Electrochemical ELISA based on *E.coli* with autodisplayed Z-domains

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Abstract

Recently, we reported autodisplayed Z-domains on *E.coli* can highly improve the sensitivity of immunoassays through the orientation control of detection antibodies [1-3]. 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, an immunoassay was prepared by immobilizing anti-HRP antibodies to *E.coli* cells with autodisplayed Z-domains and the amperometric analysis was demonstrated the quantification of bound HRP. 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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Keywords: electrochemical ELISA; autodisplay; *E.coli*; Z-domain

1. Introduction

Recently, we reported autodisplayed Z-domains on *E.coli* can highly improve the sensitivity of immunoassays through the orientation control of detection antibodies [1-3]. As shown in Fig. 1, the immunoassay can be performed on *E.coli* cells with autodisplayed Z-domains and the assay result can be estimated by using optical density (OD) measurement at a specific wavelength [3]. The application of amperometry for the conventional ELISA based on 96-well microplate was reported to be possible by reduction of chromogenic dyes, and such an amperometric analysis was known to have several advantages over the optical analysis methods [4-6]. In this work, the amperometric analysis was applied to the immunoassay based on *E.coli* cells with autodisplayed Z-domains.
2. Materials and methods

2.1 Autodisplay of Z-domains

The vector for autodisplay was constructed by the cloning of the antibody binding Z-domain from *S. aureus* with PCR amplification as described in the previous works [11-12]. *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.

2.2 ELISA procedure by using *E.coli* with autodisplayed Z-domains.

The assay was carried out according to the conventional ELISA procedure as shown in Fig. 1. For the reagent change, the *E.coli* solution was centrifugated for 5 min at 14000 rpm. For the immobilization of antibodies, the solution of *E.coli* with autodisplayed Z-domain at the OD value of 1.0 (200 ul) was centrifugated and then resuspended with anti-HRP (or CRP) antibody solution (100 ul) at the concentration of 1 μg/ml and then incubated for 1 hr.

2.3 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 [5]. The gold-film electrode was produced by sputtering of 100 nm of a gold layer on a polyetherimide (PEI) film with an area of 1mm×10mm (Goodfellow GmbH, Bad Nauheim, Germany). On the gold layer an additional acrylate layer was coated by spraying on an acrylate solution (ethylacetate and *n*-butylacetate in 1-methoxy-2-propanol). The exposed areas of the electrode and the electric contact were 1.5mm×1.0mm and 3.0mm×1.0 mm, respectively. The reference electrode was prepared by spin coating (1500 rpm, 5 min) Ag/AgCl paste (Du pont, Bristol, UK) on a PEI film. Two gold-film electrodes and an Ag/AgCl reference electrode were fixed on a printed circuit board using an electrically conductive adhesive (Elecolit 336, Panacol–Elosol GmbH, Oberursel, Germany).
3. Results and discussion

Usually, the conventional ELISA kits have been used 3,5,3’,5’-tetramethylbenzidine (TMB) as the chromogenic dye which is one of substrates of horseradish peroxidase (HRP). As shown in Fig. 2, the TMB molecule can be oxidized and reduced by amperometry, and the redox current can be correlated to the OD value at a specific wavelength (λ_{max} of TMB = 450 nm). For the amperometric analysis, the previously reported gold film electrode was used [5]. In this work, the reduction current was measured at the potential of -50 mV and it was correlated to the OD value.

![Fig. 2: The cyclic voltammogram of the TMB samples with different OD values. (a) The scanning range was from -300 to 500 mV at the scanning rate of 30 mV/s. (b) The plot of voltammetric signal versus OD of TMB samples at several fixed potentials.]

As shown in Fig. 3, 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, an immunoassay platform was prepared by immobilizing anti-HRP antibodies to E.coli cells with autodisplayed Z-domains, and HRP with a known concentration was used as a model analyte. TMB was used for the quantification of the amount of bound HRP, and then the amperometric analysis was carried out.

![Fig. 3: The correlation between amperometric signal and OD values. (n=5)
As shown in Fig. 4(a), the immunoassay based on *E. coli* with autodisplayed Z-domains (square) shows far higher sensitivity at the whole range of analyte in comparison to the conventional ELISA method based on physical adsorption of antibodies to 96-well microplate (circle). When the *E. coli* without autodisplayed Z-domains (triangle) was used for the same immunoassay, there observed no significant sensor response. These results show that the *E. coli* cells based on autodisplayed Z-domains can be used for highly sensitive immunoassay and the assay result can be reported by using amperometric analysis. For the demonstration of medical diagnosis, C-reactive protein (CRP) was detected by using electrochemical ELISA based on *E. coli* with autodisplayed Z-domains as shown in Fig. 4(b).

![Fig. 4](image)

**Fig. 4:** Amperometric analysis of (a) HRP and (b) C-reactive protein (CRP) (n=3).

**Acknowledgements**

This research was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0082188, 2009-0073809, 2009-008-1529, 2010-0020767 and 2010-0020772).

**References**


