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Bioassay of Glutathione S-transferase Antibody-Antigen Interactions Using Microcantilever Sensor†

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In this study, a real-time detection of glutathione S-transferase antigen and glutathione S-transferase antibody binding using a microcantilever sensor is reported. Glutathione S-transferase was immobilized onto microfabricated cantilever gold-coated surfaces by using thiol self-assembled monolayer. Consequently, the deflections of the microcantilever corresponding to the process of injecting anti-glutathione S-transferase antibody were generated, which was measured by using optical lever technique. An enzyme linked immunosorbent assay was used to determine the presence of an active antibody coating and to monitor the lifetime and stability of the immobilized antibody. This label-free method affords a new way to monitor various biomolecules interactions.

Key Words: Microcantilever, Biosensor, Antibody-antigen, Surface stress.

INTRODUCTION

The performance of proteins regulation is most of the biochemical processes in cells and their detection essential applications ranging from clinical diagnosis, environmental control to homeland security issues. At the same time it is a complex and challenging task to study proteins. Historically, widely used biochemical methods for protein detection, such as Western blotting and enzyme-linked immunosorbent assay, were usually time and material consuming and require multi-step protocols. Others (*e.g.* fluorescence) require the attachment of labels. Biophysical sensors, such as surface plasmon resonance and quartz crystal microbalance, offer several advantages, such as real-time, label-free detection.

Recently, emerged highly advantageous method is the microcantilever sensor. Over the last 10 years, the application of the cantilever sensor was extended to the measurements of bio-compounds in solution, resulting in a versatile biosensors[1-3]. Because of its label-free detection principle and small size, this kind of biosensor is advantageous for diagnostic applications, disease monitoring and research in genomics or proteomics. The adsorption of biochemical species on a functionalized surface of a microfabricated cantilever can cause surface stress and consequently induce cantilever bending. It has been successfully applied in the gas-sensing field, in genomics and proteomics[4,5].

In the present study, a microcantilever immunosensor for anti-GST antibody for was developed. Glutathione S-transferase was covalently side of cantilevers by using thiol self-assembled monolayer, at the same time the no immobilized antibody on the gold-coated reference cantilever.

EXPERIMENTAL

The anti- glutathione S-transferase antibody was generated by Prof. Liu Jing's group. TMB, bovine serum albumin (BSA), 11-mercaptoundecanoic acid and horseradish peroxidease (HRP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals that were of analytical grade were obtained from Beijing Chemical Reagents Co. (Beijing, China). Ninety six-wells polystyrene microtiter plates were purchased from Costar (Corning, NY, USA).

In our experiments, the dimensions of the V-shaped silicon microcantilevers (Veeco Instruments, Plainview, NY, USA) were 200 μm in length, 20 μm width for each leg and 0.5 μm in thickness (Fig.1C). A diagram of the experimental setup (Fig. 1A) is given used in this study was previously reported[5]. The cantilever is mounted in a flow-through cell of a 500 μL liquid. A diode laser is focused onto the tip of the cantilever. The deflection of the cantilever is measured by monitoring the position of a laser beam reflected from the cantilever onto a position sensitive detector. A peristaltic pump was used to flow the working solution and the analyte of interest was injected using a low pressure injection port sample loop system.

Antibody immobilization: For the selective capture experiment, cantilevers were cleaned in a solution of standard

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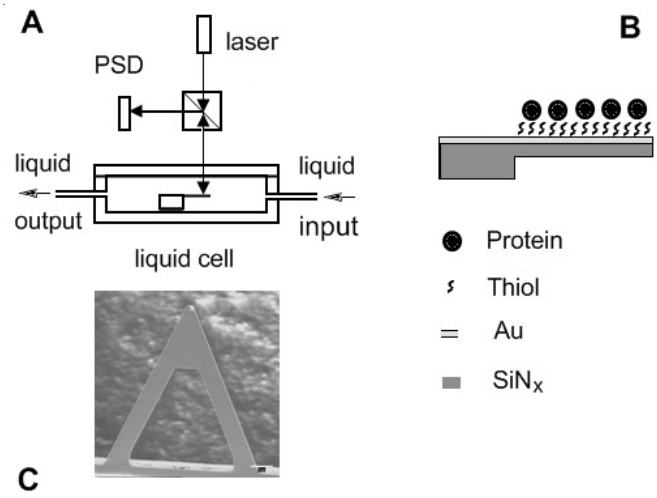

Fig. 1. Measurement setup and sensor functionalized. (A) Schematic drawing of the sensor instrument. (B) Side view of a gold-coated silicon cantilever functionalized with trypsin. (C) Top view of the microcantilever used in experiments

piranha (H_2O_2:H_2SO_4 1:3 in vol.), rinsed in deionized water, immersed in ethanol, dried with nitrogen gas. Clean microcantilevers were stored overnight in a 1 mM ethanolic solution of 11-mercaptoundecanoic acid. Then, these microcantilevers were thoroughly rinsed with distilled water before being immersed in a solution of 0.5 M NHS and 0.2 M EDC in distilled water for a time period of 30 min. This was followed by thorough rinsing of the cantilevers in deionized water to remove excess chemicals. The final step was the immobilization of the anti-GST antibody on the microcantilever. 5 µL of the FIP anti-viral antiserum was dissolved in 1 mL of PBS. The microcantilever was incubated in this solution for a period of 3 h. The microcantilever was then rinsed to remove any excess antibody. The chemically modified microcantilever was stored in buffer solution before use.

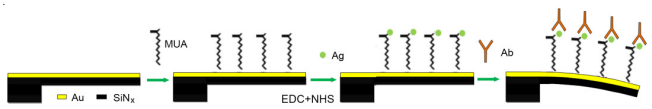

Fig. 2. Schematic diagram of the experimental for decorating antigen on Au film of a cantilever anti-GST antibody detection

The sensor cantilever was functionalized with glutathione S-transferase antibodies and the reference cantilever without antibodies and blocked with bovine serum albumin solution in running buffer. After a stable baseline was obtained, several times injections of buffer were performed to verify that there was no differential signal due to buffer injection. Glutathione S-transferase was passed the flow cell. Finally, as a control mechanism, the bovine serum albumin protein was injected at a concentration corresponding to the highest glutathione S-transferase concentration used in the experiment.

Characterization of the activity of antibody using an enzyme-linked immunosorbent assay: The functionalized microcantilever and a reference microcantilever (without immobilized antibodies) were put in the different microtiter plate wells. Then 200 µL per well of an aliquot of goat anti-mouse IgG-HRP diluted in PBS was added to per well. Followed by incubating at 37 ºC for 0.5 h, the microcantilevers were washed three times with PBST and put in another clean well separately. 200 µL per well of substrate solution was pipetted to the well. After incubated at 37 ºC for 15 min, the reaction was stopped by adding 50 µL stopping solution. The absorbance was read at 492 nm by the microplate reader.

RESULTS AND DISCUSSION

Characterization of immobilized antibody using enzyme-linked immunosorbent assay: The absorbance values of non-competitive enzyme-linked immunosorbent assay for the functionalized microcantilever and unfunctionalized one were 0.2906 and 0.026 respectively. Athough it cannot direct to confirm antibody activities and the inhibition results compared to dcELISA, the non-competitive enzyme-linked immunosorbent assay was a useful method to measure the affinity constant of antibody on the gold surface of microcantilever. Such a method can be used recognition of the immobilized antibody by the labeled secondary antibody indirectly to estimate the activity and density of the antibody rather than the direct measure of the actual inhibitory activity of the anti-GST antibody functionalized on the gold surface of microcantilever.

Fig. 3 displays the deflections of the microcantilever caused by glutathione S-transferase and anti-GST antibody binding. The cantilever was immersed in PBS with a constant flow rate before the injection of anti-GST antibody and as shown in the figure, a negative deflection (means bending toward the SiNx surface of the cantilever) generated after the injection of anti-GST antibody (1.6 mg/mL, in presence of 0.1 mg/mL of BSA). A small peak near the injection point was induced by disturbs during the injection and the time delay (about 3 min) was attributed to the time cost flowing from the place of injection to the fluid cell. It can be seen that over a short period of about 70 s, the cantilever deflection decreased and then saturated to a steady-state value. This implied that the binding reaction between the antigen and antibody has already come to an equilibrium state during this short interval. To check whether the cantilever deflection was caused by a nonspecific binding of bovine serum albumin, a new cantilever coated with glutathione S-transferase was used and detected with the injection of 0.1 mg/mL bovine serum albumin. Fig. 4 shows the experimental results. It is evident that there is no significant variation of deflections. This result indicates that the nonspecific binding of bovine serum albumin cannot induce the deflections shown in Fig. 3.

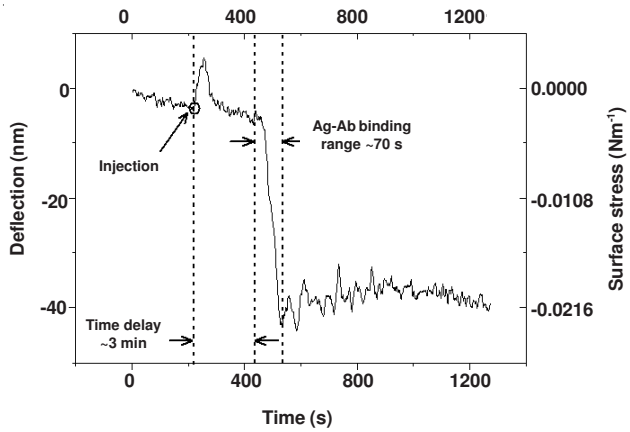

Fig. 3. Cantilever deflection versus time for injection of anti-GST antibody 1.6 mg/mL in presence of 0.1 mg/mL of bovine serum albumin

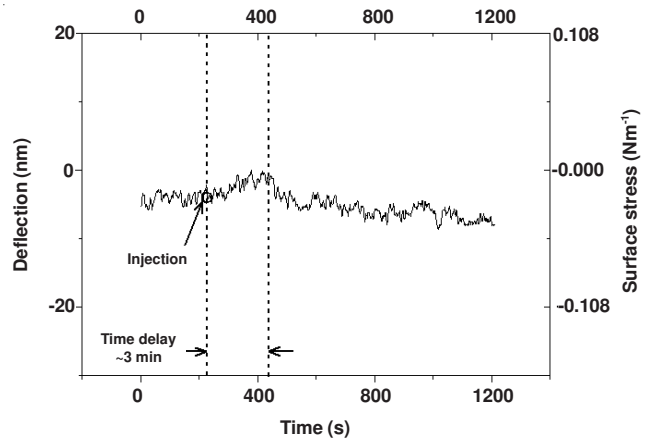

Fig. 4. Cantilever deflection versus time for injection of 0.1 mg/mL of BSA

The specific glutathione S-transferase and anti-GST antibody binding generated a negative deflection which cannot be attributed to the mass loaded on the cantilever or some other reasons other than a change of the surface stress on the coated surface. The detailed mechanism of this process is still unclear. But it is considered that the origin of this change is a result of the sum of different processes taking place at the coated surface, such as protein-protein interactions and conformation change. The specific antigen-antibody binding process is driven by the desire to minimize their total energy and the binding complexes on the crowded surface result in changes in surface charge and hydrophilicity of molecules, which leads to the change of the surface stress and a defection consequently. In addition, conformational changes within immobilized antigen upon binding of antibody may also give contributions. It is known that surface stress can be related with surface free energy[6]. From this energy view, the microcantilever technique is sufficiently general to detect many specific biomolecular interactions without the need of labels.

Conclusion

In conclusion, though self-assembled monolayer and carboxyl-activation methods, one surface of the microcantilever was immobilized with the glutathione S-transferase and antigen-antibody binding was experimentally investigated using microcantilever sensor. The results show that the specific antigen-antibody binding induces changes of surface stress and consequently generates deflections of the microcantilever. This label-free method affords a new way to monitor various biomolecules interactions.

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