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Electrochemical ELISA based on *Escherichia coli* with autodisplayed Z-domains

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ABSTRACT

Recently, we reported autodisplayed Z-domains on *Escherichia coli* can highly improve the sensitivity of immunoassays through the orientation control of detection antibodies. In this work, the amperometric analysis was applied to the immunoassay based on *E. coli* cells with autodisplayed Z-domains. For amperometric analysis, the reduction current of 3,5,3′,5′-tetramethylbenzidine (TMB) was measured at the potential of −50 mV and the correlation between the reduction current and the OD value was estimated to be 28 nA/OD and the limit of detection was calculated to be 0.07 in OD unit. In this work, the feasibility of the immunoassay based on amperometric analysis was presented by the quantification of HRP as a model analyte. For the demonstration of medical diagnosis, C-reactive protein (CRP) was detected by using electrochemical ELISA based on *E. coli* with autodisplayed Z-domains.

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1. Introduction

Recently, we reported autodisplayed Z-domains on *Escherichia coli* can highly improve the sensitivity of immunoassays through the orientation control of detection antibodies [1–3]. The autodisplay technology is an expression method of a target protein, and the autodisplayed protein is expressed on the outer membrane of *E. coli* [4,5].

In this work, Z-domain was autodisplayed as a fusion protein with an outer membrane protein called AIDA-1. As Z-domain has the antibody-binding activity by using the specific affinity towards the F_c-region of immunoglobulins, it has been used for the orientation control of antibodies. The outer membrane with autodisplayed Z-domains could be isolated, and then the outer membrane was layered on the polystyrene surface of microplates and the gold surface of a SPR biosensor. In both cases, the sensitivity of immunoassay was estimated to be far more improved by the orientation control effect of the autodisplayed Z-domains [2,3].

The *E. coli* cell with autodisplayed Z-domains itself was also applied to immunoassays for the detection of target analytes, and the sensitivity of the "direct assays" was observed to be far more improved in comparison with the immunoassays without the orientation control of antibodies [6]. For the direct immunoassay, however, *E. coli* cells with the autodisplayed Z-domains should be centriguged repeatedly for washing steps and for reagent chaging steps. The centrifugation steps during immunoassays resulted in several problems: (1) the loss of *E. coli* cells during washing steps, (2) delayed analysis time, and (3) difficulties in automation of immunoassays. In this work, the *E. coli* cell was immobilized on the surface-modified microplate for the effective immunoassay. The *E. coli* cells with autodisplayed Z-domains were immobilized on poly-lysine coated microplates for effective immunoassay. For the immobilization of *E. coli* cell with a negatively charged outer membrane [7], the microplate surface was modified to be positively charged by using parylene-H film and poly-L-lysine as shown in Fig. 1(a).

The immunoassay was carried out on the outer membrane of *E. coli* cells by using the immobilized *E. coli* cell with autodisplayed Z-domains, and the immunoassay result was reported by the enzyme reaction of horseradish peroxidase (HRP) with 3,5,3′,5′-tetramethylbenzidine (TMB) as shown in Fig. 1(b). From the enzyme reaction of HRP, TMB is known to be oxidized by two steps and the final product with a yellow color can be quantified by measuring the optical absorption at the wavelength of 495 nm [8,9]. In this work, the oxidized TMB from the enzyme reaction was measured by using amperometric analysis. Usually, the application of amperometry for the conventional ELISA based on 96-well microplate was known to be possible by reduction of chromogenic dyes of benzidine derivatives [10–12]. This electrochemical ELISA method has advantages over the optical methods in the simplicity of instrumentation and low production cost, and it can be applied to the commercial ELISA kits without any modification [10]. Such electrochemical ELISA should satisfy the following conditions: (1) the electrochemical signal should be convertible to OD at a range required for ELISA; (2) the measuring time should be appropriate for ELISA; (3) the electrode should fit to a 96-well microplate.

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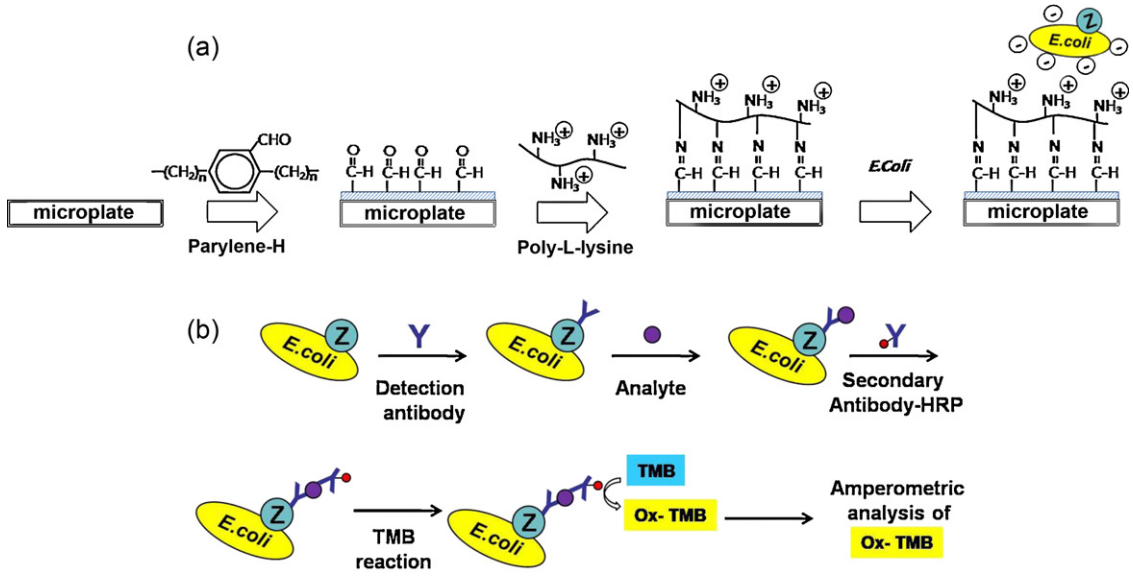

Fig. 1. Electrochemical ELISA by using immobilized *E. coli* cells with autodisplayed Z-domains. (a) Surface modification with poly-L-lysine and parylene-H film for the immobilization of *E. coli* cells. (b) Assay configuration of the electrochemical ELISA by using immobilized *E. coli* cells with autodisplayed Z-domains.

In this work, the feasibility of the immunoassay based on amperometric analysis was presented by the quantification of HRP as a model analyte. For the demonstration of medical diagnosis, C-reactive protein (CRP) was detected by using electrochemical ELISA based on *E. coli* with autodisplayed Z-domains.

2. Materials and methods

2.1. Autodisplay of Z-domains

The vector for autodisplay was constructed by cloning the antibody binding Z-domain from *Staphylococos aureus* with PCR amplification as described in the previous works [1,2]. *E. coli* cells were routinely cultured at 37 °C in Luria–Bertani (LB) broth of 10 μM EDTA, 10 mM 2-mercaptoethanol and ampicillin at the concentration of 100 mg/l. For the activity assay, *E. coli* cells transformed with the plasmid pET-Z-18-3 were grown overnight and diluted 100-fold in a freshly prepared medium. The *E. coli* cells were grown at 37 °C with vigorous shaking until the optical density (OD) reached 3.0 at the wavelength of 578 nm. After the *E. coli* cells were harvested, they were washed three times with PBS and then resuspended in PBS to a final optical density of 1.0 at the wavelength of 578 nm.

2.2. Surface modification of microplate and immobilization of E. coli cells

The surface modification of a microplate made of polystyrene from SPL Co. (Seoul, Korea) was carried out by (1) deposition of a parylene-H film with formyl groups and (2) covalent coupling of poly-L-lysine. The parylene-H film was coated on a polystyrene microplate by using a parylene coater from Femto Science Co. (Seoul, Korea) and the parylene precursors of parylene-H was supplied from Femto Science Co. (Seoul, Korea). As previously reported, the parylene films were deposited by polymerization steps: (i) evaporation of monomer at the temperature of 160 °C, (ii) pyrolysis for the production of highly reactive p-xylene radical at the temperature of 650 °C, and (iii) deposition to the microplate under vacuum condition of less than 2 Torr at the room temperature [13,14]. The thickness of the parylene-H film was controlled to be 20 nm by adjusting the initial amounts of parylene-H precursors of 50 mg. The thickness of the parylene-H coated was measured by using an atomic force microscope (XE-100) from Park System Co. (Seoul, Korea). For the immobilization of poly-L-lysine to the microplate, the poly-L-lysine solution from Sigma–Aldrich Korea (Seoul, Korea) was treated for 2 h. As shown in Fig. 1(a), the primary amine groups of poly-L-lysine are covalently linked to the formyl groups of parylene-H in the form of imine bonding. For the control experiment, the poly-L-lysine coated microplate without parylene-H film was also prepared by treatment of the poly-L-lysine solution at the same condition. The *E. coli* was immobilized to microplates by incubation of *E. coli* cells at each well of the microplates. The freshly prepared *E. coli* culture at the concentration of 10^9 cells/ml in PBS (100 μl) was washed three times with PBS, and then incubated at each well of the microplate overnight at 37 °C. Then, the microplate was treated with BSA blocking solution at the concentration of 10 mg/ml for 1 h. The microplates were washed three times with 0.1% Tween 20 in PBS.

2.3. ELISA procedure by using immobilized E. coli with autodisplayed Z-domains

Immunoassay was carried out according to the conventional ELISA procedure as shown in Fig. 1(b). For the reagent change, the *E. coli* solution was centrifuged for 5 min at 14,000 rpm. For the immobilization of antibodies, the antibody solution at the concentration of 100 μg/ml was prepared and 100 μl of the antibody solution was incubated for 2 h at room temperature. Then, the microplate was washed with 0.1% Tween 20 in PBS for three times. The HRP assay was demonstrated by the chromogenic reaction of the TMB solution which was made by dissolving 1 mg TMB (Sigma–Aldrich Co., USA) in 0.05 M phosphate–citrate buffer (pH 5.0). After quenching with 2 M sulfuric acid solution, the optical density was measured at the wavelength of 450 nm by using an ELISA reader (Vesamax, Molecular Devices, USA).

2.4. Fabrication of electrode and amperometric detection

The gold-film array-electrode was made by fixing eight sets of the three-electrode unit in a printed circuit board as previously described [10,11]. The gold-film electrode was produced by sputtering of 100 nm of a gold layer on a polyetherimide

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