Anal. Chem. **2003,** *75,* 5213−5219

A Microfluidic Electrocapture Device in Sample Preparation for Protein Analysis by MALDI Mass Spectrometry

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The design and operation of a microfluidic device for sample preparation in MALDI mass spectrometry of peptides and proteins is described. It is particularly useful for proteomics applications and for mass determination of proteins in salt- and detergent-containing solutions. The system consists of a flow channel with two conductive areas or electrical junctions where proteins and peptides are retained by means of an electric field. The microfluidic device is made of PEEK tubing, and the junctions are covered with a conductive polymeric membrane. A syringe pump connected to the device produces a flow stream, and injection of sample is carried out manually via hydrodynamic pressure. Proteolytic peptides and intact proteins in salt- and detergent-containing acidic media were captured at the cathode junction followed by exchange of the original solution to a solvent suitable for subsequent mass spectrometry. Using this principle, a significant desalting effect was obtained for tryptic peptides in mass-mapping experiments. Protein sequence coverages were high (up to 40%) at subpicomole levels with results better than those obtained using reversed-phase solid-phase extraction. In contrast to the latter technique, the microfluidic device has the capacity to efficiently remove detergents such as CHAPS before peptide mapping and protein analysis.

Over the past decade, miniaturization has become important in the design of analytical devices for high-throughput applications such as analysis of proteins and peptides in large-scale proteome characterizations.[1−5] In this respect, the combination of mass spectrometry and microfluidics offers interesting possibilities.[6−8] Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a powerful tool for protein identification and determination of posttranslational modifications[9,10] and an interesting target for microfluidics since it allows work with samples in the submicroliter range.

Microtechnology in protein identification by MALDI-MS has been tested with silicon micromachining of integrated microanalytical devices for protein digestion.[7,11] Notably, in these and other tests reported, the components making up the sample solutions are frequently selected not to interfere with the MALDI-MS analysis. However, in common practice, most protein and peptide samples contain a wide range of interfering molecules such as dyes, detergents, chelators, buffers, and salts, necessitating the use of sample cleanup.

Although MALDI-MS tolerates some degree of contamination, sample purification is required to achieve maximum sensitivity and accurate mass measurement. Salts, detergents, and other interfering molecules lower the overall signal intensity and increase spectral complexity as a consequence of the generation of many adduct peaks (e.g., alkali metal adducts). In addition to cleanup, a preconcentration step is often necessary because of the limited volume of sample that is normally mixed with matrix and spotted onto the MALDI probe.

Solid-phase extraction (SPE) has been used to preconcentrate and clean up samples in microfluidic systems.[12] However, the solid support tends to complicate the construction.[13,14] Furthermore, most analytes and contaminants require a specific solid support for efficient handling (e.g., C-4 for proteins, C-18 for peptides, and ionic resins for detergent cleanup) which lowers the flexibility of a microfluidic system based on a particular SPE material.

Microfluidic systems that combine the use of porous or semipermeable membranes and electric field have been used for sample pretreatment. Electrodialysis has been coupled to capillary electrophoresis (CE) as an on-line pretreatment technique to select desirable analytes with a molecular mass lower than the cutoff

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(1) Reyes, D.; Iossifidis, D.; Auroux; P.; Manz, A. *Anal. Chem.* **2002**, *74*, 2623−2636.
(2) Anderson, R. C.; Su, X.; Bogdan, G. J.; Fenton, J. *Nucleic Acids Res.* **2000**, *28*, E60.
(3) Khandurina, J.; Guttman, A. *J. Chromatogr., A* **2002**, *943*, 159−183.
(4) Burns, M. A.; Johnson, B. N.; Brahmasandra, S. N.; Handique, K.; Webster, J. R.; Krishnan, M.; Sammarco, T. S.; Man, P. M.; Jones, D.; Heldsinger, D.; Mastrangelo, C. H.; Burke, D. T. *Science* **1998**, *282*, 484−487.
(5) Mitchell, P. *Nat. Biotechnol.* **2001**, *19*, 717−721.
(6) Figeys, D.; Gygi, S. P.; McKinnon, G.; Aebersold, R. *Anal. Chem.* **1998**, *70*, 3728−3734.
(7) Ekström, S.; Önnerfjord, P.; Nilsson, J.; Bengtsson, M.; Laurell, T.; Marko-Varga, G. *Anal. Chem.* **2000**, *72*, 286−293.
(8) Jiang, Y.; Lee, C. S. *J. Chromatogr., A* **2001**, *924*, 315−322.
(9) Larsen, M. L.; Roepstorff, P. *Fresenius J. Anal. Chem.* **2000**, *366*, 677−690.
(10) Aebersold, R.; Mann, M. *Nature* **2003**, *422*, 198−207.
(11) Laurell, T.; Nilsson, J.; Marko-Varga, G. *J. Chromatogr., B* **2001**, *752*, 217−232.
(12) Oleschuk, R. D.; Shultz-Lockyear, L. L.; Ning, Y.; Harrison, D. J. *Anal. Chem.* **2000**, *72*, 585−590.
(13) Ocvirk, G.; Verpoorte, E.; Manz, A.; Grasserbauer, M.; Widmer, H. M. *Anal. Methods Instrum.* **1995**, *2*, 74−82.
(14) Yu, C.; Davey, M. H.; Svec, F.; Frechet, J. M. *Anal. Chem.* **2001**, *73*, 5088−5096.

10.1021/ac0300901 CCC: $25.00
Published on Web 08/30/2003

limit for the dialysis membrane. The sample is placed in a chamber outside the fluidic system, and via diffusion and electrical gradient, analytes pass through the membrane for analysis by CE.[15,16] In a different approach, a porous structure separated from, but in electrical contact with the electrophoretic channel enables electrokinetic concentration of proteins and DNA for further analysis.[17,18]

In this report, we describe a novel microfluidic device designed for desalting, cleanup, and enrichment of samples in peptide mapping and protein analysis by MALDI-MS. The technique is based on the principles described for sample concentration in CE of DNA[19] and negatively charged proteins.[20] The current construction captures positively charged peptides and proteins (1−10-μL samples) in an electric field sufficiently strong to retain them in a small volume in a micrometer-diameter fluidic channel for exchange of the salt-containing solution to a solvent suitable for mass spectrometry, followed by deposition of the concentrated and desalted sample (typically 0.4 μL) onto a MALDI target plate for analysis. The electrocapture device is easy to manufacture and does not contain chromatographic supports or frits.

EXPERIMENTAL SECTION

Reagents and Chemicals. Horse muscle myoglobin (Mb), bovine serum albumin (BSA), β-casein (Cas), alcohol dehydrogenase I (ADH), urea, Na_2HPO_4, NaCl, dithiothreitol, iodoacetamide, sodium dodecyl sulfate (SDS), α-cyano-4-hydroxycinnamic acid, and sinapinic acid were obtained from Sigma Chemical Co. (St. Louis, MO). Porcine, modified trypsin (sequencing grade) was from Promega (Madison, WI), and trifluoroacetic acid (TFA) was from Applied Biosystems (Warrington, U.K.). The 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) detergent was obtained from Pharmacia Biotech (Uppsala, Sweden). The water used was from a MilliQ water purification system (Millipore).

Protein Solutions and Trypsin Digestion. All protein stock solutions were prepared at concentrations in the range 2−5 mg/mL. To block disulfide interactions and allow efficient mass-mapping of tryptic peptides, carbamidomethylation of cysteine residues was carried out. Briefly, 25 μL of protein stock solution was mixed with 75 μL of 8 M urea, and 25 μL of this solution was mixed with 5 μL of 45 mM dithiothreitol followed by incubation at 50 °C for 15 min. After cooling to room temperature, 5 μL of 100 mM iodoacetamide was added and the mixure was further incubated for 15 min. Following the alkylation, 60 μL of 83 mM Tris-HCl buffer (pH 8) and 1 μL of trypsin solution (1 μg/μL) was added, and incubation at 37 °C for 4 h started. The resulting tryptic digest was frozen (−20 °C) and used as tryptic peptide stock solution.

Samples. For peptide analysis, the tryptic peptide stock solution was mixed with either of two buffers to yield the final concentrations: (A) 50 mM phosphate (pH 2.5), 40 mM NaCl, and 0.05% SDS and (B) 10 mM CHAPS, 50 mM phosphate (pH 2.5), and 40 mM NaCl. For protein analysis, stock solutions were mixed with buffer B to give the final concentrations indicated above.

Solid-Phase Extraction. Reversed-phase solid-phase extraction (RP-SPE) with μZipTips (C-18, Millipore, Bedford, MA) was performed after conditioning: 10 μL of sample, containing 0.1% TFA, was pipetted and washed with 0.1% TFA in water. For elution, 1 μL of 70% acetonitrile in 0.1% TFA was used. For analysis, the eluate was mixed 1:1 (v/v) with the matrix solution and 1 μL of this mixure was spotted onto the MALDI-MS target.

Crude Sample Analysis. The results from experiments using the microfluidic device or SPE were compared with those from direct analysis of untreated crude sample by mixing 1:1 (v/v) the crude sample containing 0.1% TFA with the MALDI matrix solution. Aliquots of 1 μL were spotted onto the MALDI-MS target and analyzed.

Mass Spectrometry. All mass spectra were obtained with a Voyager DE-PRO MALDI time-of-flight (TOF) mass spectrometer (Applied Biosystems) operated in the positive-ion mode. For peptides, reflector mode was used at 20-kV accelerating voltage, 71% grid voltage, 0.005% guide wire voltage, and 180-ns delayed extraction. Proteins were analyzed in linear mode using 20-kV accelerating voltage, 91% grid voltage, 0.2% guide wire voltage, and 750-ns delayed extraction. The matrix for peptides was α-cyano-4-hydroxycinnamic acid (almost saturated solution in 70% acetonitrile/0.1% TFA), while for proteins sinapinic acid was used. Database searches employed the MS-Fit software (http://prospector.ucsf.edu) and the SwissProt protein sequence database.

Device Fabrication. The microfluidic device was manufactured as schematically outlined in Figure 1. Two small openings were made on a piece of PEEK tubing (Upchurch, Oak Harbor, WA) having the dimensions 127-μm i.d. and 512-μm o.d., and the distance between the gaps was 1.6 cm. The openings were covered with a conductive tubular cation-selective membrane of poly(tetrafluoroethylensulfonate) material (Permapure Inc., Toms River, NJ) having the dimensions 330-μm i.d. and 610-μm o.d. The two electrical junctions were placed in separate electrode chambers made from 500-μL plastic tubes (Eppendorf, Hamburg, Germany) filled with 0.05% TFA. Electrodes of platinum wire were connected to a high-voltage power supply (Spellman, Plainview, NY). Current was monitored using a homemade current-to-voltage converter circuit digitally converted by a PCMCIA acquisition card (CyberResearch, Branford, CT) employing a Notebook-PC with Labtech Software (Laboratory Technologies Co., Wilmington, MA). A syringe pump (Harvard Apparatus, Holliston, MA) equipped with a 500-μL gastight syringe (Hamilton, Reno, NV) that was filled with the washing solution produced a flow in the PEEK channel. The microfluidic device was connected to the sample injection system through a six-port valve (Upchurch) with Teflon tubing having the dimensions 256-μm i.d and 1.58-mm o.d. that was carefully drilled to ensure good connection with the PEEK tubing coming from the capture device. To optimize the capture and release conditions, a UV absorbance detector was coupled to the outlet of the system in the first set of experiments. This was accomplished via epoxi-gluing a fused silica capillary (100 μm i.d. and 360 μm o.d.; Polymicro Technologies, Phoenix,

(15) Buscher, B. A. P.; Tjaden, U. R.; van der Greef, J. *J. Chromatogr., A* **1997**, *764*, 135−142.
(16) Buscher, B. A. P.; Tjaden, U. R.; van der Greef, J. *J. Chromatogr., A* **1997**, *788*, 165−172.
(17) Khandurina, J.; Jacobson, S. C.; Waters, L. C.; Foote, R. S.; Ramsey, J. M. *Anal. Chem.* **1999**, *71*, 1815−1819.
(18) Wei, W.; Yeung, E. *Anal. Chem.* **2002**, *74*, 3899−3905.
(19) Park, S.-R.; Swerdlow, H. *Anal. Chem.*, in press.
(20) Astorga-Wells, J.; Swerdlow, H. *Anal. Chem.*, in press.

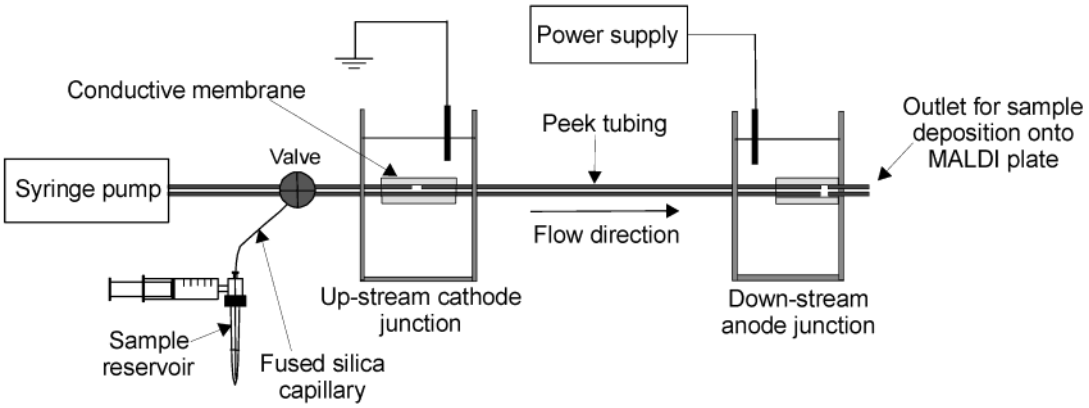

Figure 1. Schematic representation of the microfluidic electrocapture device. For details of the construction and operation, see text. A PEEK tubing with two gaps covered by conductive membrane was connected via a valve to the sample injection system, and the syringe pump distributed the washing solution.

AZ) to the outlet of the capture device. An optical window was made by removing the polyimide coating and an absorbance detector (ISCO UV/VIS detector model 229, Lincoln, NE) was used to monitor proteins and peptides eluting from the device. Depending on the flow rate (0.2−1.0 μL/min), 100−1000 V was applied between the gaps in such a manner that the electrode in the upstream chamber was negative relative to the electrode in the downstream chamber. Once the capture and release conditions were optimized, the outlet was left open for direct deposition of samples onto the MALDI target plate.

Injector Setup. A 250 μL Eppendorf tube was used as sample reservoir (Figure 1). The tube was airtight sealed to a homemade T-connection using a stainless steel nut. At the opposite port, a fused silica capillary tube (100 μm i.d. and 360 μm o.d.; Polymicro Technologies, Phoenix, AZ) was inserted in a manner that it almost reached the bottom of the sample tube. To produce a hydrodynamic pressure driven injection, a plastic syringe was attached to the perpendicular port of the T-connection, and the capillary and the syringe were both airtight sealed using microtight fittings from Upchurch. The capillary going out from the T-connection was connected to the six-port valve in the microfluidic device via a PTFE tubing (300 μm i.d. and 1.6 mm o.d.; Upchurch) in a manner to allow switching between washing solution from the syringe pump and sample from the sample reservoir. Using this configuration, a pressure applied with the plastic syringe will push the sample solution through the capillary and inject it into the six-port valve (Figure 1).

System Operation. The general operation of the microfluidic electrocapture device involves four steps: (I) injection, (II) washing, (III) analyte release, and (IV) MALDI plate spotting. (I) The device is filled with washing solution using the syringe pump, and the power supply is turned on. At the same time, to begin the sample injection into the valve (in waste position), a gentle pressure is applied to the plastic syringe. After a few seconds, the valve is switched to inject position. (II) Once the sample is injected, the valve is switched back again to the washing solution position. For peptides, the washing solution is 0.1% TFA, and for proteins 1% acetic acid. (III) The voltage is turned off when the current is similar to that for the washing solution alone. (IV) Fractions corresponding to ~0.4 μL are directly spotted onto the MALDI target and mixed on-plate with matrix (1:1, v/v).

RESULTS AND DISCUSSION

A microscale capture device has been constructed, and a method for sample cleanup in MALDI-MS of peptides and proteins has been developed. The device consists of a microfluidic channel where an electric field captures charged molecules that pass through the system (Figure 1). Once captured, the original buffer is replaced by injecting another buffer/solvent with good analytical performance. Disconnection of the electric field releases the captured molecules, which are now in the new buffer/solvent and can be collected for further analysis.

Using this principle, proteins and peptides in salt- and detergent-containing solutions were captured and prepared for MALDI-MS. The polypeptides were dissolved at pH 2.5, where they normally have a positive net charge. If under these conditions an appropriate combination of electric field strength and flow rate is applied, the positively charged peptides and proteins will resist the hydrodynamic sweeping force and will be captured at the cathode junction (Figure 1). Since the ionization process in MALDI is enhanced by acidic low-salt conditions, the original buffer was changed to 0.1% TFA or 1% acetic acid.

System Optimization. *Practical Considerations.* Since flow rate is the factor dominating sample throughput, optimal conditions were established to allow fast and robust sample cleanup. As the system captures positively charged polypeptides via the interaction between hydrodynamic and electrical forces, an increase in the flow rate has to be followed by an increase of the electric field. Such an increase leads potentially to bubble formation because of excessive Joule heating, thereby constituting a limiting factor of the sample throughput. Due to the relatively large volume between syringe, injector, valve, and device, flow rates below 0.2 μL/min were not used in order to keep the processing time at an acceptable level.

Theoretical Optimization. To make a rational estimate of optimal voltage and flow rate values, the following theoretical considerations were made. In electrophoresis, the velocity of an ion in an electric field is given by the relation

$$V_e = \mu_e E \qquad (1)$$

where V_e is the electrophoretic velocity (cm/s), μ_e is the electro-

phoretic mobility ($cm^2/V \cdot s$), and E is the electric field strength (V/cm). To capture polypeptides in the flow stream, the magnitude of the counteracting V_e vector has to be equal to or higher than the linear velocity of the hydrodynamic flow, V_H (cm/s). From eq 1, we can theoretically establish that the minimum E necessary to capture analytes (when $V_e = -V_H$) is the ratio between V_H and μ_e. This means that, to comply with the condition of capture ($V_e = -V_H$), any increase in the flow rate has to be followed by an increase in E. From experience with CE and from the expected sample characteristics (content of salts and detergents that increase the conductivity), we can predict a considerable Joule heating when an E higher than 200 V/cm is used. With this value and eq 1, we can estimate the practical maximum value for V_e (V_{emax}), and the practical upper limit for V_H (V_{Hmax}), when $V_e = -V_H$ and $E = 200$ V/cm:

$$-V_{Hmax} = \mu_e\ (200\ \text{V/cm}) \qquad (2)$$

To obtain μ_e for a model protein, such as Mb, we used the equations given by Basak and Ladisch,[21] which correlate electrophoretic mobilities of proteins and peptides with their charge and mass values. The value of μ_e was calculated for experimental conditions of 1% acetic acid, (pH 3.0), and the net charge of Mb was estimated using the web site http://www.up.univ-mrs.fr/wabim/d_abim/index.html. For the model protein, μ_e was found to be of the order 2.5×10^{-4} $cm^2/V \cdot s$, which is in accordance with experimental data obtained for other proteins at a similar pH.[22] Using eq 2, the resulting V_{Hmax} is 0.05 cm/s, which in a capillary with 127-μm inner diameter represents a flow rate of 0.38 μL/min. Even though individual proteins have different μ_e values, we expect that at low pH most will be in the same range.

Peptides are expected to have lower μ_e values than those for proteins, in the range 0.7×10^{-4}–2.0×10^{-4} $cm^2/V \cdot s$.[23,24] In this case, a lower V_{Hmax} is expected. For example, typical μ_e values for tryptic peptides of human growth hormone are of the order 1.5×10^{-4} $cm^2/V \cdot s$ at pH 2.35.[25] Using eq 2, the V_{Hmax} for these peptides is then 0.03 cm/s, which corresponds to a flow rate of 0.23 μL/min.

Experimental Optimization. In order not to work close to the highest practical flow rate, 0.3 μL/min was used for proteins and the voltage was set to a value giving good protein recovery. A flow rate of 0.3 μL/min was also reported to give robust results in capturing of DNA and proteins in low-salt-containing solutions.[19,20]

Since the conductive membrane (Figure 1) is transparent when hydrated, the use of a colored protein such as Mb allows visualization of the protein behavior during operation of the system. Already a few seconds after the voltage was turned on, Mb was observed at the upstream cathode junction. After turning the voltage supply off, a substantial amount of the visualized protein was released from the conductive membrane, and reversing the polarity of the electric field for 2 s before turning the voltage off was sufficient to release all of the visible protein from the conductive membrane (data not shown).

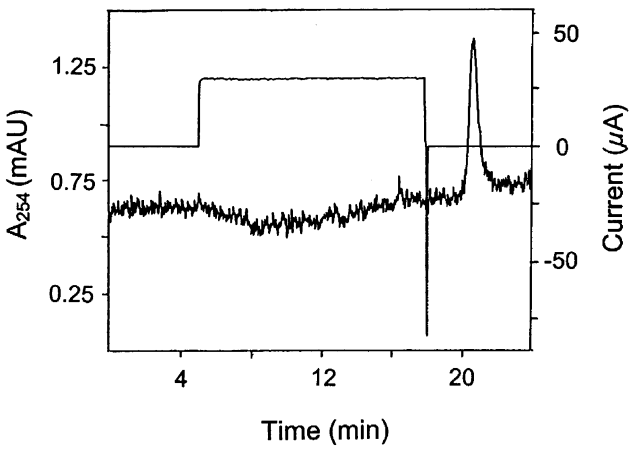

Figure 2. Overall current (upper trace) and UV absorption (254 nm, lower trace) versus time at the outlet of the microfluidic electrocapture device. Myoglobin (0.2 mg/mL) in 1% acetic acid was injected continuously into the system, captured and concentrated (5–18 min), and finally released (from 18 min, following a 2-s reversal of the polarity resulting in a negative spike in the current graph). A distinct absorbance peak corresponding to myoglobin is clearly visible ~2 min after the voltage was turned off.

For a more accurate determination of the conditions for capture, an absorbance detector (UV at 254 nm) was used to monitor protein levels at the outlet of the capture device. Different voltages were tested using continuous injection of Mb in 1% acetic acid. Since the protein is continuously passing the detection window, a slight decrease in the UV baseline will result from protein accumulation in the system after turning the power supply on (Figure 2), indicating that the protein is being captured. To efficiently release the protein into the flow stream after turning the voltage supply off, the polarity of the electric field is at the end of the capturing period switched for 2 s, resulting in elution of a sharp peak corresponding to 400 nL (Figure 2).

Since the conductive membrane has a pore size of a few angstroms,[26,27] we assume that proteins and peptides merely stick to the membrane surface and do not pass through. In addition, since cross-contamination between samples was not observed, we conclude that proteins and peptides are efficiently removed after each analysis.

Protein recovery was obtained indirectly by comparison between theoretical and experimental preconcentration factors. In the experiment shown in Figure 2, 3.9 μL of a 0.2 mg/mL Mb solution was injected (13-min injection at 0.3 μL/min and 120 V/cm) and released in a sharp band of 0.4 μL, giving a theoretical preconcentration factor of ~10. A comparison of the absorbance reading for the protein solution and that for the preconcentrated sample (represented by the peak of the released protein) with that for the solvent background (1% acetic acid) gives a preconcentration factor in the range 8–9 for a 0.2 mg/mL Mb solution. Using these numbers, we can calculate a protein recovery of 80–

(21) Basak, S.; Ladisch, M. R. *Anal. Biochem.* **1995**, *226*, 51–68.
(22) Bergmann, J.; Jaehde, U.; Schunack, W. *Electrophoresis* **1998**, *19*, 305–310.
(23) Janini, G. M.; Metral, C. J.; Issaq, H. J. *J. Chromatogr., A* **2001**, *924*, 291–306.
(24) Adamson, N. J.; Reynolds, E. C. *J. Chromatogr., B* **1997**, *699*, 133–147.
(25) Rickard, E. C.; Strohl, M. M.; Nielsen, R. G. *Anal. Biochem.* **1991**, *197*, 197–207.
(26) Bookman, P.; Nicholson, J. In *Developments in Ionic Polymers*; Wilson A., Prosser H., Eds.; Elsevier Applied Science Publishers: London, 1986; Vol. 2, pp 269–283.
(27) Koter, S. *J. Membr. Sci.* **2000**, *166*, 127–135.

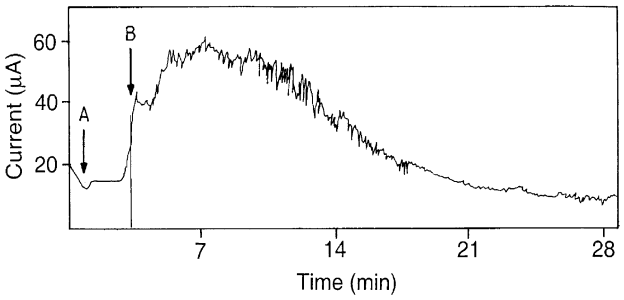

Figure 3. Overall current versus time for a salt-containing protein sample. Conditions: sample, 0.2 mg/mL myoglobin in 50 mM phosphate (pH 2.5), and 40 mM NaCl; flow rate, 0.3 μL/min; electric field, 120 V/cm. (A) Sample injection. (B) Washing solution injection. The slight decrease in current at (B) (shoulder) is caused by a disturbance of the flow rate due to valve switching.

90% for Mb under the experimental conditions described (Figure 2).

With these results, the optimal conditions for capturing proteins were found to be 0.3 μL/min and 120 V/cm. In another set of experiments, using 214 nm for UV absorbance detection, the capture conditions for peptides were optimized in the same manner as for proteins. As expected, peptides required a higher *E* and a lower flow rate to be efficiently captured. The optimal conditions for peptides were found to be 168 V/cm at 0.2 μL/min. The general applicability of these conditions was demonstrated via analysis of tryptic peptides and a protein mixture by MALDI-MS (below).

To assess the time required to perform the buffer/solvent exchange, a plug of salt solution containing the protein was injected and the current was monitored over time. A plot of current versus time for a salt-containing sample typically reveals an increase in the value for the current over that for the washing solution (1% acetic acid) and indicates that the salt-containing solution is entering into the electric field (Figure 3). On the other hand, a decrease in the overall current value in relation to that for the washing solution means that the salt-containing solution has passed through the system and is exchanged for the washing solution. For proteins, the procedure takes ~20 min at 0.3 μL/min (Figure 3) and for peptides ~30 min at 0.2 μL/min.

The capture device is easy to build (~30 min) and can be used for several months if properly cleaned and moderate currents are used (<200 μA). In fact, all the experiments described in this report were carried out using the same device.

Evaluation of Sample Cleanup and Comparison with a Standard Method. *Peptide Analysis.* MALDI-TOF mass spectra resulting from analysis of tryptic peptides in 50 mM phosphate buffer containing 40 mM NaCl and 0.05% SDS (Figure 4) normally present several sodium adduct peaks (Figure 4A). For digests of small proteins (e.g., Mb,16 kDa), the number of peptides is fairly low, and in the resulting spectra, it is usually possible to discern between sodium adducts and the corresponding $[M + H]^+$ peaks. However, for spectra of higher complexity that result from digestion of large proteins (e.g., BSA, 67 kDa), the task is difficult, time-consuming, and sometimes impossible due to overlaps between $[M + Na]^+$ and $[M + H]^+$ signals (Figure 4A). Using

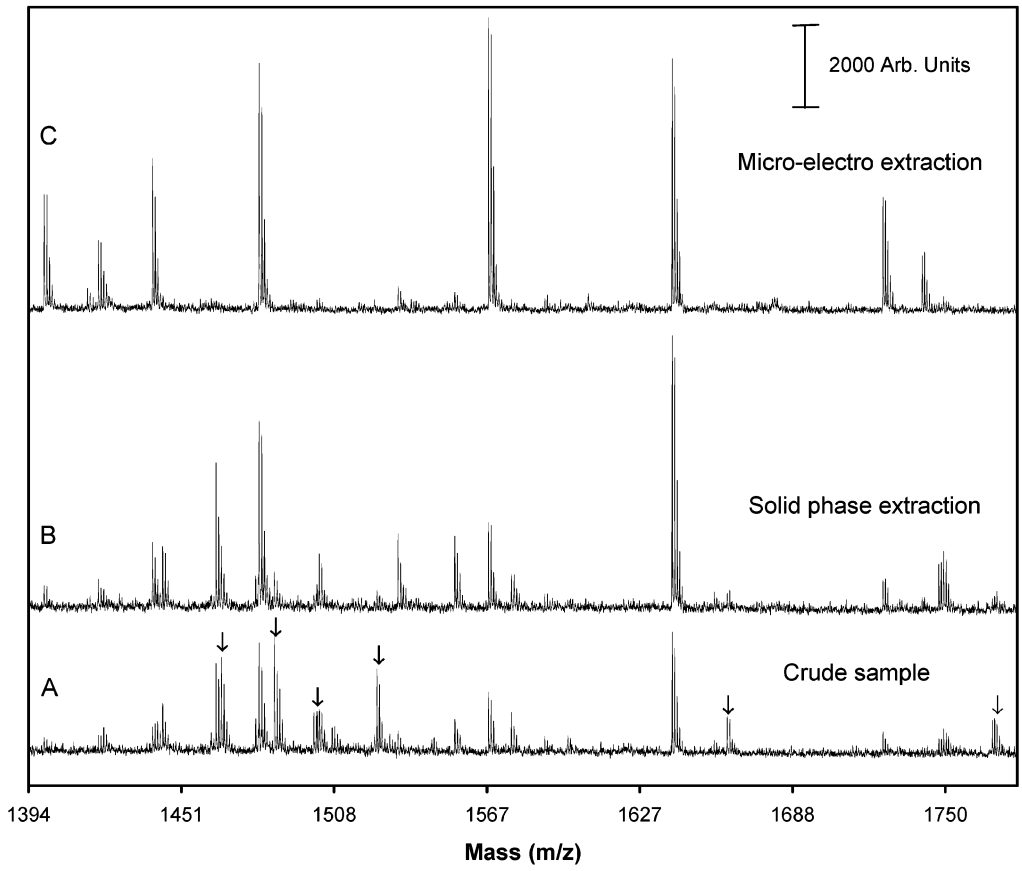

Figure 4. MALDI-TOF spectra for BSA tryptic digest (0.1 pmol/μL) containing 50 mM phosphate buffer, 40 mM NaCl, and 0.05% SDS. (A) Crude sample without desalting, (B) sample desalted using conventional C-18 solid-phase extraction, and (C) sample desalted using the microfluidic electrocapture device. Signals from peptide/sodium adducts are indicated by arrows. Matrix was α-cyano-4-hydroxycinnamic acid.

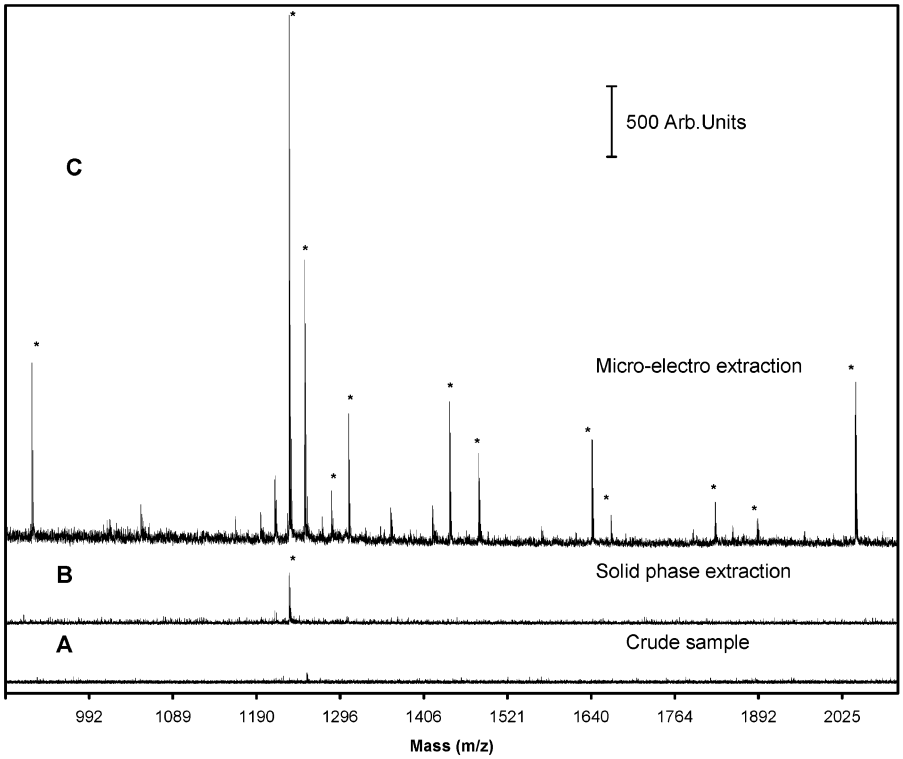

Figure 5. MALDI-TOF spectra of BSA tryptic digest (0.1 pmol/μL) containing 50 mM phosphate buffer, 10 mM CHAPS, and 40 mM NaCl. (A) Crude sample without desalting, (B) sample desalted using conventional C-18 solid-phase extraction, and (C) sample desalted using the microfluidic electrocapture device. Asterisks denote signals corresponding to BSA tryptic peptides. Matrix was α-cyano-4-hydroxycinnamic acid.

the microfluidic electrocapture device, a significant desalting effect was observed and the sodium adducts were essentially removed after the buffer exchange (Figure 4C). In addition, more intense peptide signals were always observed in the desalted/preconcentrated samples (Figure 4C).

A comparison of the mass spectrum resulting from analysis of 0.5 pmol of BSA tryptic peptides treated by RP-SPE (Figure 4B) with the mass spectrum generated from 0.2 pmol of BSA tryptic peptides treated by the electrocapture device (Figure 4C) reveals that signal intensities are the same or even higher after microfluidic electrocapture although less than half the amount of protein digest was applied. Therefore, we can conclude that the peptide recovery is similar to and even better than that obtained in RP-SPE using the current technique, i.e., significantly greater than 70%, which has been reported for C-18 μZipTips,[28] but further studies using a wider range of proteins are needed to confirm this point. In addition to these observations, slight differences regarding the spectral profiles were also seen. They are likely to reflect the different principles the methods are based on. The device described uses charge to retain the peptides in the system, while in RP-SPE, hydrophobic interaction is employed. In general, using the electrocapture device, protein digests can be routinely processed down to, and even below, the 100-fmol-level, and tests with tryptic peptides in this range resulted in high-quality mass spectra with signal-to-noise ratios of up to 50:1. In the mass mapping experiments, for 0.5 pmol of Mb, 0.2 pmol of BSA, and 0.5 pmol of ADH, 30, 38, and 22% of the protein sequences were covered, respectively, using a mass accuracy of 0.1 Da. The crude samples did not give a positive identification. RP-SPE resulted in similar coverages (32, 35, and 20%, respectively, within 0.1 Da).

Although 3−4 times less intense, peptide signals could be observed in the presence of phosphate and NaCl at millimolar concentrations, but the situation changed dramatically in the presence of CHAPS detergent (Figure 5). No significant signals were obtained for the crude sample (Figure 5A), and C-18 SPE did not improve the analysis very much (Figure 5B). In contrast, treatment in the microfluidic electrocapture device successfully removed CHAPS and resulted in excellent MALDI-TOF spectra (Figure 5C). It should be noted that, in addition to CHAPS removal, it was possible with the device to desalt the sample to such an extent that in the resulting spectrum essentially no sodium adduct peaks were observed (Figure 5C). Sequence coverage in the MALDI-TOF analysis of BSA and Mb tryptic digests using ~1 pmol protein was in the range 30−45% (mass accuracy 0.1 Da). Control samples treated with RP-SPE prior to MALDI-MS did not generate a positive identification.

Protein Analysis. A protein mixture composed of BSA, myoglobin, and β-casein in 50 mM phosphate buffer with 10 mM CHAPS and 40 mM NaCl was tested in MALDI-MS without prior desalting, after SPE treatment, and following microfluidic electrocapture (Figure 6). The results reveal no signals after application of the crude sample (Figure 6A) and only very faint signals after SPE (Figure 6B), while a significant improvement is achieved using the capture device (Figure 6C). For protein analysis, CHAPS removal appears to be crucial to obtain a useful spectrum. SPE does not remove CHAPS sufficiently well, and MALDI-MS gives a spectrum with weak protein signals (Figure 6B). The data generated after microfluidic electrocapture indicate efficient

(28) Stewart, I. I.; Thomson, T.; Figeys, D. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 2456−2465.

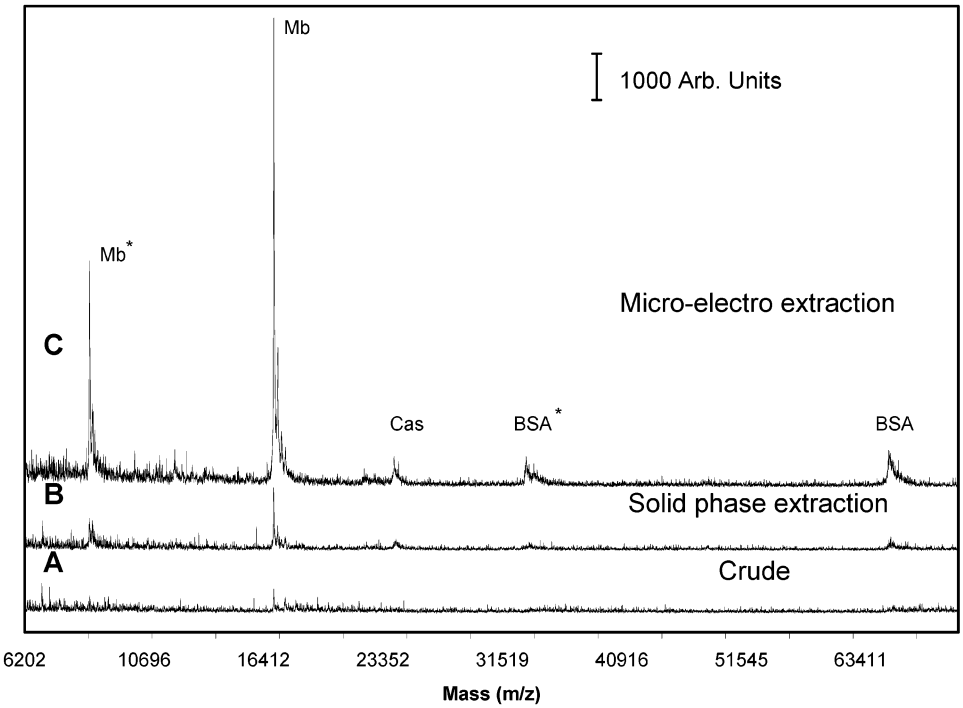

Figure 6. MALDI-TOF spectra of proteins. Mb 0.7 pmol/μL, Cas 2.6 pmol/μL, and BSA 13 pmol/μL in 50 mM phosphate buffer containing 10 mM CHAPS and 40 mM NaCl. (A) Crude sample, (B) solid-phase extraction (C-18) for sample cleanup, and (C) sample treated in the microfluidic electrocapture device prior to MALDI-MS. Asterisk indicates that the signal corresponds to the doubly charged species. Matrix was sinapinic acid.

CHAPS removal allowing adequate determination of the molecular weights for the proteins analyzed (Figure 6C).

In conclusion, a novel microfluidic electrocapture device for sample preparation in protein and peptide analysis by MALDI-MS has been developed. A significant advantage of the capture device over SPE was seen. The conditions for capture were set to allow a wide range of salt concentrations for highest flexibility and general applicability. The system is easily implemented into microfluidic channels and is suitable for incorporation into microchips for single-channel or multichannel sample preparation.

ACKNOWLEDGMENT

This work was supported by grants from the Swedish Research Council, the Swedish Cancer Society, Vinnova, and Karolinska Institutet. We are grateful to Dr. Harold Swerdlow, Solexa Ltd., Essex, U.K., for fruitful discussions.

Received for review March 6, 2003. Accepted July 8, 2003.

AC0300901