MICROCHIP ELECTROPHORESIS OF OLIGOSACCHARIDES IN 'SINGLE' STRAIGHT CHANNEL

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ABSTRACT

The applicability of an online sample preconcentration technique, large volume sample stacking with electroosmotic flow pump (LVSEP), to microchip zone electrophoresis (MCZE) for the analysis of oligosaccharides was investigated. Since the sample stacking and separation proceeded successively without polarity switching in LVSEP, a single 'straight' channel microchip could be employed. To suppress the sample adsorption onto the channel surface in the MCZE analysis of oligosaccharides, the straight microchannel was modified with poly(vinyl alcohol) (PVA). Although it is well-known that the PVA coating can suppress the electroosmotic flow (EOF), an enhanced EOF occurred temporarily in a low ionic strength sample solution, which worked as the driving force to remove the sample matrix in LVSEP. To evaluate the analytical performance of LVSEP–MCZE, oligosaccharides were analyzed in the PVA-coated straight channel. As a result, both glucose ladder and oligosaccharides released from bovine ribonuclease B were well enriched and separated with up to a 2900-fold sensitivity enhancement compared with a conventional MCZE analysis. By applying the LVSEP technique to MCZE, a voltage program for fluidic control could be simplified from four channels for two steps to two channels for one step.

KEYWORDS: Microchip Electrophoresis, Single Channel Analysis, Online Sample Preconcentration, Oligosaccharide

INTRODUCTION

Recently, microchip zone electrophoresis (MCZE) has attracted much attention for the rapid analysis of oligosaccharides, which play very important roles in the living body such as cell recognition, cell communication, and cell proliferation. However, the poor sensitivity of MCZE with the conventional pinched injection (PI) technique, have prevented the practical use. To overcome this issue, several online preconcentration techniques have been applied [1]. However, such approaches often required a complicated voltage program for the fluidic control and reduced the effective separation length due to the large-volume sample injection, resulting in poor reproducibility, inconvenient procedure, and low resolution. Hence, we focused on an online preconcentration technique developed in capillary electrophoresis, large-volume sample stacking with electroosmotic flow (EOF) pump (LVSEP) [2], which permits both the separation and the stacking of analytes injected throughout the capillary without polarity switching. Therefore, a simple combination of single 'straight' channel microchip with a constant voltage application is expected to provide both the high sensitivity and good resolution. In this paper, we investigated the mechanism of LVSEP–MCZE using a microchannel coated with poly(vinyl alcohol) (PVA), and the analytical performance of LVSEP–MCZE was evaluated in the analysis of oligosaccharides.

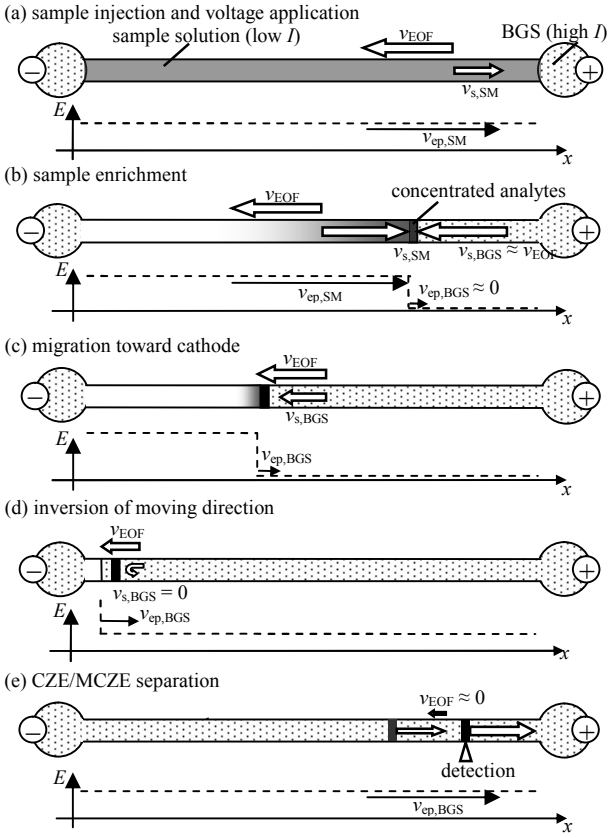

Fig. 1. Concept of LVSEP in the PVA-coated microchannel. v_{ep}, v_{EOF} and v_s denote the electrophoretic velocity of the analyte, the EOF velocity, and the apparent velocity of the analyte, respectively.

THEORY

In LVSEP–MCE, the PVA-coated straight channel is filled with a low ionic strength (*I*) solution containing anionic analytes. After the inlet and outlet reservoirs are filled with a high *I* background solution (BGS), a constant voltage is applied (Figure 1a). The analytes are concentrated around the anodic-side sample/BGS boundary according to the difference in the electric field (*E*) strength between the two zones (field-amplified sample stacking, Figure 1b). At the same time, a temporarily enhanced EOF occurs due to the low *I* sample solution, in spite of the EOF-suppressed channel surface. The enhanced EOF transports the focused sample toward the cathode, introduce the BGS from the anodic reservoir into the channel, and remove the sample matrix from the cathodic channel end (Figure 1c). After the low *I* solution without the analytes is removed out of the channel, the EOF is suppressed again due to the high *I* BGS, and *E* in the BGS increases drastically (Figure 1d). The concentrated analytes then start moving toward the anode and are separated by microchip zone electrophoresis (MCZE) (Figure 1e). It should be noted that almost the same separation

length as that in conventional MCZE is available in LVSEP–MCZE because the concentrated analytes are transported to near cathodic channel end before the MCZE separation.

EXPERIMENTAL

A poly(dimethylsiloxane) (PDMS) microchip device was fabricated by the conventional soft lithography technique. The straight channel microchip had a single straight channel (50 μm width × 50 μm depth) with a total separation channel length of 80 or 40 mm. The cross-channel microchip consisted of three 5 mm long channels and a 40 mm long separation channel (50 μm width × 50 μm depth). The surfaces of PDMS channel substrate and a lid plate were activated by O_2 plasma to be bonded with each other. Immediately after bonding, 2% PVA (M_w = 80 000, 88% hydrolyzed) was introduced into the microchannel and then left for 15 min. The solution was removed , and the microchip was heated at 110 °C for 15 min. The injection of the PVA solution and the heating of the microchip were repeated three times, where the temperature at the third heating was 140 °C.

In LVSEP–MCZE, 40 and 80 mm long channels were employed in fluorescence imaging of the preconcentration process and the separation of oligosaccharides derivatized with 8-aminopyrene-1,3,6-trisulfonic acid, respectively. A sample solution was introduced into the entire channel by using a syringe manually. The two reservoirs were filled with 10 μL of 10 or 25 mM HEPES buffer (pH 8.0). The electric field strength of 500 V/cm was applied through two platinum electrodes immersed in the two reservoirs. In fluorescence imaging, detection was performed by Hg lamp and CCD camera as the light source and detector, respectively. In the separation of oligosaccharides, detection was performed at 5 mm from the anodic channel end by laser-induced fluorescence (excitation/emission wavelengths of 488/520 nm). In the conventional PI–MCZE, the cross channel PDMS microchip with a total separation length of 40 mm was used. There were four reservoirs at the end of each channel. Three reservoirs connected to the loading channel were for BGS (B), sample (S), and sample waste (SW), whereas that to the separation channel was for BGS waste (BW). In the first step of the PI, the applied voltages were 1.5, 1.5 and 2.5 kV at the S, B and SW, respectively, while the BW was grounded. After 30 s, the voltage was switched to the separation mode with 1.0, 0.0, 1.0 and 2.5 kV for the S, B, SW and BW, respectively. Detection was carried out at the distance of 5 mm from the BW reservoir.

RESULTS AND DISCUSSION

To verify the mechanism of LVSEP–MCZE, the moving preconcentration boundary was traced from the anodic channel end as shown in Figure 2. After applying the voltage, the analyte was stacked from the anodic side. The concentrated analyte moved toward the cathode by the enhanced EOF (Figures 2a and 2b). The observed velocity of the stacked analytes remained almost constant until reaching near the cathodic end of the channel. When the analytes reached the channel position of 3 ~ 4 mm, the analytes decelerated drastically (Figures 2c and 2d), and then the moving direction of the concentrated analyte was inverted to the anode at the position of 2.3 mm (Figure 2e). After the turn, the analyte migrated with almost the same velocity until being removed out from the anodic channel end (Figure 2f). Such migration behavior was observed at the HEPES concentration ranging from 0 to 0.2 mM in the sample solution. The result agreed well with the U-turn model of LVSEP described above. The observed inversion position was more than 92% of the whole channel length from the anodic end. This suggested almost all the channel length could be used as an effective separation length, resulting in no loss of resolution.

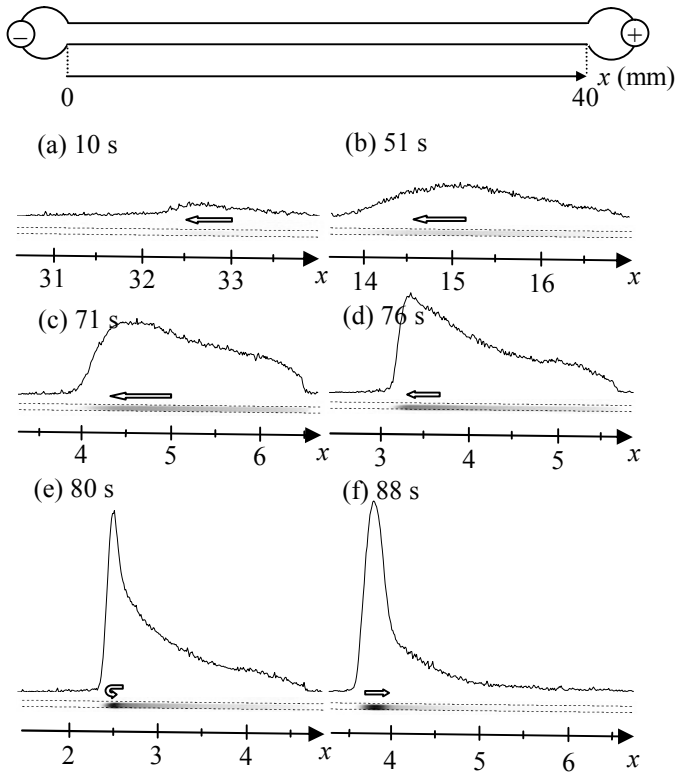

Fig. 2. Fluorescence images and intensity profiles of fluorescein concentrated by LVSEP in a 40 mm-long straight channel. The abscissa axis represents the distance from the anodic channel end. The length of the arrow is proportional to v_s.

To evaluate the analytical performance, the LVSEP–MCZE and conventional PI–MCZE analyses of oligosaccharides were performed on the 80 mm-long straight channel microchip and a cross channel microchip with the 40 mm-long separation channel, respectively. Linear glucose ladder and dendritic sugar chains obtained from bovine ribonuclease B were used as the model and real samples, respectively. When a bare PDMS microchip was employed, the oligosaccharides were seriously adsorbed onto the channel surface and could not be separated in the PI–MCZE analysis. Thus, the PVA-coated microchip was applied to suppress both the EOF and the sample adsorption. In the PI–MCZE analysis of the glucose ladder, G1 ~ G10 were well separated but longer oligomers than G10 could not be detected as shown in Figure 4a. On the

other hand, G1 ~ G20 were well concentrated and separated in the LVSEP–MCZE analysis (Figure 4b). It should be emphasized that the effect of the anionic electrolytes in the BGS on the preconcentration efficiency was not considerable but the conductivity of the BGS was significant. Among several buffer components (phosphate, acetate, HEPES, MES and MOPS), we found that 25 mM HEPES was optimal in the LVSEP–MCZE analysis of oligosaccharides. The sensitivity enhancement factor (SEF), which was calculated by comparing the peak height obtained in the LVSEP condition with that in the conventional PI–MCZE taking into account the dilution factor regardless of the injection volume of the sample solution, was estimated to be 930 ~ 2900. In the analysis of the real sample, only M5 and M6 were detected in the PI–MCZE analysis (Figure 5a). On the other hand, M5 ~ M9 could be detected and resolved in LVSEP–MCZE with the SEFs ranging from 1900 to 2200 (Figure 5b). As far as we know, such high SEF values over 1000 have not been reported in the previous papers on the preconcentration of carbohydrates in CE. The run-to-run repeatabilities of the migration time and peak height were good with the relative standard deviations of 1.1% and 7.2%, respectively, which were better than those in normal MCZE. These results indicate that LVSEP on the PVA-coated microchannel is one of the ideal preconcentration and separation techniques for analyzing biomolecules such as proteins and nucleic acids.

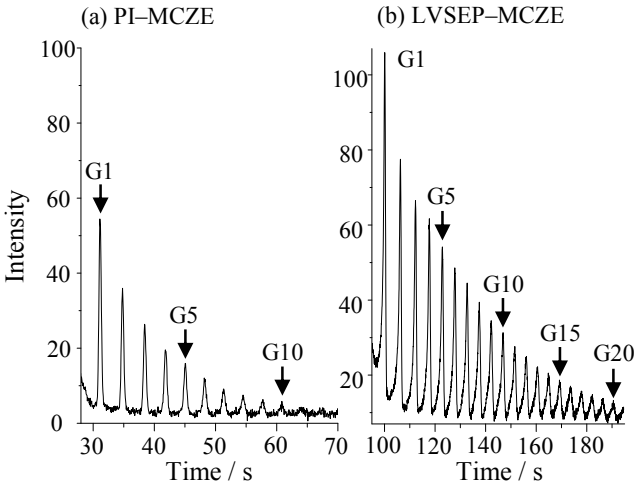

Fig. 3. Electropherograms of glucose ladder obtained with (a) conventional PI–MCZE and (b) LVSEP–MCZE. Concentration of glucose ladder; (a) 160 ppb, (b) 320 ppt.

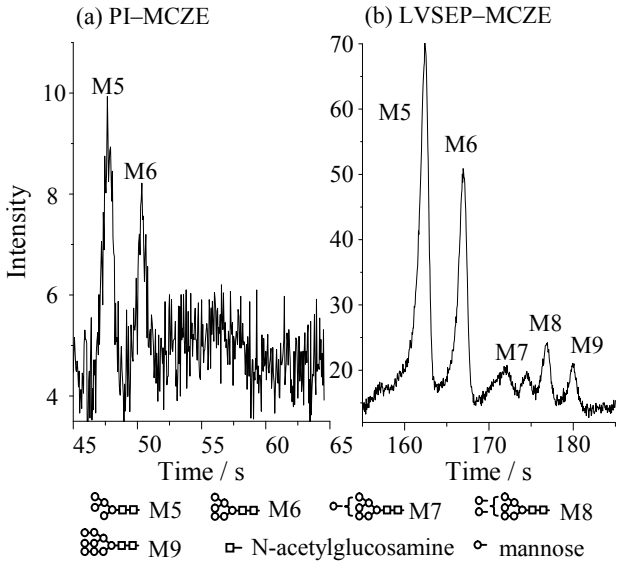

Fig. 4. (a) Conventional PI–MCZE and (b) LVSEP–MCZE analyses of oligosaccharides released from bovine ribonuclease B. Sample concentration in LVSEP–MCZE was 200-fold lower than that in PI–MCZE.

CONCLUSION

The mechanism of LVSEP on the EOF-suppressed straight channel microchip was investigated on the basis of the theoretical model and fluorescence imaging. In the LVSEP–MCZE analysis of oligosaccharides, both sample preconcentration and separation were achieved with up to a 2900-fold increase in the sensitivity compared with the conventional PI–MCZE analysis. The straight channel geometry and the simplification of the voltage program for fluidic control should be effective for high throughput analysis. High analytical performance of the LVSEP–MCZE technique will contribute to more practical analyses not only for oligosaccharides but also anionic biomolecules, e.g., DNA, peptides, proteins, organic acids, metabolites, and so on.

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