okadaic acid (OA) is a key component of diarrheal shellfish poison (DSP). As a common type of toxin produced by Prorocentrum lima, OA is often enriched in the digestive glands of many marine organisms and accumulates during the food chain transfer process. Acute diarrhea poisoning occurs after the consumption of seafood contaminated with OA, and the symptoms are easily confused with those of bacterial gastroenteritis [1]. The long-term accumulation of OA in the body inhibits protein phosphatase activity and leads to protein phosphorylation and interference with enzymatic activity in the DNA replication and repair process, thus ultimately resulting in a high likelihood of human organ malformations. Moreover, it is recognized as an apoptosis-inducing agent in a variety of cells and as a potential tumor promoter or carcinogen [2]. The long-term toxicity effects of OA on the human body have caused widespread concern worldwide. European standards (CE No. 853/2004) stipulate that the maximum content of OA in seafood must not exceed 160 μg/kg (of shellfish meat) [3].

A single instance of consumption of OA contaminated seafood can cause acute diarrhea poisoning, and the long-term accumulation of OA in the body is likely to be harmful. Hence, a highly sensitive and rapid detection method for OA is needed. At present, several commonly used biological and chemical methods for OA detection are available [4], such as mouse bioassay (MBA) [5], enzyme-linked immunosorbent assay (ELISA) [6], high performance liquid chromatography-fluorescence detection (HPLC-FLD) and liquid chromatography mass-spectroscopy (LCMS) [7]. However, these methods each have limitations. For example, MBA, an in-vivo assay, has been used as a standard method for OA quantitative detection because it indicates actual toxicity in the sample. However, mouse sex and health, and low affinity of the receptors in vivo for the toxins inevitably affect the results, thus leading to poor accuracy and replicability. Moreover, ethical issues, complex operation procedures and long detection cycles also limit the application of MBA [4]. ELISA, based on antigen-antibody binding, has the advantages of high specificity, efficiency, low cost and safety. However, the production of antibodies is complicated, and the detection process also requires labeling. Moreover, production of antibodies against small molecules rather than large molecules is more difficult; consequently, these antibodies are less easily obtained and may not be successfully produced [8]. HPLC-FLD can be used to determine various toxic components with precision and accuracy. However, there are strict requirements for the operation and pre-treatment and procedures, and the
equipment is expensive. With LCMS, although almost all DSP toxins can be detected precisely and efficiently, the sample purification procedure must be extremely strict to avoid high interference in detection. All the above methods, when applied to OA detection, do not enable highly sensitive and rapid detection. Therefore, developing a highly sensitive and rapid OA detection technology is crucial.

In the past few years, microcantilever sensing technology, with the benefits of convenience, high sensitivity and quantification, has rapidly developed, and array sensing has increasingly appeared in microcantilever sensing field. Compared with single cantilevers, array sensing can eliminate the non-specific signals caused by different environments in multiple experiments, thus producing more credible experimental results [9]. This technology has been applied to the label-free and real-time detection of cancer [10], pesticides [11], heavy metal ions [12], drugs [13] and allergens [14]. The two working modes of microcantilever sensors are dynamic and static [15]. In the dynamic mode, the resonance frequency of the microcantilever decreases because of the increase in mass caused by the binding of the target molecule. Usually, this mode is appropriate for detecting macromolecules [16,17]. In static mode, changes in surface stress, caused by intermolecular interactions between the probe molecule and the target molecule, result in deflection of the microcantilever. The intermolecular interactions are quite complex, and the stress change may be caused by conformational changes, electrostatic interaction, hydrogen bonding and base stacking [13]. The dynamic mode of the microcantilever sensor depends on the mass increase caused by binding the target molecule, however because OA has a low molecular weight, it may theoretically have a smaller detection range and lower sensitivity in dynamic mode and thus was not suitable to achieve our goals. Our previous research has shown that the binding of small molecules and probe molecules may cause large stress changes on the microcantilever surface [18]. By contrast, static mode was more conducive to quantitative detection of the small molecule OA.

Aptamer, probe molecules, consist of oligonucleotide sequences screened in vitro through systematic evolution of ligands by exponential enrichment (SELEX) [19,20]. Compared with traditionally used protein antibodies, aptamers have higher affinity and specificity, are easy to synthesize and store, and have low cost and good stability [21]. Aptamers have successfully been used to identify cells [22], small molecules [23], antibiotics [24] and chemical materials [25]. The superior properties of aptamers have led to their widespread use as substance detection probes. In previous reports, several aptamers that bind OA have been screened, and OA34 has been found to have the best binding performance [26].

Aptamers bind macromolecules as well as micromolecules and subsequently undergo multiple intermolecular interactions such as conformational changes. When an aptamer is immobilized on a microcantilever and the target macromolecule is added, a change in surface stress results, thus enabling detection of the macromolecules. Antibodies are also often used as probes to immobilize molecules [27–30]. However, compared with the changes associated with aptamers, the steric hindrance of antibodies and the conformation changes are relatively small. In addition, during synthesis, the terminus of the aptamer can be modified with a thiol group, thus enabling the orientation and stable immobilization of the aptamer on the microcantilever in one step and simplifying the immobilization process. However, the orientation of the antibody occurs at random. Achieving oriented immobilization of the antibody is difficult and complicated, and may impair the activity of the antibody [18]. Therefore, aptamer-based sensors may be superior to antibody-based sensors in terms of detection sensitivity and convenience.

Here, we developed an aptamer-based microcantilever array sensor for highly sensitive, rapid and label-free detection of OA in seafood for the first time. We then realized the first application of enzyme-catalyzed enlargement to a microcantilever array and verified successful functionalization of the microcantilever. This enabled us to achieve rapid detection of OA with the microcantilever array sensor. We immobilized the aptamer on the microcantilever array in one step and detected different concentrations of OA. We additionally verified the applicability of microcantilever array sensors to detect OA in food samples. Finally, we tested the specificity of the sensor toward OA among a variety of diarrheal shellfish toxins.

2. Materials and methods

2.1. Materials and reagents

OA sodium salt from proroceutcent concavum (0.5 μg mL−1), 6-mercapto-1-hexanol (MCH) and 3,3’,5,5’-tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The sequence of the OA aptamer [26] was 5’-(SH)-(CH2)n-GGT CAC CAA CAG GGA GCG CTA CGC GAA GGG TCA ATG TGA GGT CAT CCG GAT GTG TGG-3’, and the sequence of the Biotin-aptamer was 5’-(SH)-(CH2)n-GGT CAC CAA CAG GGA GCG CTA CGC GAA GGG TCA ATG TGA GGT CAT CCG GAT GTG TGG-3’-Biotin. A reference aptamer (OAA18) [26] that has low affinity to OA had the sequence 5’-(SH)-(CH2)n-GGC GCG AGA GAC AAC AAG GAT ATA TAT TAT TCG GTT GGT GTA GTG TGG-3’. These aptamers were all synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The avidin and horseradish peroxidase (avidin-HRP) reagent was acquired from Sangon Bioengineering Co., Ltd. (Shanghai, China). Shellfish toxins analogs dinophysistoxin-1 (DTX-1), dinophysistoxin-2 (DTX-2) and saxitoxin (STX) were provided by the National Research Council Canada (Ottawa, ON, Canada). All other reagents were acquired from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The buffer (pH = 7.5) comprised 50 mM Tris-HCl, 150 mM NaCl and 2 mM MgCl2. Microcantilever arrays consisted of eight silicon cantilevers provided by Micromotive GmbH (Mainz, Germany). The top layer of the microcantilever (500 μm long, 90 μm wide and 1 μm thick) was covered by a 20 nm gold layer, and the 2 nm titanium layer under the gold layer served as a bonding agent.

2.2. Measurement device

The 96-well polystyrene microtiter plates were provided by Costar (Corning, NY, USA). A microplate reader (Multiskan MK3) was purchased from Thermo (Vantaa, Finland). The microcantilever array sensor device based on optical fibers was designed by our laboratory (Fig. 1) [9]. The volume of the liquid cell in which the microcantilever array was placed was 200 μL, and the bottom was equipped with a Peltier element for controlling the temperature of the liquid cell with an accuracy of 0.02 °C. Eight semiconductor lasers were used as light sources to align with the optical fibers, and the laser beams were transmitted through the optical fibers. The other ends of the fibers were kept parallel to one another and maintained the same spacing as the microcantilevers. An aspheric lens was used to avoid spherical aberration and focus the laser beams on the tips of the microcantilevers. The real-time deflection signal at the tip of the microcantilever was detected with a position sensitive detector (PSD; accuracy of 0.1 μm). The flow velocity of the liquid was controlled with a peristaltic pump. The measurement device was placed indoors and maintained at 25 °C with air conditioning.

2.3. Microcantilever array functionalization

The process of aptamer immobilization on the microcantilever array was performed in microplate wells, with a new microplate well at each step. First, the microcantilever array was washed in piranha solution (V (98% H2SO4): V (30% H2O2) = 3:1, 200 μL) for 5 min, rinsed with deionized water four times and blown dry with nitrogen. Afterward, the microcantilever array was immersed in 200 μL OA aptamer solution (1 μM) and incubated at indoor temperature for 2 h. When the modification was completed, the microcantilever array was washed four times with buffer and blown dry with nitrogen. Finally, the microcantilever array
was blocked with 200 µL MCH alcohol solution (1 mM) for 1 h. The array modified with the aptamer and MCH was washed four times with buffer and blown dry with nitrogen again. The control microcantilever array was also functionalized with the same steps, except that the modified aptamers were different.

### 2.4. Enzyme-catalyzed enlargement assay

The enzyme-catalyzed enlargement assay was also performed in microplate wells, with a new microplate well at each step. The microcantilever arrays were immersed in piranha solution (V (98% H2SO4): V (30% H2O2) = 3:1, 200 µL) for 5 min, flushed carefully with deionized water four times, and then blown dry with nitrogen. Then the microcantilever arrays were immersed in 200 µL Biotin-aptamer solution (1 µM) and incubated for 2 h (step 1 in Fig. 3). After modification, the arrays were flushed carefully with buffer (50 mM Tris-HCl, 150 mM NaCl and 2 mM MgCl2, pH 7.5) four times. Next, the microcantilever arrays were immersed in 200 µL MCH alcohol solution (1 mM) and blocked for 1 h. After being blocked, the arrays were very carefully flushed with buffer and blown dry with nitrogen. Then the microcantilever array was immersed in 100 µL avidin-HRP solution (2 mg mL⁻¹) for 30 min and then flushed with buffer four times again. Then, 100 µL TMB solution was added to each well, and plates were kept in the dark for 15 min. After 15 min reaction, 100 µL concentrated sulfuric acid was added to stop the reaction. The microplates were placed in a microplate reader, and the absorbance B was read at 450 nm. The control microcantilever array had the same functionalization steps except that there was no biotin aptamer modification step.

### 2.5. Deflection measurements of the microcantilever

The functionalized microcantilever array was fixed in the liquid cell. Buffer was added to the liquid cell, and a peristaltic pump was started to evacuate the bubbles in the liquid cell. The temperature of the liquid cell was controlled to 25 ± 0.02 °C, and the room temperature was controlled to 25 ± 0.2 °C. The peristaltic pump was set to flow at a steady flow rate of 0.5 µL s⁻¹. Simultaneously, the PSD began to monitor the deflection of the microcantilever array and converted it into a real-time signal curve through the platform software. When the microcantilever array was stabilized in a buffer at a steady flow rate (0.5 µL s⁻¹), the OA standard solution (1.5 mL) was added to the liquid cell. The real-time deflections of the microcantilever arrays were recorded. A new array microcantilever array was used for each test experiment.

### 2.6. Detection of OA in food samples

Referring to reported work on food sample processing [31,32], we used standard addition methods to test the feasibility of the sensors in food sample detection. Clams were purchased from Qingdao, and 2 g of clam meat was stirred to a homogenate and then mixed with different amounts of OA standard solution (0.5 µg mL⁻¹) and 10 mL buffer solution to produce mixed solutions with final concentrations of 5 and 500 pg mL⁻¹. The mixed solution was shaken for 15 min and allowed to stand for 30 min. Filter papers with different pore sizes (30, 10 and 5 µm) were used to filter the solution several times. The final filtered solution was used as the food sample and detected with the developed sensor.

### 3. Results and discussion

#### 3.1. Feasibility of the microcantilever array sensor in OA detection

The specific DNA aptamer of OA (OA34) was previously screened from related studies [26]. OA34 consists of a 58 base sequence and is modified with a thiol-group at the 5' end during synthesis. OA34 was immobilized in one step on the gold surface of the microcantilever array through the thiol-group (step 1 in Fig. 1), and the microcantilever array was blocked with MCH. The functionalized microcantilever was fixed in
the liquid cell. We first tested a high concentration of OA standard solution (500 pg mL\(^{-1}\)). After all microcantilevers in one array were stabilized in buffer, OA standard solution (500 pg mL\(^{-1}\)) was injected into the liquid cell. Fig. 2 shows the deflections of all eight microcantilevers in an array, as a result of specific binding of OA and the OA specific aptamer in real time. Here, we defined a deflection to the gold surface as positive and a deflection to the silicon surface as negative. After the injection of the OA standard solution, the eight microcantilevers bent toward the silicon side simultaneously until the sample was exhausted in approximately 70 min. Deflections of the eight microcantilevers were similar (170.4 ± 7.7 nm at 70 min). The experimental results showed that the eight microcantilevers in an array with OA aptamer functionalization had good corresponding consistency, which is extremely important for enabling array sensing detection. Sensor has obvious deflection signal when used for OA detection. The aptamer that we used was a single-stranded oligonucleotide with a stable three-dimensional structure. For the aptamers to bind the target modules, the corresponding spatial structure was altered to expose the binding site. After the binding of the OA aptamer and OA molecules, various intermolecular interactions such as conformational changes, electrostatic interaction, hydrogen bonding and base stacking may occur [33]. The mechanical signals transmitted by these intermolecular interactions caused changes in surface stress on the gold side of the microcantilever, thus resulting in the deflection of the microcantilever (step 2 in Fig. 1). Therefore, the microcantilever showed deflection in the detection experiment.

3.2. Verification of successful functionalization of microcantilever

When we used the microcantilever array sensor to detect OA, the successful immobilization of the synthetic aptamer on a microcantilever array was a crucial step. However, there was no simple way to verify whether the aptamer was successfully immobilized on the microcantilever array. The reported work of microcantilever array also did not carry out corresponding verification and exploration in this respect. Therefore, we applied a method based on enzyme-catalyzed enlargement to the micro-cantilever array to determine whether the aptamer was successfully immobilized on the gold surface of the microcantilever array (Fig. 3 (A)). The OA aptamer was modified with Biotin at the 3’ end during synthesis. If the Biotin-aptamer was successfully immobilized on the microcantilever, all aptamers had a partial different base sequence from that of OA34 and has low affinity to OA; the experiment used a high concentration of OA standard solution (5000 pg mL\(^{-1}\)). In another control group, the microcantilever array was functionalized with OA34, and an experiment was performed in solution with only buffer added but no OA. The microcantilever array functionalized with OA18 showed relatively small deflection even with high concentrations of OA standard solution (5000 pg mL\(^{-1}\)), whereas the microcantilever array functionalized with OA34 had no clear deflection after only buffer was added. These results indicated that the deflection of the microcantilever array was due to specific binding of OA aptamer and OA. Thus, our microcantilever array sensor successfully provided real-time and quantitative detection of OA in real time.

The intermolecular interactions generated during aptamer binding to OA micromolecules are complex and diverse. When an aptamer binds OA, the aptamer begins to fold into a α-spiral conformation, thus producing a wider groove; this extended conformation allows the aptamer to provide more sites for binding OA [33,34]. In the present study, when aptamers were immobilized on a microcantilever, all aptamers had a linear spiral conformation. However, when the aptamers bound OA, an extended conformational change in each aptamer occurred over the entire gold surface; the gold surface area was limited, and the aptamers subsequently began to compete with and repel one another (step 2 in Fig. 1). This conformational change occurred when aptamers bound OA, as confirmed in previous studies [26]. The repulsive force between all aptamers gradually increased. This increased repulsive force, acting on the surface of the microcantilever, caused deflection to the silicon.

3.3. Relationship between deflection and OA concentration

To achieve quantitative detection of OA, we measured the average deflection of the microcantilever array with different concentrations of OA (1 pg mL\(^{-1}\) to 5000 pg mL\(^{-1}\)). The results are shown in Fig. 4(A). After the arrays were stabilized in buffer, the addition of OA standard solution at different concentrations caused the microcantilever to deflect toward the silicon surface to different degrees. The average deflection values of OA at concentrations of 1, 5, 20, 50, 100, 500, 1000 and 5000 pg mL\(^{-1}\) at 70 min were 32, 50, 75, 96, 130, 170, 213 and 238 nm, respectively. The average deflection of the microcantilever arrays increased as the OA concentrations increased. In one control group, the microcantilever array was functionalized with OA18, which has a partially different base sequence from that of OA34 and has low affinity to OA; the experiment used a high concentration of OA standard solution (5000 pg mL\(^{-1}\)). In another control group, the microcantilever array was functionalized with OA34, and an experiment was performed in solution with only buffer added but no OA. The microcantilever array functionalized with OA18 showed relatively small deflection even with high concentrations of OA standard solution (5000 pg mL\(^{-1}\)), whereas the microcantilever array functionalized with OA34 had no clear deflection after only buffer was added. These results indicated that the deflection of the microcantilever array was due to specific binding of OA aptamer and OA.

Fig. 2. With the addition of 500 pg mL\(^{-1}\) OA standard solution, the eight microcantilevers in an array displayed consistent real-time deflection.
surface. In addition, electrostatic interactions among charged groups may also influence the deflection of the microcantilever [35]. Because of the unstable chemical properties of OA, the OA standard solution was made of OA sodium salt. When OA sodium salt is ionized in buffer (pH = 7.5), OA has a negative charge. Because both the aptamer and OA are negatively charged, when OA binds aptamers, the negative charge of each aptamer increases, thereby increasing the repulsive force between aptamers. Superposition of conformational changes and electrostatic interactions enhance aptamer repulsion, thus potentially explaining the high sensitivity observed. In the reported work of detecting microcystin, the author believes that when microcystin and aptamer are combined, the repulsive force caused by conformational changes and electrostatic interaction is in the opposite direction, thus limiting the detection limit of microcystins to the nanogram level [23]. Our picogram-level detection limit for OA also proves the speculation in the reported work from another angle. These results inspired us that the design of a microcantilever sensor should in which the force that deflects the microcantilever is controlled to be in the same direction while avoiding the force acting in the opposite direction. We reasoned that the consistency of conformational changes and electrostatic effects might endow the microcantilever array sensors with greater deflection and higher sensitivity for OA detection.

Fig. 4. (A) Different real-time average deflections of the microcantilever array, caused by the addition of OA standard samples at different concentrations (1–5000 pg mL⁻¹). After the microcantilever was functionalized with OA18, the addition of high concentration OA standard samples (5000 pg mL⁻¹) did not cause significant deflection. A microcantilever functionalized with OA34 showed no deflection after only buffer was added. (B) Fitting function curve of the average deflection of the microcantilever array at different OA concentrations.
least three experiments were performed at each concentration. Every point on the fitted curve represents the average deflection of arrays at each concentration. The error bars represent the standard deviation of deflection values of eight microcantilevers in an array.

Although our microcantilever array is single-use, the storage stability of the micro-cantilever array is still very important after being functionalized. The storage stability of the microcantilever functionalized by the antibody can be maintained for 1–7 days. Considering that compared with antibodies, aptamers have good spatial linearity on the micro-cantilever surface and in previous literatures, the storage stability of the biosensor when stored at −20 °C does not change significantly within 4 months [36]. Therefore, under low-temperature storage conditions, the storage stability of the microcantilever array functionalized by the aptamer should be maintained for at least 7 days. Further investigations on the storage stability of microcantilever arrays functionalized by the OA-specific aptamer are required.

3.4. Application in food sample detection

We used clams to evaluate the applicability of sensors in food samples. Each experiment used a new array. The average deflection for the two concentrations of food samples (5 pg mL⁻¹ and 500 pg mL⁻¹) is shown in Fig. 5. After the microcantilever array was stabilized in buffer, the food sample was added, and the aptamer binding OA in the food sample caused deflection of the microcantilever array. The average deflection of the microcantilever array in the 5 pg mL⁻¹ and 500 pg mL⁻¹ OA food samples at 70 min was 41 nm and 174 nm, respectively (Fig. 5), values similar to the average deflection of the microcantilever array at the corresponding concentration of OA standard solution (Fig. 4 (A)). In the control group, comprising the food sample without added OA standard solution, the microcantilever array did not show clear deflection. Additionally, recoveries of OA-spiked clam samples were ranged from 85.2% to 102.3%. These results illustrate the applicability of the microcantilever array sensor for detection of OA in food samples.

Simultaneously, we observed that the detection phase for food samples had significantly higher noise, because the tested food sample solution had been filtered only several times, and many tiny impurities could not be filtered out. However, our microcantilever array sensor is extremely sensitive, and tiny impurities caused fluctuations in the detection signal.

3.5. Comparison with existing detection methods

In previous reports, several detection methods using aptamer have included impedimetric aptasensor/gold electrode and [Fe(CN)₆]⁴⁻/⁻-redox probe (70 pg mL⁻¹) [26], a dual fluorescence resonance energy transfer aptasensor (50 pg mL⁻¹) [37] and fluorometric determination using a truncated aptamer (39 pg mL⁻¹) [38]. Our method has a detection limit (1 pg mL⁻¹) 39–70 times lower and a wider dynamic range (1 to 5000 pg mL⁻¹) than these methods. More importantly, the gold electrode of impedimetric aptasensor is expensive and requires the assistance of Fe(CN)₆⁴⁻/⁻-redox probe. The modification process of the resonance energy transfer aptamer sensor is more complicated. And the last two methods all require fluorescent labeling. This makes the detection process of these methods is tedious, uneconomical, and difficult to practically apply to the detection of OA in food samples. However, our method based on microcantilever array sensors is simple, fast, label-free, inexpensive and practical. The method using fluorophore-linked aptamer assay coupled with rolling circle amplification (RCA)/FAM fluorescent dye (1 pg mL⁻¹) [39], despite having the same limit of detection with us, this method requires continuous replication of the complementary sequence of the DNA template with the assistant of RCA. Not only that, but also requires a large number of FAM-labeled fluorescent probes to hybridize with RCA amplified repeats. In addition to being extremely complex and time-consuming, these steps also require highly trained personnel, intensive labor and very expensive equipment. These defects make this assay impossible to apply to food sample detection. But in our assay, a specific aptamer is immobilized on the microcantilever array in one step, and the detection can be started, which makes the detection label-free and convenient, rapid, pragmatic and economical. Therefore, our proposed method based on microcantilever array sensors has the advantages of being ultra-sensitive, rapid, label-free, economical and more applicable to food sample detection.

3.6. Specificity of the microcantilever array sensor for OA

OA is an ingredient of DSP that has many analogues. Therefore, the ability of the microcantilever sensors to distinguish OA from other analogs is critical in the analysis of food samples. We selected three other shellfish toxin analogs, DTX-1, DTX-2 and STX, for further experiments. Each toxin corresponded to a single experiment, and each experiment used a new microcantilever array. When the array was stabilized in buffer, we added OA standard solution (5000 pg mL⁻¹) or solution of the three other analogs (5000 pg mL⁻¹). The average deflection of the microcantilever for DTX-1, DTX-2 and STX at 70 min was −28, −30 and 31 nm, respectively (Fig. 6). In Fig. 4(A), the average deflection of the
microcantilever for OA at 70 min was –238 nm. Because of the complex intermolecular interactions in the detection of OA and three analogs, the direction of the force acting on the surface of the microcantilever differed, and consequently the deflection direction of the microcantilever also differed. The positive and negative deflection represented the deflection of the cantilever toward either the gold or the silicon surface, and the absolute value of the deflection indicated the specificity of the sensor for the detected substance. Therefore, the absolute values of the deflection of the microcantilever for OA and three analogs were compared. The absolute value of the average deflection of the microcantilever for OA, DTX-1, DTX-2 and STX (5000 pg mL−1) was 238, 28, 30 and 31, respectively. Considering that the non-specific adsorption of macromolecules may affect the deflection of the microcantilever, we have used MCH to block the microcantilever after functionalization. MCH can effectively prevent the adsorption of macromolecules and greatly reduce the impact of non-specific adsorption on the microcantilever [22]. At the same time, it can be seen from the blank control experiment that the microcantilever has no obvious deflection (Fig. 5), which indicates that the non-specific adsorption of macromolecules will not interfere with the deflection of the microcantilever. Therefore, the deflection of the microcantilever in Fig. 6 was caused by specific binding. The absolute deflection of the microcantilever for OA detection was nearly an order of magnitude greater than the absolute deflection for the three analogs. The result indicated that this sensor has excellent specificity toward OA among shellfish toxins, thus further verifying its applicability for food sample detection.

4. Conclusion

In this study, an aptamer-based microcantilever array sensor was developed for OA detection for the first time. Compared with existing OA detection methods, our method has an extremely low detection limit (1 pg mL−1) and excellent dynamic range (1–5000 pg mL−1), while providing the advantages of rapid, convenient, low cost and label-free detection. We also evaluated the applicability of microcantilever array sensors in food sample detection and the specificity of microcantilever array sensors for OA among shellfish toxin analogs. Our results verified the applicability of our method to food sample detection. The sensitive, fast, convenient, economic and label-free characteristics of this technique suggest that the assay should have broad application prospects for the detection of OA in seafood.

Declarations of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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