Asian Journal of Chemistry; Vol. 24, No. 9 (2012), 3951-3953

ASIAN JOURNAL OF CHEMISTRY

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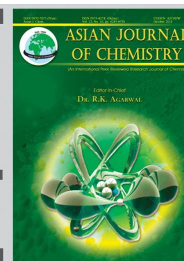

Detection of Ethyl Parathion Using Microcantilever Biosensor†

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AJC-11281

A microcantilever biosensor has been developed for label-free detection of ethyl parathion. Through self-assembled monolayer (SAM) method, anti ethyl parathion antibody was immobilized on the gold surface of the microcantilever for the measurement of ethyl parathion. The deflection of the microcantilever corresponding to different ethyl parathion concentration was real time monitored by optical lever technique. The experimental results of non-competitive enzyme-linked immunosorbent assay (ELISA) indicate that anti ethyl parathion antibody on the gold surface can keep the activity. Deflection of microcantilever is linear to ethyl parathion concentration from 500-2000 ng/mL. The limit of detection is 500 ng/mL.

Key Words: Microcantilever biosensor, Ethyl parathion, Antibody, Self-assembled monolayer.

INTRODUCTION

In recent years, the extensive use of pesticides and their effects on the environment has been become more the public concern[1]. Conventional analytical methods available are expensive, time consuming or inconvenient for *in situ* monitoring. Considerable efforts have been made to eliminate some of the drawbacks of conventional analytical methods. Biosensor, especially microcantilever biosensor, has been attracted much attention in the pesticides' detection[2]. In recent years, there was an increasing research into developing microcantilever biosensor technique as label-free detection of biomolecules. It was widely used in the biochemical, chemical and material research fields[3].

As one of the most important organophosphorus pesticides, ethyl parathion can deactivate enzyme acetylcholinesterase, disrupt nerve function and result in paralysis or even death[4]. Due to its high toxicity, it was prohibited from being applied to agriculture in many developed countries. In this paper, we have developed a microcantilever biosensor which was functionalized with self-assembled monolayer (SAM) methods, for sensitive detection of ethyl parathion.

EXPERIMENTAL

The anti ethyl parathion antibody was generated by Prof. Baomin Wang's group. Ethyl parathion, TMB, human serum albumin (HSA), bovine serum albumin (BSA), 11-mercapto undecanoic acid and horse radish 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).

Surface modification approaches: As shown in Fig. 1, each microcantilever was pretreated with piranha solution (H_2O_2:H_2SO_4 = 1:3) in one of the microtiter wells for 0.5 h, washed with deionized water (200 µL) for three times. The cleared microcantilever was immediately placed in a 50 mM 11-mercapto undecanoic acid solution (dissolved in ethanol) for 12 h at room temperature. 0.2 M of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and 0.5 M N-hydroxysuccinimide solution which has been prepared in distilled water were applied to the SAM by a micropipette (100 L) for 0.5 h. An aliquot of 200 µL of 1 µg/mL antibody solution was pipetted into the well followed by incubation for 1 h at 37 ºC. After

†Presented to The 5th Korea-China International Conference on Multi-Functional Materials and Application.

washed with PBST for three times, the microcantilever was placed in a clean well, 200 μL of 3 % non-fat dry milk coating buffer was then added and followed by incubation for 0.5 h at 37 ºC for blocking of the silicon nitride side of the microcantilevers. Later, washed with PBST for three times and the functionalized microcantilever was ready to use.

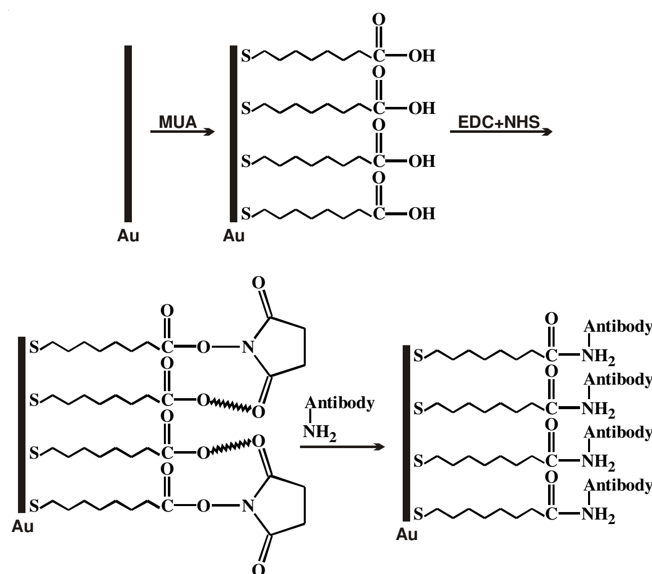

Fig. 1. Schematic diagram of the experimental for decorating antibody on Au film of a cantilever

Measurements of the microcantilever deflection in liquid environment: The microcantilever deflection was measured by the optical level technique as reported earlier[5]. All analyte solutions were prepared in buffer solvent, which was also used as a background solution. Microcantilever mounted in the flow cell was initially allowed to equilibrate in running background buffer. A constant flow rate of 0.034 mL/min was applied for reagent and exchange buffer. After obtained a stable baseline, 2 mL solution of ethyl parathion (500, 1000 and 2000 ng/mL), was injected in the flow cell.

Characterization the activity of antibody using an enzyme-linked immunosorbent assay (ELISA): 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

Characterisation of immobilised antibody using enzyme-linked immunosorbent assay (ELISA): The absorbance values of non-competitive ELISA for the functionalized microcantilever and unfunctionalized one were 0.3583 and 0.036 respectively. Athough it cannot direct to confirm antibody activities and the inhibition results compared to dcELISA, the non-competitive ELISA 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 antibody functionalized on the gold surface of microcantilever.

Measurement of ethyl parathion by microcantilever biosensor: The deflections of microcantilevers for different concentration ethyl parathion solutions (500, 1000 and 2000 ng/mL) injected to the flow cell were shown in Fig. 2, which indicated the deflection of the microcantilever was increasing with increasing concentration of solution of ethyl parathion. Moreover, to identify the specific binding, the nonspecific deflection of ethyl parathion to the reference microcantilever was also shown in Fig. 2. No significant signal was observed when BSA at a concentration of 1 mg/mL was injected. This indicated little interferences from nonspecific absorption in the experiment. The interaction force which generated during the specific binding between monoclonal antibodies on the gold surface of microcantilever and ethyl parathion in solution only happened in the gold surface of microcantilever. Much higher free energy change was lead as specific binding between molecules than nonspecific binding.

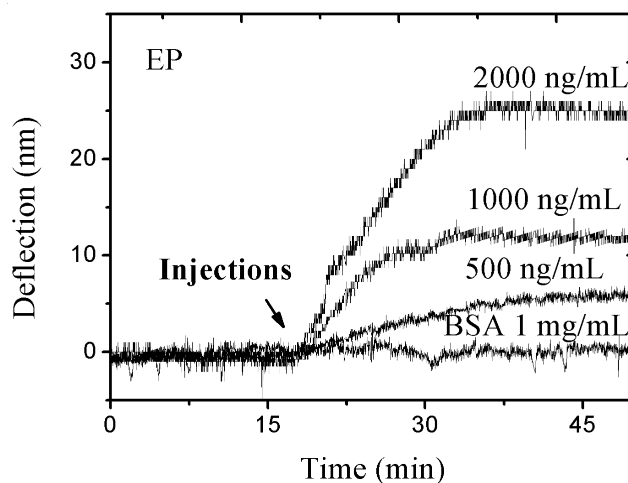

Fig. 2. Deflections of anti ethyl parathion (EP) antibody functionalized microcantilever at varying concentrations of ethyl parathion in PBS ranging from 500-2000 ng/mL

In this paper, a microcantilever biosensor is developed and functionalized with self-assembled monolayer method for sensitive and quantitative detection of ethyl parathion. Microcantilever biosensor detection requires no labels or external probes, is a rapid detection method of highly sensitive, specific and portable. These characteristics make this method appealing for environmental monitoring applications for further research.

ACKNOWLEDGEMENTS

This work was supported by the Foundation for Young Talents in College of Anhui Province (2012SQRL053ZD), the Natural Science Research of Key Projects of Anhui Provincial

Universities (KJ2010B316) and Doctor Foundation of Anhui University of Science and Technology.

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