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Hybrid Dynamic Coating with *n*-Dodecyl β-D-Maltoside and Methyl Cellulose for High-Performance Carbohydrate Analysis on Poly(methyl methacrylate) Chips

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Hybrid dynamic coating using *n*-dodecyl β-D-maltoside (DDM) and methyl cellulose (MC) has been developed for suppression of analyte adsorption and electroosmotic flow (EOF) in a poly(methyl methacrylate) (PMMA) channel. The adsorption of APTS-labeled sugars in a PMMA channel was obviously suppressed with DDM dynamic coating; however, EOF was reduced only by a factor of ∼25%, resulting in irreproducible separations. In contrast, both analyte adsorption and EOF in a PMMA channel were efficiently minimized with MC coating; however, concentrated MC above 0.3% was required to achieve high-performance separations, which greatly increased viscosity of the solution and caused difficulties during buffer loading and rinsing. In addition, *n*-dodecyltrimethylammonium chloride did not show observable effects on reducing analyte adsorption, although it has the same hydrophobic alkyl chain as DDM. These results strongly indicated that the polysaccharide moiety of surface modifiers has a specific affinity to surface charges and is crucial to achieving efficient and stable dynamic coating on the PMMA surface. Hybrid dynamic coating with 0.25% DDM and 0.03% MC was found to minimize both analyte adsorption and EOF in a PMMA channel to a negligible level, while still keeping a low viscosity of the solution. High-speed and high-throughput profiling of the N-linked glycans derived from α1-acid glycoprotein, fetuin, and ribonuclease B was demonstrated in both single-channel and 10-channel PMMA chips using DDM−MC hybrid coating. We propose that DDM−MC hybrid coating might be a general method for suppressing analyte adsorption and EOF in polymer MCE devices. The current MCE-based method might be a promising alternative for high-throughput screening of carbohydrate alterations in glycoproteins.

Micro total analysis systems (μ-TAS) have attracted much attention during the last two decades and have provided fast and parallel tools for performing a wide variety of chemical and biological assays with reduced cost and increased reliability.[1−3] Microchip capillary electrophoresis (MCE) devices, as one of the earliest successful demonstrations of a μ-TAS concept, are finding numerous applications in various emerging fields of life sciences, especially in the fields of genomics and proteomics.[4−9] Some potential applications in medical diagnostics and drug discovery may require disposable devices to avoid any risk of cross-contamination.[10−12] To address these growing needs, MCE devices have to be fabricated with high reproducibility in mass and at low cost. Although glass-based materials are well-used in fabrication of early MCE devices due to their excellent optical properties, high chemical and mechanical stability, and well-established

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surface chemistry, MCE devices on glass-based substrates must be serially produced using hazardous chemicals. In addition, the cost associated with fabrication processes including standard photolithography, wet etching, and thermal bonding is too high to produce disposable devices on a cost-effective basis. Thus, much effort has recently been devoted to development of polymer MCE devices. A variety of polymer materials, such as poly(dimethylsiloxane) (PDMS),[13] poly(methyl methacrylate) (PMMA),[14] poly(ethylene terephthalate),[15] and polycarbonate,[16] have been used in mass fabrication of MCE devices, showing tremendous potential in reaching disposable MCE devices.

Polymer materials provide advantages over glass materials in the following points: low cost, ease of fabrication, and diversity of mechanical and chemical properties as well as a wide choice in fabrication techniques, such as injection molding,[17] hot embossing,[18] casting or imprinting,[13,19] laser ablation, and plasma etching[20] for mass production of MCE devices.[10,11] However, the surfaces of many polymer materials, such as PMMA and PDMS, are quite hydrophobic and not compatible with aqueous separation media, thereby causing poor separation performance due to serious analyte adsorption on the channel surface. Thus, surface modification is a critical issue for polymer MCE devices in electrophoresis applications.[12,21] Various approaches for either chemical or physical modification of polymer surfaces[22−25] are reported to manipulate electroosmotic flow (EOF) and minimize analyte−wall interactions in polymer MCE devices. Chemical treatments are often regarded as the most effective way to modify surface chemistry and electrokinetic properties of polymer devices, especially in the cases of affinity CE, on-line MS coupling and integrated chemical reactions such as PCR where surface modifiers in separation buffers for dynamic coating may be detrimental. However, chemical surface modification is generally a time-consuming and labor-intensive process and might accordingly be less attractive for disposable MCE devices. On the other hand, dynamic coating represents a simple and convenient surface modification technique, in which surface modifiers, such as polymers or surfactants dissolved in running buffers, physically adsorb on microchannel walls to form a coating layer for regulation of EOF and suppression of analyte adsorption. In our previous studies,[24,25] we demonstrated effectiveness of cellulose polymers as dynamic coating additives for suppression of analyte adsorption and EOF in a PMMA channel. However, difficulties are often experienced during buffer loading and rinsing because the presence of cellulose polymers greatly increases viscosity of the buffer solution. Indeed, increased viscosity of a running buffer is a serious problem for a microfabricated capillary array electrophoresis (μ-CAE) chip.[26,27] Recently, Huang et al.[28] reported that *n*-dodecyl β-D-maltoside (DDM) might be an efficient coating additive for reducing nonspecific adsorption of proteins on the PDMS surface. However, separation applications in detail were not performed.

In the current work, we developed a hybrid dynamic coating method including DDM and methyl cellulose (MC) for suppression of both analyte adsorption and EOF in a PMMA channel. Hybrid dynamic coating with 0.25% DDM and 0.03% MC allows suppression of both analyte adsorption and EOF in a PMMA channel to a negligible level and a comparative low-viscosity of the buffer solution that is easy to handle in PMMA MCE devices. High-speed and high-throughput separation of the N-linked complex glycans released from α1-acid glycoprotein (AGP), fetuin, and ribonuclease B was performed in both single-channel and 10-channel PMMA chips with an effective separation channel of 30 mm using a 20 mM phosphate buffer containing 0.25% DDM and 0.03% MC.

EXPERIMENTAL SECTION

Reagents and Buffer Solution. Bovine ribonuclease B, fetuin from fetal calf serum, human AGP, maltotriose (G_3), DDM, and MC (viscosity of 2% aqueous solution at 20 °C, 4000 cP) were purchased from Sigma Chemical Co. (St. Louis, MO). Peptide N-glycosidase (PNGase F, EC.3.6.1.52 recombinant) with 5× reaction buffer (250 mM sodium phosphate, pH 7.5), denaturation solution (2% SDS, 1 M β-mercaptoethanol), and Triton X-100 (15% solution) were obtained from ProZyme (San Leandro, CA). Highly purified 8-aminopyrene-1,3,6-trisulfonate (APTS) was acquired from Molecular Probes (Eugene, OR). Dextrin 15 was purchased from Fluka (Buchs, Germany). Sodium cyanoborohydride, tetrahydrofuran, *n*-dodecyltrimethylammonium chloride (DTAC), and all other chemicals were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan).

Running buffers containing MC, DDM, or DTAC were prepared by adding the appropriate amount of additives to a 20 mM phosphate buffer (pH 6.93) and stirring slowly until the solution appeared homogeneous and transparent. Double-deionized water was used for the preparation of buffers and samples.

Sample Preparation and Derivatization. Glycans in glycoproteins was released using the methods described previously.[25,29] Typically, 200 μg of glycoprotein (AGP, fetuin, or ribonuclease B) was mixed with 10 μL of 5× reaction buffer, 2.5 μL of denaturation solution, and 35 μL of water in a 500-μL screw-capped microcentrifuge tube. The solution was heated at 100 °C for 5 min to denature the proteins. After cooling, 2.5 μL of Triton X-100 and 2 μL of PNGase F solution were added to the solution. After

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incubation of 24 h at 37 °C, the mixture was heated at 100 °C for 3 min to terminate the reaction. The mixture was mixed with 150 μL of cold ethanol to precipitate protein, immediately followed by centrifugation at 14 000 rpm at 4 °C for 3 min. The supernatant was collected in a 500-μL screw-capped microcentrifuge tube and evaporated to dryness by a centrifugal vacuum evaporator at room temperature. The residue was dissolved in 50 μL of 2 M aqueous acetic acid and kept at 80 °C for 2 h to remove sialic acids from glycans. After lyophilization, the residue or 2-nmol standard carbohydrates was mixed with 5 μL of 0.1 M APTS in 15% acetic acid solution and 10 μL of 0.5 M $NaBH_3CN$ in tetrahydrofuran. The mixture was kept at 55 °C for 1.5 h, diluted with water to 200 μL, and stored at − 20 °C. An aliquot of the above solution was diluted to the desired concentrations with double-deionized water prior to analysis.

PMMA Microchips. A PMMA i-chip was purchased from Hitachi Chemical (Tokyo, Japan), microfabricated on a PMMA substrate of 85 mm × 50 mm with three simple cross-channels of 100-μm width and 30-μm depth. The distances from the channel crossing point to the sample, sample waste, buffer, and buffer waste reservoirs were 5.25, 5.25, 5.75, and 37.5 mm, respectively.[24,25]

The fabrication procedure for 10-channel μ-CAE PMMA chips has been described previously.[26,27] Briefly, the μ-CAE chips containing 10 independent separation channels with a rectangular cross section of 50-μm width, 50-μm depth, and 100-μm pitch, 31 reservoirs of 2.0 mm in diameter, and a sacrificial channel network of 50-μm depth and 100-μm width were fabricated simultaneously in a 27 mm × 65 mm PMMA substrate by injection molding. The bonding of μ-CAE PMMA chips was achieved manually by lamination of a pressure-sensitive sealing sheet (3M advanced polyolefin microplate sealing tape, 3M Co.) at room temperature.

Apparatus and Electrophoresis. The MCE experiments were performed on an SV1100 Microchip CE system (Hitachi Electronics Engineering Co., Ltd., Tokyo, Japan) equipped with a light-emitting diode confocal fluorescence detector (exciting at 470 nm and collecting fluorescence at >580 nm). The buffer solution was introduced into the microchannels of the i-chip via the buffer waste reservoir using a syringe. Sample injection was accomplished by applying 300 V at the sample waste reservoir and grounding other three reservoirs for 30 s. Separation was performed at a field strength of 200 V/cm by applying 900 V at the buffer waste reservoir, 150 V at the sample and sample waste reservoirs, and grounding the buffer reservoir for 180 s.[25]

The μ-CAE chip experiments were carried on the stage of an inverted fluorescence microscope (Olympus IX70, Olympus, Tokyo, Japan) with LIF detection, as described previously.[26,27] Briefly, the 488-nm laser beam (~5 mW) from a diode solid-state laser (Sapphire 488-25 CDRH, Coherent, Santa Clara, CA) was expanded into ~2 cm using a set of expander optics and then focused vertically on the detection part of 10 separation channels using a cylindrical lens (f = 150 mm; Melles Griot). A 10×/0.3 NA objective lens (Olympus, Tokyo, Japan) and a band-pass filter (D520/20 nm, Chroma Technology Corp., Rockingham, VT) were used for fluorescence imaging. A HV488 high-voltage sequencer (LabSmith) was used to provide voltages for μ-CAE experiments. Fluorescence images of a separation were captured by a CCD camera (C5985; Hamamatsu Photonics, Hamamatsu, Japan) and analyzed by an image-processing software (Aquacosmos 2.5, Hamamatsu photonics).

Measurement of Electroosmotic Flow. The EOF in a PMMA microchannel was measured using the current monitoring method.[30] Briefly, the reservoirs and the fluidic channels of a PMMA chip were filled with a 20 mM phosphate buffer, and the contents of the buffer waste reservoir were then replaced with a 5 mM phosphate buffer. The current variation in the fluidic channel was monitored after an electrical field was applied to the channel using a HV488 high-voltage sequencer, and the time required for the current to reach a constant level was recorded. The electroosmotic mobility, μ_{EOF}, was calculated by dividing the channel length by the buffer replacement time and the electric field strength.

RESULTS AND DISCUSSION

Hybrid Dynamic Coating with DDM and MC. Although widely used in fabrication of MCE devices, PMMA has a very hydrophobic surface, thereby causing strong adsorption of analytes, such as fluorescence dyes and proteins, on the PMMA surface.[22−25] As shown in Figure 1A, the separation of APTS-labeled G_3 was impossible in an uncoated PMMA chip due to strong interactions between APTS and the PMMA surface. Therefore, development of simple surface modifications to minimize analyte adsorption is crucial to performing electrophoresis on PMMA MCE chips. In the previous studies,[24,25] we found that the use of cellulose derivatives such as hydroxypropylmethyl cellulose, hydroxyethyl cellulose, and MC as buffer additives greatly reduces analyte adsorption on PMMA chips; however, high concentrations of cellulose derivatives greater than 0.3% are required to obtain high-performance separations. The greatly increased viscosity due to the presence of concentrated cellulose derivatives in a running buffer makes handling quite difficult, especially for high-density multichannel chips. In contrast, very small amounts of DDM above 0.06% in running buffers have shown considerable effects on suppressing nonspecific adsorption of APTS-labeled G_3 on the PMMA surface, resulting in symmetry peaks without fronting or tailing (Figure 1A). Because DDM is a nonionic surfactant with a low molecular weight, minute amounts of less than 1% DDM do not change the properties of a running buffer such as viscosity and ionic strength, which makes DDM more attractive than polymer additives for surface modification of PMMA chips.

The performance of DDM dynamic coating was evaluated by the number of theoretical plates and reproducibility of migration times and is summarized in Table 1. The results show that satisfactory and nearly constant column efficiencies were obtained with DDM of greater than 0.12% in a 20 mM phosphate buffer; however, relatively large RSD values of migration times were observed throughout the concentration range of DDM used in the current work, indicating that no further improvement in separation performance can be achieved using higher concentrations of DDM in a running buffer. The RSD values with DDM dynamic coating were ~4-fold larger than that with MC coating, and the column efficiencies were lower than that with MC coating by a factor of ~17%. The difference in separation performance between MC coating and DDM coating appears to reflect the weaker ability of DDM to block charge sites on the PMMA surface as compared to MC, which results in unstable EOF and, hence,

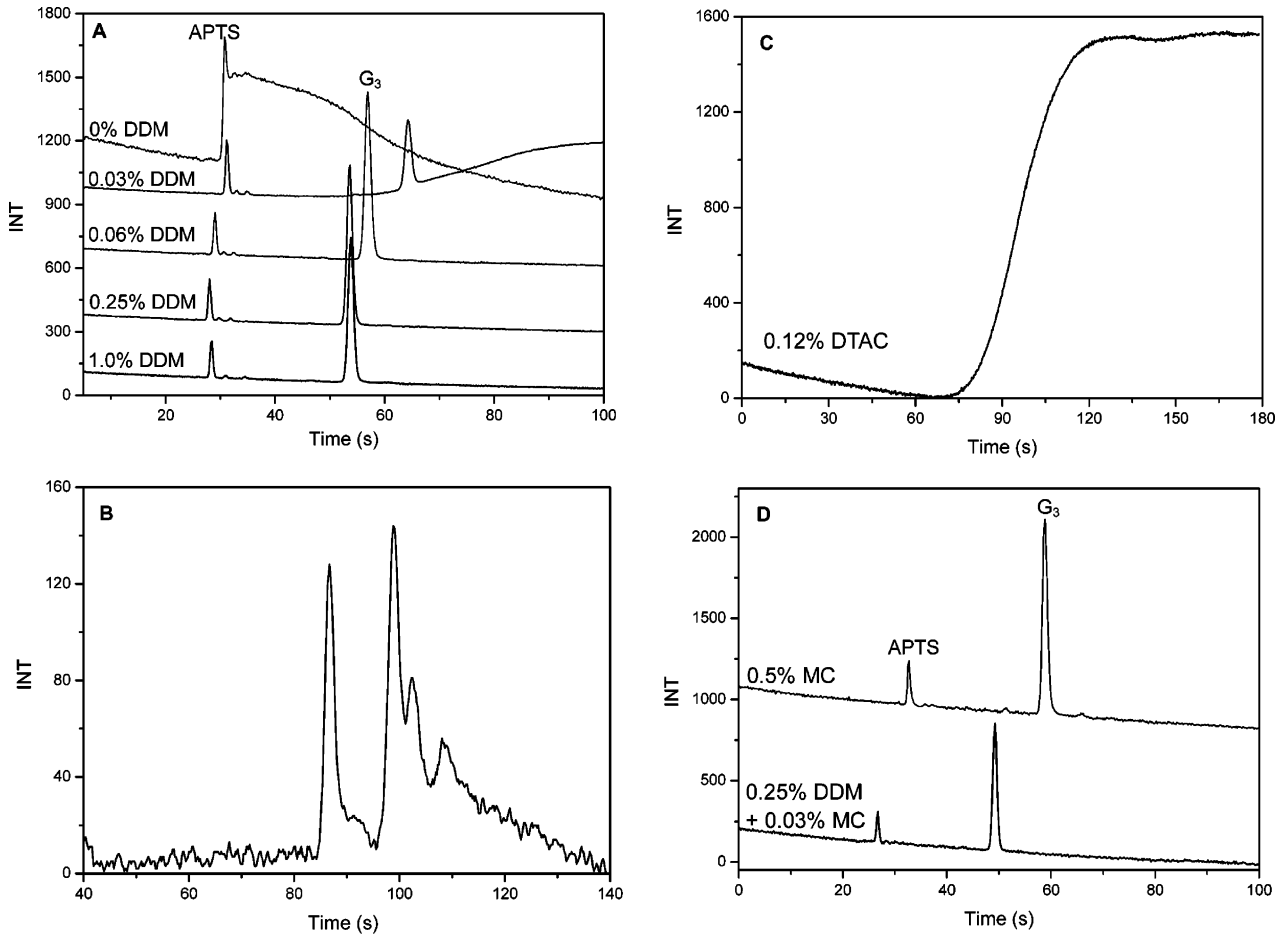

Figure 1. (A) Microchip electropherograms of APTS-labeled G_3 in PMMA channels dynamically coated with various concentrations of DDM. (B) Microchip electropherogram of APTS-labeled glycans from AGP in a PMMA channel dynamically coated with 0.25% DDM. (C) Microchip electropherogram of APTS-labeled G_3 in PMMA channels dynamically coated with 0.12% DTAC. (D) Microchip electropherograms of APTS-labeled G_3 in PMMA channels dynamically coated with 0.5% MC and 0.25% DDM−0.03% MC. Conditions: E_{sep} = 200 V/cm; 20 mM phosphate buffer, pH 6.93; 10^{-7} M APTS−G_3 in water.

Table 1. Column Efficiencies and Migration Time Reproducibilities for APTS-labeled G_3

surface modifiers	migration time (s)[a]	RSD (%)	total plates ($\times 10^4$)[a]
0.06% DDM	58.27	3.28	1.03
0.12% DDM	55.24	2.95	1.25
0.25% DDM	54.59	2.59	1.26
0.50% DDM	55.19	2.74	1.23
1.0% DDM	54.76	2.83	1.21
0.50% MC	58.06	0.68	1.49
0.25% DDM and 0.03% MC	49.66	0.72	1.36

[a] Data were calculated from independent separations on four channels. MCE conditions were the same as in Figure 1.

relatively larger RSD values of migration time and lower column efficiencies. In addition, DDM coating was also sensitive to the handling procedure. For example, rinsing a PMMA channel with 10-μL of a 20 mM phosphate buffer containing 0.25% DDM lead to a ~10% decrease in column efficiency and a ~2-s increase in migration time (data not shown). More seriously, poor separation of the complex glycans derived from glycoproteins such as AGP was often obtained on a PMMA channel dynamically coated with DDM (Figure 1B). These findings suggest that DDM dynamic coating is not stable and efficient enough to allow high-performance separation of real biological samples with a wide range of molecular weight in a PMMA channel.

The EOF mobilities of a 20 mM phosphate buffer (pH 6.93) in an untreated PMMA channel and a 0.25% DDM-coated PMMA channel were $(1.68 \pm 0.08) \times 10^{-4}$ and $(1.26 \pm 0.05) \times 10^{-4}$ $cm^2 \cdot V^{-1} \cdot S^{1-}$, respectively, and the direction of EOF was from anode to cathode (data obtained with 4 independent measurements). The EOF mobility of a 20 mM phosphate buffer (pH 6.93) was negligible in a 0.50% MC-coated PMMA channel because the variation in current during EOF monitoring is too small to obtain any statistically meaningful measurements. The small EOF mobility and strong analyte adsorption observed in an untreated PMMA channel clearly indicated that there are more nonspecific hydrophobic sites and fewer charge sites on the PMMA surface. Surface modifier molecules bind to the PMMA surface via hydrophobic, electrostatic, and other interactions to form a dynamic coating layer for reduction of analyte adsorption and EOF. DDM is a nonionic surfactant with a long hydrophobic alkyl chain and a short hydrophilic oligosaccharide chain. A ~25% reduction in EOF mobility (from 1.68×10^{-4} to 1.26×10^{-4} $cm^2 \cdot V^{-1} \cdot S^{1-}$) and satisfactory separation of APTS-labeled G_3 with 0.25% DDM dynamic coating suggested that DDM molecules adsorbed to the

PMMA surface primarily via hydrophobic interactions rather than via electrostatic interactions due to its short hydrophilic oligosaccharide chain. On the other hand, MC molecules adsorbed to the PMMA surface mostly via electrostatic interactions rather than via hydrophobic interactions because the polysaccharide chain of MC has a high affinity to surface charges but a low affinity to hydrophobic sites on the PMMA surface. Thus, highly concentrated MC is required to cover dominant hydrophobic sites and to minimize analyte adsorption. The good separation performance and a ~100% reduction in EOF mobility obtained with MC dynamic coating indicated that an efficient and stable dynamic coating layer was formed in the surface of a PMMA channel, most likely caused by electrostatic or hydrogen-bonding interactions between polysaccharide chains of surface modifier molecules and surface charges. As shown in Figure 1C, DTAC, an ionic surfactant, did not show observable effects on suppressing adsorption of APTS-labeled G_3 in a PMMA channel. Note that no reproducible separations were obtained with DTAC dynamic coating due to serious analyte adsorption, even using higher concentrations of DTAC. Because DTAC has the same alkyl chain as DDM, the significant difference in coating performance between DDM and DTAC should result from their hydrophilic ends. The hydrophilic end of DDM molecules with multiple-hydroxyl groups (maltoside) facilitates hydrogen-bonding interactions with surface charges, whereas the hydrophilic end of DTAC molecules with charged ammonium groups favors electrostatic interactions in a 20 mM phosphate at pH 6.93. Therefore, we deduced that the oligosaccharide moiety of surface modifiers has a high affinity to charge sites on the PMMA surface primarily via hydrogen-bonding interactions rather than electrostatic interactions, which plays a critical role in forming stable dynamic coating on the PMMA surface.

The ideal surface modifiers should have an alkyl chain and an oligosaccharide chain in structure with an optimal ratio. Because such an optimal ratio between an alkyl chain and an oligosaccharide chain can be obtained by adjusting concentrations of DDM and MC in a running buffer, a hybrid dynamic coating method using DDM and MC is explored in the following work. As expected, high-performance separation of APTS-labeled G_3 was obtained in a PMMA channel hybrid-coated with 0.25% DDM and 0.03% MC in a 20 mM phosphate buffer (Figure 1D). For a 3-cm-long separation channel, the number of theoretical plates and the RSD value of migration time were 1.36×10^4 and 0.72, respectively. These values are comparable to those obtained with 0.5% MC coating and much better than those with 0.25% DDM coating (Table 1), thereby indicating a stable hybrid dynamic coating layer formed on the PMMA surface. The EOF mobility of a 20 mM phosphate buffer (pH 6.93) in a PMMA channel hybrid-coated with 0.25% DDM and 0.03% MC was also found to be negligible. A decrease in migration time of APTS-labeled G_3 from 54.59 s with 0.25% DDM coating to 49.66 s with 0.25% DDM−0.03% MC coating is a direct consequence of further EOF reduction in a PMMA channel using DDM−MC hybrid dynamic coating, because APTS-labeled sugars migrate against EOF. Note that no separation can be obtained due to serious analyte adsorption on a PMMA channel dynamically coated with 0.03% MC without DDM.[24] Because very small amounts of DDM and MC can efficiently minimize both analyte adsorption and EOF in a PMMA channel, indeed, a running buffer containing 0.25% DDM and 0.03% MC is still a dilute and low-viscosity solution (smaller than 30 cP at 25°C) that is easy to handle in PMMA MCE devices. Although DDM−MC hybrid coating in this work was developed for surface modification of a PMMA MCE chip, it might become a general coating procedure for many polymer MCE devices thanks to its excellent ability of blocking nonspecific hydrophobic and charge sites on the surface as well as of providing a low-viscosity solution.

High-Speed and High-Throughput Analysis of Carbohydrates Released from Glycoproteins. Glycoproteins are ubiquitous components of extracellular matrixes and cellular surfaces, and their carbohydrate moieties are recognized to play pivotal roles in a wide range of cell−cell and cell−matrix recognition, signal transduction, and other important biological functions.[31,32] Glycans derived from glycoproteins are often complex compounds with subtle differences in their structure, such as carbohydrate sequence, types of glycosidic bonds, and number and position of various branched chains, which makes analysis of glycans from glycoproteins challenging. So far, conventional CE has demonstrated the highest separation efficiency and resolution for analysis of the complex glycans released from glycoproteins as labeled with suitable fluorescent reagents among all reported methods.[33−35] Although separation efficiency obtained in MCE is as high as or slightly higher than that in conventional CE, the obtainable resolution is often lower than that in conventional CE due to shorter channel length for separation. Therefore, analysis of complex glycans from glycoproteins by MCE has not been well-developed yet. Here, we explored capacity of the current MCE-based method for carbohydrate analysis using several samples derived from glycoproteins that are well-characterized by conventional CE.

Parts A and B in Figure 2 present fast separations of N-linked asialoglycans derived from AGP and fetuin in a PMMA channel hybrid-coated with 0.25% DDM and 0.03% MC. Human AGP is a plasma glycoprotein of 41−43 kDa in molecular mass, consisting of ~45% carbohydrate attached in the form of five complex-type N-linked carbohydrate chains. The carbohydrate moiety of AGP has been thoroughly investigated because AGP is one of the few serum glycoproteins that contains diantennary, triantennary, and tetraantennary N-linked glycans.[29,36] Figure 2A shows that the five N-linked glycans from AGP were well-resolved within 80 s in a 3-cm PMMA channel. The resolutions of two triantennary N-linked glycans (AII and AIII) and two tetraantennary N-linked glycans (AIV and AV) were 0.984 and 0.896, respectively, although small difference was found in their structure with one fucose unit (Figure 2C). Fetuin contains one diantennary glycan (AI) and two triantennary glycans (AII and FII), of which AII and FII are two positional isomers with the Galβ1−4GlcNAc-branch and Galβ1−3GlcNAc-branch. Figure 2B shows that the N-linked asialoglycans derived from fetuin were resolved into two peaks due to the comigration of two triantennary N-linked asialoglycans, indicating that the current MCE-based method cannot separate positional

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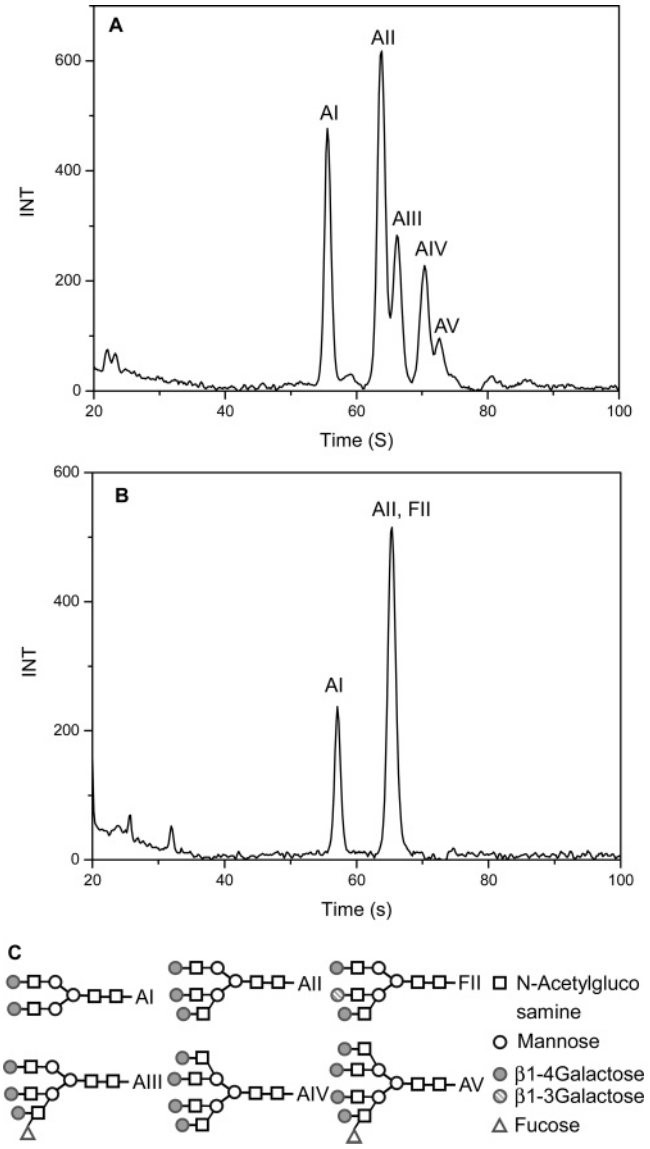

Figure 2. (A) Microchip electropherogram of N-linked asialoglycans derived from AGP. (B) Microchip electropherogram of N-linked asialoglycans derived from fetuin. (C) Structures of N-linked asialoglycans from AGP and fetuin. Conditions: E_{sep} = 300 V/cm; 0.25% DDM and 0.03% MC in 20 mM phosphate buffer, pH 6.93.

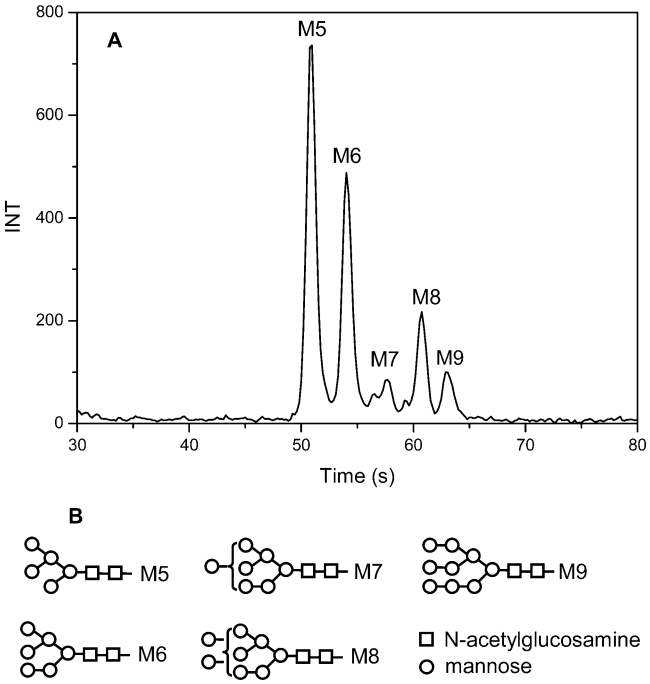

Figure 3. (A) Microchip electropherogram of N-linked glycans derived from ribonuclease B. (B) Structures of N-linked glycans from ribonuclease B. Conditions as in Figure 2.

sugar isomers as reported previouly.[25] Because N-linked asialoglycans from glycoproteins have specific interactions with lectins, a further effort is underway to exploit lectins as a buffer additive to resolve such positional sugar isomers in a PMMA microchannel.

Figure 3A presents a 70-s separation of high-mannose-type glycans released from ribonuclease B in a PMMA channel hybrid-coated with DDM and MC. The peak profiling obtained in MCE was similar to that in conventional CE,[37,38] and all glyans from M5 (M5GlcNAc2, 7 sugar units) to M9 (M9GlcNAc2, 11 sugar units) were well-resolved in a 15-s separation window. Interestingly, M7 also appeared as three peaks in the MCE electropherogram like conventional CE because M7 consists of three positional isomers (Figure 3B). Because the current MCE-based method has shown limited potential for resolving positional sugar isomers as demonstrated in separation of AII and FII, we cannot verify whether these three peaks are consistent with three positional isomers of M7 without further investigation using other analytical techniques

We investigated high-throughput screening of carbohydrates using a high-density 10-channel PMMA chip (Figure 4A) and a LIF detection system recently developed in our laboratory.[26,27] Figure 4B shows a high-speed and high-throughput separation of an APTS-labeled maltooligosaccharide ladder on a 10-channel μ-CAE PMMA chip. Because EOF in PMMA channels hybrid-coated with 0.25% DDM and 0.03% MC was negligible, a maltooligosaccharide ladder was baseline-resolved from glucose to maltodecaose in the order of degree of polymerization within 80 s. The migration times were consistent with RSD values of less than 1.26% in all 10 channels, which was comparable to those obtained in Hitachi PMMA i-chips (Table 1). Figure 4C presents a fast separation of APTS-labeled N-linked glycans derived from AGP on a 10-channel μ-CAE PMMA chip. The separation performance of μ-CAE PMMA chips was obviously better than those obtained on Hitachi PMMA *i*-chips; for example, the resolution of AII and AIII was 1.171 ($N = 10$), and the resolution of AIV and AV was 1.104 ($N = 10$). This is understandable considering that μ-CAE PMMA chips have smaller rectangular channels (50 $\times$ 50 μm) than Hitachi PMMA i-chips (100 $\times$ 35 μm), providing smaller longitudinal diffusion and thus higher separation performance. The above results demonstrated several advantages of the current MCE-based method over conventional CE in profiling of complex glycans derived from glycoproteins, including short analysis time, high throughput, and high reproducibility. Since both single-channel and multichannel PMMA chips are mass fabricated with high reproducibility using injection molding, we propose that the

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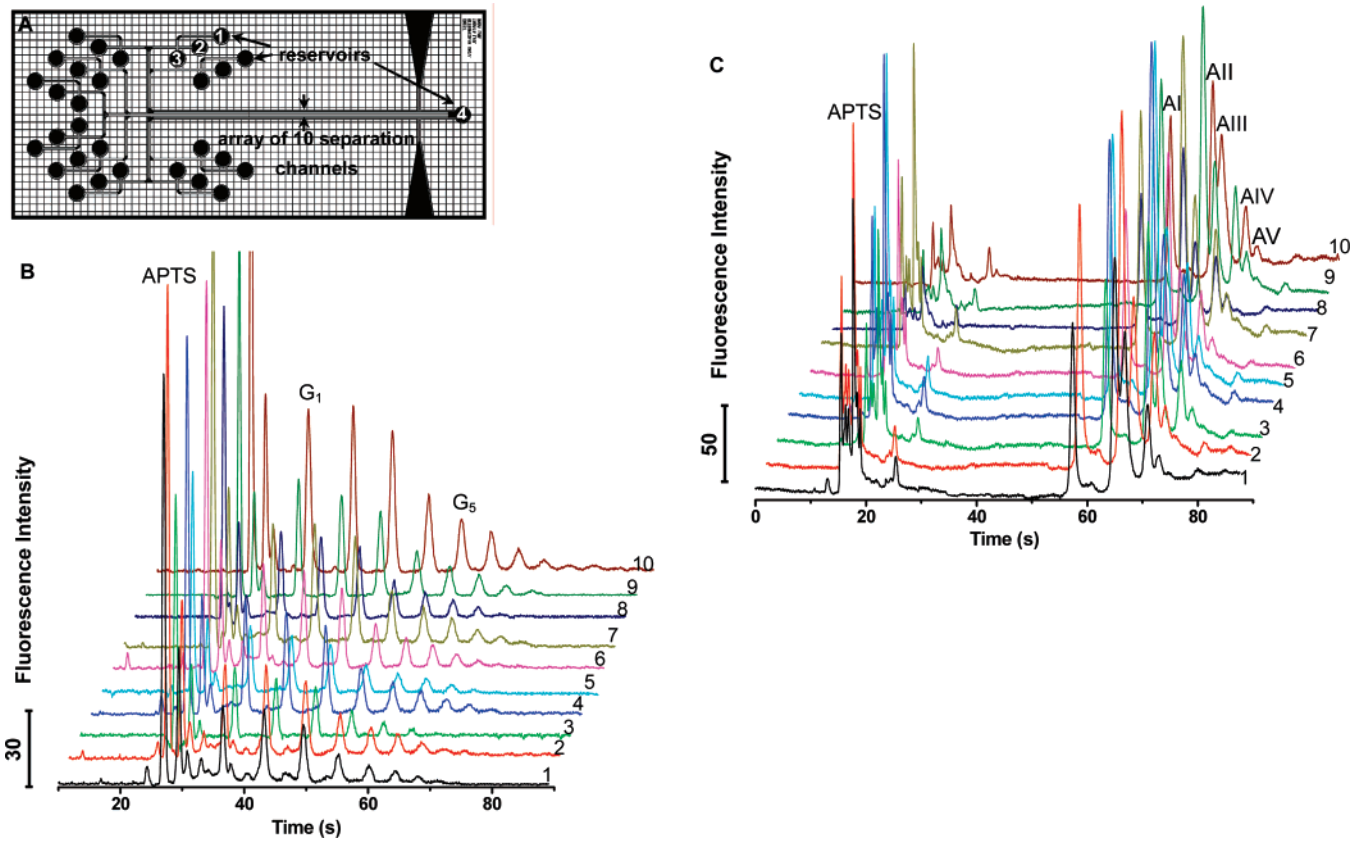

Figure 4. (A) Schematic representation of a 10-channel μ-CAE PMMA chip. (B) Electropherograms of a maltooligosaccharide ladder on a 10-channel μ-CAE PMMA chip at a field strength of 200 V/cm. (C) Electropherograms of N-linked asialoglycans from AGP on a 10-channel μ-CAE PMMA chip at a field strength of 300 V/cm. Conditions: 0.25% DDM and 0.03% MC in a 20 mM phosphate buffer, pH 6.93.

current MCE-based method might become a promising analytical tool for carbohydrate analysis.

CONCLUSIONS

Comparing the dynamic coating performance of DDM, MC, and DTAC with their molecular structures, we found that polysaccharide chains of surface modifiers have a specific affinity to surface charges on a PMMA channel primarily via hydrogen-bonding interactions, which is crucial to achieving efficient and stable dynamic coating on the PMMA surface. Ideal surface modifiers should show a high affinity to both hydrophobic and charge sites with a hydrophobic alkyl chain and a hydrophilic oligosaccharide chain in structure. Thus, a hybrid dynamic coating using 0.25% DDM and 0.03% MC was developed for suppression of both analyte adsorption and EOF in a PMMA channel while still keeping a low-viscosity solution. High-speed and high-throughput profiling of the N-linked complex glycans released from AGP, fetuin, and ribonuclease B was achieved in both single-channel and multichannel PMMA chips using hybrid dynamic coating with DDM and MC. Further work will focus on applying the current MCE-based method for high-throughput screening of carbohydrate alterations in glycoproteins such as AGP in respond to pathophysiological conditions including inflammation or infection, cancer, pregnancy, and so on, which allows better understanding of pathophysiological significance of carbohydrate alterations in protein glycosylation.

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