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Short communication

Label-free detection of single-stranded DNA binding protein based on a cantilever array

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ABSTRACT

We report a simple and sensitive method for label-free detection of single-stranded DNA-binding protein (SSBP) based on an array of microfabricated cantilevers. The single-stranded DNA (ssDNA) was immobilized on the surface of the sensing cantilevers to detect SSBP, while the reference cantilevers were modified with 6-mercapto-1-hexanol to detect any unwanted cantilever deflection. The differential deflection signals that reveal specific SSBP–ssDNA binding have been found to depend on the SSBP concentration. Using the cantilever array sensor we can detect SSBP in the concentration range from 0.01 to 7 μg mL^{-1}. Other proteins, such as thrombin or bovine serum albumin induced no significant deflection of the cantilevers. Our results show the potential for the application of cantilever array sensor system as a powerful tool to detect proteins with high sensitivity and specificity.

1. Introduction

Proteins and nucleic acids are two kinds of the most important biological macromolecules for all living organisms. The interaction of proteins–nucleic acids is involved in many aspects of cellular metabolism processes, such as the replication, repair, transcription, and packaging of DNA and the translation of RNA. Among proteins that can interact with nucleic acid, single-stranded DNA-binding protein (SSBP) is a kind of protein which can bind preferentially to single-stranded DNA (ssDNA) without sequence specificity. The SSBP monomer contains four ssDNA binding sites and exists as a stable homotetramer in solution [1]. It binds the oligonucleotide-binding domains of ssDNA through a combination of electrostatic and base-stacking interactions to protect the ssDNA from premature annealing and digestion by nuclease [2–4]. Thus it can be seen that the SSBP plays an important role in the replication, recombination, and repair of DNA [5,6]. So far, a growing interest in developing new methods for the detection of SSBP has culminated for biochemical investigation and clinical use. However, some conventional study methods such as electrochemical approaches [7], fluorescent titration method [8] and isothermal titration calorimetry [9,10] always involve labeling procedures, which are time consuming and non-instantaneous. Therefore, a more simple and effective approach to detect SSBP is important to aid in their utilizations and the deeper understanding of SSBP–ssDNA binding mechanism.

Cantilever sensor system has attracted much attention in recent decades as a label-free, real-time and highly sensitive approach to detect target molecules [11–13]. When the receptors immobilized on one side of a cantilever bind to the target molecules, a nanomechanical bending of the cantilever will occur due to the surface stress-change. Then the nanomechanical motion can be detected optically. Recent experiments using cantilever sensors have demonstrated the wide applications of this label-free detection approach, such as heavy metal ion detection [14], DNA hybridization [15], detection of single-nucleotide mismatches in DNA [16], protein–ligand binding [17] and protein–DNA interaction [12].

In this paper, we demonstrate a label-free method to detect SSBP based on the change in surface stress of the cantilevers. The basic principle of SSBP detection is illustrated in Fig. 1. Briefly, the sensing cantilevers (blue) were modified with self-assembled monolayers (SAMs) of ssDNA to detect SSBP. While the reference cantilevers (yellow) were functionalized with 6-mercapto-1-hexanol (MCH) SAMs. They were used to eliminate the influence

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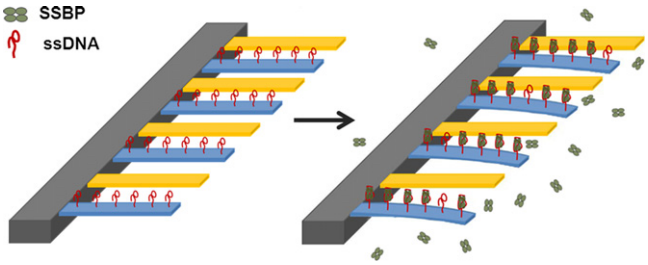

Fig. 1. An illustration of the basic principle of SSBP detection is shown. Cantilevers 1, 3, 5, 7 (blue) are coated with ssDNA SAMs to detect SSBP, while cantilevers 2, 4, 6, and 8 (yellow) are coated with MCH SAMs to serve as references. The schematic illustrates how the target SSBP will bind to the ssDNA that immobilized on the particular cantilevers. This binding generates a compressive surface stress, which causes the sensing cantilevers to bend downward compared with the reference cantilevers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of environmental disturbances, such as nonspecific adsorption, changes in pH, ionic strength, and temperature. Upon contact between the sensor surface and SSBP, SSBP–ssDNA bindings created a differential surface stress, which is relieved by the cantilever in turn bending toward or outward the gold side. The high selectivity of the cantilever array sensor was evaluated through measuring the response of the cantilever array when it was exposed to thrombin (TMB) or bovine serum albumin (BSA).

2. Experimental

2.1. Reagents and buffers

Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was bought from Alfa Aesar. MCH was purchased from Sigma. SSBP was purchased from Promega Corporation (USA). The ssDNA (5′-HS–$(CH_2)_6$–T_{70}-3′) was synthesized in Sangon Biotech Co., Ltd. (Shanghai, China). All other chemicals were of analytical reagent grade and used as received without further purification. Pure water (18.2 MΩ cm) used in the experiment was obtained from a Milli-Q system (Millipore). In addition to the special statement, the running buffer (pH 7.5) consisted of 10 mM potassium phosphate, 100 mM NaCl, and 10 mM $MgCl_2$. The ssDNA was dissolved in the potassium phosphate buffer (10 mM potassium phosphate, 100 mM NaCl, 1 mM TCEP, pH 7.0) and stored at −20 °C until use. The TCEP was used to allow the free-sulfhydryl group of ssDNA to remain in reduced form.

2.2. Cantilever functionalization

The cantilever arrays are consisted of eight identical silicon cantilevers (500 μm × 100 μm × 1 μm) with a 20 nm layer of gold coated on the top side (Concentris GmbH, Switzerland). Before using, the cantilever array was cleaned with ethanol and pure water followed by a UV–ozone cleaning cycle for 20 min. The functionalization of the sensing cantilevers was performed by inserting four of the cantilevers into an array of microcapillaries that was filled with 1 μM ssDNA in potassium phosphate buffer for 3 h. Then the cantilever array was immersed in MCH solution (2 mM concentration) for 1 h to remove nonspecific ssDNA adsorption on the sensing cantilevers and form MCH SAMs on the other four reference cantilevers. Lastly, the array was washed consecutively with ethanol and pure water three times, and dried under a stream of nitrogen gas.

2.3. Measurements and apparatus

All of the experiments were carried out on the commercial Cantisens sensor platform (Concentris GmbH, Switzerland) equipped with a measurement cell of 5 μL, an automated liquid handling system and an integrated temperature control with sample preheating stage. Temperature of the experiments was controlled at 25.0 ± 0.01 °C. The cantilever array was initially placed into the measurement cell and equilibrated in the running buffer at a constant buffer flow of 0.42 μL s^{-1} until a stable baseline was obtained. Then it was exposed to SSBP solution for 10 min followed by a flow in running buffer environment. The resulting nanomechanical deflection of each cantilever was measured in real time by monitoring the position of a laser beam reflected off the tip of the cantilever onto a four-quadrant photodiode. The bending of cantilever toward or outward the gold side was defined as the positive or the negative deflection.

3. Results and discussion

3.1. Cantilever deflection due to SSBP binding

The functionalized cantilever array, which consisted of four sensing cantilevers with ssDNA SAMs and four reference cantilevers with MCH SAMs, was firstly placed in the measurement cell, and allowed a few hours for equilibration to establish a stable baseline. Subsequently, SSBP was introduced into the measurement cell at a concentration of 5 μg mL^{-1} (shaded area, Fig. 2). The response of the

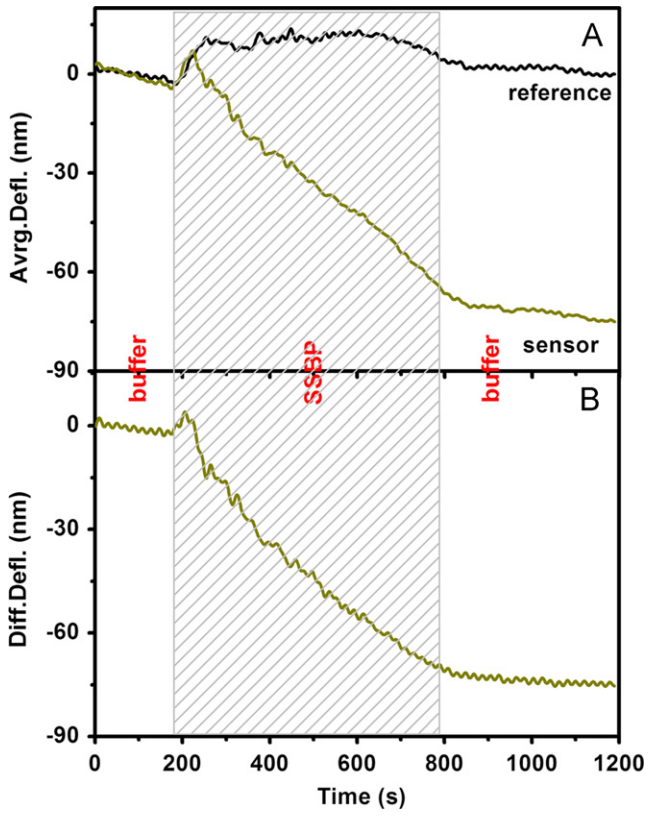

Fig. 2. Detection of SSBP with ssDNA SAMs modified cantilever array. (A) Averaged deflections (Avrg. Defl) response of ssDNA-coated cantilevers (shown in dark yellow) and MCH-coated cantilevers (shown in black) against time. Upon injection of SSBP the deflection of each cantilever was measured in situ. The shaded area corresponds to the injection of 5 μg mL^{-1} SSBP solution. (B) Corresponding differential deflections (Diff. Defl) derived by subtracting the MCH references. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

cantilever array can be detected in real time. The averaged deflection signal in Fig. 2A represents the average measurements for the identically functionalized cantilevers within an array. Corresponding differential deflection representing the specific interactions between SSBP and ssDNA is derived by subtracting the MCH reference (Fig. 2B).

As shown in Fig. 2, when the cantilever array was exposed to SSBP (at 170 s), the averaged deflection signals exhibited a noticeable transient positive deflection for all cantilevers. However, this positive deflection could not be observed in the differential signal, indicating the effect was caused by the nonspecific interactions and adsorption of SSBP [18]. Then, the sensing cantilevers started to exhibit strong negative averaged and differential deflections at about 220 s, which implies a sudden change from tensile to compressive stress. There are two possible reasons which could be responsible for the change. On one hand, the increased lateral forces acting between the neighboring SSBP–ssDNA complexes chains play a very important role. The long continuous ssDNA chain can interact with the subunits of the SSBP molecules, and wrap entirely around them to form SSBP–ssDNA complexes chain [2]. It is reasonable to consider that the formation of the SSBP–ssDNA complexes chains can make the surface of the cantilevers become more crowded. In order to get more free space, the SSBP–ssDNA complexes chains were trying to expand and the gold surface was subjected to a compressive stress. On the other hand, the lateral electrostatic forces between the SSBP–ssDNA complexes chains will also generate surface stress change. It is well documented that the isoelectric point of the SSBP is about 6.0 [19]. At the working pH of 7.5, the SSBP molecules are negatively charged. Therefore, binding of charged SSBP molecules to ssDNA can cause stronger repulsion due to the presence of additional negative charge. After the injection of SSBP (at 800 s) the liquid cell was rinsed with running buffer only. However, the deflection signal did not return to baseline level but stabilized to reach a differential equilibrium value of 75 nm, which indicates the SSBP has an extremely high affinity of the ssDNA.

3.2. Relationship between deflection amplitudes and the concentrations of SSBP

Since the bending of the cantilever is caused by the molecular binding events on the cantilever surface, the magnitude of differential deflection is indicative of the SSBP–ssDNA binding degree. Different concentrations of SSBP sample were added to examine the effect of protein concentration on the cantilever deflection. For each concentration, the sensing experiments were repeated at least three times to assess the reproducibility of the experimental measurements. The relative standard deviation of our experiment was within 5%. Fig. 3 shows the differential deflection response as a function of time for different concentrations of SSBP. As can be seen, the differential deflection of the cantilevers changed more quickly and had greater bending amplitude with the increase of SSBP concentration. The linear relationship between the differential deflection and SSBP concentration is shown in Fig. 3 (Inset). Under the experiment conditions, the cantilever array sensor displays a good linear response ranging from 0.01 to 7 $\mu g\ mL^{-1}$, with a correlation coefficient of 0.993. The linear progress equation is $Y=12.61+12.92X$, where Y is the differential deflection (nm) and X is the concentration of SSBP ($\mu g\ mL^{-1}$). The limit of detection (LOD) of the SSBP was 0.008 $\mu g\ mL^{-1}$ ($S/N=3$).

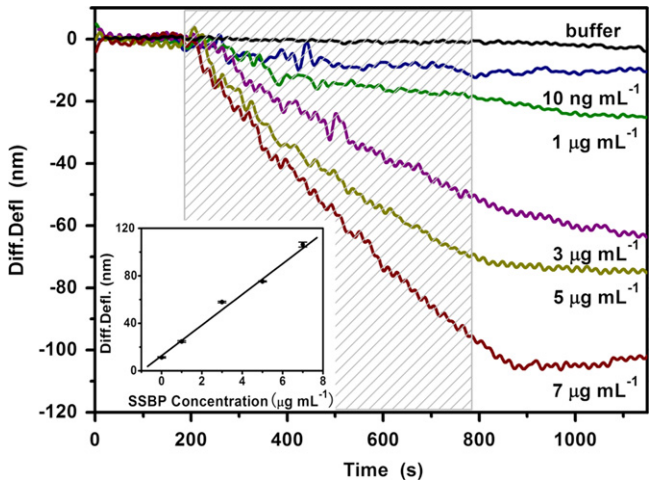

Fig. 3. Differential deflection response of the cantilever array sensor to different concentrations of SSBP as a function of time. The inset is the corresponding calibration plots in the concentration range from 0.01 to 7 $\mu g\ mL^{-1}$.

3.3. Specificity of the cantilever array sensor

For an excellent analytical experiment, high specificity is a matter of necessity. To investigate the specificity of the SSBP recognition using cantilever array sensor, we exposed the cantilever array to TMB and BSA with the same concentration (3 $\mu g\ mL^{-1}$). The differential deflections of the cantilevers are compared with the

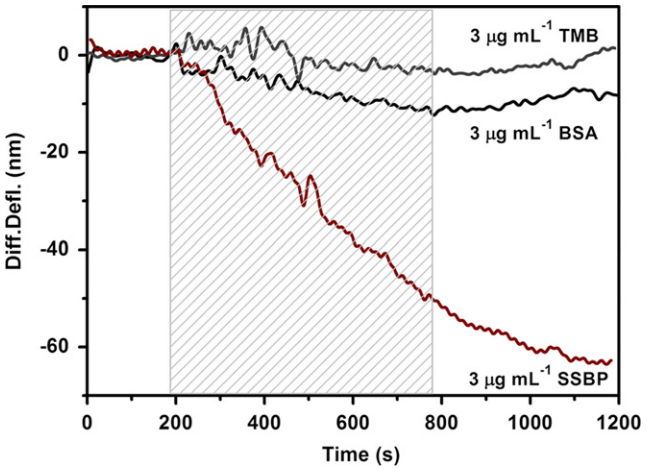

Fig. 4. Differential deflection response of the cantilever array sensor to BSA and TMB at the same concentration (3 $\mu g\ mL^{-1}$).

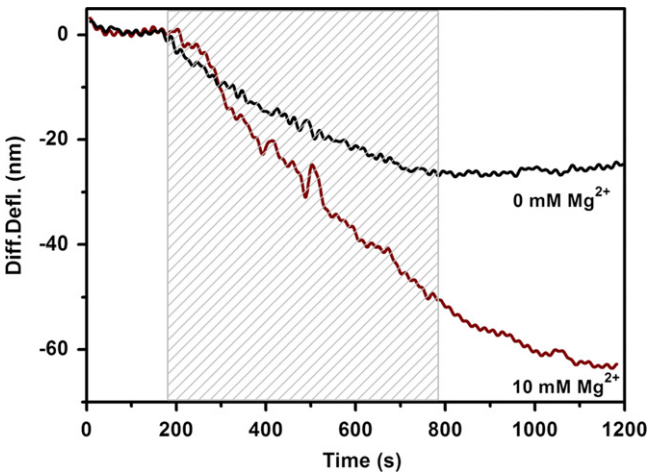

Fig. 5. Differential deflection response of the cantilever array sensor under different Mg^{2+} concentrations against time.

responses to 3 μg mL^{-1} SSBP. As shown in Fig. 4, no significant differential deflection response to TMB or BSA was observed. The result suggests that the cantilever array sensor is of high selectivity for SSBP detection.

3.4. Influence of Mg^{2+} on the SSBP binding

Studies show Mg^{2+} can influence the specific recognition between ssDNA and SSBP [20–22]. It can be assumed that the differential deflection response of the cantilever array sensor can also be affected by the concentration of Mg^{2+} in solution. Fig. 5 shows the bending response of the cantilever as the concentration of Mg^{2+} is varied. As can be seen, the differential deflection in the presence of Mg^{2+} is twice as large as that without Mg^{2+}, which is consistent with the previous report [20]. Since the SSBP molecules were negatively charged in the potassium phosphate buffer (pH 7.5), the positively charged Mg^{2+} could bind SSBP molecules to the phosphate groups of ssDNA tightly as a bridge ion [23]. Therefore, the interactions between SSBP and ssDNA are much stronger and cause greater bending amplitude in the presence of Mg^{2+}.

4. Conclusion

In summary, we present a facile method for label-free detection of SSBP based on a cantilever array sensor system, which utilizes ssDNA as receptor. The cantilever responses are quantitatively dependent on the concentration of SSBP, and can be detected in real time within minutes. Under the experimental conditions, the cantilever array sensor can selectively respond to SSBP at concentration as low as 0.008 μg mL^{-1}. The sensitivity of SSBP detection using cantilever array sensor system is comparable to some traditional methods [8]. These results provide evidence that the cantilever array sensor can be applied successfully to detect trace amounts of SSBP. At present, experiments are under way to develop new sensors for other proteins using cantilevers modified with different molecular recognition materials. The technology will become an outstanding platform for label-free and high sensitivity analysis of more proteins.

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