SIMPLE AND HIGHLY-SENSITIVE ENZYME ACTIVITY ASSAY BASED ON REAGENT-RELEASE CAPILLARY - ISOELECTRIC FOCUSING (RRC-IEF) TOWARDS THE DEVELOPMENT OF MULTI ANALYTE SENSING MICRO DEVICE CAPABLE OF DETECTING BOTH PROTEINS AND ENZYME ACTIVITIES

Y. Nogawa*, H. Yokoyama, K. Kawamura, T. Endo, and H. Hisamoto
Osaka Prefectutre University, JPN

ABSTRACT
We developed a simple and highly-sensitive enzyme activity assay method based on reagent-release capillary – isoelectric focusing (RRC-IEF), that can be applied for the development of multi analyte sensing device allowing simple and highly sensitive multi assay of both proteins and enzyme activities.

KEYWORDS: capillary-assembled microchip (CAs-CIP), isoelectric focusing, enzyme activity assay

INTRODUCTION
Enzyme activity and protein abundance in cell lysate are the most typical analytical targets in the field of drug screening. However, existing methods need independent analysis for proteins and enzyme activities, respectively, since the analytical procedures are different. It prevents the progress of drug screening analysis from developing more efficient and highly integrated analytical device allowing simultaneous analysis of both proteins and enzyme activities. On the other hand, recently we developed a simple and rapid immunoassay based on RRC-IEF. In this case, fluorescently-labeled antibody was immobilized with carrier ampholyte by physical adsorption, thus, highly-sensitive protein analysis within 10 minutes was achieved by simply introducing sample solution by capillary action and subsequent electric field application [1]. Therefore, if the RRC-IEF is applicable for enzyme activity assay, development of capillary array micro device capable of detecting both enzyme activity and proteins with simple, rapid, and highly sensitive manner in small amount of cell lysate is expected. Here, we focused on the use of fluorescent enzyme substrate based on rhodamine 110 for physical adsorption. This molecule reacts with protease to form fluorescent rhodamine 110. Since rhodamine 110 possess amino and carboxyl groups, this molecule is expected to be concentrated by the principle of IEF. In this work, trypsin activity detection was carried out as a proof of concept.

EXPERIMENTAL
First, we prepared a reagent-release capillary (RRC) by covalently immobilizing poly (dimethylacrylamide) (PDMA) followed by physical adsorption of fluorescent enzyme substrate, carrier ampholyte (CA), and additives (Figure.1). This capillary was assembled on a PDMS chip [2]-[4]. Sample enzyme solution was introduced by capillary action. After addition of anolyte (0.02M H₃PO₄) and catholyte (0.02M NaOH) to reservoirs, high voltage was applied (450V/cm).

Figure 1: General concept of simple and highly-sensitive enzyme activity assay based on reagent release capillary - isoelectric focusing (RRC-IEF)
RESULTS AND DISCUSSION

Conventional IEF of rhodamine 110 revealed that the preconcentration by capillary isoelectric focusing (CIEF) was successful. Detection limit of rhodamine 110 was found to be down to $5 \times 10^{-9}$ M by using conventional fluorescence microscope equipped with CCD camera (Figure 2). Next, RRC was prepared as described above. When a sample trypsin solution was introduced into RRC, fluorescence was observed inside RRC down to 200ng/ml. However, when we carried out RRC-IEF, we could observe two fluorescent spots along the capillary. One of the spots was rhodamine 110, another was monoamide derivative. This enzyme reaction has two steps. First step is producing monoamide. Second is producing rhodamine 110 by creaving two amide bonds. Therefore, both species were concentrated in each pI position. When the spot of rhodamine 110 was used as enzyme activity signal, detectability was found to be down to 100pg/ml. On the other hand, when the spot of monoamide was used, detectability was lowered to 10pg/ml (Figure 3).

Figure 2: Calibration curve for rhodamine 110 by conventional CIEF

Figure 3: Detection of trypsin activity by RRC-IEF

CONCLUSION

In conclusion, RRC-IEF using fluorescent enzyme substrate based on rhodamine 110 was successfully applied for enzyme activity assay. Present method has 5000 times higher sensitivity for detecting trypsin activity than that for conventional assay [5]. Furthermore, simple sample handling by capillary action and extremely small amount of sample made the present method user-friendly and useful for cell lysate analysis, especially for non-specialist users. Present method has a significant potential for developing a micro device capable of detecting both enzyme activity and proteins by simply arraying together with RRCs for immunoassay previously developed by our group [1].

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CONTACT
H. Hisamoto, tel: +81-72-2549285; hisamoto@chem.osakafu-u.ac.jp