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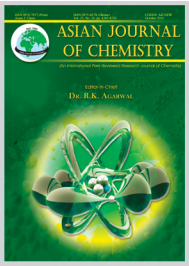

Denaturation of Protein Using Microcantilever Sensor†

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A real-time detection denaturation of the trypsin using microcantilever sensor platform with optical lever technique was reported. The trypsin was indirectly immobilized on the cantilever gold surface by thiol self-assembled monolayer and the activated carboxy group of the thiol by EDC/NHS. Consequently, the deflections of the microcantilever corresponding to the process of injecting different concentration of GuHCl were generated.

Key Words: Microcantilever, Denaturation, Surface stress.

INTRODUCTION

It is well known that the biological activity of a protein is closely related to its conformation. The denaturation and renaturation of proteins is one of those basic problems[1]. Consequently, studying the mechanisms of denaturation of protein may be helpful in developing new method to learn the protein fold and its conformation. Spectral analysis was the useful tool research on the thermal denaturation and chemical denaturation. To date, there is no available means to detect the mechanics behave in the process of denaturation.

In recent years there are increasing researches into developing microcantilever biosensor techniques[2] as label-free detection of biomolecules without labeling by radioactive or fluorescent. As a novel biosensor, it can be applied to recognize a variety of analytes using immobilized biomolecular on the functional surface, such as prostate-specific antigen (PSA)[3], single-chain antibody fragments[4], heavy-metals, *etc*.

Trypsin enzyme acts to degrade protein, often referred to as a proteolytic enzyme or proteinase, is commonly used in biological research by the different techniques. In this paper, we showed a new way to detection denaturation of the protein using microcantilever sensor.

EXPERIMENTAL

Trypsin, 11-mercapto undecanoic acid was purchased from (Sigma-Aldrich, 99 %). EDC and NHS were obtained from Shanghai Medpep Company. Microtiter plate with 16 wells was purchased from Nunc. All the other reagents used were of analytical grade.

In present 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 such as those used in AFM that is placed on a temperature controller. 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 while the analyte of interest was injected using a low pressure injection port sample loop system.

Functionalization of the cantilever surface: A typical EDC/NHS surface procedure for protein immobilization was used. The microcantilevers were thoroughly cleaned with piranha solution (1:3 H_2O_2:H_2SO_4) and rinsed with deionized water. The microcantilever was immersed in a 1 mM solution of 11-mercapto undecanoic acid (MUA) in ethyl alcohol for 12 h and rinsed in EtOH, next, the microcantilevers were immersed in PBS buffer solution containing 0.2 M/L EDC and 0.05 M/L NHS for 0.5 h at room temperature, then immersed in a solution containing trypsin 0.05 M/L for 1 h in 37.5 ºC.

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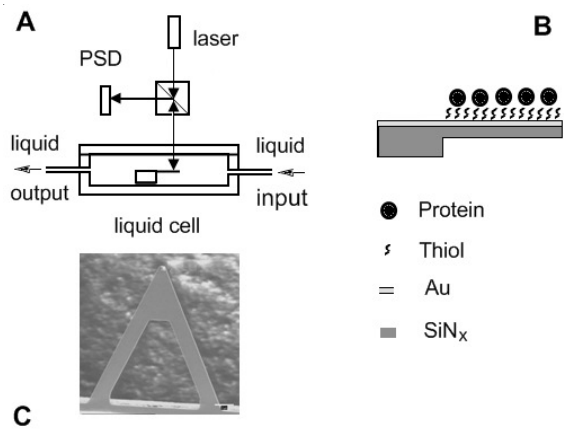

Fig. 1. Measurement setup and sensor functionalized. (A) Schematic drawing of the sensor instrument. (B) Side view of a gold-coated silicon cantilever functionalized with trypsin. (C) Top view of the microcantilever used in experiments

The microcantilevers were then rinsed with TPBS solution and store in buffer waiting to use.

Deflection measured with trypsin coated microcantilever: The chemically modified microcantilevers were placed in flow cell. The working solution phosphate buffer solution, were run through the low pressure injection port sample loop system into the flow cell using peristaltic pump. The flow rate of the solutions flowing through the fluid cell was maintained at a constant rate of 4.5 mL/h throughout the experiment[6,7]. The analyte was injected until a baseline was obtained and the deflection of the position-sensitive detector was set as background corresponding to 1 nm. The bending of the cantilever was quantified using the instrument described above (Fig. 1A). Analyte containing different concentrations of GuHCl dissolved in phosphate buffer solution were injected through the injection port sample loop system which allows for the continuous exposure of the cantilever to the desired solution.

RESULTS AND DISCUSSION

Bending responses of the trypsin modified MCL: The data repetitiveness was a crucial for experiment. So we used the same concentration of 0.75 M GuHCl and PBS buffer to doing three times detections. The result as shown in the Fig. 2, we got the repetitive and reversible microcantilever bending covered with trypsin when switching between GuHCl and PBS.

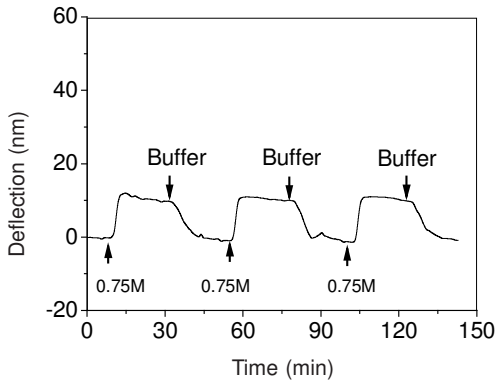

Fig. 2. Three replicates curves of bending responses as a function of time for a trypsin modified MCL following injection of 0.75 M GuHCl

Fig. 3 compares the cantilever deflection profiles when the modified cantilever is exposed to a PBS under different concentrations of GuHCl. In these experiments, buffer before and after injection were about the same. The deflection signals by the introduction of the GuHCl showed positive values, indicating the upward bending of the cantilever. After stopping the injection of GuHCl to the cantilever, then the inject buffer to the flowing cell after 20 min. It was observed that the cantilever bending amplitude in higher concentrations solution was higher in solutions with lower one. The larger bending amplitude suggested a larger conformational change of cantilever in larger concentrations of denaturant and *vice versa*.

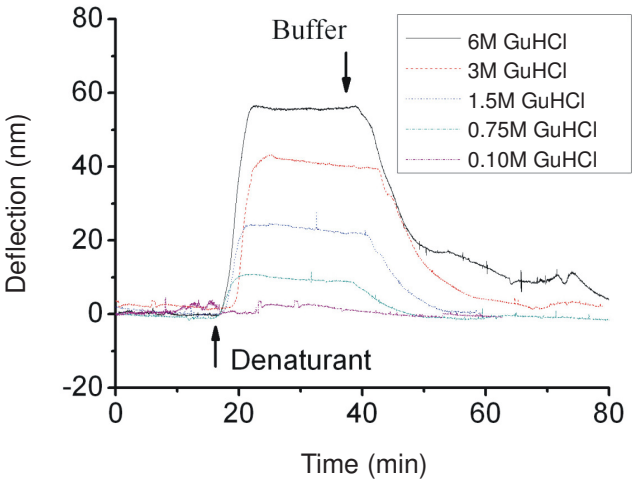

Fig. 3. Steady-state bending response *versus* time for trypsin coated microcantilever, upon injection cycling between the buffer and GuHCl at the different concentrations in PBS buffer

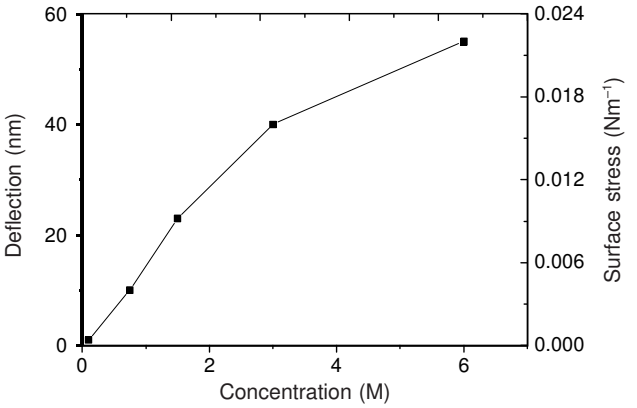

Fig. 4. Maximum bending amplitude and surface stress change for trypsin modified microcantilever as a function of the change in concentration of GuHCl in buffer solution

Conclusion

Microcantilever-based surface stress measurements provide a tool to probe the denaturation of proteins on solid surfaces. This strategy would make it possible to develop a sensor with both high sensitivity and continuous monitoring denaturation of target molecules. Such knowledge is of critical importance in biotechnology applications involving the protein fold and its conformation. A quantitative description of the origin of the changes in surface stress on adsorption and how

this can be related to the microscopic, conformational changes occurring in the proteins is as yet not available.

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