

RAPID AND HIGHLY SENSITIVE ELECTROPHORETIC IMMUNOASSAY DEVICE BASED ON THE ON-LINE CONCENTRATION OF ENZYME-LABELED ANTIBODY USING HYDROGEL IMMOBILIZING FLUORESCENT SUBSTRATE

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ABSTRACT

This paper reports the development of a rapid, highly sensitive and simple electrophoretic immunoassay device using a hydrogel immobilizing fluorescent substrate. To achieve the proposed electrophoretic immunoassay, electrophoretic behavior of enzyme-labeled antibody and subsequent reaction with fluorescent substrate immobilized within hydrogel were investigated. Especially, it was confirmed that a novel detection scheme based on an on-line concentration of enzyme-labeled antibody using the hydrogel could accelerate the enzyme reaction, providing a rapid and highly sensitive detection with a simple procedure.

KEYWORDS: Electrophoretic filtration, ELISA, Hydrogel immobilizing fluorescent substrate

INTRODUCTION

Enzyme linked immunosorbent assay (ELISA) is widely used for highly selective protein assay, while ELISA requires labor-intensive, time-consuming operations and amount of much reagents. Many researchers have developed microfluidic ELISA devices to reduce the analysis time and the reagents, while it still needs the tedious procedure [1]. To solve these problems, in our laboratory, single-step immunoassay microdevice using hydrogel immobilizing fluorescent substrate was developed [2]. However, a short optical pathlength and the slow diffusion in the hydrogel cause a low detectability and long analysis time, respectively. Here, we propose a novel immunoassay device using a reagent-release capillary (RRC) [3], electrophoretic separation, and on-line concentration of enzyme-labeled antibody.

GENERAL CONCEPT

Figure 1 shows the concept of proposed electrophoretic immunoassay. A sample solution is introduced into a “sampling capillary” containing enzyme-labeled antibodies by capillary action. After the sampling capillary is connected with a “separation and detection capillary”, voltage is applied. Enzyme-labeled antibody can reach the hydrogel immobilizing fluorescent substrate (detection gel), whereas the immuno-complex cannot, since “separation gel” in front of the detection gel interferes the migration of the immuno-complex by molecular sieving effect. The free enzyme-labeled antibody is then concentrated nearby the interface between the detection and separation gels, resulting in the enhancement

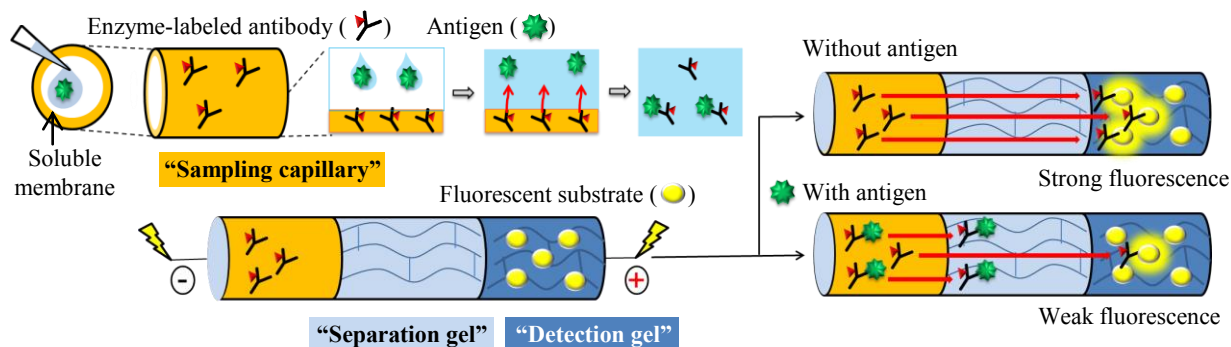


Figure 1: Concept of proposed electrophoretic immunoassay.

of fluorescence intensity by the acceleration of enzyme reaction. Therefore, the intensity of the obtained fluorescence at the detection gel depends on the amount of antigen in the sample solution which forms the immuno-complex, allowing a quantitative analysis of antigens.

EXPERIMENTAL

To evaluate the performance of the detection hydrogels, 12%(w/v) acrylamide hydrogel immobilizing fluorescent substrate (Figure 2) was prepared in a capillary, and electrophoresis device was then fabricated (Figure 3). Alkaline phosphatase (ALP)-labeled immunoglobulin G (IgG) solution and HEPES buffer (pH 8.0) were introduced into the solution reservoirs, and the voltage was then applied.

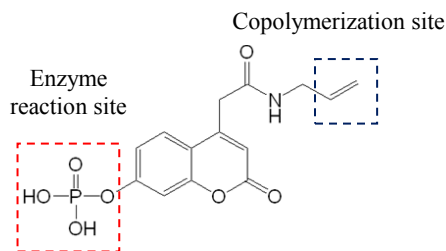


Figure 2: Fluorescent substrate monomer for alkaline phosphatase.

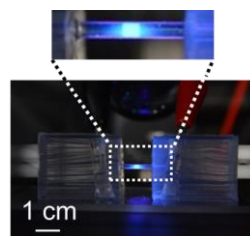


Figure 3: Electrophoresis device integrating a capillary containing detection gel.

To investigate the effect of applying voltage on enzyme reaction, fluorescence intensity changes by electrophoresis and molecular diffusion of ALP-labeled IgG was investigated. The dependence of fluorescence intensity on the concentration of ALP-labeled IgG was also evaluated.

RESULTS AND DISCUSSION

When the ALP-labeled IgG was electrokinetically introduced into the capillary containing the hydrogel immobilizing fluorescent substrate, the increase in the fluorescence intensity was observed only nearby the hydrogel interface (Figure 4, Figure 5). It suggests that the on-line concentration of ALP-labeled IgG by electrophoretic filtration nearby the hydrogel interface provided the efficient enzyme reaction.

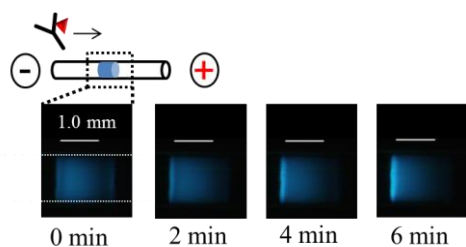


Figure 4: Fluorescence image of hydrogel immobilizing fluorescent substrate during electrophoresis of ALP-labeled IgG.

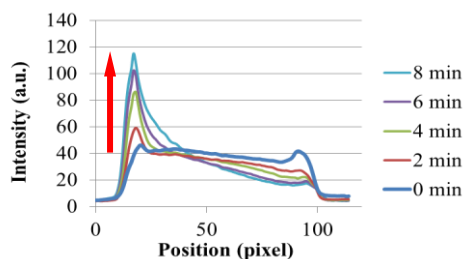


Figure 5: Changes of fluorescence intensity profile during electrophoresis of ALP-labeled IgG.

To evaluate the effect of electrophoretic filtration on enzyme reaction, observation of the fluorescence intensity nearby the hydrogel interface was carried out with/without applying the voltage. As a result, it was confirmed that the fluorescence intensity was increased only during the voltage application. These results indicate that the electrophoretic migration and on-line concentration of ALP-labeled IgG could accelerate the enzyme reaction (Figure 6).

When the fluorescence intensity was measured with varying the concentration of ALP-labeled IgG, it was found that the observed fluorescence intensity clearly increased upon increasing concentration of ALP-labeled IgG. In addition, the recognizable difference in the signal was obtained within 6 minutes (Figure 7).

Consequently, it was confirmed that the prepared “detection gel” with electrophoresis provided rapid and sensitive detection as compared to the method using some detection gel in previous our work (Table 1).

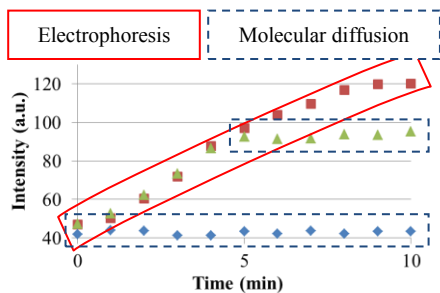


Figure 6: Effect of applying voltage on fluorescence intensity nearby the hydrogel interface.

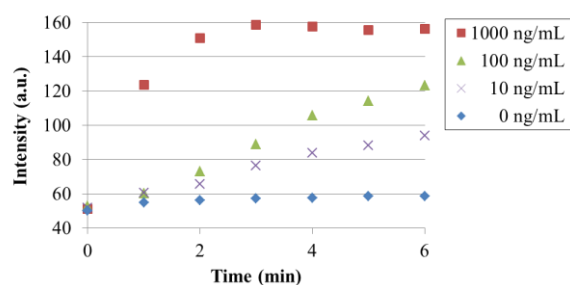


Figure 7: Dependence of the fluorescence intensity on ALP-labeled IgG concentration.

Table 1: Comparison of present work with previous work.

	Previous work [2]	Present work
Analysis time (min)	60	6
Fluorescence intensity change (a.u.)	16 (∇ 190 ng/mL)	70 (∇ 100 ng/mL)

CONCLUSION

It is confirmed that the prepared hydrogel immobilizing the fluorescent substrate provides the rapid and highly sensitive detection of the ALP-labeled IgG by using electrophoretic concentration. During electrophoresis, ALP-labeled IgG was concentrated nearby the interface of hydrogel immobilizing fluorescent substrate (detection gel), which resulted in the acceleration of enzyme reaction. As a result, the detection gel realized a rapid and sensitive detection as compared to the method using some detection gel in previous our work. The proposed electrophoretic immunoassay device will be developed by the combination of the sampling capillary with the capillary containing the separation and detection gels.

ACKNOWLEDGEMENTS

This work is partially supported by the Grant-in-Aid for Young Scientists (B) (No. 24750068) and Grant-in-Aid for Scientific Research (C) (26410159) from Japan Society for the Promotion of Science.

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