DESIGN AND SYNTHESIS OF FLUORESCENT ENZYME SUBSTRATE MONOMER AND ITS APPLICATION TO THE DEVELOPMENT OF HYDROGEL-BASED SINGLE STEP IMMUNOASSAY MICRODEVICE

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ABSTRACT

Here, we report the development of hydrogel-based single step immunoassay microdevice, which is expected to detect the disease marker proteins rapidly in medical diagnoses. In the present study, we designed and synthesized an enzyme substrate monomer for alkaline phosphatase (ALP), which was immobilizable to hydrogel by copolymerization, and preliminary investigation for the development of single step immunoassay microdevice using this monomer was carried out.

KEYWORDS

Immunoassay, Hydrogel, Microdevice, Single step, Fluorescent enzyme substrate

INTRODUCTION

Enzyme linked immunosorbent assay (ELISA) is widely used for highly sensitive biomarker detection. However, ELISA usually needs complicated and time-consuming operation steps involving the premixing of sample (antigen) with reagents (enzyme labeled antibody, fluorescent enzyme substrate molecule) and washing to separate unreacted species. Therefore, simple and rapid method of measuring biomarker is required. We have been investigated the ELISA devices using capillary format [1]-[4]. However, these works still needs improvement in operation steps. In order to solve the problems, we have newly designed a single step immunoassay by using a hydrogel covalently immobilizing fluorescent enzyme substrate and a soluble coating containing enzyme labeled antibody (Figure 1). In this case, sample introduction by capillary action allows enzyme labeled antibody to be released and react with antigen. Since antigen-antibody complex has larger molecular weight, permeation through gel is restricted, thus fluorescence intensity decreases with increasing antigen concentration.

In order to realize this concept, enzyme substrate which cannot be leaked out from gel during the enzyme reaction is necessary. Here, we synthesized a fluorescent enzyme substrate monomer having enzyme reaction site, fluorescence intensity change moiety after enzyme reaction, and copolymerization site with gel by copolymerization, and evaluated the immuno sensing performance of the hydrogel bearing the synthesized monomer molecule.

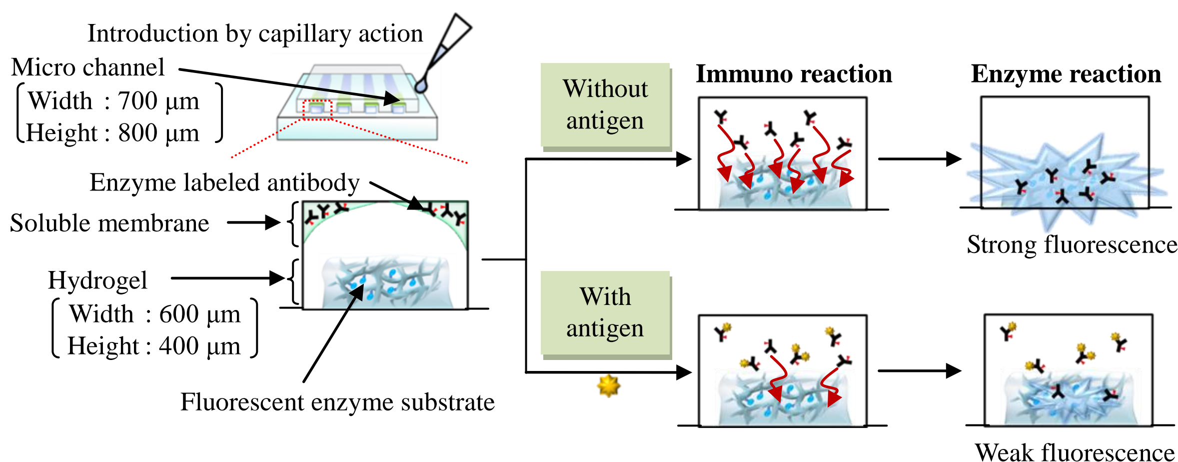

Figure1. Single step immunoassay microdevice using a hydrogel immobilizing fluorescent enzyme substrate

EXPERIMENTAL

First we synthesized a substrate monomer for ALP (Figure 2), and investigated the fundamental characteristics of this molecule (enzyme reaction kinetics, fluorescence intensity, immobilization capability in hydrogel by copolymerization). In the next step, the synthesized fluorescent substrate monomer was immobilized to poly (acrylamide) gel by copolymerization, and fabricated a sensing hydrogel for immunoassay on slide glass coated with 3-(trimethoxysilyl)propyl methacrylate (Figure 3). As a preliminary investigation, this sensing hydrogel was soaked in Tris-HCl buffer containing ALP labeled anti-Human IgG together with Human IgG or without Human IgG, and investigated the difference of fluorescence intensity.

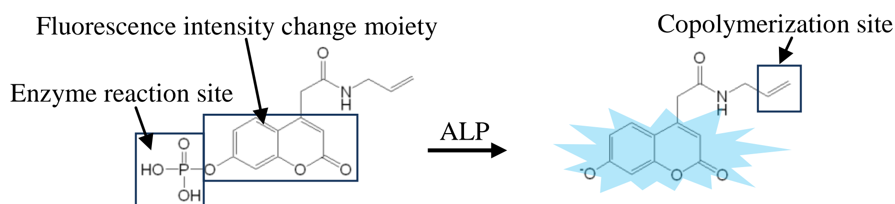

Figure2. Molecular design concept of enzyme substrate monomer for ALP

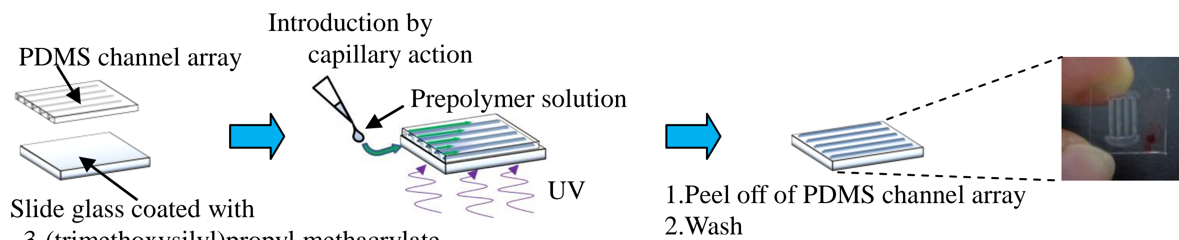

Figure3. Fabrication of a sensing hydrogel for immunoassay

RESULTS AND DISCUSSION

First, we confirmed that fluorescence intensity of the synthesized substrate monomer for ALP was changed after enzyme reaction according to ALP concentration (Figure 4(a)). In order to evaluate the effect of covalent immobilization, two hydrogels prepared using synthesized monomer and similar molecule without copolymerization site were compared. As shown Figure 4(b), hydrogel prepared by synthesized monomer showed that the copolymerization site actually worked for preventing a monomer from diffusing out of hydrogel. These results indicate that this monomer has a potential to be applied for the development of new hydrogel-based single step immunoassay device.

Next, we fabricated a sensing hydrogel for immunoassay on slide glass, and carried out preliminary investigation. When this sensing hydrogel was soaked in Tris-HCl buffer containing ALP labeled anti-Human IgG together with Human IgG, low fluorescence intensity was confirmed. In contrast, when this sensing hydrogel was soaked in Tris-HCl buffer containing ALP labeled anti-Human IgG without Human IgG, high fluorescence intensity was observed (Figure 5(a)). Furthermore, fluorescence intensity was decreased upon increasing Human IgG concentration (Figure 5(b)). These results indicated that we succeeded in developing a new scheme of “sample to answer” immunoassay, by using the synthesized enzyme substrate monomer.

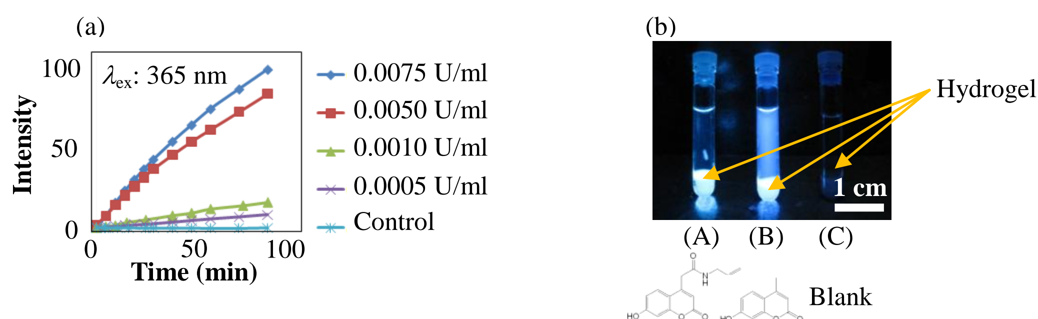

Figure4. The fundamental characteristics of enzyme substrate monomer for ALP

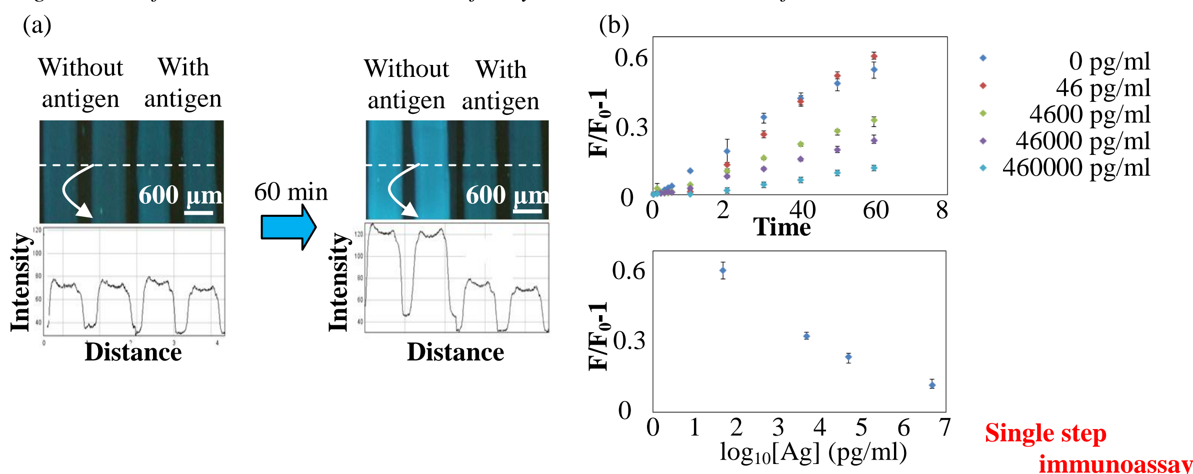

Figure5. Preliminary experiments for single step immunoassay microdevice

CONCLUSION

Fluorescent substrate monomer molecule was newly designed and synthesized. A hydrogel prepared by using the monomer showed fluorescence response to ALP-labeled antibody without leaching out from gel. Since the fluorescence intensities were the function of antigen concentration, development of the single step immunoassay device using soluble coating containing ALP-labeled antibody is expected.

ACKNOWLEDGEMENTS

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REFERENCES

[1] T. G. Henares, F. Mizutani, R. Sekizawa, and H. Hisamoto, *Multiple enzyme linked immunosorbent assay system on a capillary-assembled microchip integrating valving and immuno-reaction functions*, Anal. Chim. Acta, 589, pp. 173–179, (2007)

[2] T. G. Henares, S. Funano, S. Terabe, F. Mizutani, R. Sekizawa, and H. Hisamoto, *Characterization of capillary immunosensor for capillary-assembled microchip (CAs-CHIP) integration*, Sens. Mater, 19(4), pp. 249-256, (2007)

[3] T. G. Henares, E. Tsutsumi, H. Yoshimura, K. Kawamura, T. Yao, and H. Hisamoto, *Single-step ELISA capillary sensor based on surface-bonded glucose oxidase, antibody, and physically-adsorbed PEG membrane containing peroxidase-labeled antibody*, Sens. Actuators B, 149(1), pp. 319-324, (2010)

[4] E. Tsutsumi, T. G. Henares, S. Funano, K. Kawamura, T. Endo, and H. Hisamoto, *Single-Step Sandwich Immunoreaction in a Square Glass Capillary Immobilizing Capture and Enzyme-linked Antibodies for Simplified Enzyme-linked Immunosorbent Assay*, Anal. Sci, 28(1), pp. 51-56, (2012)

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