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Use of a Mixture of *n*-Dodecyl-β-D-maltoside and Sodium Dodecyl Sulfate in Poly(dimethylsiloxane) Microchips To Suppress Adhesion and Promote Separation of Proteins

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Dynamic modification of poly(dimethylsiloxane) channels using a mixture of *n*-dodecyl-β-D-maltoside (DDM) and sodium dodecyl sulfate (SDS) is able to suppress analyte adsorption and control electroosmotic flow (EOF). In this mixed surfactant system, the nonionic surfactant DDM functions as a surface blocking reagent, whereas the anionic surfactant SDS introduces negative charges to the channel walls. Changing the DDM/SDS mixing ratio tunes the surface charge density and the strength of EOF. Using 0.1% (w/v) DDM and 0.03% (w/v) SDS, Alexa Fluor 647 labeled streptavidin can be analyzed according to the charges added by the fluorophores. Protein molecules with different numbers of fluorophores are well resolved. DDM and SDS also form negatively charged mixed micelles, which act as a separation medium. The low critical micellar concentration of DDM/SDS mixed micelles also allows the use of SDS at a nondenaturing concentration, which enables the analysis of proteins in their native state. The immunocomplex between a membrane protein, β_2 adrenergic receptor, and anti-FLAG antibody has been fully separated using 0.1% (w/v) DDM and 0.03% (w/v) SDS. We have also analyzed the composition of light-harvesting protein−chromophore complexes in cyanobacteria.

In recent years, microfluidic devices have been demonstrated as an invaluable tool for integrating sample manipulation and analysis.[1−3] Among various analysis techniques that have been implemented in microfluidic platforms, capillary electrophoresis (CE) is one of the earliest and is still highly attractive.[4−6] In earlier times, many microfluidic devices were made of glass substrates, allowing the direct implementation of CE methods previously developed in fused-silica capillaries. With the emergence of low-cost, easy-to-fabricate polymer substrates such as poly(methyl methacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS), microfluidics-based analysis has gained ever increasing popularity. Unfortunately, these polymer materials are usually hydrophobic and do not support a stable electroosmotic flow (EOF).[7,8] Their hydrophobicity also causes the nonspecific adsorption of analytes. Therefore, surface modifications are usually required in order to enable CE analysis.[9,10] The change of surface properties can be static, using chemical reaction such as plasma oxidation,[11] polymer grafting/coating,[12,13] or prepolymer additives.[14] Alternatively, dynamic modification can be performed by adding to the sample solution reagents that adsorb on the surface. In comparison with static modification, dynamic modification is usually simpler if the surface modification additive does not interfere with the application. Surfactants are the most widely used dynamic coating reagent.[15] Other coating reagents include polyelectrolytes[16] and proteins.[17]

Among the dynamic modification reagents, we have demonstrated that *n*-dodecyl-β-D-maltoside (DDM), a nonionic surfactant, is highly efficient in reducing nonspecific protein adsorption on hydrophobic substrates.[18] The formation of a monolayer of this nonionic surfactant shields the surface charges and thus reduces the EOF, but this suppression of EOF is not sufficient to support electrophoresis-driven separation.[14,19] It has been reported that

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(1) Whitesides, G. M. *Nature* **2006**, *442*, 368−373.
(2) Janasek, D.; Franzke, J.; Manz, A. *Nature* **2006**, *442*, 374−380.
(3) Psaltis, D.; Quake, S. R.; Yang, C. H. *Nature* **2006**, *442*, 381−386.
(4) Harrison, D. J.; Fluri, K.; Seiler, K.; Fan, Z. H.; Effenhauser, C. S.; Manz, A. *Science* **1993**, *261*, 895−897.
(5) Zhang, L. H.; Dang, F. Q.; Baba, Y. *Electrophoresis* **2002**, *23*, 2341−2346.
(6) Huang, B.; Wu, H. K.; Bhaya, D.; Grossman, A.; Granier, S.; Kobilka, B. K.; Zare, R. N. *Science* **2007**, *315*, 81−84.
(7) Kirby, B. J.; Hasselbrink, E. F. *Electrophoresis* **2004**, *25*, 187−202.
(8) Kirby, B. J.; Hasselbrink, E. F. *Electrophoresis* **2004**, *25*, 203−213.
(9) Belder, D.; Ludwig, M. *Electrophoresis* **2003**, *24*, 3595−3606.
(10) Makamba, H.; Kim, J. H.; Lim, K.; Park, N.; Hahn, J. H. *Electrophoresis* **2003**, *24*, 3607−3619.
(11) Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M. *Anal. Chem.* **1998**, *70*, 4974−4984.
(12) Xiao, D. Q.; Van Le, T.; Wirth, M. J. *Anal. Chem.* **2004**, *76*, 2055−2061.
(13) Wu, D. P.; Luo, Y.; Zhou, X. M.; Dai, Z. P.; Lin, B. C. *Electrophoresis* **2005**, *26*, 211−218.
(14) Luo, Y. Q.; Huang, B.; Wu, H.; Zare, R. N. *Anal. Chem.* **2006**, *78*, 4588−4592.
(15) Badal, M. Y.; Wong, M.; Chiem, N.; Salimi-Moosavi, H.; Harrison, D. J. *J. Chromatogr., A* **2002**, *947*, 277−286.
(16) Liu, Y.; Fanguy, J. C.; Bledsoe, J. M.; Henry, C. S. *Anal. Chem.* **2000**, *72*, 5939−5944.
(17) Linder, V.; Verpoorte, E.; Thormann, W.; de Rooij, N. F.; Sigrist, M. *Anal. Chem.* **2001**, *73*, 4181−4189.
(18) Huang, B.; Wu, H. K.; Kim, S.; Zare, R. N. *Lab Chip* **2005**, *5*, 1005−1007.
(19) Dang, F. Q.; Kakehi, K.; Cheng, J. J.; Tabata, O.; Kurokawa, M.; Nakajima, K.; Ishikawa, M.; Baba, Y. *Anal. Chem.* **2006**, *78*, 1452−1458.

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DDM can be mixed with methyl cellulose to suppress EOF completely so that carbohydrates can be analyzed.[19]

Here we report electrophoretic analysis of proteins in a PDMS channel using the mixture of DDM and a low concentration of sodium dodecyl sulfate (SDS). The addition of the anionic surfactant SDS generates a stable and controllable EOF.[20] Moreover, the existence of the DDM−SDS mixed micelles[21] in the separation buffer facilitates the separation in a mechanism that is thought to be the same as micellar electrokinetic chromatography (MEKC).[22] In this article, we demonstrate the separation of protein charge ladders, immunocomplexes, and light-harvesting complexes from a cyanobacteria lysate.

EXPERIMENTAL SECTION

Materials and Reagents. DDM is obtained from Anatrace (Maumee, OH). SDS is purchased from Sigma-Aldrich (St. Louis, MO) as a 10% solution. Alexa Fluor 647 carboxylic acid succinimidyl ester (AX647) and Alexa Fluor 647 labeled streptavidin (AX647−SA) are from Molecular Probes (Invitrogen, Eugene, OR). Cy5 succinimidyl ester is from GE Health Care (Piscataway, NJ). Purified recombinant human β_2 adrenergic receptor (β_2AR) and anti-FLAG M1 monoclonal antibody (M1) are provided by Professor Brian K. Kobilka, Department of Molecular and Cellular Physiology and Medicine, Stanford University. Cyanobacteria *Synechococcus* sp. PCC 7942 is provided by Dr. Devaki Bhaya and Dr. Arthur R. Grossman, Department of Plant Biology, Carnegie Institution of Washington. B-PER II is purchased from Pierce Biotech.

PDMS prepolymers RTV615 A and RTV615 B are purchased from General Electric through R.S. Hughes (Santa Clara, CA). The silicon masters for PDMS microfluidics chips are produced at the Stanford Nanofabrication Facilities, and the PDMS microchips are fabricated using a standard soft lithography procedure.[6]

Sample Preparation. Anti-FLAG M1 antibody is labeled with Cy5 by incubating with Cy5 succinimidyl ester in 100 mM sodium bicarbonate with a dye-to-protein ratio of 3:1. The labeled antibody is purified using a Superdex 75 column (GE Health Care). The final dye-to-protein ratio in the purified antibody is 1.1:1 according to UV−vis absorption measurements. β_2AR is labeled with tetramethylrhodamine (TMR) maleimide[23] and stabilized using a high-affinity antagonist, carazolol.

Synechococcus cells from a liquid culture are harvested by centrifugation, washed with 20 mM HEPES buffer (pH 7.5), treated with 10 mM lysozyme in 20 mM HEPES at 40 °C for 10 min, washed again with 20 mM HEPES, then lysed by incubating with a nonionic surfactant solution, B-PER II, for 1.5 h. The lysate is used for analysis directly without further treatment.

Electroosmotic Flow Measurement. EOF mobilities are measured using a current monitoring method[24] in straight PDMS channels that are 6 cm long, 100 μm wide, and 15 μm high. The reservoirs at the two ends of the channels are filled with 20 mM and 22 mM HEPES (pH 7.5) containing specific amounts of surfactants, respectively. A picoammeter (KI6485, Keithley Instruments, Cleveland, OH) records the current change when applying 1000 V between the two reservoirs. The time for the buffer in the channel to be completely replaced is used to calculate the EOF mobility.

Electrophoresis. All microchip electrophoresis experiments are performed using standard double-T chips with 70 μm wide × 7 μm high channels. The injection channels on the chip are 5 mm long, and the separation channel is 30 mm long. The distance between the injection junction and the detection point is 20 mm. A homemade four-channel high-voltage power supply with computer interface controls the sample loading and separation. 1000 V is applied across the separation channel during the separation step, corresponding to an electric field strength of about 300 V/cm. All separation buffers and sample buffers contain 20 mM HEPES (pH 7.5), 0.1% DDM and 0.005% to ~0.05% SDS. In the case of β_2AR-M1 separation, 1 mM $CaCl_2$ is added to the buffers because M1 binding is calcium-dependent.

Laser-Induced Fluorescence Detection Using Cylindrical Optics. All experiments are performed on an inverted microscope (TE2000-U, Nikon, Melville, NY). A 638 nm diode laser (RCL-025-638, Crystalaser, Reno, NV) or a 532 nm diode-pumped solid-state laser (Compass 215M, Coherent, Santa Clara, CA) is used as the excitation source for laser-induced fluorescence (LIF) detection. We use a cylindrical optical configuration to improve the sensitivity, as explained in detail elsewhere.[6] Briefly, a cylindrical lens is used to focus the excitation laser at the back focal plane of the microscope objective (40× Achromat NA 0.65 or 100× Plan Apo NA 1.4 Oil, Nikon) so that the emerging laser from the objective forms a line-shaped focus that lies across the fluidic channel. The fluorescence emission is collected by the same objective. A dichroic mirror (400-535-635TBDR, Omega Optical, NJ) separates the excitation laser and the fluorescence emission, and a band-pass filter is used to block excitation laser in the detection light path (595AF60 from Omega Optical for detecting TMR-β_2AR and HQ675/50m from Chroma Technology, NJ, for all other analytes). Corresponding to the line-shaped excitation laser focus, a 50 μm wide slit is placed in front of a single-photon counting photomultiplier tube (PMT) detector (H6245-01, Hamamatsu, Japan) to reject out-of-focus fluorescence background. The photon counts are recorded by a digital counter−timer (PCI 6602, National Instruments, Austin, TX) with an integration time of 200 ms (for β_2AR−M1 separation) or 50 ms (all other experiments).

RESULTS AND DISCUSSIONS

Effect of *n*-Dodecyl-*β*-D-maltoside and Sodium Dodecyl Sulfate on Electroosmotic Flow. It has been reported that DDM and SDS form mixed micelles in a wide range of molar ratios owing to the strong interaction between them.[21] Therefore, we expect that they will also form a mixed monolayer on the hydrophobic PDMS surface. By varying their relative concentration, we could change the density of the negatively charged SDS molecule adsorbed on the surface, thus tuning the surface charge density and controlling the strength of EOF.[7,8] To test this hypothesis, we measure the electroosmotic mobility of 20 mM HEPES buffer (HB), pH 7.5, containing different concentrations of DDM and SDS in a PDMS channel. Figure 1 shows that DDM suppresses the EOF, whereas SDS enhances the anodic EOF in

(20) Nagata, H.; Tabuchi, M.; Hirano, K.; Baba, Y. *Electrophoresis* **2005**, *26*, 2247−2253.
(21) Zhang, R.; Zhang, L.; Somasundaran, P. *J. Colloid Interface Sci.* **2004**, *278*, 453−460.
(22) Terabe, S. *TrAC, Trends Anal. Chem.* **1989**, *8*, 129−134.
(23) Swaminath, G.; Xiang, Y.; Lee, T. W.; Steenhuis, J.; Parnot, C.; Kobilka, B. K. *J. Biol. Chem.* **2004**, *279*, 686−691.
(24) Wheeler, A. R.; Trapp, G.; Trapp, O.; Zare, R. N. *Electrophoresis* **2004**, *25*, 1120−1124.

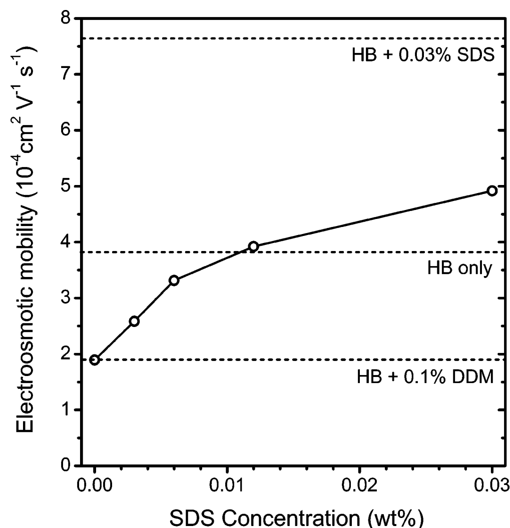

Figure 1. Electroosmotic mobilities of HB containing 0.1% DDM and different concentrations of SDS, measured in a native PDMS channel. The electroosmotic mobilities of HB without surfactants and with either one of the two surfactants are marked as the dashed lines.

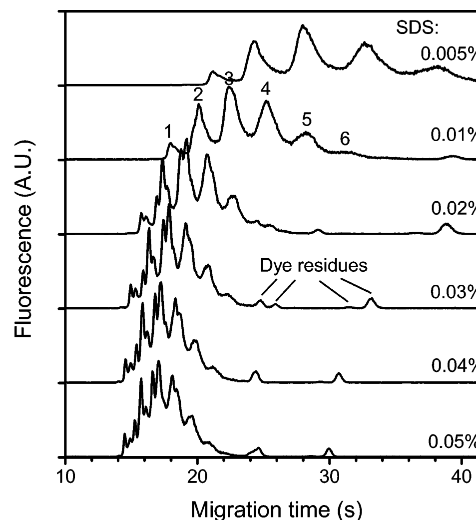

Figure 2. Separation of 200 nM AX647−SA (3.3 dye per protein) using HB containing 0.1% DDM and different concentrations of SDS. All separations use the same sample buffer (HB containing 0.1% DDM and 0.01% SDS). The electropherograms are shifted vertically for clarity. Peaks corresponding to different number of dyes on the protein and those from dye residues are marked.

a native PDMS channel. This observation is consistent with previous reports.[14,25] By changing the mixing ratio of the two surfactants, we are able to tune the strength of EOF, confirming the ability to control the surface charge density.

Separation of Dye-Labeled Proteins. To demonstrate the separation of proteins with capillary zone electrophoresis using the DDM/SDS mixed surfactant system, we analyze streptavidin (SA) labeled with an amine reactive fluorescent dye, Alexa Fluor 647 (AX647), in a PDMS chip. Because AX647 has two negative net charges,[26] the reaction of each AX647 with a primary amine on SA introduces three additional negative charges. SA labeled with multiple AX647 molecules then results in a charge ladder, which has been reported previously.[27]

Figure 2 shows that this charge ladder can be resolved with capillary zone electrophoresis using the DDM/SDS mixed surfactant system. With an average dye-to-protein ratio of 3.3, SA molecules labeled with up to six AX647 dyes can be observed. The separation of a charge ladder is confirmed by observing the change in relative peak heights in the electropherograms when varying the dye-to-protein labeling ratio, which is reported elsewhere.[28] Interestingly, the increase of SDS concentration from 0.005% to 0.03% (0.17−1 mM) while keeping the DDM concentration at 0.1% (2 mM) significantly improves the resolution of separation, which reveals the fine structures within each main peak. This observation suggests that the addition of SDS might stabilize the surface coating. Although the identities of these fine peaks are still under investigation, they could possibly be attributed to AX647 reacted with amine residues with different pK_a values.

We observed that SA under the same separation condition is able to bind fluorescently labeled biotin (data not shown), which confirms that SA is not denatured in our experiments. This result can be explained by the low SDS concentration we used and the fact that DDM can "dilute" the surface charge of mixed micelles.[21] It is also noteworthy that the surfactant concentration we used here is 10-fold higher than the critical micelle concentration (cmc) of the DDM/SDS mixture (total surfactant concentration of 0.20 and 0.26 mM when the mole fraction of SDS is 0.25 and 0.5, respectively[21]). Such a low cmc ensures that we can further reduce the concentration of surfactants if denaturation would happen. For example, we have found that decreasing the concentration of both DDM and SDS by a factor of 10 only slightly affects the separation of dye-labeled SA.

Separation of Immunocomplexes. The DDM/SDS mixed micelle is negatively charged, meaning that it migrates in the opposite direction as the EOF. The presence of these negatively charged micelles make it possible to separate analytes by utilizing their participation into these micelles, which is the principle of MEKC. Figure 3 shows the separation of the immunocomplexes between the mouse monoclonal anti-FLAG M1 antibody (M1) and a transmembrane protein, β_2 adrenergic receptor (β_2AR). β_2AR has been genetically fused with a short peptide sequence, the FLAG tag, at the N-terminus so that it binds with M1. To detect both proteins, M1 is labeled on primary amine groups using Cy5 (which has one negative net charge) with a labeling ratio of 1.1 dyes per protein. On the other hand, β_2AR is labeled on a specific cysteine residue using TMR, which has no net charge.[23] By limiting the number of fluorophores labeled on the protein and/or using fluorophores having the same charge as the reactive groups, the peak broadening or splitting introduced by the labeling procedure, as described in the previous section, can be minimized. Fluorescence from these two proteins is monitored separately in consecutive runs by switching the excitation and detection wavelengths. The immunocomplexes appear in the electropherograms as peaks having fluorescence signal from both proteins. As is required by M1−FLAG binding, 1 mM $CaCl_2$ is added to the separation buffer. Such a low concentration of bivalent Ca^{2+}

(25) Ocvirk, G.; Munroe, M.; Tang, T.; Oleschuk, R.; Westra, K.; Harrison, D. J. *Electrophoresis* **2000**, *21*, 107−115.
(26) White, S. S.; Li, H. T.; Marsh, R. J.; Piper, J. D.; Leonczek, N. D.; Nicolaou, N.; Bain, A. J.; Ying, L. M.; Klenerman, D. *J. Am. Chem. Soc.* **2006**, *128*, 11423−11432.
(27) Colton, I. J.; Anderson, J. R.; Gao, J. M.; Chapman, R. G.; Isaacs, L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1997**, *119*, 12701−12709.
(28) Kim, S.; Huang, B.; Zare, R. N. *Lab Chip*. In press.

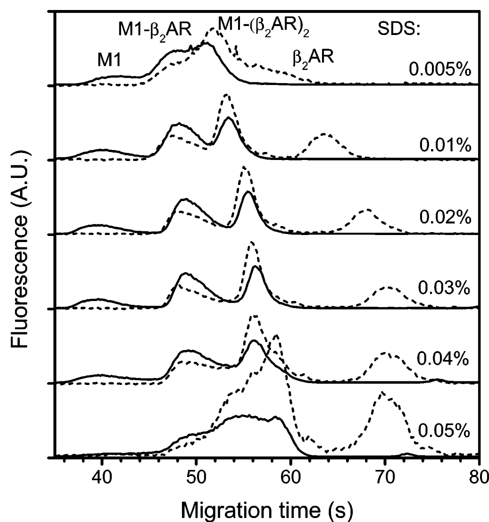

Figure 3. Separation of the immunocomplexes between β_2AR and M1 antibody. A mixture of 20 nM Cy5−M1 and 40 nM TMR−β_2AR in the sample buffer (HB containing 0.1% DDM, 0.005% SDS, and 1 mM $CaCl_2$) is separated using different separation buffers with varying SDS concentrations. The electropherograms obtained from Cy5 and TMR fluorescence are shown as solid and dashed lines, respectively. Electropherograms are shifted vertically for clarity. The enhancement in separation when increasing the SDS concentration up to 0.03% and the breakdown of the immunocomplexes with higher SDS concentrations are shown here.

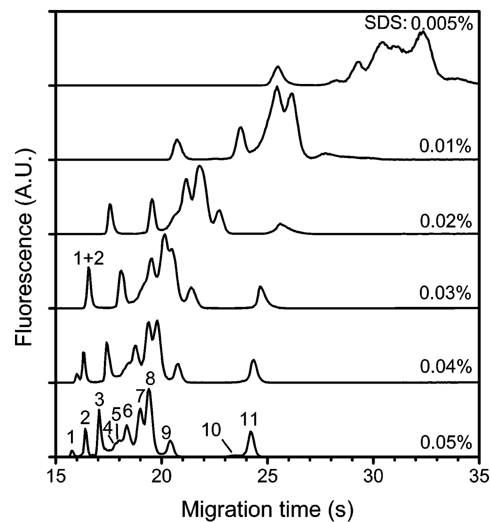

Figure 4. Separation of photosynthetic protein−chromophore complexes in *Synechococcus* cells. *Synechococcus* lysate in B-PER II is diluted 1:10 into sample buffer (HB containing 0.1% DDM and 0.01% SDS) and analyzed using HB containing 0.1% DDM and different concentrations of SDS as the separation buffer. Electropherograms are shifted vertically for clarity.

substantially decreases the EOF, although the separation efficiency is not affected significantly.

Increasing the molar ratio of SDS to DDM adds more negative charges to both the surface and the mixed micelles, causing the increase of both the cathodic EOF and the anodic electrophoresis of the mixed micelles. As can be seen in Figure 3, when increasing the SDS concentration from 0.005% to 0.03%, the migration time of M1 shortens because of the increased EOF, whereas the peak of β_2AR moves backward because of its association with the DDM/SDS mixed micelles. Consequently, all four species in the M1−β_2AR mixture can be separated with almost baseline resolution. This way of tuning the number of charges on a surfactant micelle appears to be a new result. This experiment again proves that protein analytes can maintain their functionality during electrophoresis when the SDS concentration is below 0.03%.

Analysis of Phycobiliprotein Complexes in Cyanobacteria. To demonstrate the application of CE separation using DDM/SDS mixed surfactants, we analyze the light-harvesting protein complexes of a unicellular cyanobacterium, *Synechococcus* sp. PCC 7942 (*Synechococcus* hereafter). Cyanobacteria use phycobilisome, a large protein−chromophore complex, to collect light and transfer the light energy to the chlorophyll-containing photosynthetic reaction center. The phycobilisome in *Synechococcus* has two types of pigmented phycobiliprotein subcomplexes: the phycocyanin (PC) in the periphery rod structures and the allophycocyanin (APC) in the core structure.[29] Various linker peptides in these subcomplexes tune the absorption and emission wavelengths of the chromophores so that the absorbed energy flows toward the core structure and then to photosystem II (PS II).[29] The number and composition of phycobilisomes in *Synechococcus* are regulated by many factors, including light illumination level and nutrient supply.[30]

When *Synechococcus* cells are lysed in a low ionic strength buffer, phycobilisomes are released and dissociate into smaller subcomplexes. Using the DDM/SDS separation buffer, we can separate these subcomplexes without further dissociation or denaturation and measure their relative abundances, revealing the composition of the phycobilisome (Figure 4). The identities of the peaks in Figure 4 are determined by measuring their fluorescence emission spectra,[6] which is extremely sensitive to the composition of the phycobiliprotein subcomplexes. PC complexes containing the three linker peptides (peaks 1, 7, and 9), PC complex without linker peptide (peak 8), one PC−APC complex (peak 6), both APC complexes (peaks 2 and 3), and chlorophylls in PS II (peak 11) are identified. It can be noticed that increasing SDS concentration from 0.005% to 0.05% improves the separation. The effect of DDM/SDS mixed micelles is evident in two aspects: (1) PS II (peak 11), which is a transmembrane protein complex, has a very long migration time; (2) when increasing the SDS concentration from 0.03% to 0.05%, the migration time of peak 1 (one of the PC complexes in the rods of phycobilisomes) shortens, whereas the migration time of peak 2 (the APC−L_{CM} complex that links the phycobilisome to the thylakoid membrane) shows little variation, with the result that the two peaks are completely separated.

CONCLUSION

We have demonstrated that the use of a mixture of DDM and SDS for dynamic coating in PDMS channels can suppress protein adsorption and support EOF efficiently. With the use of the mixed

(29) Glazer, A. N.; Lundell, D. J.; Yamanaka, G.; Williams, R. C. *Ann. Microbiol. (Paris)* **1983**, *B134*, 159−180.

(30) Grossman, A. R.; Schaefer, M. R.; Chiang, G. G.; Collier, J. L. *Microbiol. Rev.* **1993**, *57*, 725−749.

surfactants, protein analytes can be separated with high performance under nondenaturing conditions. Moreover, the DDM/SDS mixed micelles create an environment where proteins can be separated according to their affinity to the micelles. Considering the fact that DDM is a widely used surfactant in solubilizing membrane proteins,[31] our method is expected to have wide applications in the analysis of membrane proteins, which are important in cell functioning but often difficult to analyze because of their poor solubility in water. We believe that the DDM/SDS mixed micelle approach could be generalized to other mixtures of nonionic−ionic reagents and other hydrophobic materials for fabricating microfluidic devices.

(31) Kobilka, B. K. *Anal. Biochem.* **1995**, *231*, 269−271.

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