On-chip microextraction for proteomic sample preparation of in-gel digests

Despite the high sensitivity and relatively high tolerance for contaminants of matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) there is often a need to purify and concentrate the sample solution, especially after in-gel digestion of proteins separated by two-dimensional gel electrophoresis (2-DE). A silicon microextraction chip (SMEC) for sample clean-up and trace enrichment of peptides was manufactured and investigated. The microchip structure was used to trap reversed-phase chromatography media (POROS R2 beads) that facilitates sample purification/enrichment of contaminated and dilute samples prior to the MALDI-TOF MS analysis. The validity of the SMEC sample preparation technique was successfully investigated by performing analysis on a 10 nM peptide mixture containing 2 M urea in 0.1 M phosphate-buffered saline with MALDI-TOF MS. It is demonstrated that the microchip sample clean-up and enrichment of peptides can facilitate identification of proteins from 2-DE separations. The microchip structure was also used to trap beads immobilized with trypsin, thereby effectively becoming a microreactor for enzymatic digestion of proteins. This microreactor was used to generate a peptide map from a 100 nM bovine serum albumin sample.

Keywords: Microextraction / Microreactors / Microtechnology / Sample preparation PRO 0206

1 Introduction

Although new methods for differential protein expression analysis such as isotopic labelling in combination with mass spectrometry [1–5] are gaining ground as an alternative to 2-D gel comparisons, the backbone of proteomics research is still 2-D electrophoresis [6]. While this separation technique gives a display of the protein expression, it gives no information about the identity of the individual proteins. Protein identification can be carried out by traditional techniques like immunoblotting and chemical sequencing, but these techniques are considered too slow and labour intensive for the new proteomic needs. Currently, one of the most commonly used ways to identify and learn about the primary structure or different post-translational modifications in proteins is by the use of 2-DE and mass spectrometry, i.e. peptide mapping [7–11]. The recent development within 2-D–capillary liquid chromatography (2D-C109 LC) interfaced to electrospray MS/MS as a complement to 2-DE and the emerging field of microtechnology [12–14] have proven the benefits of miniaturization. The commercial initiatives of miniaturizing protein analysis are now also rapidly expanding, c.f. the protein separation chip recently launched by Agilent Technologies (Palo Alto, CA, USA).

The driving force for the rapid development of miniaturized technologies within proteomic and bioanalytical research is the improved possibilities to efficiently handle the actual amounts of biological material that are needed to successfully complete a protein expression study. The benefits widely sought are two-fold: (a) improved biological readout, and (b) minimized amount of starting material e.g. cell cultures or tissue samples. The development within pharmaceutical, biotechnology companies and academic research institutes in the world shows that miniaturization of analytical systems combined with intelligent use of biology is generally considered to be a strategy that will aid in overcoming the hurdles in qualitative proteome analysis.

Mass spectrometry has become the most versatile and important technique for protein analysis, owing to the high speed, sensitivity, selectivity and throughput capacity provided [15]. MALDI-TOF MS is one of the most frequently used MS techniques for biomolecules, such as peptides, proteins and oligonucleotides, due to its inherent simplicity, low sample consumption and high sensitivity [16, 17]. The sensitivity of this mass spectrometry (MS) technique allows analysis of attomole amounts
of sample molecules. MALDI-TOF MS is probably also the most contaminant tolerant MS technique. However, in order to obtain useful mass spectra from small volumes of very dilute samples (such as in-gel digests in the low nanomolar range) it is necessary to minimize the contaminants and simultaneously enrich the analyte.

Thus, there is often a need to concentrate and most importantly purify sample solutions before the MALDI-TOF MS analysis. This can be done in a number of different ways, e.g. by deploying a chromatography step prior to MS analysis, or by use of membranes [18–21] (PVDF or similar) or different monolayers [22, 23] onto which the analytes bind, followed by a washing step prior to the analysis. One of the most effective and simple ways to concentrate and purify minute amounts of proteins/peptides is by the use of reversed-phase chromatographic beads. These beads can either be added to the dilute sample solution to absorb the analytes, the beads are then deposited onto the MALDI targets [24, 25] or they can be packed into a miniature column, as is common in microscale sample purification/concentration [26–28]. The micro RP column can be made from a GelLoader tip (Eppendorf, Germany) that is carefully squeezed with a pair of pliers, to reduce the inner diameter to a diameter less than the size of the particles that one wishes to trap. This allows it to be filled with a stationary phase such as POROS R2 to a bead volume of 0.05–1 µL. Equilibration, sample, wash and elution solutions are then manually introduced with the aid of a pipette and syringe.

This technique has proven to be very useful and is today commonly used in many MS laboratories. There are also commercially available alternatives, such as the ZipTip (Millipore, Bedford, MA, USA), which together with automated robotics can be used to perform automated protein analysis. This type of sample clean-up/enrichment can often provide the “sensitivity increase” that is necessary for identification of low abundant protein spots by MALDI-TOF MS. The drawbacks of this technique is the manual work associated with making the microcolumns, handling the sample and in the case of the commercially available ZipTip the “high” cost of each tip. An alternative to the tip based microcolumns is to use a capillary [29–31] or a microstructure packed with chromatographic media. Recently Oleschuk et al. [32] presented a microstructure manufactured in Corning 0211 glass for trapping RP beads in a chip used for on-chip solid-phase extraction prior to electrophorescopraphy. The use of bead trapping microchip structures has also been presented for performing immunooassays [33, 34]. Furthermore, a polymeric microstructure prepacked with RP beads for peptide clean-up/enrichment in a rotating CD format is currently being commercialized by Gyros (Uppsala, Sweden).

The use of a microstructure for capturing beads has the advantage that it can easily be repacked and connected on-line to other equipment. Another advantage of using microstructures is that unnecessary sample transfers can be avoided, something which due to adsorption is of great importance when handling dilute protein/peptide samples. In earlier studies [35, 36] we have made a number of silicon microstructure developments that have enabled the handling of minute amounts of sample, and allowed us to obtain extended sequence information, as well as increased sensitivity. In order to obtain identities of even lower abundant proteins, these microanalysis techniques could benefit from the incorporation of a microscale sample clean-up and enrichment step [37]. Therefore we have developed a silicon microchip structure that allows trapping of various stationary phase beads for sample clean-up/enrichment prior to MALDI-MS analysis. It is also possible to use this type of microstructure as an immobilized enzyme reactor (IMER) by trapping beads that have been immobilized with enzymes, as demonstrated by Wang et al. [38].

2 Material and methods

2.1 Chemicals

DTT (Lot 45H007215), iodoacetamide (Lot 25H8452), trypsin type IX, porcine pancreas (Lot 25H0359), albumin bovine (Lot 53H00665) and α-cyano-hydroxycinnamic acid (CHCA) (Lot 51229) were all purchased from Sigma (St. Louis, MO, USA). HCl, acetone, methanol, NH4HCO3, KOH, HF, CaCl2, KH2PO4, Na2HPO4, NaCl, acetonitrile (ACN) were from Merck (Darmstadt, Germany), and used without any further purification. Angiotensin I, ACTH clip 1–17, ACTH clip 18–39 and ACTH clip 7–38 were all part of mass standard kit no: 2–3143–00 (Lot 7022801) (Perseptive Biosystems, Framingham, MA, USA). The water was purified using a MilliQ apparatus (Bedford, MA, USA). All chemicals were used without further purification and all buffers used were freshly prepared.

2.2 Microstructure fabrication

All silicon processing followed standard procedures for UV photolithography and wet etching of silicon. The silicon used was 3” p-doped (1–20 µcm) <100>-type single-side polished. The silicon micro extraction chip (SMEC) utilized a weir in the v-groove shaped flow channel as a bead trap. Briefly, the fabrication procedure was
Figure 1. Process steps for fabricating the bead trapping microchip.

as follows (Fig. 1). The wafer was wet oxidized for 4 h in 1100°C creating a 1 μm thick oxide layer on the silicon surface. The oxidized wafer was subsequently coated with photoresist by spin coating. The pattern on the glass mask, defining the channels (330 μm wide) on both sides of the weir was then transferred to the photoresist by UV exposure (Fig. 1A). The following developing step of the photoresist, removed the exposed part of the photoresist (Fig. 1B). The wafer was then exposed to buffered HF, which etched the silicon dioxide where the photoresist was developed (Fig. 1C). After cleaning the wafer with acetone and ethanol an anisotropic etching process in a KOH solution was performed forming 200 μm deep etched v-grooves channels (Fig. 1D). Another lithographic process of the silicon wafer was carried out to open up the oxide layer on top of the weir (Fig. 1E). The silicon wafer was subsequently etched in KOH, (40g KOH/100 mL water at 80°C), forming the finalized weir, defining a 10 μm flow path below the original surface (Fig. 1F). The wafers were then diced and a glass lid was anodically bonded to the original surface, creating a sealed flow channel including the bead trap (Fig. 2).

2.3 Fluid connections

Low dead-volume liquid connections were obtained by inserting capillaries (od 170 μm, id 100 μm) 2–3 mm into the SMEC inlet and outlet flow channels. These were secured by applying a small amount of epoxy resin (5 min epoxy; Devcon, Danvers, USA) to each channel. The epoxy resin was then automatically drawn into the channel by capillary force. This provided secure liquid connections that were able to endure high pressure without leaking.

2.4 Packing of beads in the microextraction chip

The microchip structures were filled with POROS 20 R2 beads (Applied Biosystems, Foster City, MA, USA), in this reversed-phase media, the bonded phase is an extremely nonpolar surface. By using a mobile phase that is polar, usually water or an aqueous solution, nonpolar or hydrophobic molecules (almost all peptides and proteins) bind preferentially to the stationary phase and the polar molecules (salts and other contaminants) remain in the mobile phase. The microstructure was packed with beads by attaching a syringe to the outlet of the microchip and slowly aspirating a dilute (1 mg/mL) suspension of POROS 20 R2 beads in methanol. The packing procedure was inspected under a microscope via the bonded glass lid. Typical bead volumes were 100–300 nL.

2.5 Microchip sample purification and concentration

Figure 3 depicts the experimental set-up that was used for investigation of the microstructures. A CMA/100 pump (CMA/microdialysis, Solna, Sweden) was used to generate the carrier flow (mobile phase 0.1% TFA). The syringe pump provided the carrier flow for wetting, equilibration, sample and elution fluids. When performing the sample clean-up/enrichment, the injection loop (volumes ranging between 1 and 50 μL) was filled with sample and injected into the microchip. Elution of the bound molecules from the RP beads was accomplished by reducing the polarity of the mobile phase, allowing the hydrophobically bound molecules to partition off the bonded phase surface. In this case, the elution was achieved by passing a water-miscible organic solvent, acetonitrile, through the column. If the matrix, necessary for the MALDI-TOF MS analysis, is added to the elution fluid the elution of the analyte molecules can be done directly onto the MALDI target plate.
2.5.1 Protocol for the sample clean-up/enrichment

(1) Wetting 50 μL of ACN/0.1% TFA, 8:2; (2) Equilibration 50 μL of 0.1% TFA, (3) Sample injection, acidified —0.1–1% TFA, (4) wash 50 μL, 0.1% TFA, (5) elution of the analytes onto the MALDI target with 1–3 μL, 70% ACN/0.1% TFA containing 0.5–5 mg/mL of CHCA. To ensure maximal binding of the analyte all samples were acidified with TFA to a final concentration of 0.1–1% TFA. Standard peptide mixtures for investigation of the microstructure performance was prepared in 0.1% TFA unless otherwise specified. For comparative reasons some samples were also analysed (prior to SMEC sample preparation) utilizing a seed-layer sample preparation [39, 40], in which the sample mixed 1:1 with matrix (50% ACN/0.1% TFA containing 10 mg/mL of CHCA) was deposited with a micro-pipette onto a thin layer of matrix (1 mg/mL of CHCA in 100% ACN).

2.6 Microchip enzyme reactor

By trapping beads that have enzyme immobilized on their surface, the microchip can be used as an enzyme reactor (IMER). Immobilization of trypsin onto 20 μm cross-linked polystyrene-divinylbenzene) beads activated with aldehyde, POROS-AL, was achieved by preparing a suspension of these beads in a solution of trypsin 4 mg/mL in 0.1 M PBS, pH 7.0. In order to reduce the imine-bindings that are formed during the coupling reaction, a small amount of NaCNBH₃ was added to the suspension. The coupling reaction, was then allowed to proceed for 16 h at room temperature. After the coupling reaction the beads were washed with 0.1 M PBS, pH 7.0 and packed into the microstructure.

2.7 In-gel digestion

Cell lysate from TGF-β1 stimulated human lung fibroblasts (HFL-1) was used as a cell model to investigate the protein expression patterns during kinetic profiling. Approximately 50–100 μg of the cell lysate was separated by 2-DE. The IEF step was performed in an IPGphor (Amersham Pharmacia Biotech, Uppsala, Sweden) and the second-dimension was carried out on 14% homogeneous Duracryl slabgels (genomic Solutions, Ann Arbor, NY, USA). The proteins were visualized with silver staining and the in-gel digestion of the excised spots was performed according to Schvchenko et al. [41]. After in-gel digestion of a protein spot, the resulting peptide solution was acidified by addition of TFA prior to the extraction/sample clean-up.

2.8 Mass spectrometry

The MALDI-TOF instrument used was a Voyager DE-PRO (Perseptive Biosystems, Framingham, MA, USA). The instrument was equipped with a linear flight tube of 1.1 m, delayed extraction ion source, and used a nitrogen laser (λ = 337 nm) with a laser focal spot diameter of approximately 100 μm. The ion acceleration voltage used was 20 kV. Peptide mass mapping was performed in the positive ion reflector mode, using the Data Explorer MS software (Applied Biosystems). Two-point internal mass calibration was used in the peptide mapping experiments with the peptide fragments (M, 842.51 and 2211.10) originating from autodigestion of trypsin. The protein identification was performed by peptide mapping using the database search program MASCOT [42]. The database search allowed for 50 ppm mass accuracy and one missed cleavage site.

3 Results

3.1 Enrichment/sample clean-up of model peptides

The experimental set-up described in Section 2.5 was used for investigation of the microstructures. The sensitivity of the SMEC sample preparation was investigated with a dilution series of four standard peptides (angiotensin I, ACTH Clip 1–17, ACTH Clip 18–39 and ACTH Clip 7–38) in 0.5% TFA. The samples were loaded onto
Figure 4. Mass spectra resulting from the MALDI-TOF MS analysis of a 10 nM peptide mixture containing 2 M urea in 0.1 M PBS. (A) Mass spectrum obtained without microchip sample clean-up/enrichment, and (B) mass spectrum obtained after microchip sample clean-up/enrichment of 10 μL sample solution.

Table 1. Sensitivity of SMEC sample preparation

<table>
<thead>
<tr>
<th>Conc. of the 20 μL sample loaded (fmol/μL)</th>
<th>Total amount (fmol at 100% recovery)</th>
<th>Detected in MALDI analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>200</td>
<td>all 4 observed</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>all 4 observed</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>all 4 observed</td>
</tr>
</tbody>
</table>

the microextraction chip according to the given protocol and eluted in one spot using a solution of 0.5 mg/mL CHCA in 70% ACN/0.1% TFA. The result is presented in Table 1. Using a 1 nM peptide mixture and a loading of 20 fmol, all four peptides were clearly detected in the MS analysis. Although it should be noted that, using peptide solutions with concentrations below 1 nM, the SMEC sample preparation resulted in no or poorly reproducible mass spectra. The mass spectra in Fig. 4 shows the MS analysis of a 10 nM peptide mixture containing 2 M urea in 0.1 M PBS. Figure 4A shows the sample solution as deposited prior to microextraction on the MALDI target plate by the dried droplet seed-layer technique, where no peptides could be detected. Figure 4B shows the corresponding mass spectrum when the SMEC preparation was used.

3.2 Application to proteomic samples

One of the main uses of this type of sample clean-up/enrichment is in the analysis of in-gel digested 2-DE spots. Two previously identified gel spots (actin and ATP synthase, beta chain) were excised from the gel and subjected to the SMEC sample preparation. After in-gel digestion of the protein spots with trypsin, 1 μL of the resulting peptide solution was used to make a seed-layer sample preparation. The remaining solution was acidified by the addition of 0.1% TFA (100 μL total volume) and subjected to extraction/sample clean-up on the microchip. The mass spectra resulting from the analysis of the ATP synthase spot is presented in Fig. 5 and that of the

Figure 5. (A) Mass spectrum obtained by seed-layer sample preparation of the digest supernatant before micropurification, where no peptides could be observed. (B) Mass spectrum resulting from the microchip sample clean-up/enrichment of the in-gel digested ATP synthase spot. The spot was correctly identified by database search as human ATP synthase, beta chain (SWISS-PROT acc. No. P06576).
actin in Fig. 6. In neither case did a seed-layer sample preparation made prior to dilution 0.1% TFA provide identification of the proteins. After the microchip sample clean-up/enrichment, the spots could be correctly identified by database search.

In a specific study that was undertaken to investigate the SMEC performance using biological samples derived from 2-D gels, twenty spots were excised from a 2-D gel separation of human fibroblast cell lysate, for details see Section 2.7. Extracts (~20 μL) of the gel supernatants were loaded on the SMEC, washed and eluted onto the MALDI target plate with matrix (0.5 mg/mL CHCA in 70% ACN/0.1% TFA) as discrete spots of 0.5 mm diameter. Proteins were identified by the software, MASCOT, where a MOWSE score above 62 (significant p < 0.05) was considered to provide a correct identity. The identification results are shown in Table 2.

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein id.</th>
<th>Acc. No.</th>
<th>MOWSE score</th>
<th>No of id. peptides</th>
<th>Sequence cov. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A12</td>
<td>Sorting Nexin 4</td>
<td>O952119</td>
<td>52</td>
<td>6</td>
<td>18.2</td>
</tr>
<tr>
<td>B12</td>
<td>Calumenin precursor</td>
<td>O43852</td>
<td>97</td>
<td>10</td>
<td>21.3</td>
</tr>
<tr>
<td>C12</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D12</td>
<td>Ubiquitin carboxy-terminal hydrolase L1</td>
<td>AAD09172 (P09939)</td>
<td>83</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>E12</td>
<td>Cathepsin D precursor</td>
<td>P07339</td>
<td>97</td>
<td>10</td>
<td>29.4</td>
</tr>
<tr>
<td>F12</td>
<td>Phosphoglycerate mutase</td>
<td>P18669</td>
<td>127</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>G12</td>
<td>Triosephosphate isomerase</td>
<td>P00938</td>
<td>138</td>
<td>11</td>
<td>34.4</td>
</tr>
<tr>
<td>H12</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>A11</td>
<td>Vimentin</td>
<td>P08670</td>
<td>159</td>
<td>14</td>
<td>48.3</td>
</tr>
<tr>
<td>B11</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C11</td>
<td>Glutathione transferase omega 1</td>
<td>P78417</td>
<td>49</td>
<td>4</td>
<td>30.8</td>
</tr>
<tr>
<td>D11</td>
<td>NUCELAR CHL</td>
<td>O00299</td>
<td>67</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>E11</td>
<td>Serum albumin</td>
<td>P02769</td>
<td>142</td>
<td>15</td>
<td>39.5</td>
</tr>
<tr>
<td>F11</td>
<td>NA</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>G11</td>
<td>RHO GDP-Dissociation inhibitor (RHO GD1)</td>
<td>P52565</td>
<td>62</td>
<td>6</td>
<td>17.1</td>
</tr>
<tr>
<td>H11</td>
<td>Annexin V (Lipocortin V)</td>
<td>P08758</td>
<td>62</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>A10</td>
<td>Annexin V</td>
<td>P08758</td>
<td>162</td>
<td>13</td>
<td>41.9</td>
</tr>
<tr>
<td>B10</td>
<td>Annexin V</td>
<td>P08758</td>
<td>83</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>C10</td>
<td>40S Ribosomal protein (40P)</td>
<td>P08865</td>
<td>82</td>
<td>12</td>
<td>16.8</td>
</tr>
<tr>
<td>E10</td>
<td>NA</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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</tbody>
</table>
As can be seen from Table 2, 13 (65%) of the proteins were identified with a score above 62, as denoted by the software MASCOT. Out of the seven unidentified spots, two of these displayed mass spectra that were close to significant identities, and three spots gave peptides but were not identified. Only two spots did not result in any peptides at all. The database search was performed with a peptide mass tolerance of 50 ppm. Most of the identified proteins were determined at an average mass accuracy level of 10 ppm. Some examples of proteins and their corresponding mass spectra are shown in Fig. 7 a–c.

![MALDI spectra of Cathepsin D precursor, Annexin V, and 40 S Ribosomal protein. T, trypsin autolysis internal calibrants; *, identified peaks.](image-url)
3.3 Microchip enzyme reactor

The ability to easily pack microchip structures with beads opens the possibility to use them as immobilized enzyme reactors (IMER) by the trapping of enzyme activated beads. A microchip IMER was made by packing the microchip with POROS beads immobilized with trypsin. The microchip IMER was then used to digest a 1 μL sample of reduced and alkylated BSA at a concentration of 100 nM in 25 mM Tris, 1 mM CaCl₂, pH 8.0. The 1 μL sample plug was injected and a carrier flow of digestion buffer transported the plug through the microchip IMER, the eluate was collected in an Eppendorf tube and then subjected to microscale sample clean-up/enrichment on a microchip packed with POROS 20 R2 as described in Section 2.4. The resulting mass spectrum is presented in Fig. 8.

4 Discussion

There are several positive aspects associated with the use of a microstructure to capture beads for sample clean-up/enrichment. The microchip provides similar performance as the methodology where a GelLoader tip (Eppendorf) is used to trap the beads, but requires less manual work and the microchip is reusable since it can be unpacked and repacked. Unpacking and repacking also makes the microchip a cheaper solution in the long run than the commercially available pipette tips packed with chromatography media.

During initial experiments it became evident that packing the microstructure with beads by injecting the bead material was not the best procedure, since the beads tended to precipitate from the solution. Thus, any difference in size between the fluid connections led to the packing of beads in the tubing interconnects or valve instead of the bead-trapping zone of the microstructure. This problem was avoided by suction loading of the beads. The disadvantage with the suction loading of the current design of the microchip is that it requires the attachment of a syringe to the inlet to elute the sample and is difficult to automate. One problem with the presented SMEC is that the eluted sample plug becomes rather long due to dispersion in the structure. This could probably be corrected by development of new designs of the microchip with less pressure-drop over the bead-trapping zone and matching the channel size to that of the outlet capillary. Also, the sample deposition of the eluted analyte could probably be improved by combination with microdispenser deposition [36] or prestructured MALDI targets [43], providing a higher sensitivity. The SMEC sample preparation was successfully applied to samples in the 1 nM range, but was unable to reproducibly handle samples containing concentrations below 1 nM. This is probably due to surface adsorption of the analytes, which becomes critical in the very low nanomolar range. There are at least two ways to counter the surface adsorption, either by surface modification of the tubing and microstructure or better by deploying the same methodology as where beads are added directly to the sample solution. The beads with “trapped” analyte can then be packed into the microstructure. By this methodology the analyte is protected from adsorption during the transfer to the bead-trapping zone, i.e. the adsorption of analyte to the bare silica walls of the capillary and microchip is minimized. But with the microchip the deposition of beads on the MALDI target that might give rise to low resolution (if the beads are large) is avoided and it is also easier to achieve a small spot size containing all the eluted analyte. Another advantage of the microchip is that it can be integrated with other on-chip sample manipulations for further increase of
sensitivity. As demonstrated by the digestion of BSA, the microstructure can be used as a microreactor. It is also possible to couple several of these microchip structures in serial, each containing a different type of bead (ion exchange material, metal chelate beads or beads immobilized with antibodies or enzymes).

In the peptide mapping experiment 13 out of 20 gel spots were identified. This corresponds to a 65% identification rate, which is what can be expected in typical cases [44]. The sensitivity tests with the standard peptides indicate that lower sensitivities can be reached, especially with the implementation of the discussed improvements of the system.

5 Concluding remarks

We have demonstrated that the use of a microchip to trap beads for sample clean-up and enrichment of contaminated sample solutions containing low concentrations of analyte offers several advantages. The easy packing of the beads in the microchip enables a multitude of different applications for this type of sample preparation, and the on-line format and modular design allows coupling to other microstructures either by tubing or in integrated form. It is foreseeable that with some improvements this type of microchip structure may become a flexible and efficient tool in sample preparation for mass spectrometry.

This work was supported by grants from: The Carl Trygers Foundation, The Crafoord Foundation, and The Swedish Research Council and The Swedish Foundation for Strategic Research.

Received December 20, 2001

6 References


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