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Protein identification platform utilizing micro dispensing technology interfaced to matrix-assisted laser desorption ionization time-of-flight mass spectrometry

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Abstract

An integrated protein microcharacterization/identification platform has been developed. The system has been designed to allow a high flexibility in order to tackle challenging analytical problems. The platform comprises a cooled microautosampler, an integrated system for microcolumn HPLC, and a capillary reversed-phase column that is interfaced to matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) system via a low internal volume flow-through microdispenser. The chromatographic separation is continuously transferred onto a MALDI target plate as discrete spots as the dispenser ejects bursts of droplets of the column effluent in a precise array pattern. A refrigerated microfraction collector was coupled to the outlet of the flow-through microdispenser enabling enrichment and re-analysis of interesting fractions. The use of target plates pre-coated with matrix simplified and increased the robustness of the system. By including a separation step prior to the MALDI-TOF-MS analysis and hereby minimizing suppression effects allowed us to obtain higher sequence coverage of proteins compared to conventional MALDI sample preparation methodology. Additionally, synthetic peptides corresponding to autophosphorylated forms of the tryptic fragment 485–496 (ALGADDSYYTAR) of tyrosine kinase ZAP-70 were identified at sensitivities reaching 150 amol.

Keywords: Instrumentation; Matrix-assisted laser desorption ionization mass spectrometry; Interfaces, LC–MS; Proteins

1. Introduction

The fast development in protein identification is strongly driven by the mapping of the human genome program. Commercial initiatives currently claim the HUGO (human genome) project to be completed within 2 years thanks to the rapid development of novel technologies. Today's pathophysiological findings form the basis for a lot of research activities undertaken in order to localize

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and identify regulatory pathways. Additionally, biomolecules responsible for biological actions related to diseases are currently a highly active research field. Analyzing protein expressions derived from a cell culture, a tissue or some other biological material of relevance is of increasing interest to gain better understanding of biological key events. In the field of proteomics protein regulations are analyzed at a given time in a given biological sample [1]. Not only is the total protein expression and its regulation of interest and importance in drug discovery and development, but also identifying regulating proteins with key functions that are considered as targets. This can be made in a solubilized cell extract by ''target fishing'' using affinity techniques, e.g., antibodies for a specific group of proteins, whereby the proteins of interest are isolated [2,3]. Inherently protein technology as such is more complex than DNA-based technology, not only due to the larger number of amino acids compared to four nucleotides, but also the fact that there are so many ways in which proteins can be modified after they have been synthesized. Post-translational modifications, e.g., the addition/subtraction of phosphate, carbohydrate, sulfate, methyl, acetyl or lipid groups, and the fact that there are proteins that never will be coded, increase the biological complexity dramatically [4]. In order to obtain high sensitivity and to some extent high-throughput, an increasing number of technologies based upon quantitative analysis of protein expressions are being developed with a clear focus on miniaturized liquid chromatography coupled to mass spectrometry. Mass spectrometric techniques will allow the use of quantitative protein level measurements of gene expression in order to understand the mechanisms of protein regulation. Comparing theoretical masses of peptide map fingerprints in sequence databases currently available with measured masses from enzymatically or chemically digested proteins obtained by mass spectrometry, are frequently used in the identification process of proteins [5]. A pre-requisite is that the origin of the protein is known. Unknown proteins can still be found in the large sets of expressed sequence tags available in public, and company databases. At the current state peptide mapping by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is an attractive technique offering high throughput, biocompatibility and sensitivity. The MALDI technique is currently reaching the confidence level comparable to sequencing techniques due to improved mass accuracy and sample preparation methodologies available [6,7]. The development of droplet dispensers for sample handling is currently making its way into analytical chemistry [8]. The development is fueled by rising demands for speed in high throughput screening systems and the overall trend toward miniaturized analysis systems. The technology has evolved from the conventional drop-on-demand ink-jet printing technology, where droplets are generated by employing a pressure pulse from an enclosed volume of liquid terminated by an orifice [9]. A common pressure generating principle in these systems is based on a piezo-ceramic element, which changes dimension upon applying a voltage and thereby creating a pressure pulse resulting in droplet ejection. In a previous work we interfaced microcolumn liquid chromatography to MALDI-TOF-MS utilizing a piezoelectric flow-through microdispenser [10]. Here we report on the development of an integrated protein identification platform with an enhanced flexibility compared to the previous system. The miniaturized chromatographic separation is coupled on-line to a flow-through microdispenser. By ejecting bursts of droplets in a precise array pattern as subfractions of the column effluent, the chromatographic separation is continuously transferred onto a target plate, enabling MALDI-TOF-MS analysis of the discrete spots obtained. The effluent that is not dispensed onto the target plate is collected into a refrigerated microfraction collector, hereby giving the option of enrichment and re-analysis of the collected fractions. Furthermore, the collected sample can be subjected to complementary analytical techniques, e.g., electrospray ionization tandem mass spectrometry (ESI-MS–MS) or enzyme-linked immunosorbent assay (ELISA). Pre-coating of the target plate with a thin and homogenous layer of matrix–nitrocellulose simplified the automation of the analytical procedure.

2. Experimental

2.1. Materials

α-Cyano-4-cinnamic acid (α-CHCA) and 2,5-

dihydroxybenzoic acid (DHB) were obtained from Aldrich (Steinheim, Germany). Lysozyme was purchased from ICN (Costa Mesa, CA, USA). Goat serum albumin, cytochrome *c*, angiotensin I adrenocorticotropic hormone fragment 18-39 (ACTH 18-39) and ammonium hydrogencarbonate were all purchased from Sigma (St. Louis, MO, USA). Dithiothretiol, iodoacetic acid and trifluoroacetic acid (TFA) were obtained from Fluka (Buchs, Switzerland). Trypsin (sequence grade) was obtained from Boehringer Mannheim (Mannheim, Germany). Phosphorylated/non-phosphorylated synthetic peptides corresponding to the tryptic fragment 485–496 (ALGADDSYYTAR) of ZAP 70 were custom synthesized by Sigma and dissolved in water containing 0.1% (v/v) TFA. All solutions were prepared using Milli-Q water filtered with a 0.2-μm nylon membrane filter from Millipore (Bedford, MA, USA). Nitrocellulose membranes were obtained from Bio-Rad (Richmond, CA, USA). Acetone and 2-propanol were from Fisher Scientific (Loughborough, UK), while gradient-grade acetonitrile (ACN) was obtained from Merck (Darmstadt, Germany).

2.2. Chromatographic system

The capillary high-performance liquid chromatography (HPLC) separations were performed using an Ultimate LC system (LC Packings, Amsterdam, The Netherlands) coupled to a Famos micro autosampler (LC Packings). The separations were carried out on a 15 cm×300 μm I.D. capillary LC column packed with 3 μm C_{18} stationary phase (PepMap, LC Packings) at a flow-rate of 4 μl min^{-1}. For all separations, eluent A consisted of 0.1% TFA in ACN–water (5:95, v/v) and eluent B of 0.08% TFA in ACN–water (90:10, v/v). The gradient applied for the different tryptic digest solutions was from 2 to 50% B in 45 min. All the separations were performed at ambient temperature and the injection volumes were 5 μl. Prior to the automated on-line dispensing onto the MALDI target the separations were monitored using UV detection at 214 nm. The detector cell of the Ultimate system consisted of a Z shaped capillary flow cell (35 nl) with a 10 mm path length ensuring high sensitivity UV tracing. A refrigerated microfraction collector, CMA model 200 (CMA/Microdialysis AB, Solna, Sweden), was connected to the outlet of the UV detector.

2.3. Tryptic digestion

The digestion was performed according to a modified protocol from Shevchenko et al. [11] where 1 μl of a 0.1 μ*M* solution of dithiothretiol was added to 50 μl of 25 m*M* ammonium hydrogencarbonate buffer containing the protein at the total concentration of 2 pmol μl^{-1}. The reduction took place on an Eppendorf Thermomixer (Hamburg, Germany) at 48°C during 30 min. The proteins were later alkylated during 90 min in darkness after the addition of 2.5 μl 0.2 μ*M* iodoacetic acid. The digestion proceeded at 37°C overnight after an addition of 10 μl of a 0.025 μg ml^{-1} trypsin solution in 25 m*M* of ammonium hydrogencarbonate. Adding 5 μl 5% TFA to the solution terminated the digestion.

2.4. MALDI-MS and sample preparation

The MALDI-TOF-MS instrument was a Voyager DE-PRO (Perseptive Biosystems, Framingham, MA, USA) with built-in delayed extraction and a linear path of 1.1 m. It was equipped with a video camera system to ensure precise focusing of the 337 nm nitrogen laser, having a focused laser spot diameter of approximately 100 μm. The sample plate could be moved with high precision using an *x–y* stage with a minimal increment step of 3.2 μm. The mass spectra was acquired in the reflector mode. The MALDI targets were laboratory-manufactured stainless steel plates (45×45 mm) mounted in an adapter holder from Perseptive Biosystems. The matrix consisted of 10 mg ml^{-1} α-CHCA and 5 mg ml^{-1} nitrocellulose membrane, dissolved in acetone–2-propanol (80:20, v/v) and mixed 1:1. The stainless steel plates were sprayed with a thin and uniformly distributed matrix–nitrocellulose solution, into which we also added 1 μg of angiotensin I per ml solution as an internal standard. The MALDI target plate was placed on a motor-driven *x–y* stage (Vexta, Oriental Motor, Tokyo, Japan) and controlled by a laboratory-developed software based on LabVIEW (National Instruments, Austin, TX, USA). Furthermore, the software enabled adjustment of the spot pattern, resolution between discrete spots, droplet size, frequency of droplet burst and the duration of the dispensing time. In order to enhance evaporation of the dispensed droplets, the target holder was heated to 38°C. Visualization of the sample spotting was

obtained by using a microscope (Leitz, Wetzlar, Germany) equipped with a video camera (CCD-72EX, DAGE-MTI, Michigan City, IN, USA). Typical pulse conditions, obtained using a pulse generator (Model 8111A, Hewlett-Packard, Palo Alto, CA, USA) were 20–50 Hz, using an applied voltage of 9 V given by a d.c. power supply (Model E3612A, Hewlett-Packard).

3. Results and discussion

3.1. Interfacing microcolumn HPLC with MALDI-TOF-MS

Peptide maps generated from proteins after enzyme digestions are mandatory information needed for primary structure information of proteins. Small sample volumes necessitate miniaturization of analytical systems in order to obtain this information at fmol levels. It is of great value for both characterization and identification of the recombinant field as this will circumvent losses of proteins and peptides due to adsorption. Fig. 1a illustrates the automated protein identification platform operated in the automated ''on-line mode''. It comprises the microcolumn LC separation system coupled on-line to the MALDI interface, i.e., the piezoelectric flow-through microdispenser. The microdispenser transferred the separated tryptic digest onto the matrix pre-coated MALDI target plate in a precise array pattern. The rest of the column effluent was fractionated into a refrigerated microfraction collector. The system was operated fully automated starting with the sample being injected from the 96-well plate by the microautosampler. After concentrating the sample on top of the analytical reversed-phase column, the gradient is applied and the elution of the analytes starts. The most polar peptides in the sample are eluted first and the elution order will increase with increasing hydrophobicity. The separated sample will pass the UV detector to certify the efficiency of the chromatographic separation whereafter it is eluted into the flow-through dispensing unit. The outlet of the UV detector consisted of a 50 μm I.D. fused-silica capillary where the end was slid into a 250 μm I.D. PTFE tubing (LC Packings) which made it fit the 1/16 in. (1 in.=2.54 cm) silicone rubber tube at the inlet of the microdispensor with virtually a zero-dead volume connection. The microdispenser comprised a 500-nl flow-through cell where the dead volume measured from the inlet to the nozzle was 250 nl. The thin channel wall of the dispenser bends when a voltage is applied to the piezoelectric element whereby a droplet is pressed through the nozzle in the opposing wall. The resulting droplets formed are in the range of 60 pl and are deposited onto the MALDI target plate which is placed in a motor-driven high-resolution *x–y* stage which is computer controlled by laboratory-developed routines written in the LabVIEW software. The generated bursts of droplets are accurately dispensed as discrete spots by moving the *x–y* stage in a pre-defined array pattern. The part of the flow that pass through the dispenser is fractionated into a refrigerated microfraction collector (6°C) in volumes of 4–12 μl/fraction, depending on the flow-rate and the fraction collection time, thereby avoiding loss of any sample. The fractions from the separation are stored in the fraction collector for future use. The dispensing unit can also be operated in the ''static mode'' as shown in Fig. 1b. The ''static mode'' refers to the off-line operation of the microdispenser for discrete sample processing. This system set-up enabled the handling of sample volumes down to 2 μl. In a previous paper the sample/matrix handling followed a different scheme; making a two step deposition on the bare gold, or stainless steel plate surface by spotting the sample followed by the matrix deposition [12]. We have improved the sample deposition alignment as well as simplified the two-step deposition method to a single-step methodology. As previously described we have developed an air-brush technique where a thin and homogenous matrix–nitrocellulose film can be evenly sprayed on the target plate [10]. The entire peptide map is spotted as small dots onto the target surface. Upon reaching the MALDI target surface, the liquid droplet wets the polymer surface and dries in seconds whereupon the sample crystal layer is formed as illustrated in Fig. 2. The spot diameters will vary somewhat with the viscosity of the mobile phase. Typical crystal spot diameters obtained range between 400 and 700 μm. Fig. 3 illustrates a magnified portion of the target plate covered with α-CHCA–nitrocellulose containing the dispensed sample spots. The repeatability of the spot diameters

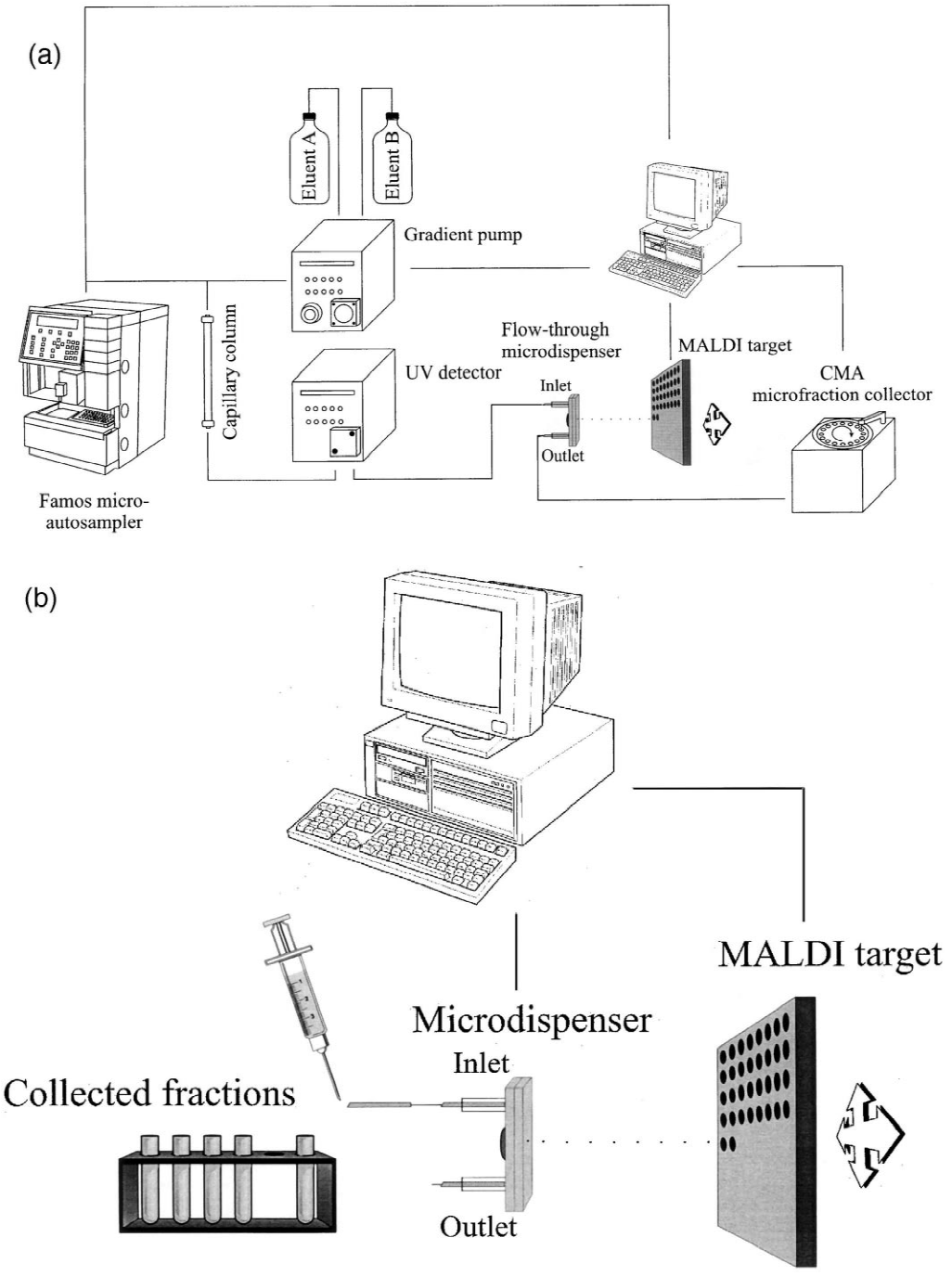

Fig. 1. Schematic overview of (a) the automated protein identification system operating in the ''on-line mode''. The flow-through microdispenser is the interface, coupling the chromatographic separation to MALDI-TOF-MS. Furthermore, the column effluent is stored by using a refrigerated microfraction collector giving the option of re-analysis of interesting fractions; (b) the ''static mode'' enabled complementary studies or enrichment of collected fractions correlated to the deposited sample spots on the MALDI target plate. Sample volumes down to 2 μl could be handled.

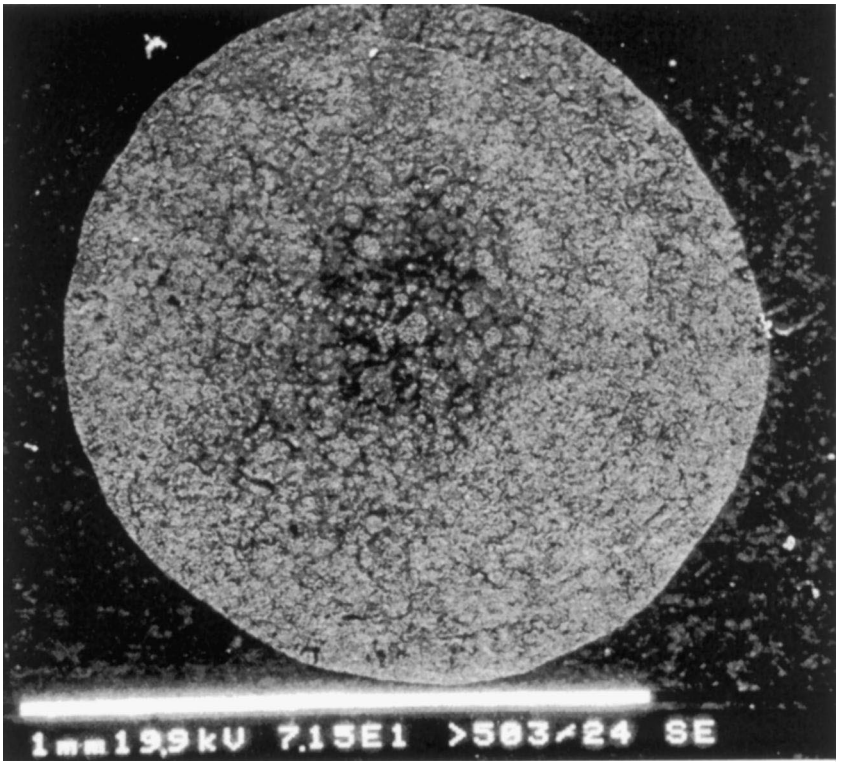

Fig. 2. Scanning electron micrograph illustrating an overview of a dispensed sample spot surface on an air-brush preparation of CHCA–nitrocellulose matrix. The crystals are formed after the sample has been dispensed.

was good, and the relative standard deviations (RSD) obtained were typically within 10%. Three different proteins, namely, goat serum albumin, cytochrome *c* and lysozyme were digested using trypsin as the tryptic enzyme followed by a subsequent high-resolution peptide separation. Separations of these proteins typically generated 15–60 eluting peaks. In most cases co-elution of unresolved peptides were obtained, peaks containing two peptides and in some cases even four peptides were identified by MALDI-MS. The chromatogram depicted in Fig. 4 shows a huge frontal eluting peak which contain interfering matrix components, e.g., buffer salt and autodigested fragments of trypsin, as well as the most polar peptides of the digested protein. Restrictions in peptide mass identity were suspected to occur at this frontal peak due to the ion suppression effects in the MALDI-MS analysis. The most straightforward method to identify a protein is to cleave it either enzymatically and/or chemically. The resulting mass spectrum of this cleavage is the peptide mass map consisting of a set of peptides unique for the specific protein. Identification is then employed by comparing the theoretically expected peptide mass map of every protein in a sequence database to the measured one. Trypsin cleaves selectively at Arg and Lys residues but the purity of the enzyme is critical in order to obtain strict Arg and Lys cleaved peptide peak labels. These peptide mass maps of proteins are usually analyzed by utilizing MALDI-TOF-MS [13]. However, even if it is possible to analyze protein digestions directly with MALDI a separation step is often necessary for unambiguous identification of complex mixtures [14]. Additionally, suppression of the ion signal of analytes in mixtures is often the case [15,16]. In our approach we interface a micro-column LC system with a flow-through microdispenser, hereby achieving a chromatographic separation of the tryptic digest prior to the MALDI-TOF-MS analysis and thus reduce suppression effects. The

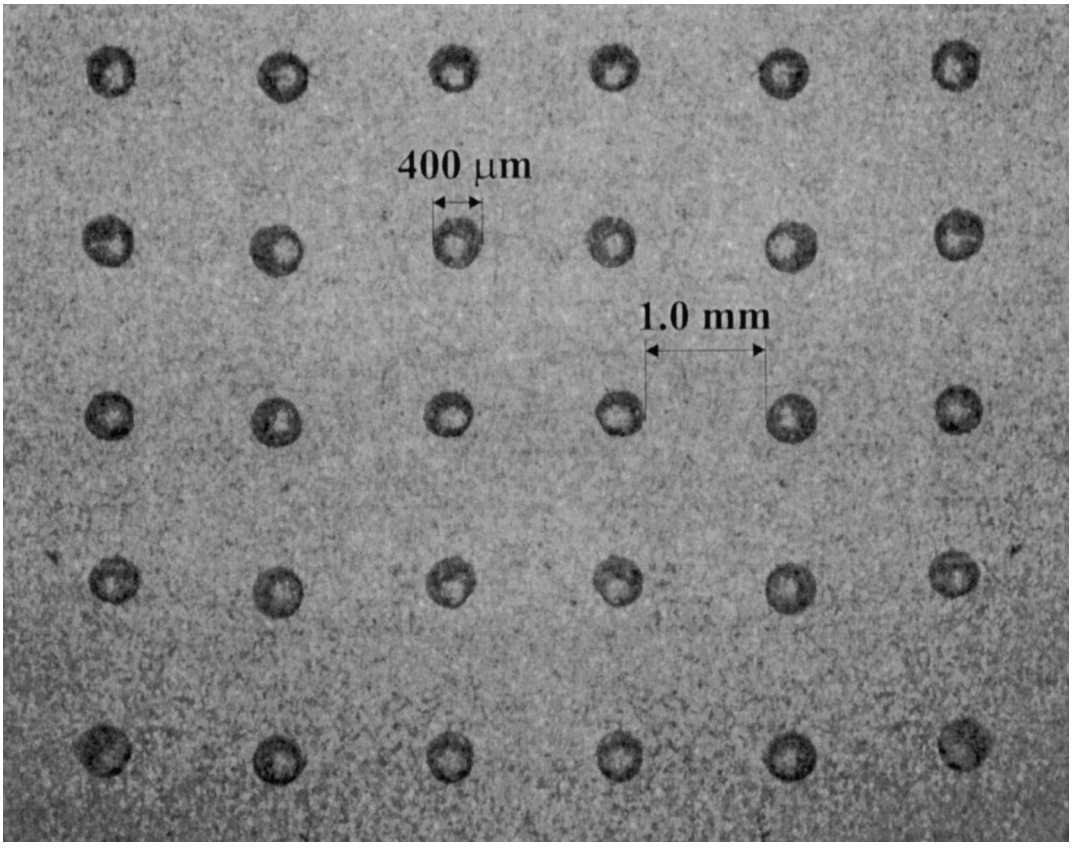

Fig. 3. Magnification of spot positions on a small portion of the MALDI target plate. The spot density depends on the pre-set distance of the *x*–*y* stage. Typically the *x*/*y* distance between discrete spots was set to 1 mm which hereby generated an array pattern consisting of about 1500 discrete spots. The diameter of the spots generated varied between 400 and 700 μm depending on the deposition rate and the total amount of droplets dispensed. A manuscript describing the air-brush technique in detail is in preparation.

robustness of the overall system is dependent on obtaining reproducible chromatography performance. The repeatability of the chromatographic separation was investigated by running 10 consecutive runs of trypsin cleaved myoglobin (10 pmol injected). Fig. 5 depicts an overlay of the first, fifth, and tenth consecutive run. Calculating the RSD of the retention times of five peaks covering the major part of the elution time gave the value of 8%. This indicates that the gradient formation is precise and that enough time (20 min) is given for equilibrating the microcolumn between runs. For details of the chromatographic conditions see Fig. 5.

3.2. Improved sequence coverage

In order to compare our developed protein identification system with traditional MALDI sample preparation, the same sample was subjected to both methodologies using a mass accuracy of 50 ppm. The seed layer approach described in an earlier work [17] was used as the reference method and Table 1 illustrates the results obtained for goat serum albumin, lysozyme and cytochrome *c*, respectively. Table 2 contains the results obtained utilizing our system. The sequence coverage of goat serum albumin was significantly higher when including a separation step, 37 tryptic peptide fragments were identified compared to 22 identifications when using the seed layer method. This is almost a doubling of the number of peptides that are sequence specific for this protein resulting in a MOWSE (molecular mass search) score which was about two-orders of magnitude higher. Lysozyme and cytochrome *c* did not

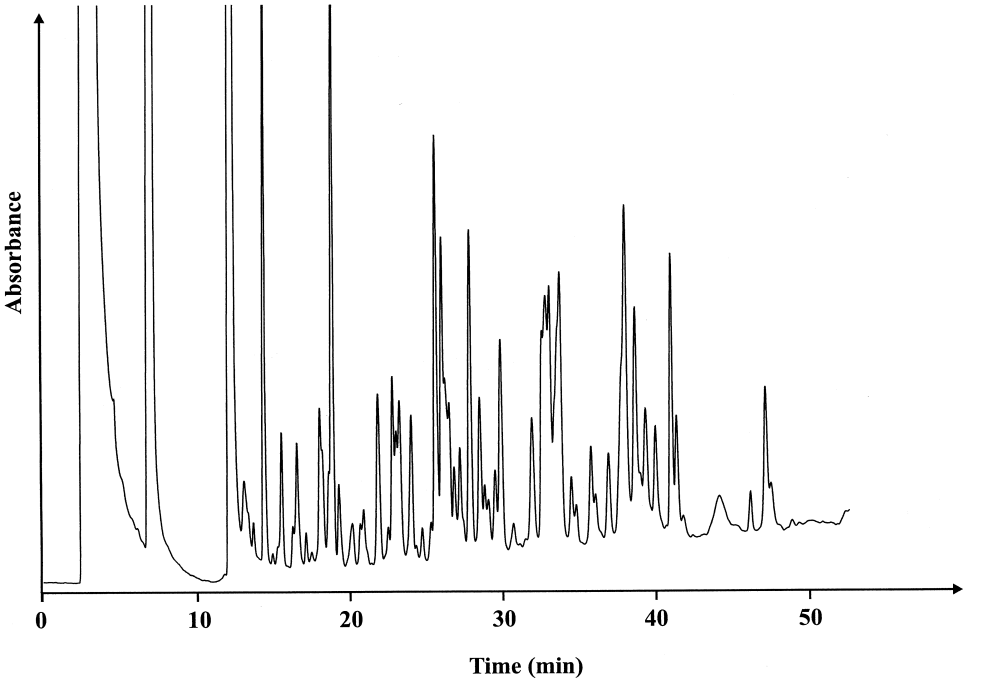

Fig. 4. Tryptic digest of goat serum albumin separated on a 300 μm I.D. reversed-phase column. In this case 10 pmol was injected onto the microcolumn (5 μl injection volume). The mass accuracy was set to 50 ppm when the dispensed peptide fragments were identified by MALDI-TOF-MS and entered into the database search.

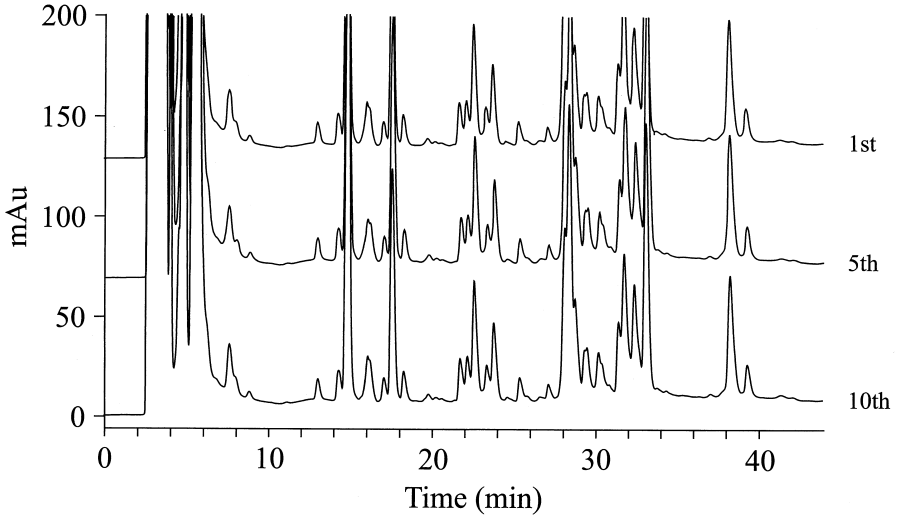

Fig. 5. Separation of a tryptic digest of myoglobin on a PepMap microcolumn, 15 cm×300 μm, 3 μm porous Si particles, pore size 100 Å. Eluent A: 0.1% TFA in ACN–water (5:95, v/v). Eluent B: 0.08% TFA in ACN–water (90:10, v/v). Elution: linear gradient, 0–50% B in 45 min, equilibration time was 20 min. Detection wavelength: 214 nm. For other details see Experimental.

Table 1
Results obtained from conventional MALDI-MS analysis of various tryptic digests using the seed layer sample preparation technique

Protein	Submitted mono-isotopic masses	Masses matched	Sequence coverage (%)	MOWSE score
Serum albumin (goat)	46	22	34	$3.2 \cdot 10^{10}$
Lysozyme (chicken)	21	12	76	$2.2 \cdot 10^{7}$
Myoglobin (horse heart)	25	8	59	$3.4 \cdot 10^{3}$

show the same magnitude of increase in sequence coverage when run on our system. As can be seen from the data in Tables 1 and 2, respectively, the total monoisotopic masses of peptides submitted for the database search was twice as high for all the proteins when including a separation prior to the MALDI analysis. We suspect that the reason to the more modest increase in sequence coverage of lysozyme and cytochrome *c* is correlated to their significantly smaller molecular size. Less peptide fragments are hereby generated which in turn may reduce ion suppression. Furthermore, it is difficult to assign the correct monoisotopic molecular mass within the mass accuracy of 50 ppm of peptides with higher molecular masses ($\geq$3000) since the mass resolution of the MALDI instrument was limited to approximately 4000. Peptide fragments of masses around 3000 were more frequently found with our system as compared to the seed layer technique, but as mentioned above it was difficult to take advantage of this in order to obtain an even higher sequence coverage.

3.3. High sensitivity peptide mapping

Protein kinases regulate a multitude of physiological processes by transfer of phosphate residues. This group of enzymes plays a central role, e.g., in control of growth, division, and differentiation of cells. The protein tyrosine kinase ZAP-70 is a critical mediator of T-cell receptor signaling [18], why the phosphorylation state during kinetic studies is of mandatory importance. In order to identify post-translational modifications such as phosphorylation patterns with high sensitivity, a feasibility study using the static mode operation of the system was applied to synthetic peptides. These peptides corresponded to the tryptic fragment 485–496 (ALGADDSYYTAR) of ZAP-70, containing Y-493, a well-known regulatory phosphorylation site [19]. The Y-493 position can result in the following four peptide alternatives upon autophosphorylation: –yy– (non-phosphorylated), –pyy– (monophosphorylated), –ypy– (monophosphorylated), and –pypy– (diphosphorylated). These four peptides, namely, the non-phosphorylated, the two isomers of the monophosphorylated and the diphosphorylated were dispensed onto a MALDI target plate pre-coated with α-CHCA–nitrocellulose matrix. Angiotensin I was included in the matrix as an internal standard, increasing the mass accuracy to about 50 ppm.

Fig. 6a shows a MALDI mass spectrum obtained when dispensing a sample containing 20 fmol μl^{-1} of the four peptides, i.e., 2 fmol was dispensed onto the target plate since 500 droplets were deposited on every spot and each droplet had a volume of about 60 pl. Spectra with monoisotopic resolution were generated for the four peptides. Angiotensin I appeared at the m/z of 1296.68 (internal standard), the non-phosphorylated peptide at 1302.57, the two

Table 2
Results obtained when including chromatography prior to the MALDI-MS analysis by using the flow-through microdispenser

Protein	Submitted mono-isotopic masses	Masses matched	Sequence coverage (%)	MOWSE score
Serum albumin (goat)	76	37	57	$7.7 \cdot 10^{12}$
Lysozyme (chicken)	46	15	85	$9.8 \cdot 10^{7}$
Myoglobin (horse heart)	50	12	66	$9.2 \cdot 10^{3}$

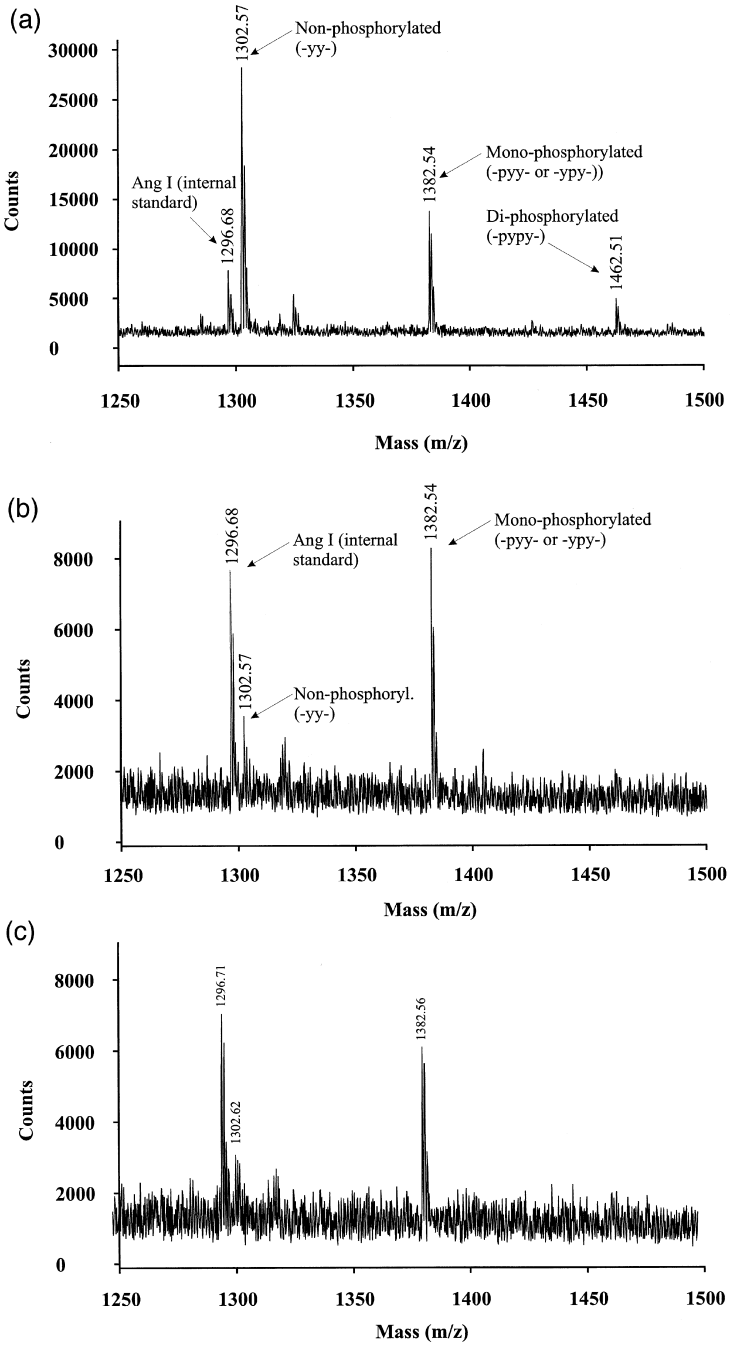

Fig. 6. MALDI mass spectrum of (a) four synthetic peptides corresponding to phosphorylated/non-phosphorylated tryptic fragment 485–496 (ALGADDSYYTAR) of ZAP-70. The total amount of peptides deposited on one spot was 2 fmol, originating from a sample concentration of 20 fmol μl^{-1}; (b) the same peptides mentioned above but at a lower concentration. The total amount of peptides deposited on each spot was in this case 150 amol, originating from a sample concentration of 5 fmol μl^{-1} (total sample volume available was 5 μl). The diphosphorylated peptide could not be detected at this level; (c) the same sample spot as in (b) analyzed after storing the target plate in darkness at room temperature for 1 week.

isomers of the monophosphorylated at 1382.54, and the diphosphorylated at 1462.51. However, discrimination of the two isomeric forms of the monophosphorylated peptide could not be obtained purely by MALDI-MS. A separation step needs to be included and ongoing work has proven that LC–MALDI-MS and LC–ESI-MS can distinguish both the two isomeric forms of the monophosphorylated peptide (data not shown). In Fig. 6b we reached a sensitivity of 150 amol of the non-phosphorylated and the monophosphorylated peptide. The total peptide concentration of the sample was 5 fmol μl^{-1} and 500 droplets were deposited on each spot using a deposition rate of 15 Hz. The diphospho peptide signal is lost at this lower concentration level and our experience is that phosphorylated peptides have lower sensitivity when analyzed by either MALDI or ESI since they are more difficult to ionize. The stability of the air-brush technique was investigated by analyzing the very same target plate and spot after a period of 1 week. A spectrum obtained from the stored sample spot is shown in Fig. 6c. A comparison between the two spectra showed in Fig. 6b and c, respectively, clearly indicates that the signals obtained are similar. Ongoing work will elucidate the storage capabilities in more detail. Absolute detectability for the two monophosphorylated forms with a signal response typically 6000 counts is in our opinion a level that can be further decreased by at least a factor of two.

4. Conclusions

With the aim of achieving a powerful and versatile protein identification system capable of handling both low analyte concentrations and minute sample volumes, we have interfaced microcolumn HPLC separations to MALDI-TOF-MS by using a low internal volume flow-through microdispenser. Including a chromatographic step prior to the MALDI-TOF-MS analysis resulted in higher sequence coverage of protein digests. Identification of synthetic phosphopeptides correlated to tryptic fragments of the protein tyrosine kinase ZAP-70 was achieved, reaching sensitivities of 150 amol. The use of MALDI target plates pre-coated with matrix simplified automation and increased the robustness of the system. Furthermore, the storage capability of these air-brushed target plates was found to be excellent. It was demonstrated that re-analysis of the same spot on a one week old plate, onto which we had dispensed trace levels of phosphopeptides (150 amol), gave virtually the same MALDI signal when stored in darkness at room temperature. A microfraction collector coupled to the outlet of the microdispenser enhanced the flexibility of the system and enabled the ''static mode'' configuration, i.e., the chromatographic separation can be fraction collected and the flow-through microdispenser can be operated in an off-line system set-up. This configuration made it possible to confirm structural information and enrich collected fractions.

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