Extended Abstracts of the 2018 International Conference on Solid State Devices and Materials, Tokyo, 2018, pp639-640

A rapid protein 2D-fingerprinting device using gel-free separation materials and label-free UV detection for proteomics

Toshihiro Sakamoto, Junpei Shinohara, Chiaki Shido, Kenichi Nishiuchi, Hiroyuki Tanaka, Masaaki Hayama and Hiroaki Oka [1]

[1] Sensing Solution Development Center, Engineering Division, Automotive & Industrial Systems Company, Panasonic corp.
1006 Kadoma, Kadoma City, Osaka 571-8506, Japan
Phone: +81-50-3687-0426, E-mail: sakamoto.to@jp.panasonic.com

Abstract

We developed a rapid protein fingerprinting device with two-dimensional fast separation depending on their isoelectric point and polarity using gel-free materials, and with a detection by label-free UV imaging method for proteome analysis. We demonstrated the formation of fingerprint pattern within 30minutes with 7 different proteins using a cellulose and porous-silica membranes.

1. Introduction

Proteomics has a high profile for many applications. The correlation between phenotype and fingerprint pattern obtained by protein is a good tool for understanding the physical condition of body. Some application in proteomics have been expected in areas such as food-safety, skincare and healthcare [1-2]. Typically, the fingerprints are obtained by two-dimensional gel-electrophoresis (2DGE). However, it has a limitation for R&D-use due to requirement of complicate operation and long analysis time. In this paper, we propose ease-to-use and rapid 2D fingerprinting device and demonstrate the concept to open up new applications including for point-of-care testing and diagnosis tools.

2. The basic principle and design of developed device

Device concept

Table 1 shows a comparison between conventional 2DGE and our proposal analysis. To realize rapid and ease-of-use fingerprinting device, two key technologies have been developed: (1) protein separation with gel-free materials, and (2) protein detection with UV imaging method. In our method, 2D separation by iso-electric-focusing (IEF) and polarity in protein is performed in a short time using membrane materials and their miniaturization whereas separation in 2DGE by IEF and molecular weight using poly-acrylamide-gel taking more than 120 min.

Gel-free 2D protein separation device

Figure 1 shows a concept drawing of our 2D fingerprinting device. As a 1st axis material, a cellulose acetate (CA) membrane is utilized. Electrophoresis is conducted after dipping proteins solution to the membrane mixed with carrier ampholyte which forms pH gradient from 3.5 to 9.5 without rehydration. Proteins are separated depending on their charge. As a 2nd axis material, an octadecylsilyl-porous-silica thin layer on glass substrate (thin layer chromatography: TLC) is used. The lower area of the TLC is attached to the CA membrane in which proteins have been separated and solvent is dipped at the bottom edge. Then, the proteins are transferred from the membrane to the TLC together with the solvent and separated to upper area by capillary force through hydrophobic interaction with the silica-surface. Finally, 2D fingerprint pattern is obtained. Each length of 1st and 2nd axis is designed to be 20 and 24 mm respectively. These length are about one-fifth of conventional 2DGE, which enables lower-voltage operation and higher speed separation.

Label-free protein detection

Figure 2 is a concept drawing of our detection method without any staining by UV imaging technology to detect native-fluorescence in protein. It is known that tryptophan (Trp) which is aromatic-amino-acid in protein excited by 280nm UV-Light and emits native-fluorescence around 360nm. In our system, high power UV-LED (λ_c=280 nm, power density: 1.5 mW/cm^2) is adopted as a UV excitation light, and a camera using 16 mega-pixel MOS image-sensor (MN34230AL: Panasonic) with peltier forced-cooling (-15ºC) and fused-silica based lens and band-pass-filter (λ_{pass}=340~420 nm) is used as an imager.

3. Results

Label-free detection and detection limit determination

Two different proteins, lysozyme (Lys) and bovine serum albumin (BSA) solution changed the concentration from 1 μg/μL to 4 ng/μL, were spotted on TLC with 1μL, and the image was captured with 1 second LED exposure as shown in Fig. 3. The contents of Trp in Lys and BSA are 3.4 and 0.4 wt% respectively. We analyzed the image (Fig. 3a) and plotted average luminance vs protein concentration for each spot (Fig. 3b). The detection limit was determined as 5ng for BSA and 1ng for Lys respectively by comparing the average between the fluorescence and +3σ of background. The sensitivity was ~50 times higher than that of conventional labeling method such as CBB (Coomassie Brilliant Blue).

Iso-electric focusing on CA membrane for 1st axis

Electrophoresis in CA membrane was performed using 4 different colored proteins to evaluate IEF property. Several bands of proteins were focused within 15 minutes under applying ~400 V to platinum electrode at both ends. Figure 4a is a photograph of the membrane after IEF and a graph plotted by moving distance vs known isoelectric-point (PI) for each protein [3]. It was confirmed that the proteins were quickly moved and the moving distance was almost laniary changed (R=0.99) with respect to their pI in the range of pH3~10.

Polarity separation on TLC for 2nd axis

Chromatogrphy on TLC was conducted using proteins having 9 different polarity to optimize separation in 2nd axis. Figure 4b is an image on TLC and a graph plotted by moving distance vs polarity which we have measured by conventional HPLC (high performance liquid chromatography) for each protein after eluted for 15 minutes using optimized solvent; 2-propanole : acetic acid : water = 40:5:55. It was confirmed that the proteins were quickly moved and the distance was almost laniary changed (R=0.86) with respect to their polarity.

Demonstration of 2D-fingerprint formation and detection

Finally, gel-free 2D-separation and label-free detection for 7 proteins which have different charge (pI) and polarity were demonstrated. Figure 5 shows captured image after 1st and 2nd separation and the names of proteins are indicated. The 7 proteins were separated, and 2D pattern was formed depending on their charge and polarity (Fig.5a). The 2D separation including 1st (IEF) and 2nd (TLC) separation, and the detection were carried out in 30 minutes. Whereas the conventional method takes 135 min for total analysis (Fig.5b). Thus, our device concept was successfully demonstrated.

4. Conclusions

The rapid protein fingerprinting device with 2D fast separation depending on their charge and polarity using gel-free separation materials and the pattern detection by label-free UV imaging were proposed. Formation of 2D fingerprint pattern within 30 minutes using 7 different proteins with CA cellulose membrane electrophoresis and TLC was demonstrated.

References

[1] N. Hayashi, Expected materials for the future (Japanese). **11** (2011) 42.

[2] Y. Sawada et al, *Proceeding of European Conference on Artificial Intelligence,* (2016) 1586.

[3] Bio-Rad Laboratories, Inc, Product web-site: http://www.bio-rad.com/ja-jp/sku/1610310-ief-standards?ID=1610310

[4] Y. Tani et al, J. Proteomics Bioinform, **7** (5) (2014) 108.

Table.1 Comparison between conventional and proposal analysis for proteomics using 2D separation technology

	Conventional	**Proposal**
Method	2DGE	**Gel-free 2D fingerprint**
Separation	1st : Charge / 2nd : MW	**1st : Charge / 2nd : Polarity**
Materials	Polyacrylamide-gel	**Gel-free materials**
Detection	Silver / CBB stain	**UV native fluorescence**
Analysis time	>120 min	< 30 min

MW: Molecular Weight

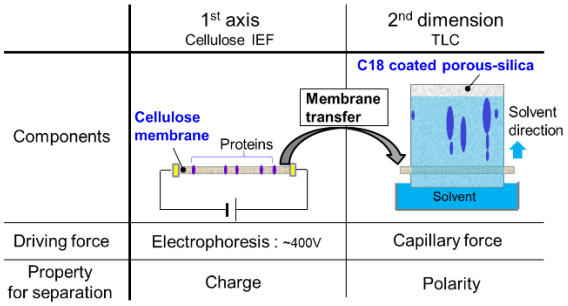

Fig. 1 Concept schematic of the gel-free 2D finger printing device

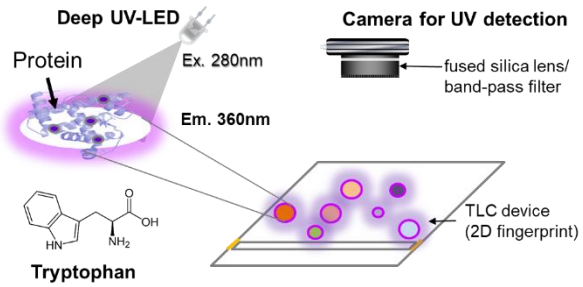

Fig. 2 Concept schematic of label-free protein detection using UV-sensitive camera

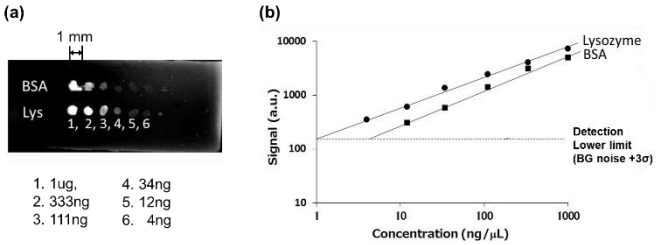

Fig. 3 Characterization of detection limit for the label-free UV detection (a) observed image (b) plots to determine detection limit

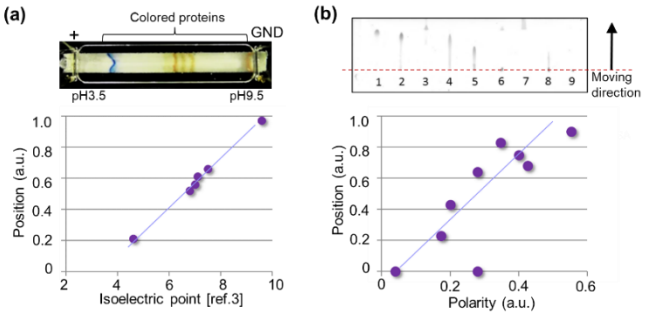

Fig. 4 Characterization of protein separation in 1st and 2nd axis. (a) IEF (b) TLC

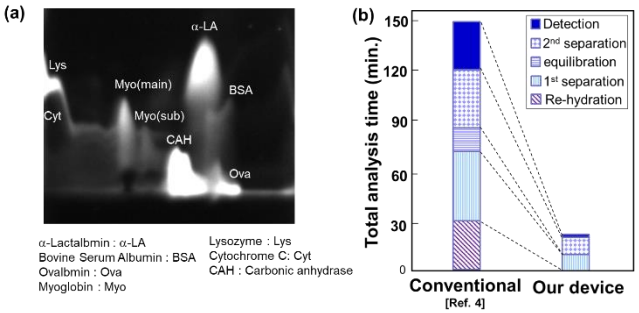

Fig. 5 Demonstration of 2D fingerprint formation using conventional 7 different proteins:
(a) captured image by label-free detection after 2D separation.
(b) comparison of analysis time between conventional and proposed method.