On-Chip IEF Peak Manipulation for 2D Protein Separation and MS Coupling

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Abstract

Multidimensional separation techniques, such as protein two-dimensional (2D) gel electrophoresis, are generally required for analyzing complex protein samples such as cell extract or serum. One of the key problems in realizing microfluidic multidimensional analysis systems is the interference between heterogeneous separation columns and buffer environments. We developed an on-chip protein peak manipulation method to integrate two different separation technologies with different buffer requirements, such as isoelectric focusing (IEF) and capillary gel electrophoresis (CGE) or capillary electrophoresis (CE), by the microfluidic valve control process. This method could become a generic strategy for various integrations between different microfluidic components such as IEF, CE, and mass spectrometry (MS).

Keywords: 2D protein separation, isoelectric focusing, microfluidic, valve control

1. Introduction

To analyze complex protein samples such as cell extract or serum where more than thousands of proteins could be present, multidimensional separation techniques, such as protein 2D gel electrophoresis, should be used. While it is highly desirable to implement multidimensional protein electrophoresis on a microchip, integration of two different separation techniques on a single microfluidic chip has been challenging. So far, microfluidic 2D separation has been achieved without active sample peak control or isolation between different separation columns.[1, 2]

In this work, we developed a new method for integrating two heterogeneous separation components with potentially incompatible buffer requirements. This new strategy was demonstrated by integrating IEF, a charge based separation, and CGE in a microfluidic system. First, we established a pH gradient from pH 3 to 10 for IEF, as shown in Figure 1. Then, the first dimension IEF-concentrated peaks were transferred by the operation of a set of microfluidic valves, made by the multilayer polydimethylsiloxane (PDMS) stacking process. The microfluidic valves in this device prevent intermixing between the two separation buffers (ampholyte and CGE gel buffer). We believe that this new strategy could be used in the microfluidic systems integration, where heterogeneous components should be connected without compromising the operations of one another.

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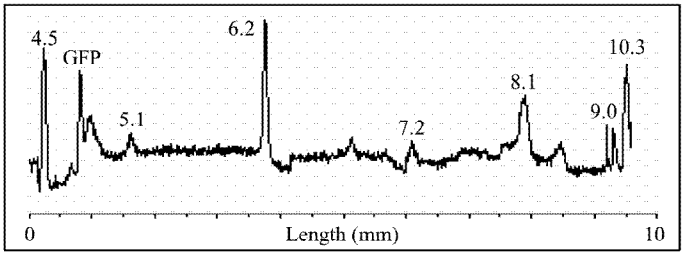

Figure 1. Intensity plot of GFP and fluorescent IEF-markers focused by their pI values in 1 cm by 100 μm by 20 μm PDMS microfluidic channel.

2. Experimental

To isolate and control the two separation media and sample peaks, microfluidic devices with control valves were fabricated and tested. Multilayer microfluidic valve designs, used in this work, have been reported previously[3]. The schematic diagram of the device is shown in Figure 2-(a).

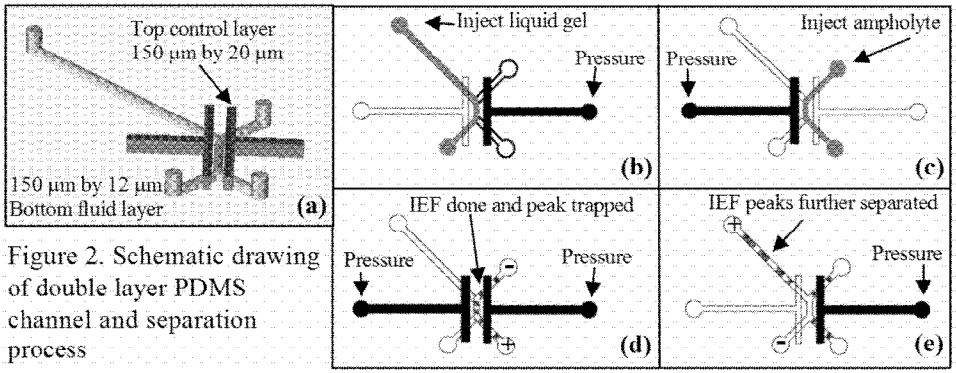

Figure 2. Schematic drawing of double layer PDMS channel and separation process

Two sets of valves (top control layer in Figure 2-(a)) were designed to isolate different separation media and to transfer IEF peaks. First, the right valve control line was pressured to close the fluid channel below it, and liquid was injected without contaminating (Figure 2-(b)). Then, left valve was pressurized to isolate liquid gel. After closing left valves, right valves were released for the injection of ampholyte and sample mixture (Figure 2-(c)). The IEF separation was achieved by applying an electric potential between anode and cathode reservoirs. After the IEF of proteins was established, one can isolate any pI region of interest from the other peaks by closing the right valves. When the left valves are open, isolated protein peaks focused within this region will reacquire charges and be sent to the second dimension separation channel by electrophoresis (Figure 2-(d)). Since protein IEF is maintained until the transfer of peaks, the resolution obtained from the IEF separation is maintained, and the dispersion of peaks caused by

coupling two separation techniques was minimized. During this migration, proteins will be separated based on the separation column chosen for the second dimension (charge-to-size ratio for CE column, for example)

It is essential to prevent protein adsorption problem and decrease electroosmotic flow (EOF) in microfluidic protein separation devices. In our experiment, we chose polyacrylamide coating to prevent protein adsorption. Besides, to grow acryamide polymer on PDMS surface, we first coated PDMS channel with 3-(Trimethoxysilyl) propyl methacrylate. Then, polyacrylamide solution was mixed with tetramethyl ethylene diamine (TEMED) and ammonium persulfate (APS) and introduced into the microfluidic channel for polymerization chain reaction. After thirty minutes reaction time, 0.5% methylcellose solution was used as pre-treatment coating material to interfere with protein-wall ion-exchange mechanism.

3. Results and discussion

In this experiment, thermoelectrically cooled CCD camera (SensiCam, Cooke, Co.) was used for fluorescence imaging. One naturally fluorescent GFP (aprox. pI 5.6, MW 26k) and three fluorescein or Alexa Fluor 488 (Molecular Probes) labelled proteins were used: ovalbumin (aprox. pI 5.1, MW 45k), low density lipoprotein (aprox. pI 5.11, MW 179k), and trypsin inhibitor (aprox. pI 4.6, MW 20k) were used as pI and MW markers. The fluorecsien or Alexa Fluor dyes will replace the lysine residues of proteins[4]. Because of the possibility of nonuniform labelling, labelled proteins generally yield broad IEF bands (Figure 3 (a)). This problem could be resolved by detecting proteins after the second dimension separation, possibly using postcolumn labelling technique[5].

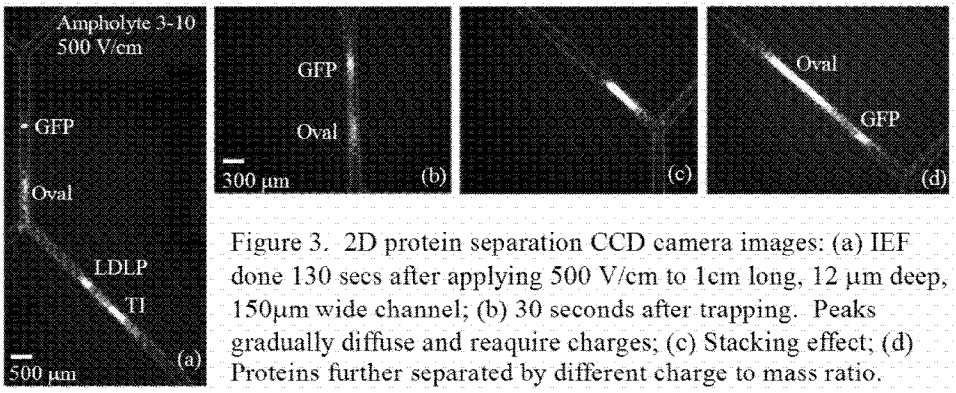

Figure 3. 2D protein separation CCD camera images: (a) IEF done 130 secs after applying 500 V/cm to 1cm long, 12 μm deep, 150μm wide channel; (b) 30 seconds after trapping. Peaks gradually diffuse and reaquire charges; (c) Stacking effect; (d) Proteins further separated by different charge to mass ratio.

After the focusing, we trap target proteins, open left valves to mix ampholyte with CE separation media (Figure 3(b)). Then, 200 V/cm electrical fields were applied to initiate second dimension GCE. In Figure 3(c), the stacking of protein bands occurs when low

conductivity ampholyte solution is being mixed with 0.4M high-conductivity Tris-Cl CE buffer. As a result, protein mixtures in certain narrow pI range were separated again based on different charge to mass ratio (Figure 3(d)). The whole separation process was finished in 10 minutes. The intensity charts are also shown in Figure 4.

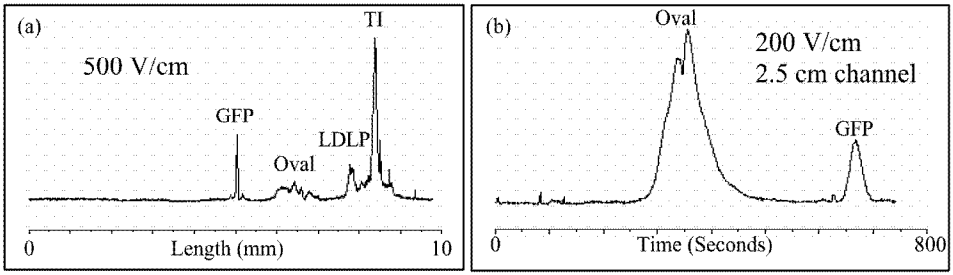

Figure 4. 2D separation intensity chart: (a) whole column intensity chart after IEF, 3-10 ampholyte; (b) Second dimension CGE (fixed point detection).

4. Conclusions

Using microfluidic valves, we successfully demonstrated the ability to couple chip base capillary IEF and CGE together. This strategy will be useful in realizing the microfluidic 2D protein separation device, coupled with appropriate labelling and detection techniques. Coupling of the device with the MS will be studied in the future too.

Acknowledgements

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