"ELISA-CIEF" USING CAPILLARY-BASED MICRODEVICE: HIGHLY-SENSITIVE ELISA BASED ON CAPILLARY-ISOELECTRIC FOCUSING OF ENZYME REACTION PRODUCT

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ABSTRACT

Sensitivity enhancement of enzyme-linked immunosorbent assay (ELISA) in capillary-based microdevice was achieved by the concentration of fluorescent final enzyme reaction product using capillary-isoelectric focusing (CIEF). In order to realize this, fluorescent substrate molecule, which can change its structure into ampholyte ion after an enzyme reaction, was newly synthesized and used. Approximately seven-fold enhancement of sensitivity and 1-2 orders of magnitude lower the detection limit were achieved.

KEYWORDS

Capillary isoelectric focusing, Enzyme-linked immunosorbent assay (ELISA), Sensitivity enhancement, Square capillary

INTRODUCTION

Enzyme-linked immunosorbent assay (ELISA) is an established method of protein analysis using enzyme labeled antibody. It is widely used for highly sensitive and selective clinical diagnostic tools. However, detection of low concentration marker proteins still remains as a problem. On the other hand, capillary isoelectric focusing (CIEF) is a well-known method for the separation and concentration of target molecules possessing isoelectric point, such as proteins and peptides, by using pH gradient formed inside the capillary. CIEF has an advantage that all target molecules can be concentrated at a certain point of capillary. Thus, if the final fluorescent product of an enzyme reaction in ELISA is concentrated, development of highly-sensitive ELISA exceeding the present sensitivity and detection limit is expected. In this research, design and synthesis of novel fluorescent substrates of alkaline phosphatase (ALP) which can change its structure into ampholyte ion after an enzyme reaction was carried out and applied to ELISA-CIEF in capillary-based microdevice.

EXPERIMENT

Synthesis of rhodamine type-ALP substrate (RD-DP) was carried out. ELISA using the synthesized substrates was performed inside a capture antibody (anti-human IgG)-immobilized square glass capillary (I.D.100μm

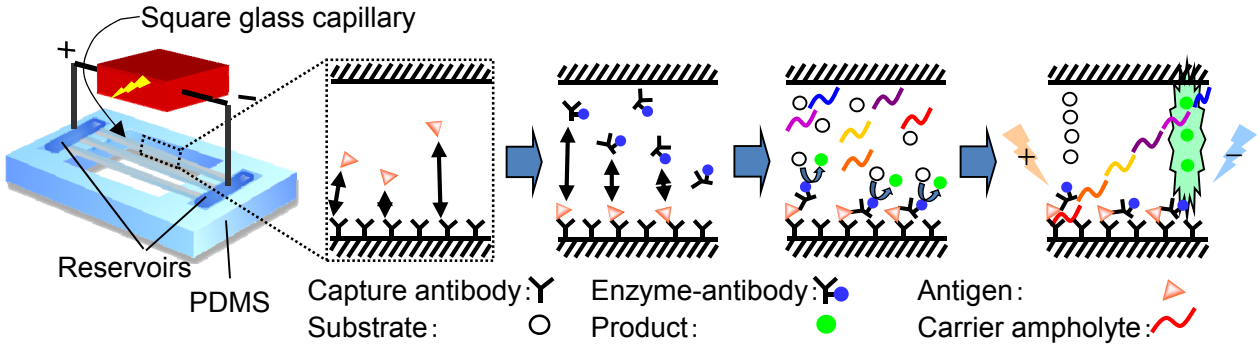

Figure 1 General concept of ELISA-CIEF on a capillary-based microdevice

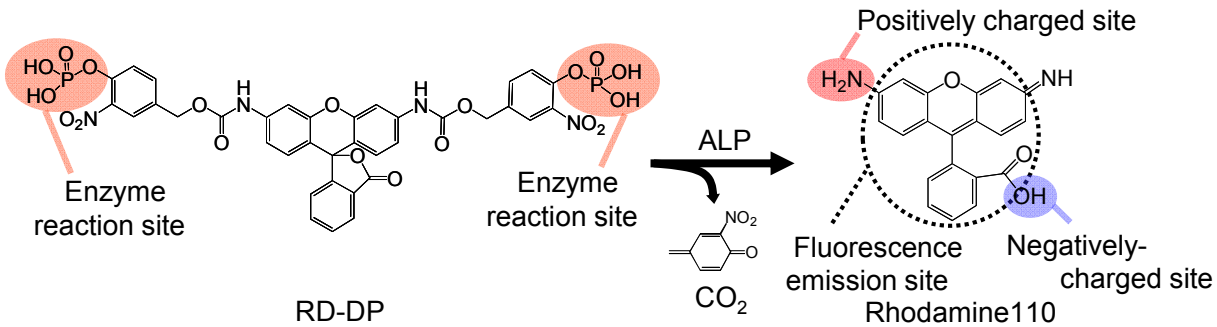

Figure 2 Newly-synthesized fluorescent substrate, RD-DP, for use in ELISA-CIEF

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(flat-to-flat), length: 4cm) by the conventional ELISA procedure using antigen (Human IgG) solution, ALP-labeled secondary antibody solution, and synthesized fluorescent substrate solution containing carrier ampholyte. According to the previous report, incubation times for immunoreactions were set to 20 minutes. [1] After immunoreactions, final enzyme reaction was carried out by introducing 50 mM Tris buffer (pH9) containing RD-DP (1.0×10^{-5} M), carrier ampholyte (Biolyte 3/10, 6 v/v %), and Tween 20 (0.1 w/v %). Enzyme reaction time was set to 60 minutes. After the enzyme reaction, capillary was set to the PDMS device shown in Figure 1, then, reservoirs were filled with acid (H_3PO_4: 0.02 M, 0.2 M, 2 M) and base solutions (NaOH: 0.02 M, 0.2 M, 2 M). Then, CIEF was carried out by applying voltage of 0.6 kV (150 V/cm). Fluorescence images were acquired by fluorescence microscope equipped with CCD camera.

RESULTS AND DISCUSSION

RD-DP shown in Figure 2 was designed as a fluorescent substrate, which can be used for CIEF, and synthesized successfully. RD-DP, which is generally a non-fluorescent compound, has enzyme reaction sites which can be cleaved by ALP to give fluorescent Rhodamine 110. Since the Rhodamine 110 has amine and carboxylic groups, CIEF concentration is possible.[2] Figure 3 shows a typical result of preconcentration of Rhodamine 110 by CIEF in poly(dimethylacrylamide)-coated capillary using 0.02 M acid and base solutions as reservoir solutions.

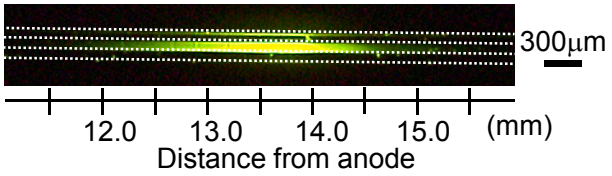

Figure 3 Concentration of Rhodamine 110 by CIEF

Since the present concept involves the CIEF of enzyme reaction product in antibody-immibilized capillary, instability of the concentrated peak due to the effect of electroosmotic flow (EOF) was suspected. In the case of CIEF using antibody-immobilized capillary, successful preconcentration of Rhodamine 110 and no significant drift of concentrated peak were observed. However, concentrated band was very broad compared with the result shown in Figure 3. This may be attributed to the adsorption of Rhodamine 110 to the protein surface and relatively slow acid-base reaction of Rhodamine 110 with H^+ and OH^- introduced into capillary by electrophoresis, because amine and carboxylic groups in Rhodamine 110 were both aromatic ones. Thus we investigated the concentration of acid and base solutions in the reservoirs and found that the experiment using higher concentrations of reservoir solutions (2 M each) gave relatively narrow peaks. Thus, we applied this experimental condition to ELISA-CIEF.

Figure 4 shows the fluorescence images of capillaries after conventional ELISA procedure (conventional ELISA) and further applied the electric field to concentrate the final enzyme reaction product (Rhodamine110) by CIEF (ELISA-CIEF). As expected, results of conventional ELISA showed fluorescence increase upon increasing antigen concentrations and response ranges of more than tenth of ng/ml level were observed. On the other hand, concerning

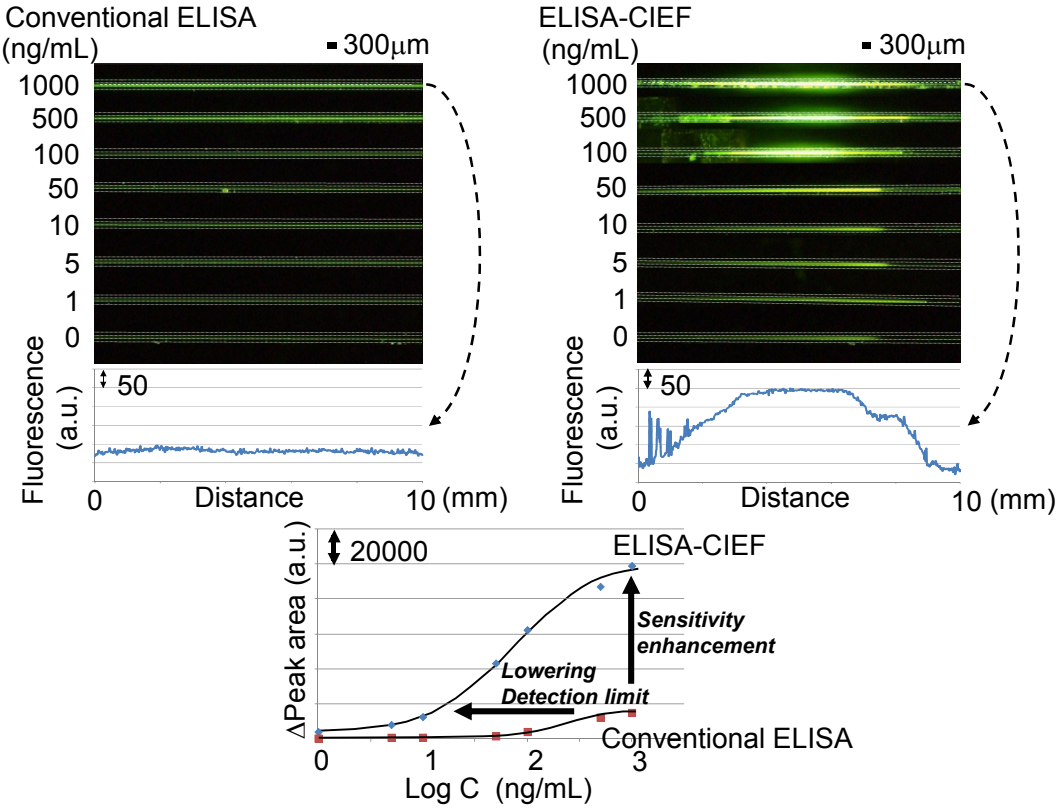

Figure 4 Comparison of the response for ELISA-CIEF and conventional ELISA

the ELISA-CIEF, although the concentrated peaks were still broad, fluorescence intensities were enhanced by concentration. Figure 4 also shows the response curves for conventional ELISA and ELISA-CIEF by using peak area. In this case, peak areas obtained from 1cm capillary length shown in Figure 4 were plotted. Compared to conventional ELISA, ELISA-CIEF showed higher sensitivity by approximately seven-fold and 1-2 orders of magnitude lower detection limit.

Thus present study clarified the new concept shown in Figure 1 actually worked and provided the possibility of detecting low concentration proteins in microdevice format.

CONCLUSION

Sensitivity enhancement and lowering the detection limit were achieved by the concept of ELISA-CIEF. Present results clarified that the enzyme substrates producing fluorescent ampholyte ion was quite useful for the highly-sensitive detection of proteins based on the principles of ELISA and CIEF. Present concept is expected to be expanded to multiple detection of different important proteins by using the capillary-array IEF format.[3]

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