

DRY REAGENT PAPER-COUPLED ELECTROPHORESIS MICROCHIP TOWARDS MULTI ASSAY OF BIOLOGICAL COMPONENTS

Yuta Miyahara, Naoki Funauchi, Tatsuro Endo and Hideaki Hisamoto

Osaka Prefecture University, Japan

ABSTRACT

Here we focused on the paper filter impregnating fluorescent substrate reagents and its combination with microchip electrophoresis (MCE). Dry reagent paper-coupled electrophoresis microchip was developed to demonstrate simple and multiple detection of different enzyme activities including alkaline phosphatase (ALP), β -galactosidase (β -Gal) and trypsin (Tryp).

KEYWORDS

Dry reagent chemistry, Enzyme activity, Microchip electrophoresis, Multiple detection

INTRODUCTION

In the drug discovery field, selective detection of various biomolecules from a cell lysate by using the various analytical reagents is routinely carried out. However, requirement of large amounts of sample and reagent, and necessity of individual analyses for each target are still the problems. Therefore development of analytical tool which can rapidly analyze various biomolecules at the same time by using a small amount of sample and reagent are necessary. On the other hand, MCE has been well-known as an analytical device which has advantages of high-speed (seconds order), small volume (nL order) for analysis, and multiple detection capability by high performance separation [1]. We focused on the use of the high performance separation capability of MCE for selectively analyzing multiple components. When the multiple analytical reagents are directly fixed at the sample reservoir, simply dropping the sample solution to the reservoir and subsequent electrophoresis would allow us analysis of multiple component in a very small sample easily and rapidly. In order to demonstrate this concept, dry reagent chemistry that uses paper filter or film immobilizing dried reagents is convenient [2]. In this case, paper filters possessing analytical reagents can be easily prepared by drying up after immersion of paper filters in reagent solutions. Therefore, fixing the paper filter to sample reservoir of electrophoresis microchip would facilitate the preparation of the device. In addition, fixing various combinations of paper filters immobilizing different kinds of analytical reagents is expected to demonstrate rapid and simultaneous analysis of multiple components of interest by electrophoretic separation.

In our research, we focused on the use of paper filter impregnating various analytical reagents together with the electrophoresis microchip (Fig.1). In this case, various paper filters required for analysis are loaded into a sample reservoir. After filling all the channels and other reservoirs by buffer, sample solution is dropped into the sample reservoir loaded with paper filters. After the reaction, application of high voltage would allow us simple and rapid multiple analysis with very small amount of sample and reagent. Here, we combined paper filters impregnating fluorescent substrates of ALP, β -Gal and Tryp, with electrophoresis microchip, and investigated the individual and the multiple detection of ALP, β -Gal and Tryp.

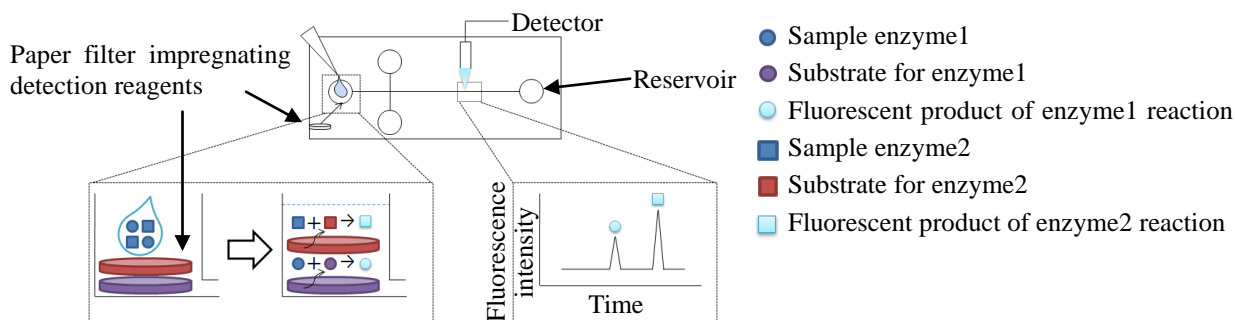


Fig.1 General concept of multi assay using electrophoresis microchip combined with paper filter impregnating detection reagents

EXPERIMENT

Poly (dimethylsioxane) (PDMS)/glass microchip was prepared by conventional soft lithography procedure. Paper filters were prepared by impregnating fluorescent substrates by individually soaking paper filter in each enzyme substrate solution (ALP: fluorescein diphosphate, β -Gal: fluorescein β -D-galactopyranoside, Tryp: Rhodamine 110 diamide derivative) and following drying procedure (Fig.2). Fig.3 shows PDMS/glass microchip with paper filter impregnating reagent. First, we loaded each paper filter impregnating fluorescent substrate into sample reservoir of the chip independently. After filling phosphate buffer solution into microchannel by capillary action, we dropped 50 μ l enzyme solution (ALP, β -Gal and Tryp) into the sample reservoir. After 10 minutes, high voltage was applied

to separate the reaction products. Next, we loaded three paper filters for ALP, β -Gal and Tryp into the sample reservoir and then carried out the same experiment. Detection was performed by SELFOC μ - fluorescence detector.

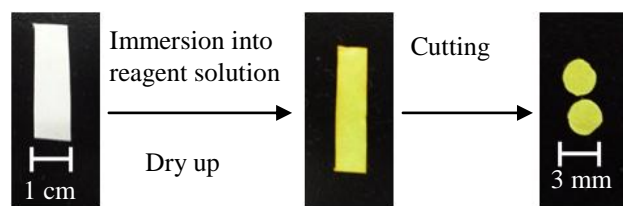


Fig.2 Preparation of paper filter impregnating detection reagents (Reagent: Fluorescein)

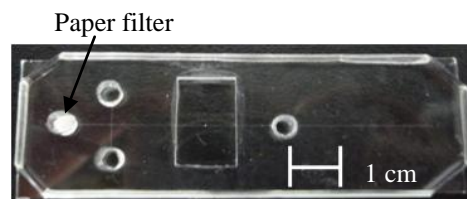


Fig.3 Microchip loaded with paper filter impregnating reagents

RESULTS AND DISCUSSION

Fig.4 shows the dissolving profile of impregnated fluorescein as a test reagent. Approximately 10 minutes were required for complete dissolution of the reagents, thus reaction time was fixed to 10 minutes. Fig.5 shows repeatability of fluorescence intensity and migration time of each paper filter impregnating fluorescein. In between each experimental run, channel surface was rinsed by 1.0% poly(dimethyldiallylammonium chloride) solution to stabilize the electroosmotic flow (EOF). This result shows good repeatability in reagent release and following electrophoresis. For the detection of ALP and β -Gal, two peaks of fluorescein (F) and intermediate (fluorescein monophosphate: FMP or fluorescein mono- β -D-galactopyranoside: FMG), and for the detection of Tryp, peak of Rhodamine 110 (R110) were observed, respectively, depending on the experimental condition. For the simultaneous detection of ALP, β -Gal and Tryp, peaks of FMP, F, FMG and R110 were successfully separated (Fig.6). Based on this result, ALP and β -Gal both of which produce same fluorescein molecule as a final product could be analyzed at the same time because FMP and FMG have different electrophoretic mobility from fluorescein. Therefore, by using the peak of intermediate, simultaneous analysis of different enzyme using fluorescein-based substrates is possible. These results suggested that loading various paper filters impregnating different reagents of interest into a sample reservoir would allow us simple and rapid multiple analysis with very small amount of sample and reagents.

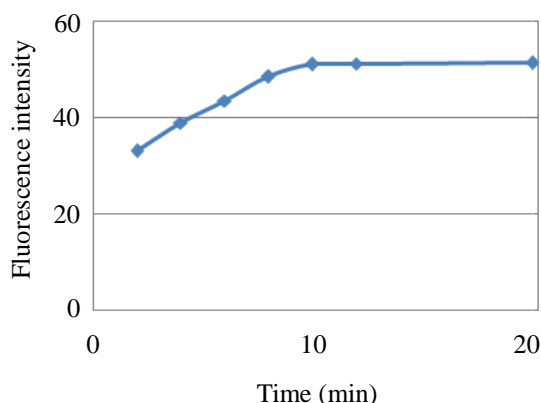


Fig.4 Dissolution profile of impregnated reagents

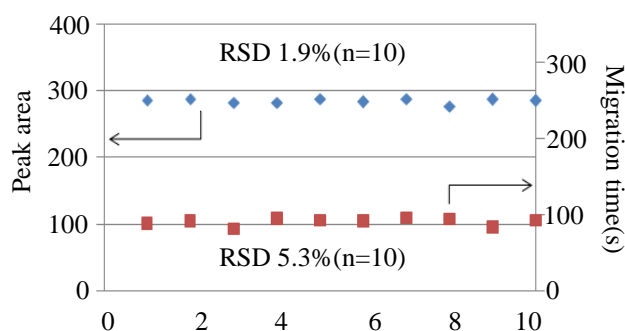


Fig.5 Repeatability of dissolution-electrophoresis experiments using 10 different paper filters impregnating fluorescein

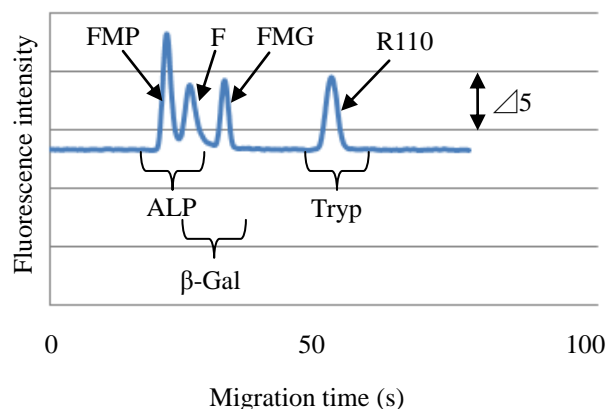


Fig.6 Simultaneous detection of β -Gal, Tryp and ALP

CONCLUSION

In conclusion, we prepared paper filters impregnating fluorescent substrates of β -Gal, Tryp and ALP. These paper filters were combined with electrophoresis microchip, and the individual detection of enzyme activities of β -Gal • Tryp • ALP and the multiple detection of these enzyme activities were successfully performed. Dry reagent paper-coupled electrophoresis microchip presented in this work could allow us simple and rapid multiple analysis with very small amount of sample and reagent.

ACKNOWLEDGEMENTS

This work is partially supported by the Grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Funding Program for Next Generation World-Leading Researchers from the Government of Japan.

REFERENCES

- [1] Verpoorte E, *Microfluidic chips for clinical and forensic analysis*, *Electrophoresis*, 23(5), pp. 677–712, (2002)
- [2] Walter B, *Dry Reagent chemistries in clinical analysis*, *Anal. Chem*, 55(4), pp. 498A-514A, (1983).

CONTACT

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