RAPID SEPARATION OF PROTEIN DIGESTS ON SU-8 BASED CAPILLARY ELECTROPHORESIS-ELECTROSPRAY IONIZATION MASS SPECTROMETRY MICROCHIPS

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ABSTRACT

De novo peptide sequencing is one of the key methods in modern bioanalysis. In this study, we have developed a rapid microchip analysis for complex protein digests. Monolithically integrated, fully SU-8-based capillary electrophoresis-electrospray ionization (CE-ESI) microchips were used for on line mass spectrometric (MS) identification of the peptides. The microchips showed good performance in separation of the peptides within 3-5 minutes, and ESI-MS detection could be done efficiently.

KEYWORDS: Protein digest, Electrophoresis, Electrospray ionization, Mass spectrometry

INTRODUCTION

Microfabricated devices are rapidly becoming a convenient platform to execute liquid-phase analysis, and concequently, a variety of techniques have been implemented [1]. In microfabricated devices the flow rates are of the order of 1-300 nl/min. These values are a close match to the flow rates necessary to operate micro/nano ESI sources [1]. Chip-based analysis of protein digests has typically been performed utilizing either CE separation with fluorescence (FL) or off-line MS detection or MS analysis without prior separation [1-3]. Microchip liquid chromatography (LC)-MS separations have also been reported [4], but they often require relatively long analysis times. In this work, we have developed a rapid microchip analysis for complex protein digests. Our microchips are fabricated fully of SU-8 with a simple process using standard photolithography. The ESI-MS detection can be done efficiently due to the accurate nozzles microfabricated monolithically at the end of the separation channel. The straightforward integration of sample introduction, separation unit and ESI emitter on a single microchip ensures no dead volumes and peak broadening.

EXPERIMENTAL

The microchips (Figure 1a and b) were fabricated of epoxy photoresist SU-8 using UV-lithography and bonding techniques as reported earlier [5], and utilized for

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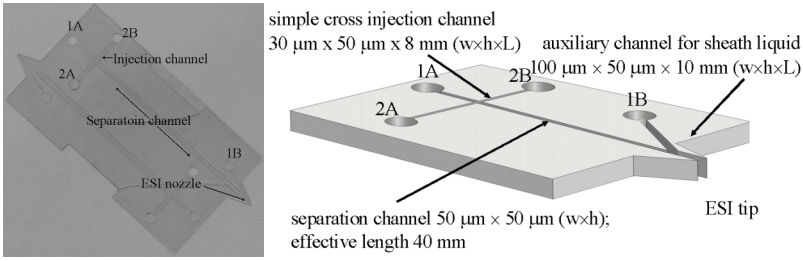
rapid CE-ESI/MS analysis. Bovine serum albumin (BSA), cytochrome C, α-lactalbumin, β-lactoglobuline and ovalbumin were incubated off-chip with trypsin in 0.1 M Tris HCl (pH 9.2) buffer containing 10% acetonitrile at +37°C for 18 h. The enzyme:protein ratio was 1:29. The peptides formed during the digestion were separated on the SU-8 based CE-ESI/MS microchip and detected on an ion trap mass spectrometer. Injection was performed in pinched injection mode (1000 V/cm; 15-25 s) (Figure 1b). The ESI voltage, applied through an auxiliary channel, was +3.6 kV (relative to MS) and the electric field strength applied for the separation was 500 V/cm.

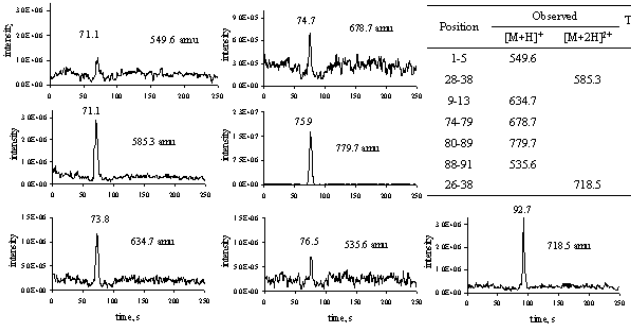


(a) *(b)*

Figure 1. a) Optical photograph of the SU-8 CE-ESI/MS microchip on which two separation channels can be seen next to each other. b) Schematic view of the CE-ESI/MS microchip middle layer. The injection voltages were 1A 0.5 kV, 1B floating, 2A 0.8 kV and 2B grounded. The separation voltages were 1A 5.6 kV, 1B 3.6 kV, 2A 5.1 kV and 2B 5.1 kV relative to MS. The excess current was led to ground from the auxiliary channel through a 50 MΩ resistor coupled in parallel with the ES voltage supply.

RESULTS AND DISCUSSION

Good resolution of the peptides was typically achieved within 3-5 minutes. The separation efficiency and detection sensitivity were very good. The number of theoretical plates was 1700-8200 per 4-cm channel length and typical sample concentrations were at low femtomole level for the tryptic peptides. The peak widths at half-height (w_h) were typically 3-6 s but peaks with w_h smaller than 1 s were also detected. Figure 2 shows a separation of the cytochrome C digest and the peptides detected from the BSA tryptic digest are listed in Table 1. For the BSA digest, over 30 tryptic peptides were observed by MS (Table 1). For α-lactalbumin, β-lactoglobuline and ovalbumin 8, 17 and 18 tryptic peptides were observed respectively. This enabled significantly better identification of the protein than CE separation combined with FL detection. For instance, in-house performed microchip-CE-FL analysis of a fluorescent labeled BSA digest on a commercial glass microchip showed only 13-15 BSA peptides as separate peaks due to the co-migration of numerous peptides. In CE-ESI/MS, the final resolution can be achieved on a mass spectrometer and most importantly, fluorescence derivatization of the analytes is not needed. On the other hand, CE separation prior to MS is preferred over direct infusion MS analysis in order to distinguish between peptides that appear at the same mass-to-charge ratio but origin from either singly or multiply charged peptides.



Position	Observed		Theoretical mass
	$[M+H]^+$	$[M+2H]^{2+}$	
1-5	549.6		546.3
28-38		585.3	1167.6
9-13	634.7		633.4
74-79	678.7		677.4
80-89	779.7		778.4
88-91	535.6		532.3
26-38		718.5	1432.8

Figure 2. Extracted ion chromatograms of the observed fragments of cytochrome C tryptic digests. Injection time was 20 s, separation buffer was 20 mM ammonium acetate with 50% methanol and sheath liquid was 80% methanol with 1% acetic acid.

Table 1. List of the observed tryptic digest fragments of bovine serum albumin with their migration times, theoretical masses (Mascot® search engine) and positions. Injection and separation conditions as specified in Figure 1 and 2.

Migration time (s)	Observed $[M+H]^+$	Observed $[M+2H]^{2+}$	Theoretical mass	Position	Migration time (s)	Observed $[M+H]^+$	Observed $[M+2H]^{2+}$	Theoretical mass	Position
75.6		699.9	1398.7	569-580	235.4	649.6		648.3	223-228
103.8	665.6		664.4	156-160	235.5		513.1	1023.4	499-507
207.0	545.6		544.3	101-105	235.8		577.6	1152.7	257-266
207.0	689.6		688.4	236-241	235.8	789.7		788.5	257-263
210.0	821.0		819.5	229-235	235.8	1002.8		1001.6	598-607
211.8		720.9	1438.8	360-371	236.5	509.5		508.3	558-561
224.4		1023.6	2044.0	168-183	236.7		502.1	1001.6	25-28
230.7		752.6	1503.9	549-561	237.0	1146.9		1144.6	236-245
231.1		1017.8	2033.1	588-607	237.6	1024.7		1023.4	499-507
231.6	1309.8		1307.7	558-568	240.0	462.5		461.2	152-155
232.8	1479.9		1478.8	421-433	240.0	508.9		507.2	229-232
233.4	712.6		711.4	29-34	240.0	660.6		659.3	490-495
233.4	1480.9		1481.8	483-495	241.2		946.2	1889.8	101-117
233.7		571.6	1141.7	548-557	241.2	1014.8		1013.6	549-557
233.7		740.8	1478.8	421-433	242.4	927.7		926.5	161-167
233.7		741.5	1481.8	483-495	259.8	960.8		959.5	210-218
234.1	517.5		516.3	281-285	264.6	751.8		751.4	341-346

CONCLUSIONS

In this study, a rapid, sensitive, and efficient analysis of protein digests for reliable identification of selected protein standards was developed. A monolithically integrated, SU-8 CE-ESI/MS microchip was shown to be an efficient tool in fast and sensitive analysis of protein digests.

REFERENCES

[1] Lazar, I., et al., *Mass Spectrom. Rev.* **2006**, *25*, 573-594.
[2] Cong, Y., et al., *J. Sep. Sci.* **2008**, *31*, 588-594.
[3] Mouradian, S., *Curr. Opin. Chem. Biol.***2002,** 6, 51-56.
[4] Lazar, I.M., et al., *Anal. Chem.* **2006,** 78, 5513-5524.
[5] Sikanen, T.; et al., *Anal. Chem.* **2007**, *79*, 9135-9144.