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Coupling Sodium Dodecyl Sulfate–Capillary Polyacrylamide Gel Electrophoresis with MALDI-TOF-MS via a PTFE Membrane

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Abstract

Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) is a fundamental analytical technique for proteomic research, and SDS–capillary gel electrophoresis (CGE) is its miniaturized version. Compared to conventional slab-gel electrophoresis, SDS-CGE has many advantages such as increased separation efficiency, reduced separation time and automated operation. SDS-CGE is not widely accepted in proteomic research primarily due to the difficulties in identifying the well-resolved proteins. MALDI–TOF–MS is an outstanding platform for protein identifications. Coupling the two would solve the problem but is extremely challenging because the MS detector has no access to the SDS-CGE resolved proteins and the SDS interferes with MS detection. In this work we introduce an approach to address these issues. We discover that poly(tetrafluoroethylene) (PTFE) membranes are excellent materials for collecting SDS-CGE separated proteins. We demonstrate that we can wash off the SDS bound to the collected proteins and identify these proteins on-membrane with MALDI-TOF-MS. We also show that we can immunoblot and Coomassie-stain the proteins collected on these membranes.

INTRODUCTION

New analytical technologies are greatly desired to attack proteomic problems. SDS-CGE could be one of the alternatives. SDS-CGE has been performed in capillaries[1,2] and on microchips.[3,4] Typically, the separated proteins are detected in-column by either an Ultraviolet (UV) absorbance or a laser-induced fluorescence (LIF) detection system. A common issue associated with SDS-CGE is that these detection techniques can monitor the well-resolved protein bands but cannot identify them, and this is the primary reason that SDS-CGE has not been widely accepted in the proteomic community.

MALDI–TOF–MS is an excellent detector for sensitive and accurate analysis and identification of proteins.[5] A marriage of the two would be ideal but allowing the MALDI-MS to access the SDS-CGE resolved proteins and preventing the interference of SDS with MS detection remain major problems. People have tried to overcome these problems but mostly failed. In one attempt,[6] SDS-CGE was first carried out in a microchannel, and after separation the microchannel was physically opened to allow a MS detector to access the resolved proteins. Owing to the constraints of the experimental setup practical applications of this method is limited. In addition, the method had issues for analysis of large proteins (>10kD) because SDS bound these proteins strongly and suppressed MS signals severely.

Polyvinylidene fluoride (PVDF) membranes have been used to collect proteins separated by capillary electrophoresis (CE), a legacy of Western blotting practice.[7–10] However, membrane collection of SDS-CGE resolved proteins has never been done, much less a

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coupled analysis by MALDI-TOF-MS. We had initially tested PVDF membranes for collections of SDS-CGE separated proteins. After numerous difficulties we succeeded in collecting the proteins but failed analyzing the collected proteins by MALDI-TOF-MS (signals undetectable for most of the proteins). In the above effort to couple SDS-CGE with MALDI-TOF-MS, the importance of removing the SDS bound to the proteins and increasing the hydrophobicity of the membrane became clear and led us to the discovery of poly(tetrafluoroethylene) (PTFE) membranes for this work.

PTFE membranes have been used as a sample support for MALDI-TOF analysis of proteins11 and peptides,12 and improved sensitivities were obtained. The sensitivity improvement is particularly effective for high molecular weight proteins.13 With introduction of an aqueous wash step, the method can tolerate high concentrations of salt additives.11 In this work we demonstrate the use of a PTFE membrane to collect and fractionate the SDS-CGE separated proteins, remove the SDS bound to the collected proteins, and identify these proteins by MALDI-TOF-MS. We also demonstrate Coomassie Brilliant Blue (CBB) staining and immunoblotting of the proteins collected on PTFE membranes.

EXPERIMENTAL

Reagents and Materials

Acrylamide, cross-linker (Bis, [N,N'-Methylene Bisacrylamide]), bi-functional reagent [(3-Methacryloxypropyl)-Trimethoxysilane], TEMED (N,N,N',N'-Tetramethylethylenediamine), APS (ammonium persulfate), and the broad molecular weight (MW) protein marker were obtained from Bio-Rad Laboratories (Hercules, CA). The low MW protein marker was purchased from GE Healthcare (Piscataway, NJ), and it includes α-Lactalbumin (14.4 kD), Trypsin inhibitor (20.1 kD), Carbonic anhydrase (30 kD), Ovalbumin (45 kD), Albumin (66 kD) and Phosphorylase b (97 kD). Other proteins that include lysozyme, trypsinogen and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO). Brilliant Blue R-250 was purchased from Fisher Scientific (Pittsburg, PA). PTFE membrane was manufactured by Taega Technologies, Inc. (High Point, NC). Other chemicals and PVDF membrane were obtained from VWR (Radnor, PA). Fused silica capillaries were bought from Molex Inc. (Phoenix, AZ). All solutions were prepared with ultra-pure water purified by a NANO pure infinity ultrapure water system (Barnstead, Newton, WA).

Preparation of cross-linked polyacrylamide (CPA) coated capillaries

The procedure for the coating process has been described previously,14 and it was used in this work with minor modifications. Briefly, a capillary was first flushed with a 1.0 M NaOH solution for ~1 h to activate its surface, and then rinsed with water for 20 min and acetonitrile for 10 min. After being dried with helium, the activated surface was reacted with a 1.0% bi-functional reagent for ~1 h, rinsed with acetonitrile and dried with helium. The capillary was then flushed with a degassed solution containing 4%T and 0.60%C (%T stands for acrylamide concentration and %C represents the Bis concentration relative to the acrylamide), 0.1% (v/v) TEMED, and 0.025% APS at ~0 °C for 4 min. The capillary was ready to use after the residual solution was rinsed out.

Preparation of replaceable cross-linked polyacrylamide (rCPA) solution

The procedure for preparing rCPA has been described previously.15 Briefly, 0.8 g of acrylamide and 24 mg of Bis were dissolved in 10 mL of DI water. This solution was mixed with 10 mL of a buffer solution containing 0.12 M Tricine, 0.042 M Tris and 0.50% SDS. After the solution was vacuum-degassed for ~5 min, polymerization reaction was initiated

by adding 10 μL of 10% APS and 2 μL of TEMED in the vial. The reaction was allowed to proceed overnight at room temperature.

Preparation of Protein Samples

The low MW protein size marker was prepared by adding 300 μL sample buffer (0.060 M Tris-0.060 M TAPS pH 8.35, 2.5% SDS, 0.20 M dithiothreitol (DTT) to a vial containing the proteins (575 μg total proteins/vial, GE HealthCare). The solution was heated at 100 °C for 5 min, and cooled in an ice bath before SDS-CGE separation. A real-world protein, AcrA, was obtained from Professor Zgurskaya's group in the Department of Chemistry and Biochemistry at University of Oklahoma. The nature and preparation of this protein is described in the literature.16 This sample had a concentration of 2.59 mg/mL, and was stored at −20 °C in 200 mM Tris-HCl buffer (pH 8.0), 500 mM NaCl, 1.0 mM phenylmethylsulfony fluoride (stabilizer) and 0.030% dodecyl maltoside (detergent). To prepare AcrA for SDS-CGE, 10 μL of its solution was mixed with 3.0 μL of 60 mM Tris-60 mM TAPS, pH 8.35, with 2.5% SDS. The mixture was cooked at 95 °C for 10 min, and mixed with lysozyme, trypsinogen and phosphorylase b. The final concentrations of all proteins in this mixture are the same, 0.50 mg/mL.

Apparatus

Figure 1a presents a schematic diagram of the apparatus for SDS-CGE and protein collection. A key component of this setup is a membrane collector, and Figure 1b shows an exploded version of the collector. To assemble the collector, a piece (0.5 × 5 cm^2) of PTFE or PVDF membrane was soaked first in methanol and then in water to wet the membrane. After the wet membrane was sandwiched by two wet filter papers and secured by the two frames as shown in Figure 1b, it was inserted into the grounding reservoir containing 100 mM Tricine – 35 mM Tris (pH7.6) and 1.0% methanol. The filter papers were utilized to prevent the membrane from being scratched by the capillary tip. The two frames were made from ¼-inch-thick acrylic sheet. In the middle of each frame there was a 2-mm-wide slit. The slits allowed the capillary tip and anode electrode to be placed directly in contact with the filter papers from the opposite sides, and also facilitated to keep them in position during the collector mobilization. As protein-SDS complexes were electrophoretically driven out of the capillary, they were forced to (but could not) pass through and collected onto the membrane. A stepper motor was used to move the membrane collector.

A capillary gel electrophoresis setup similar to that described previously15 with a Linear UVIS 200 absorbance detector was used for SDS-CGE without protein collection.

SDS-CGE and Protein Collection

After rCPA was pressurized into the separation capillary, its cathode end was inserted into the cathode reservoir containing 60 mM Tricine – 21 mM Tris with 0.25% SDS (pH7.6) and its grounding end was inserted into an anode reservoir containing 100 mM Tricine – 35 mM Tris and 1.0% methanol, as shown in Figure 1a. A protein sample was usually electrokinetically injected into the capillary from the cathode end. Occasionally, pressure injection (at 0.5~1 bars for 5~15 s) was used. The electrophoresis was normally carried out by applying an electronic field first at 220 V/cm and the membrane was moving upwards for protein collection. A step size of 0.7~1.4 mm/step and a frequency of 3~6 steps/min were used to collect proteins for Coomassie Brilliant Blue staining, while a step size of 1.4~2.8 mm/step and a frequency of 2~3 steps/min were used to collect proteins for MALDI-TOF-MS analysis.

Coomassie Brilliant Blue (CBB) R-250 Staining

After the protein collection, the membrane was taken out of the collector assembly and stained in a solution containing 0.10% Brilliant Blue R-250 in 50% methanol, 40% water and 10% acetic acid. The membrane was de-stained in the same solution without the staining dye for visualization of the protein spots.

Immunochemical Blotting

After the protein collection, the membrane was taken out of the collector assembly and treated with 5.0% milk powder in a buffer containing 0.40 M NaCl-0.10 M Tris-HCl (pH 8.0) and 0.05% Tween 20 for 1 h. After the membrane was then washed three times with the buffer solution, it was reacted with 10 mL of a 1:50,000 diluted antibody (AntiAcrA) solution for 1 h. The membrane was washed with the buffer solution again for three times before reacting with 10 mL of a 1:30,000 diluted secondary antibody (Anti-Rabbit IgG – Alkaline Phosphatase antibody) solution for 1 h. After three washes with the buffer solution, the membrane was reacted with a 10 mL of NBT-BCIT solution to detect the AcrA mutant collected on the membrane for 30 min, and the reaction was terminated by adding 0.20 mL of 0.50 M EDTA (pH 8.0) solution into the reaction solution. The NBT-BCIP solution was prepared freshly by mixing 100 μl of 30 mg/mL NBT (nitro-blue tetrazolium chloride)/DMF solution and 100 μl of BCIT (5-bromo-4-chloro-3-indolylphosphate toluidine salt)/70% DMF with 10 mL Alkaline Phosphatase buffer (0.10 M Tris at pH=9.5, 0.10 M NaCl and 5.0 mM $MgCl_2$).

MALDI-TOF-MS Analysis

To prepare for MALDI-TOF-MS analysis of proteins on a stainless steel target plate (Plate), 0.50 μL of a protein solution (0.20 μg/μL) was deposited in a well of the Plate and allowed to dry at ambient temperature. Then, 0.50 μL of α-cyano-4-hydroxycinnamic acid (CHCA) – the MALDI matrix – solution (10 mg/mL CHCA in 1:1 acetonitrile:water with 0.1% TFA in it) was introduced to the protein spot. After the solvent was evaporated, another 0.50 μL of the solution was added. The sample was ready for MS analysis once the spot was dried.

To prepare for MS analysis of proteins without SDS on a PTFE or PVDF membrane, the membrane was first wetted with methanol and then with water. The wet membrane was transferred to a wetted filter paper on a glass slide. After a small dent was created on the membrane with a pipette tip, 0.50 μL of protein sample (0.20 μg/μL) was delivered to the dented position. The solution was gradually penetrated through the membrane and absorbed by the filter paper. The membrane was then attached to the Plate with 3M tape 9713. As the membrane became partially wet and partially dry, 0.50 μL of the CHCA solution was delivered to the dented spot. After the solvent was evaporated, an additional 0.50 μL of the matrix solution was added to the same spot. It was ready for MS analysis when the membrane was dried.

To prepare for MS analysis of proteins with SDS on a PVDF membrane, an SDS-protein sample was first made by mixing 5.0 μL of protein (10 mg/mL) with 5.0 μL of sample buffer (60 mM Tris-60 mM TAPS with 2.5% SDS), cooking the solution at 100 °C for 5 min, and diluting the solution by 25 times with DI water. Then, 0.50 μL of this sample was delivered to a wet PVDF membrane on a wetted filter paper, as described above. After the solution was penetrated through the membrane and absorbed by the filter paper, the membrane was placed in a 20 mL vial containing 10 ml of 0.40% Tergitol NP Type 40 at 40 °C for 60 min to remove the SDS associated with the protein. The membrane was rinsed with DI water to remove Tergitol. Attaching the membrane to the Plate and loading the MALDI matrix were the same as described in the preceding paragraph.

The same procedure was followed for SDS-proteins deposited on a PTFE membrane, except for the SDS-removal process. To remove SDS the PTFE membrane was placed in 10% methanol at 40°C for 60 min. Because the membrane floated on surface of the solution, the side of the membrane with proteins should be facing down to the solution.

To prepare for MS analysis of the proteins collected by a PTFE or PVDF membrane, the collector assembly was taken out of the SDS-CGE system and dissembled. The membrane was carefully taken out of collector for SDS-removal. Dented marks were visible on the membrane for where the proteins were collected. These marks were created by the anode end of capillary during protein collection. After SDS removal, the rest of the operation was the same as for the proteins directly deposited on the membranes.

MALDI-TOF-MS analysis was performed on an ABI 4800 MALDI-TOF-MS Analyzer in the linear mode. The molecular range was set at the range of 5 to 30 kD for smaller proteins (≤ 30 kD) or 5 to 70 kD for molecular weight markers. The laser intensity is typically 6000, which is higher than that (3000 to 4000) for on-plate MS analysis.

RESULTS AND DISCUSSION

Figure 2a presents the image of a PTFE membrane with CBB-stained proteins separated by SDS-CGE collected on it. Figure 2b presents an electrophoregram of the SDS-CGE separation. The reproducibility can be appreciated from the fact that Figures 2a and 2b were obtained from two different SDS-CGE runs. Note also a PVDF membrane will also allow results as in Figure 2a.

PTFE membranes are much better than PVDF membranes when MALDI-TOF-MS was utilized to analyze proteins on membranes. To demonstrate this we performed an experiment by depositing proteins without SDS on three different surfaces (stainless steel, PVDF and PTFE), introducing a matrix solution to the proteins, and measuring the MS signals of these proteins. The results are exhibited in Figure 3A. Proteins on PTFE membrane and stainless steel produced comparable signals, while proteins on a PVDF yielded much lower signals. These results are in good agreement with literature reported results.[11,13]

In MALDI-TOF-MS analysis of proteins collected after SDS-CGE, the SDS must first be removed. In this case a PTFE membrane is vastly superior over a PVDF membrane: a PTFE membrane can retain the proteins well, but the proteins on a PVDF membrane are largely lost. Figure 3B presents the results of an experiment where the same amount of SDS-protein adducts were deposited on both membranes, subjected to the SDS-removal process, and subjected to MALDI-TOF-MS analysis. The PTFE membrane provided vastly superior results.

In the above tests the SDS-washing protocols were different because they were optimized for the membranes separately. In the initial stage of this project we experimented intensively on PVDF membranes, and therefore optimized the protocol to remove SDS from PVDF. With the unsatisfactory results from PVDF, we switched to PTFE membranes and re-optimized the SDS-washing protocol for PTFE membranes.

A particularly attractive possibility with the present approach is to perform a coarse separation (resolution 1~2 kD) with SDS-CGE followed by a high resolution (<1 kD) separation with MALDI-TOF-MS. To demonstrate the utility of such an approach, we deliberately took a sample with proteins of similar size. As indicated by the separation trace in Figure 4a, cytochrome C (12.8 kD), ribonuclease A (13.8 kD) and lysozyme (14.3 kD) could not be resolved by SDS-CGE. But when the single protein spot, collected on a PTFE membrane, was subjected to MALDI-TOF-MS, baseline-resolved peaks were obtained in

the MS spectrum (see Figure 4b). The SDS-CGE-PTFE membrane collector serves here as an excellent tool for fractionation /micro-sample preparation ahead of MALDI-TOF-MS. Here the SDS-CGE along with the PTFE membrane collection served as a fractionation and micro-preparative tool for MALDI-TOF-MS. The peaks at m/z=14.4, 13.8 and 12.5 kD are assigned to lysozyme, ribonuclease A and cytochrome C, while the peaks at m/z=7.2, 6.9 and 6.2 kD are assigned to the double-charged ions of these proteins, respectively. The peak at m/z=5.8 kD comes from insulin.

Normally the resolutions of the proteins collected on PTFE membrane will improve as the step-size of the collector movement is reduced. The drawback of a small step-size is the diminished protein quantity on each spot, resulting in decreased protein detection sensitivities. Thanks to the high resolving power of MS, it is unnecessary for us to retain all the resolutions of SDS-CGE separations. In Figure 2, for example, when we collected proteins on the PTFE membrane we did not particularly try to minimize the step-size to maximize the resolutions.

To demonstrate utility in the analysis of real sample analysis, we received an Escherichia coli derived protein sample, AcrA, from our colleagues; this protein has been of much interest for sometime in elucidating mechanisms of multidrug resistance and transmembrane drug efflux.16-18 The protein is normally recovered in high concentrations of SDS, glycerol and inorganic salts.16 In this particular case the AcrA (2.59 mg/mL) came in a matrix of 200 mM Tris-HCl buffer (pH 8.0), 500 mM NaCl, 1.0 mM phenylmethanesulfonylfluoride (a serine protease inhibitor), and 0.03% dodecyl-β-d-maltoside (a detergent). When directly analyzed by MALDI-TOF-MS, little or no signal could be observed. SDS-CGE readily separated the AcrA from lysozyme, trypsinogen, and phosphorylase b. Figure 5A presents the electropherogram. There is an excellent correlation between known protein MW and migration time t (s), with the regression equation being $\log(\text{MW, kD}) = -32/t + 3.8$, $r^2 = 0.9995$. On this basis, the MW of the AcrA was estimated to be 43.4 kD. In a separate run, the SDS-CGE-resolved proteins were separated and collected on a PTFE membrane. After the SDS bound to the collected proteins was removed, these proteins were analyzed by MALDI-TOF-MS. Figure 5B presents a series of MS spectra from 8 consecutive spots (#9-#16) on the membrane (other mass spectra collected before and after these spots did not have any usable signals above background). In Figure 5B, we see MS signals from only lysozyme in the first two spectra (green, spots #9, #10). The trypsinogen signals appear primarily in traces 3 and 4 (blue) that also contains some lysozyme signals. Trace 5 (black) is an example of when only the background is observed. The AcrA signals show up in the last three traces (red). Phosphorylase b was not detectable under our experimental conditions. An image of a PTFE membrane with lysozyme, trypsinogen and AcrA, with the protein spots on the membrane are aligned approximately with their corresponding spectra appear as an inset on the left panel of Figure 5B (in yellow). Inset II of Figure 5B appears as a white back panel and displays the mass spectrum for trace 6 in Figure 5B (first red trace): it contains the AcrA ions with +1, +2, +3, +4 and +5 charge. From these MS data we obtain a value of 41.5 kD, that is 1.9 kD lower than the approximate value predicted by SDS-CGE. The MW of AcrA was estimated to be 43.4 kD from light scattering analysis, and 40.6 kD based on their amino acid compositions.19

PTFE membranes are also capable of collecting SDS-CGE separated proteins for immunoblotting – representing an exact miniaturization of the Western blot. Details of the experiment are described in Supporting Information. Briefly, after protein collection, the PTFE membrane was taken out of the membrane collector and treated with 5.0% milk powder in 0.40 M NaCl-0.10 M Tris-HCl and 0.050% Tween 20 (pH 8.0). It was then treated with the primary antibody of AcrA and the secondary antibody (Anti-Rabbit IgG-Alkaline Phosphatase) followed by staining with nitro-blue tetrazolium chloride - 5-

bromo-4-chloro-3-indolylphosphate toluidine salt. Figure 3a presents the immunoblotted results. The three spots in the middle of the membrane show the immunoblotted AcrA. Figure 3b provides a comparison of the proteins collected by a PTFE membrane and stained with CBB R-250. The arrows indicate the immunoblotted and Coomassie stained spots of AcrA.

In summary, we have demonstrated an effective approach for coupling SDS-CGE to MALDI-TOF-MS. The SDS-CGE separated proteins can be collected on a PTFE membrane, the SDS bound to the collected proteins removed and then an on-membrane MALDI-TOF-MS analysis of these proteins performed. SDS-CGE-PTFE membrane collection is utilized as an extraordinarily powerful tool for preseparation and sample cleanup tool for MALDI-TOF-MS, the first separation stage providing only moderate resolution (1~2 kD) is needed. Proteins that are partially resolved by SDS-CGE can then be further resolved by the MS. The PTFE membranes collection of SDS-CGE separated proteins also is an attractive platform for subsequent Coomassie Blue staining and immunoblotting and is a very useful addition to the current tool set for proteomic research. However, there is a limitation of the present method: incapable of identifying larger proteins. We have an on-going project to attack this problem by collecting the proteins from the SDS-CGE, digesting the collected proteins on-membrane, and analyzing the digested products for protein identifications. The results of this work will be published elsewhere.

Acknowledgments

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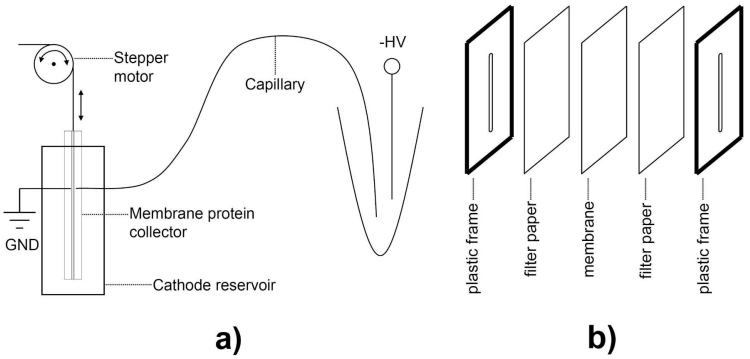

Figure 1.
Schematic diagram of experimental apparatus. (a) SDS-CGE setup with membrane collector; (b) split-view of membrane collector.

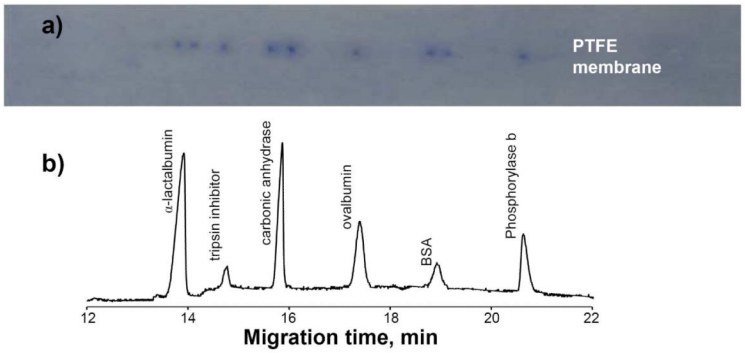

Figure 2.

Collection of SDS-CGE resolved proteins using a PTFE membrane. A 40-cm long (35-cm effective) and 150-μm i.d. CPA-coated capillary was used for SDS-CGE. The separation matrix was 4%T-3%C CPA in 60 mM Tricine-21 mM Tris (pH 7.6) and 0.25% SDS. The sample was a low molecular weight (LMW) marker, containing ~ 1.0 mg total proteins per milliliter. The sample was electrokinetically injected by applying 7 kV for 3 s, and separated under the same field strength (175V/cm). UV absorbance was measured at 280 nm. The membrane mobilization was started at 750 seconds and mobilized at 1.5 mm/step and 18 s/step. (a) Image of a PTFE membrane with protein collected and stained on it. (b) An SDS-CGE electropherogram with protein peaks being aligned approximately corresponding to their spots on the PTFE membrane.

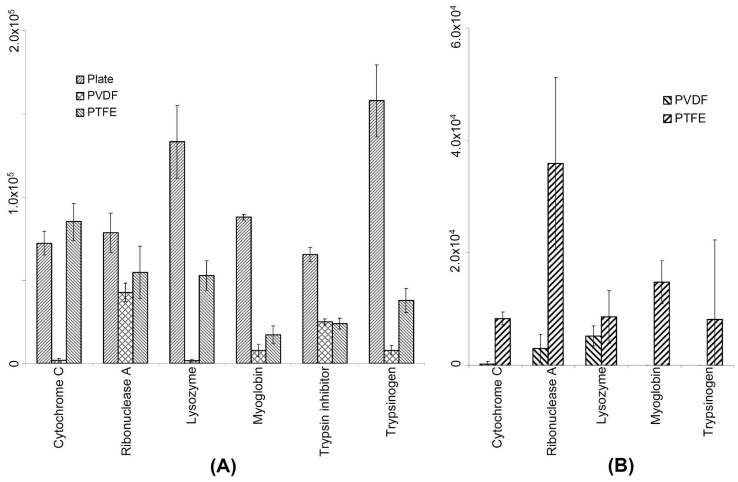

Figure 3.

Effect of support surface on protein MS signals. Y-axes are MS signals in number of ion counts. (A) MS signals from proteins on metal plate, PTFE and PVDF. An aliquot of 0.5 μl of 0.2 mg/mL protein (in water) solution was loaded onto the surface of a pre-wetted membrane or metal plate surface. Two aliquots of 0.5 μl of matrix solution containing 10 mg/mL CHCA in 50% acetonitrile and 0.1% TFA were sequentially added onto each sample spot. MALDI-TOF measurements were carried out after the matrix was dried. Five duplicate tests were performed to obtain the standard deviations (error bar values). (B) MS signals from protein-SDS adducts on PTFE and PVDF. The procedure was the same as (A) except for the sample differences and the addition of an SDS-removal process. The protein samples had final concentrations of 0.2 mg/mL protein in 2.4 mM Tris-2.4 mM TAPS and 0.1% SDS. To remove SDS, the PTFE membrane was placed in 10% methanol at 40 °C for 60 min. Myoglobin and trypsinogen on PVDF were undetectable. Trypsin inhibitor on both PVDF and PTFE was undetectable.

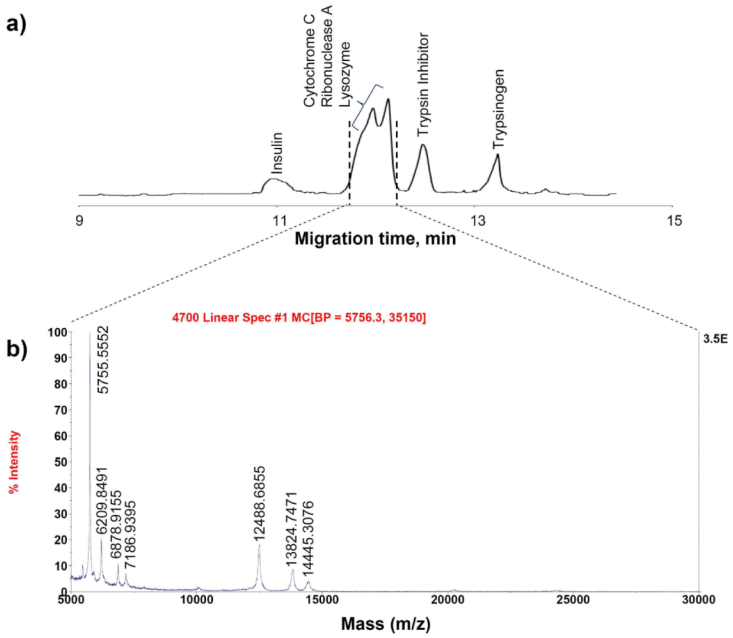

Figure 4.

SDS-CGE along with the membrane collector as a micro-preparation and fractionation tool for MALDI-TOF-MS. The sample contains 0.50 mg/mL Insulin, 1.0 mg/mL Cytochrome C, 1.0 mg/mL Ribonuclease A, 1.0 mg/mL Lysozyme, 1.0 mg/mL Trypsin Inhibitor and 1.0 mg/mL Trypsinogen. The membrane mobilization started at 650 seconds and mobilized at 2.1 mm/ step and 20 s/step. All other conditions were the same as in Figure 2. (a) SDS-CGE trace. (b) MS spectrum of the three unresolved proteins.

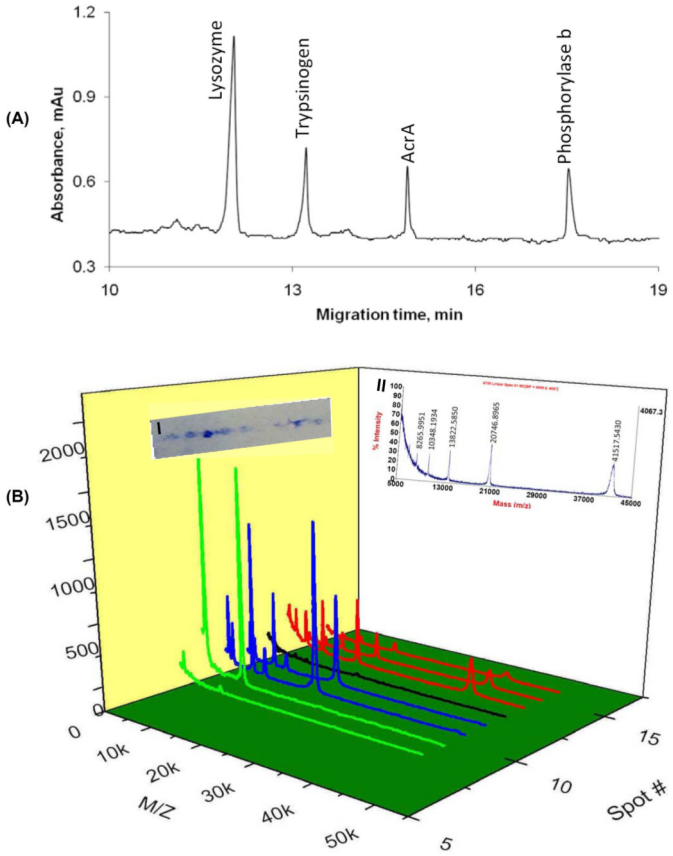

Figure 5.

Typical results. The sample contained 0.10 mg/mL Lysozyme, 0.20 mg/mL AcrA and 0.10 mg/mL Phosphorylase b. All other conditions were the same as in Figure 4. (A) SDS-CGE trace with UV absorbance detection, and (B) MS spectra of PTFE collected proteins. Inset I shows an image of a PTFE membrane with proteins collected/stained on it, and Inset II is an expanded MALDI-TOF-MS spectrum [identical to that of spot #14 in (B)].

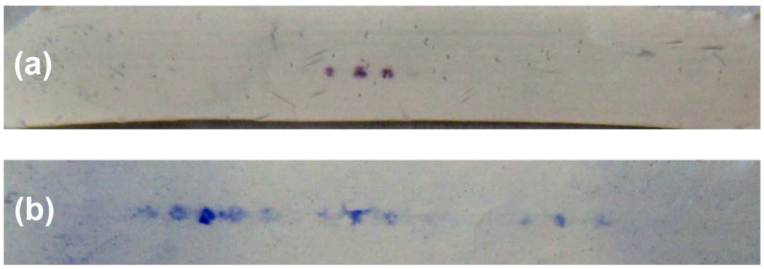

Figure 6.

PTFE membrane collection of SDS-CGE separated proteins. Sample separation and collection protocols were the same as in Figure 5. (a) A PTFE membrane showing three immunoblotted spots for AcrA. The test was performed by first treating the membrane in 10 mL of 5% dry milk powder, 0.4 M NaCl-0.10 M Tris-HCl (pH 8.0) and 0.05% Tween 20 for 1 h and washing the membrane with 0.4 M NaCl-0.10 M Tris-HCl (pH 8.0) and 0.05% Tween 20. The membrane was then treated with a primary antibody of AcrA mutant (AntiAcrA), a secondary antibody (Anti-Rabbit IgG – Alkaline Phosphatase antibody), and a NBT-BCIP solution. (b) A PTFE membrane showing Coomassie stained spots of lysozyme, trypsinogen, AcrA and phosphorylase b. Coomassie Staining was carried out by treating the PTFE membrane with a Coomassie staining solution for 5 min, followed by destaining in a destaining solution for 1 min. The three arrow-pointed spots are from AcrA.