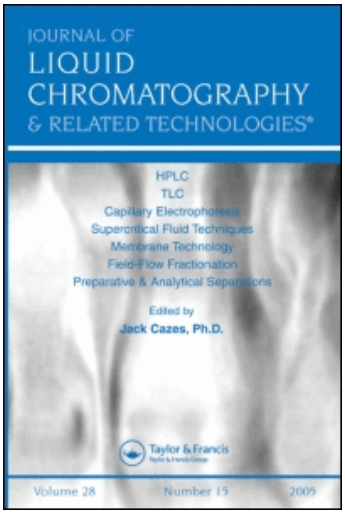

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A Strategy for Sequencing Peptides from Dilute Mixtures at the Low Femtomole Level Using Membrane Preconcentration-Capillary Electro-Phoresis-Tandem Mass Spectrometry (MPC-CE-MS/MS)

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A STRATEGY FOR SEQUENCING PEPTIDES FROM DILUTE MIXTURES AT THE LOW FEMTOMOLE LEVEL USING MEMBRANE PRECONCENTRATION-CAPILLARY ELECTROPHORESIS-TANDEM MASS SPECTROMETRY (mPC-CE-MS/MS)

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ABSTRACT

A unique method using membrane preconcentration capillary electrophoresis (mPC-CE) on-line with tandem mass spectrometry (mPC-CE-MS/MS) for sequencing <100 fmols of a series of MHC class I synthetic peptides is described. We show how this methodology in conjunction with transient isotachophoresis (tITP) after analyte elution from the membrane improves conventional CE-MS strategies by permitting the analysis of large sample volumes (>100 µL) while maintaining high analyte resolution and separation efficiencies that are typically afforded by CE. Instrumental parameters, including the internal dimensions of the mPC-CE capillary, collision gas pressure, collision gas type, and collision energy, are all shown to have substantial effect on the abundance of product ions produced in tandem MS/MS spectra when attempting to sequence peptides at the sub 100 femtomole level. Furthermore, we demonstrate that the physico-chemical properties of these analytes can also affect the MS/MS product ion abundance. In

particular, the presence of acidic residue tends to reduce mPC-tITP-CE-MS/MS sensitivity and direct fragmentation processes resulting in incomplete sequence information. This can be overcome by a simple esterification of the carboxylic acid functional group. Ultimately, we present an optimized mPC-tITP-CE-MS/MS approach that permits peptide sequence determination by consumption of ~40 femtomole of peptide analyte.

INTRODUCTION

Peptide sequence determination by tandem mass spectrometry (MS/MS) is a well established technique (1,2). However, many biologically active peptides that require sequencing by MS/MS present two major challenges. First, these analytes are usually components of exceedingly complex mixtures and are not readily isolated in pure form by standard chromatographic procedures. Second, such peptides are invariably present at extremely low concentrations (often attomoles/μL). These problems are particularly pronounced in the analysis of immunologically important peptides that bind to major histocompatibility complex (MHC) proteins, for presentation at the cell surface to cytolytic T-lymphocytes (3). For example, MHC class I peptides are reported to be extracted in low femtomole-low picomole amounts from ~5 x 10^{10} cells (4). Furthermore, it has been suggested that there are in excess of 10,000 different peptides that can be presented at the cell surface by the MHC protein (5).

We have reported the potential of on-line capillary electrophoresis-mass spectrometry (CE-MS) to effect the separation of complex mixtures of MHC class I peptides (6,7) given the recognized, high resolving capability of CE. However, it was noted that this approach proved to be problematical due to the limited loading capacity of conventional CE

capillaries for CZE separation (8). This is predominantly due to the low volumes (typically 1-2% of total capillary volume) that can normally be injected onto the capillary (9).

Subsequently we evaluated the use of a variety of commonly used sample injection techniques to increase sample loading onto the CE capillary and found transient isotachophoresis (tITP) to be of some limited success (10). Numerous other workers have also investigated the use of analyte stacking (11-15), field amplification (16) and tITP (17-23) to overcome the poor concentration limits of detection (CLOD) of CE and CE-MS in the analysis of a wide variety of compounds . However, since all of these approaches are electrophoretic and are performed within the CE capillary, they are also limited by the total volume of the CE capillary. Therefore, even in the most favorable cases, analyte resolution can only be effected by the introduction of up to 90% of the total capillary volume. This still only represents the analysis of <1 μL of sample solution. Hence these electrophoretic sample introduction techniques are often of limited use in the analysis of biologically derived peptide mixtures such as MHC class I peptides.

Following the limited success with tITP, we explored the use of on-line preconcentration-CE-MS using an adsorptive phase at the capillary inlet as a potential method to achieve our goal of analyzing and sequencing MHC class I peptides (8). On-line analyte concentration (microreactor) capillaries for use with CE were pioneered by Guzman (24-26) and subsequently developed by us (27-36) and others (37-44). Furthermore, we have, to our knowledge, demonstrated the first use and compatibility of this approach on-line with a mass spectrometer as the

detector (27,30,32,33,35,36,45). Most groups have tended to use a solid phase packing at the inlet of the CE capillary (38-41,44). However, we and others have shown compromised CE performance when using solid phase (28,33,34,38) since it necessitates from a practical perspective the use of a relatively large bed volume. This has also, in part, been attributed to an increased back pressure that reduces hydrodynamic flow within the CE capillary (34,38). In addition, ion flow may be impaired by the solid phase, resulting in reduced or anomolous electroosmotic flow (EOF). We have also previously reported that large volumes of organic solvent required to remove analytes from the solid phase within the CE capillary can also lead to reduced EOF (46,47). These factors can result in the broadening of analyte zones, compromised resolution, and loss of separation efficiency.

To reduce and ensure a consistent bed volume of adsorptive phase, we have developed and demonstrated the use of a preconcentration cartridge that contains a membrane impregnated with a suitable stationary phase (33,34), as shown in Figure 1. The cartridge fits at the inlet of the conventional CE capillary and we have termed this approach membrane preconcentration-CE (mPC-CE). We have also demonstrated the applicability of this technique on-line with mass spectrometry (mPC-CE-MS) (33,35,36). Using this approach sample contaminants (e.g., biological matrix components or chemical reagents) can be pre-eluted from the phase and washed through the CE capillary prior to analyte elution and analysis. Such on-line sample cleanup reduces sample handling and improves analyte recovery.

In the present study, we further demonstrate the applicability of mPC-CE-MS for the analysis of dilute peptide mixtures. In particular we

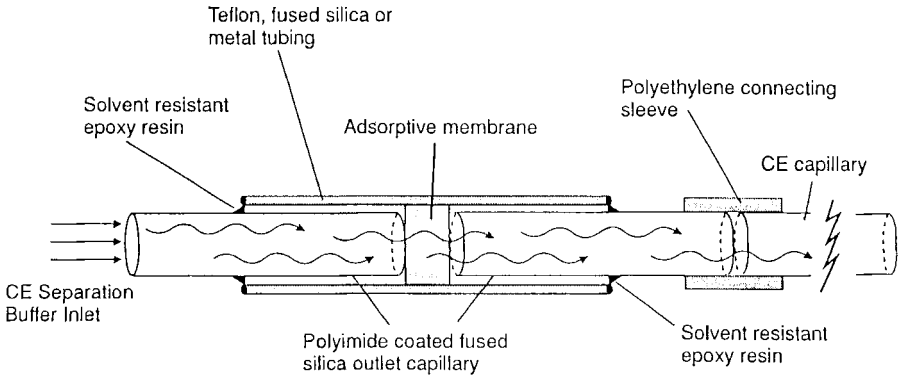

Figure 1 Schematic of a membrane preconcentration-capillary electrophoresis (mPC-CE) cartridge - (not to scale).

discuss the systematic development of mPC-CE-MS/MS for peptide sequence determination of synthetic MHC class I peptides at the low femtomole level. In this regard, it is noted that small changes in the type of collision gas, collision gas pressure, and collision energy can dramatically alter the appearance and quality of MS/MS spectra. We also discuss the improvement in spectral quality that occurs on using a smaller internal diameter CE capillary, as well as the use of chemical modification of peptides to enhance the abundance of product ions produced when glutamate and aspartate residues are present.

MATERIALS AND METHODS

Chemicals and Materials

Acetic acid (99.9%+ grade) was obtained from Aldrich Chemical Company (Milwaukee, WI). Ammonium acetate (99.9%+ grade),

ammonium hydroxide and potassium hydroxide were purchased from Sigma Chemical Company (St. Louis, MO). HPLC grade methanol, isopropanol, and water were obtained from Baxter (Minneapolis, MN). The synthetic MHC class I peptides SGINFEKL, SIINFEKL, and SIINFEKLT were synthesized by the Protein Core Facility at Mayo Clinic. The peptide mixture containing angiotensin II, bombesin, bradykinin, luteinizing hormone releasing hormone [LHRH], α-melanocyte stimulating hormone [α-MSH], leucine enkephalin, methionine enkephalin, oxytocin, and thyrotropin releasing hormone [TRH] was obtained from Biorad (Richmond, CA). Polymeric styrene-divinyl benzene copolymer (SDB) Empore™ membranes (3M, St. Paul, MN) were obtained from Varian (Harbor City, CA). Polyimide-coated fused silica capillary tubing was purchased from Polymicro Technologies (Phoenix, AZ). Teflon tubing was obtained from Chromtech (Apple Valley, MN).

mPC-CE-MS/MS

The CE capillary used in these experiments was prepared from uncoated fused silica tubing pretreated with sodium methoxide (0.5 M), methanol, and CE separation buffer. Membrane PC-CE-MS/MS studies were conducted using a cartridge system containing a SDB membrane as described previously (34) at the inlet of the CE capillary. The final dimensions of the capillary were 25 or 50 μm i.d. x 67 cm in length. After assembly, the entire PC capillary was conditioned under high pressure (20 psi) for ten minutes each with methanol and separation buffer. All subsequent capillary treatments and sample loading, washing, and elution were also carried out under high pressure (20 psi). It is noted that while the use of a mPC-cartridge reduces the rigidity of a CE capillary, this has caused no practical CE instrumentation problems in our hands.

The method of analysis included a cleaning regime of methanol (0.2 min) and separation buffer (5 min), followed by a high pressure injection of the peptide mixture (0.5-2 min injections). The capillary was then washed with separation buffer for 5 minutes and analytes were eluted from the hydrophobic membrane with a solution of methanol:water (80:20, v/v). The transient isotachophoresis (tITP) conditions were developed using a leading stacking buffer (LSB) of 0.1% ammonium hydroxide that was injected into the mPC-CE capillary prior to analyte elution. This was followed by a small volume of separation buffer (2 mM ammonium acetate:1% acetic acid in water) as a trailing stacking buffer (TSB).

Membrane PC-CE-MS/MS analyses were performed using a Beckman P/ACE 2100 instrument (Fullerton, CA, USA) modified with a Beckman-MS adapter kit for use with a mass spectrometer and coupled to a Reason Technology 486 personal computer (Rochester, MN, USA) with system control by System Gold software (Beckman). All analyses were performed on a Finnigan MAT 95Q (Bremen, Germany) of EBQ_1Q_2 configuration (where Q_1 is an rf-only octapole collision cell and Q_2 is a quadrupole mass filter). A Finnigan MAT electrospray ion source was used in positive ion mode. This source employs a spray needle that is floated to voltage (typically 3-5 kV) referenced to the accelerating voltage and a heated metal capillary (225°C) as the first stage of separation of the atmospheric pressure spray region from the vacuum of the mass spectrometer. Neither auxiliary nor sheath gas were used during these studies. The mPC-CE-MS studies were conducted at a resolution of ~1000 and a scan range of 300-1300 Da at a speed of 2 seconds/decade. Membrane PC-CE-MS/MS studies were conducted at a resolution of ~300

at MS_1 and 2-3 amu at MS_2. The scan range of MS_2 was 60-1100 Da at a speed of 0.4 s/100 amu. Parameters of collision gas and energy are discussed in the figure legends.

RESULTS AND DISCUSSION

mPC-CE-MS

The overall goal in developing mPC-CE was to ensure that the on-line preconcentration cartridge did not adversely effect CE and CE-MS performance. Hence, we found that minimizing the volume of the adsorptive phase by using an appropriately impregnated membrane made it possible to reduce the volume of elution solvent required for efficient removal of analytes from the phase (33,36). Therefore, adverse effects due to the presence of large volumes of organic solvent on CE performance could be overcome. For convenience, the membrane was installed in a cartridge system, e.g., Teflon, fused silica, or metal (stainless steel or titanium) (33), as shown in Figure 1. This facilitates the ready removal of the cartridge to allow CE capillary cleaning/conditioning and activation of the adsorptive membrane. The high loading capacity of such membranes makes it possible to load large volumes (~60-100 µL) prior to CE or CE-MS analysis (48). However, the small bed volume greatly enhances analyte recoveries using a reduced volume of organic elution solvent. Furthermore, on-line sample cleanup prior to CE-MS analysis is possible. This is particularly important for many samples derived from body fluids such as urine, where the presence of high salt concentrations can dramatically effect CE analyte separations by degrading the efficiency of contemporary CE stacking and focusing

procedures. One final advantage of mPC-CE is that a higher flow rate through the capillary is possible since impedance to flow is minimized, and this leads to faster analysis times and analyte migration is more reproducible.

mPC-tITP-CE-MS

We have previously reported that efficient recovery of peptides from the membrane requires the use of an adequate volume (>50 nL) of organic solvent (36). Hence even with mPC-CE, some peak broadening and degradation of analyte resolution may occur due to the volume of organic solvent present in order to maximize peptide recovery. Therefore we evaluated the use of tITP in conjunction with mPC-CE-MS after the peptides had been eluted from the adsorptive membrane. A nine peptide mixture consisting of angiotensin II, bombesin, bradykinin, LHRH, α-MSH, leucine-enkephalin, methionine-enkephalin, oxytocin, and TRH was loaded onto the mPC-CE cartridge. Transient ITP was effected by elution of the peptides from the membrane between zones of LSB (0.1% NH_4OH) and TSB (1% acetic acid). This method afforded baseline resolution of all of the peptides detected with theoretic plate values of 1.5×10^5 (leucine enkephalin) to 2.6×10^6 for α-MSH (see Figure 2). This example serves to demonstrate the minimal effect that mPC-CE has on CE-MS performance. The very hydrophilic peptide TRH was not detected, and we believe this is due to its poor retention on the SDB impregnated membrane.

We have also shown that variation of the LSB and TSB volumes allows manipulation of both migration time, peak width and resolution (36). More recently we have determined that the ratio of LSB to elution

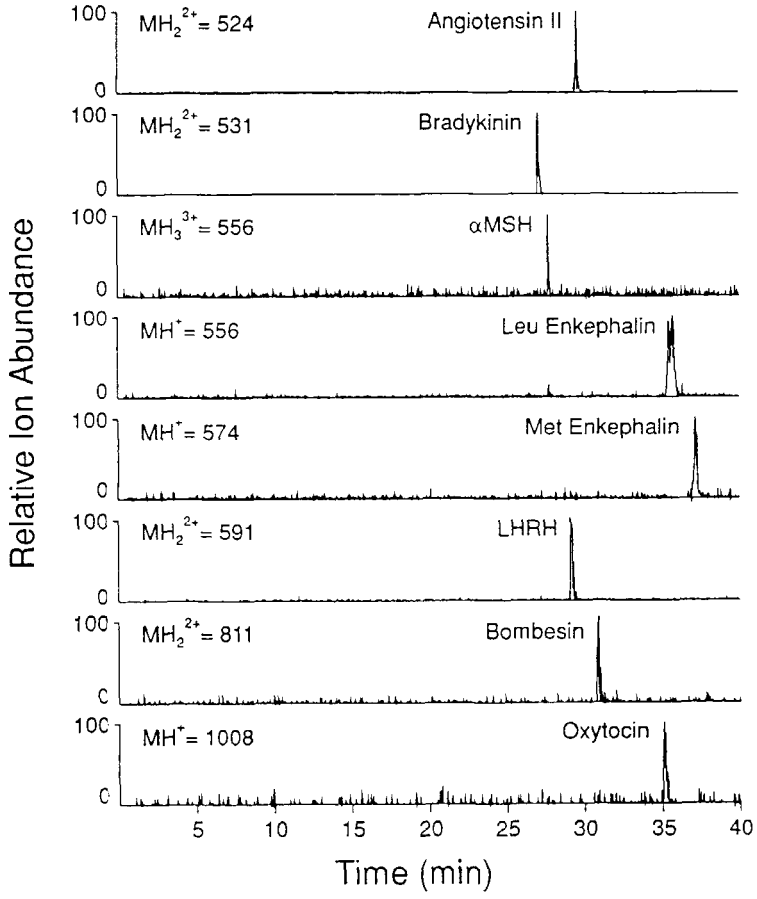

Figure 2 Ion electropherogram of a mPC-tITP-CE-MS analysis of a nine peptide mixture using a Beckman P/ACE 2100 CE connected to a MAT 95Q mass spectrometer. Separation buffer was 2 mM NH_4OAc:1% AcOH, on a 50 µm i.d. x 67 cm uncoated fused silica capillary, CE run at 30 kV. LSB was 0.1% NH_4OH (280 nL), elution solvent was 80:20 MeOH:H_2O (70 nL) and TSB was 1% AcOH (70 nL).

solvent volume is critical for optimal separation performance, and this will be reported elsewhere (48).

Conditions for mPC-CE-MS Analysis of Synthetic MHC Class I Peptides

As discussed, MHC peptides are usually present in low femto-picomole amounts. Therefore, any sample losses can easily result in a peptide concentration that is below the limits of detection. In this study, an uncoated mPC-CE capillary was used, since we have found that many of the coated CE capillaries commercially available are often not suitable for analysis of biologically derived peptides (49). In part, we believe this is due to the ubiquitous presence of trace amounts of surfactant, often used in cell lysis. The surfactants are carried through all the peptide purification steps that are undertaken prior to CE-MS analysis. Hence, while peptide-capillary wall interactions are minimized in coated CE capillaries, the hydrophobic interaction of the surfactant with the coated capillary wall, as well as the peptide with surfactant ultimately leads to peptide loss within the CE capillary. Therefore to minimize losses of biologically derived peptides, we have used the strategy of employing a carrier peptide when using an uncoated CE capillary. The carrier peptide, in this case angiotensin II, is added to the analyte peptide(s) in ~5-fold excess. The hydrophobic angiotensin II preferentially interacts with the active sites of the mPC-CE capillary minimizing analyte peptide losses. This strategy was employed in the mPC-CE-MS analysis of a mixture of three structurally similar, synthetic MHC class I peptides, namely SGINFEKL, SIINFEKL, and SIINFEKLT. The mPC-CE-MS ion abundance for all three peptides was ~30% lower when the carrier

peptide angiotensin II was not present, reflecting sizable losses to the capillary wall and possibly to the mPC-CE cartridge.

Using this approach, variable amounts of a solution containing ~200 attomoles/nL of the three MHC class I peptides were loaded onto the mPC-CE cartridge. Sample amounts of ~60 femtomoles, ~500 femtomoles and ~1.5 picomoles of the peptide mixture were subsequently analyzed by mPC-tITP-CE-MS. The mPC-tITP-CE-MS ion electropherogram for the 60 femtomole peptide mixture is shown in Figure 3. The ion electropherograms for the larger amounts of peptide loaded and analyzed by mPC-tITP-CE-MS also showed no appreciable loss of analyte resolution and quality of peak width and shape (results not shown), demonstrating the excellent dynamic range properties of mPC-CE-MS.

mPC-CE-MS/MS

The ultimate goal of peptide analysis is primary sequence determination. In this regard MS/MS affords both high sensitivity, and the most appropriate method for structural characterization of subpicomole amounts of peptide on-line with chromatographic separation techniques. Therefore, we have investigated the use of mPC-tITP-CE-MS/MS for sequencing synthetic MHC class I peptides present in a mixture at the low femtomole (<100 fmol) amounts.

Initial studies indicated that subtle changes in collision cell parameters led to substantial changes in product ion spectra. These changes appeared to be much greater for the analysis of low femtomole amounts of peptide than is normally observed at higher picomole amounts. This prompted us to systematically investigate the effects of all

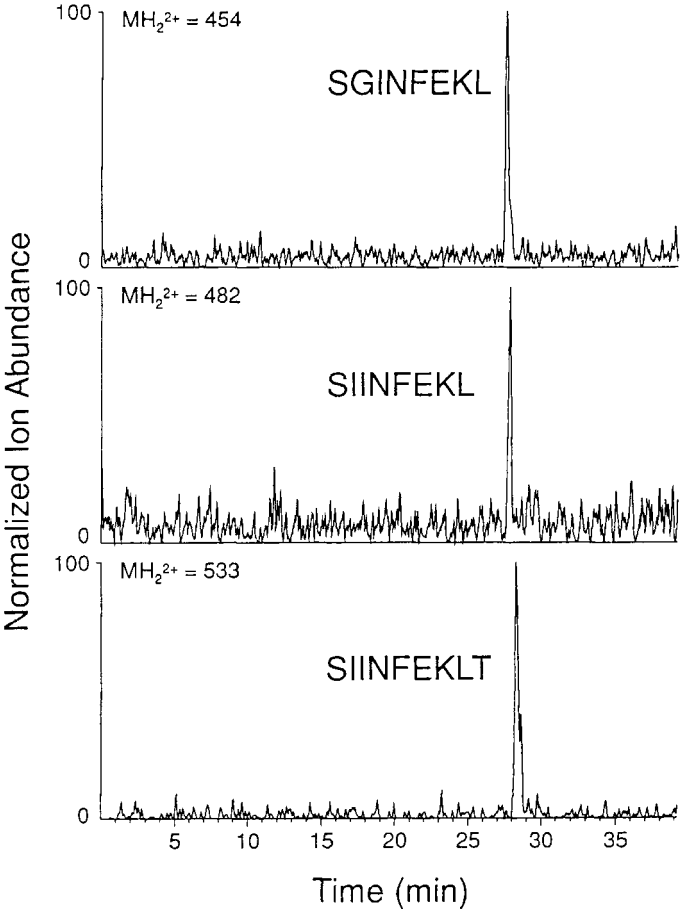

Figure 3 Ion electropherogram of a mPC-tITP-CE-MS analysis of a ~60 femtomole synthetic MHC class I peptide mixture. Conditions as described in Figure 2 except CE capillary was 25 µm i.d. x 67 cm at 20 kV, LSB was 80 nL, elution solvent was 80 nL and TSB was 30 nL.

collision cell parameters on product ion spectra obtained on less than 100 femtomoles of peptide.

Initially we optimized the pressure of the collision gas in order to minimize product ion scatter but maximize product ion formation and transmission (50). We investigated both argon and xenon as candidate target gases and found optimal gas pressures for each gas to be 2×10^{-5} mbar and 1.2×10^{-5} mbar, respectively. The differences observed in the product ion spectra after mPC-tITP-CE-MS/MS analysis of ~60 femtomoles of SIINFEKLT were substantial and are shown Figure 4. As expected from center of mass collision calculations, the larger atomic diameter of xenon gas dramatically increased the attenuation of the doubly charged precursor ion (MH_2^{2+}) (Figure 4B). This results in a substantial increase in yield of product ions detected at MS_2. From these results xenon appears to be the most appropriate gas for generating abundant sequence-related product ions.

We have also found that small variations in collision energy (5-10 eV) can result in substantial differences of the type and abundance of product ions formed in the MS/MS analysis of low femtomole amounts of peptide. This is demonstrated in Figure 5 where the product ion spectrum of SIINFEKL was obtained using xenon at a gas pressure of 1.2×10^{-5} mbar with three different collision energies of 18, 22, and 26 eV. Clearly even a collision energy of 18 eV (Figure 5A) yields a series of y and b ions that readily allows determination of the peptide sequence. However, the product ion spectrum obtained at 26 eV (Figure 5C) not only contains a more abundant y and b series of ions but also a number of new product ions that were not readily discernible at 18 eV. Since our data

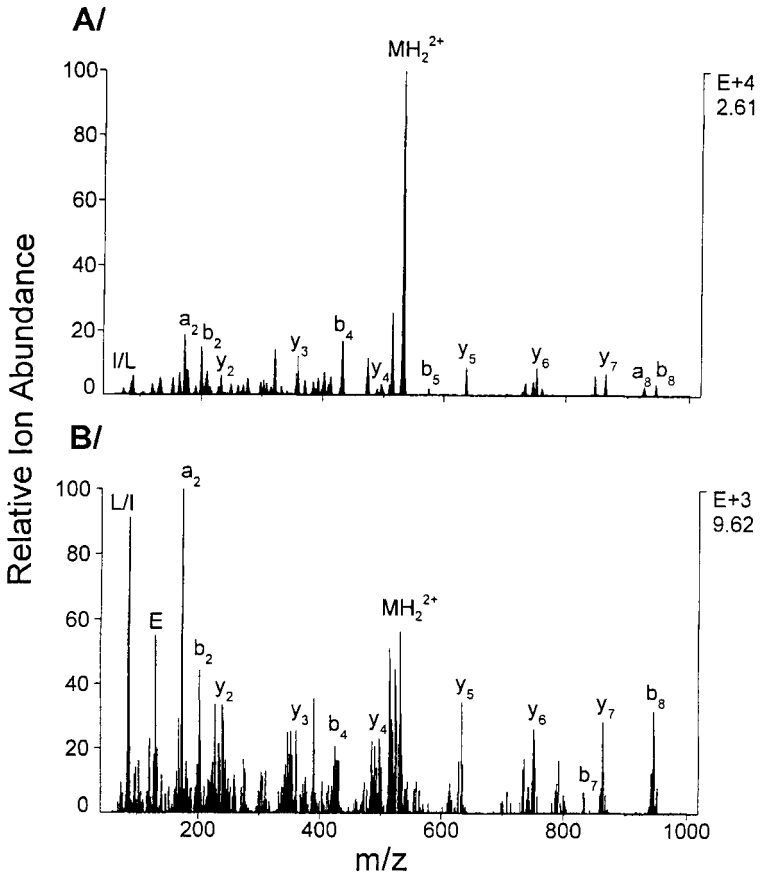

Figure 4 Product ion spectrum after mPC-tITP-CE-MS/MS analysis of ~60 femtomoles of SIINFEKLT, precursor ion selected MH_2^{2+} = 533. Conditions as for Figure 2 except separation voltage was 25 kV (plus low pressure infusion - 0.5 psi). LSB was 110 nL, elution solvent volume was 80 nL and TSB was 65 nL. For MS/MS conditions, the collision energy was 22 eV and A) argon was collision gas at a gas pressure of 2 x 10^{-5} mbar and B) xenon was collision gas at a gas pressure of 1.2 x 10^{-5} mbar.

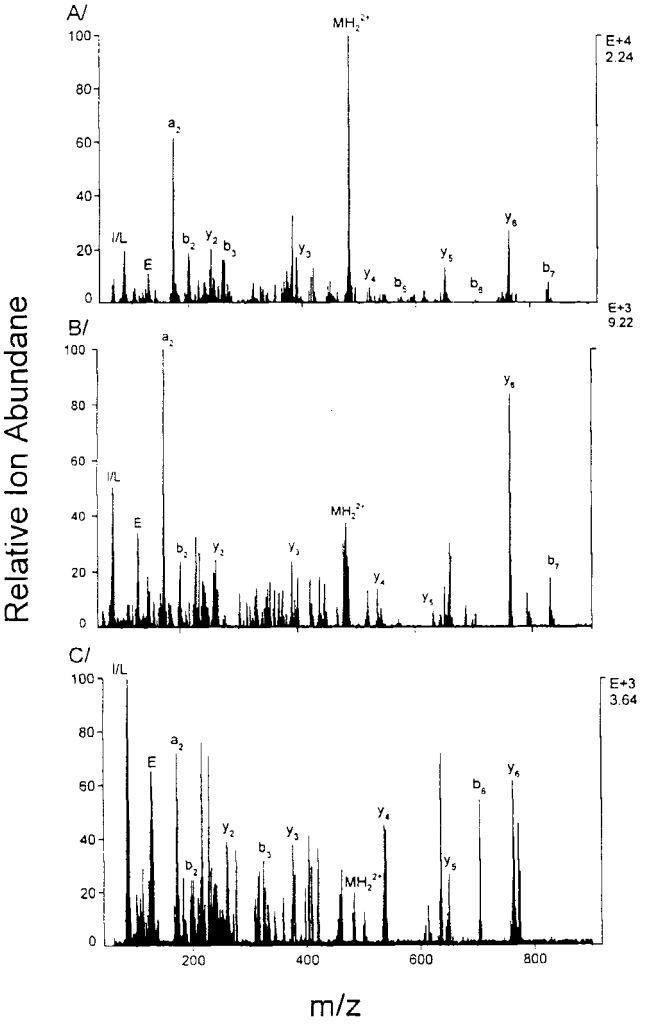

Figure 5 Effect of collision energy on the product ion spectrum of ~60 femtomoles of SIINFEKL percursor ion selected MH_2^{2+}= 482, after mPC-tITP-CE-MS/MS analysis. Conditions as for Figure 4 except xenon was used as collision gas at a gas pressure of 1.2 x 10^{-5} mbar. Collision energies used were: A) 18 eV, B) 22 eV, and C) 26 eV.

base is limited, it is difficult 'a priori' to predict an optimal collision energy for a biologically derived peptide of unknown sequence. Therefore, we suggest and are currently investigating the use of an automatic ramp of the collision energy for each MS_2 scan.

Parameters, other than those associated with the collision cell, that effect both sensitivity and quality of the peptide sequence data obtained by mPC-tITP-CE-MS/MS include the internal diameter of the mPC-CE capillary (51) and the physico-chemical properties of the analyzed peptide (52). It has been previously shown that smaller internal diameter capillaries tend to improve electrospray-MS (ESI-MS) sensitivity by the formation of smaller droplets during spray processes. Therefore, we compared the performance of mPC-CE capillaries of both 25 and 50 μm internal diameter. As expected, from the work of Smith *et al* (51), a mPC-CE capillary of reduced internal diameter greatly improved the signal:noise ratio of MS/MS product ion spectra. This is demonstrated by the analysis of ~70 fmol of the synthetic peptide SIINFEKL using mPC-tITP-CE-MS/MS (Figure 6). These results show a dramatic improvement in signal:noise ratio in the data obtained using a mPC-CE capillary of 25 μm i.d. (Figure 6B). The yield of sequence specific product ions was also enhanced in the latter spectrum, improving the completeness of the sequence information that can be derived by mPC-tITP-CE-MS/MS analysis of such small amounts of peptides.

As discussed, the ultimate sensitivity of a MS/MS method for peptide sequencing is also dependent upon the physico-chemical properties of such analytes. In particular, it is well known that both aspartic and glutamic acid amino acid residues tend to decrease ESI

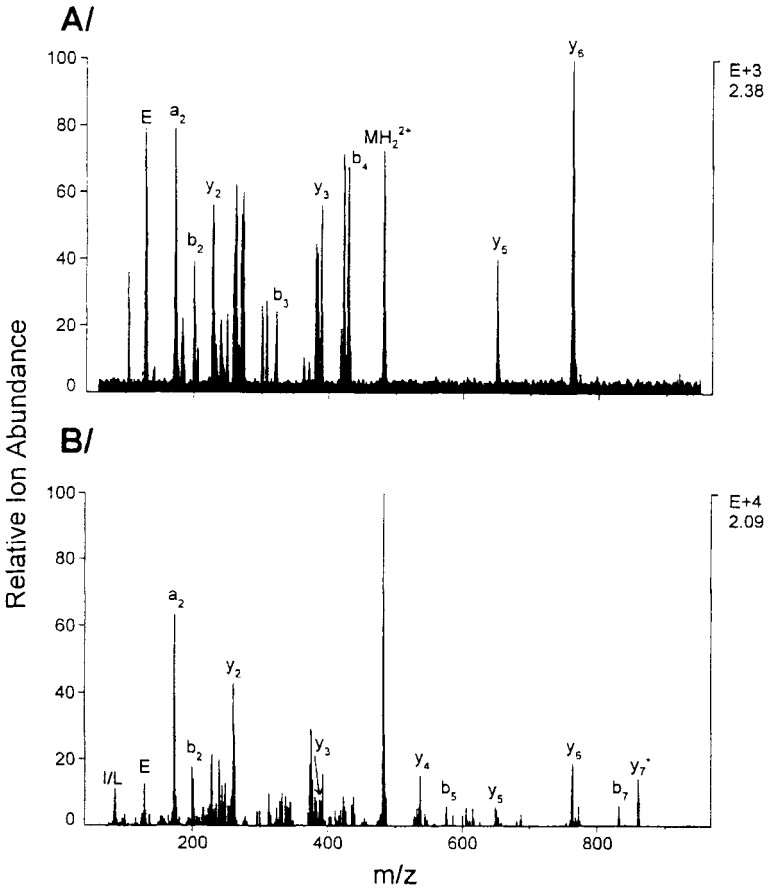

Figure 6 Effect of a change in the internal diameter of CE capillary on the product ion spectrum of ~60 femtomoles of SIINFEKL (MH_2^{2+}= 482) after mPC-tITP-CE-MS/MS analysis using 22 eV collision energy. A) Conditions as for Figure 4A using a 50 µm i.d. CE capillary. B) Conditions as for Figure 4A using a 25 µm i.d. CE capillary. LSB was 80 nL, elution solvent volume was 80 nL and TSB was 15 nL.

sensitivity in positive ion mode and also direct fragmentation processes within the collision cell. Reducing the acidic nature of these residues by esterification can minimize such effects on the MS/MS spectra (53). In this regard, since the model peptides used during these studies possessed a glutamic acid residue at position 6, we expected that methylation of both this residue and the C-terminus to improve the sensitivity of our mPC-tITP-CE-MS/MS methodology. Figure 7 shows a comparative analysis of native and methylated analogs of SIINFEKL while these data yielded only partial sequence by consumption of ~80 fmol of the native peptide (Figure 7A) an almost complete sequence was derived from ~40 fmol of its methylated analog (Figure 7B).

In summary, we have shown in this report that careful consideration of good chromatographic practices can lead to an efficient method of on-line preconcentration for CE and CE-MS methodology. Hence, minimizing the bed volume of a suitably impregnated adsorptive membrane can overcome the many limitations experienced by solid phase preconcentration-CE methods. In particular, while mPC-CE methods yield the advantages of large sample (>60 µL) analysis, it has almost no effect on the CE separation processes when used in conjunction with tITP conditions. Furthermore, the combination of mPC-tITP-CE on-line with a mass spectrometer as the detector leads to a much higher mass sensitivity when compared to conventional UV detectors. In addition, the mass spectrometer is currently the only convenient method of choice for the structural characterization with on-line chromatographic separation. However, we have also shown here and previously (27) that ultimate MS and MS/MS sensitivity is only achieved

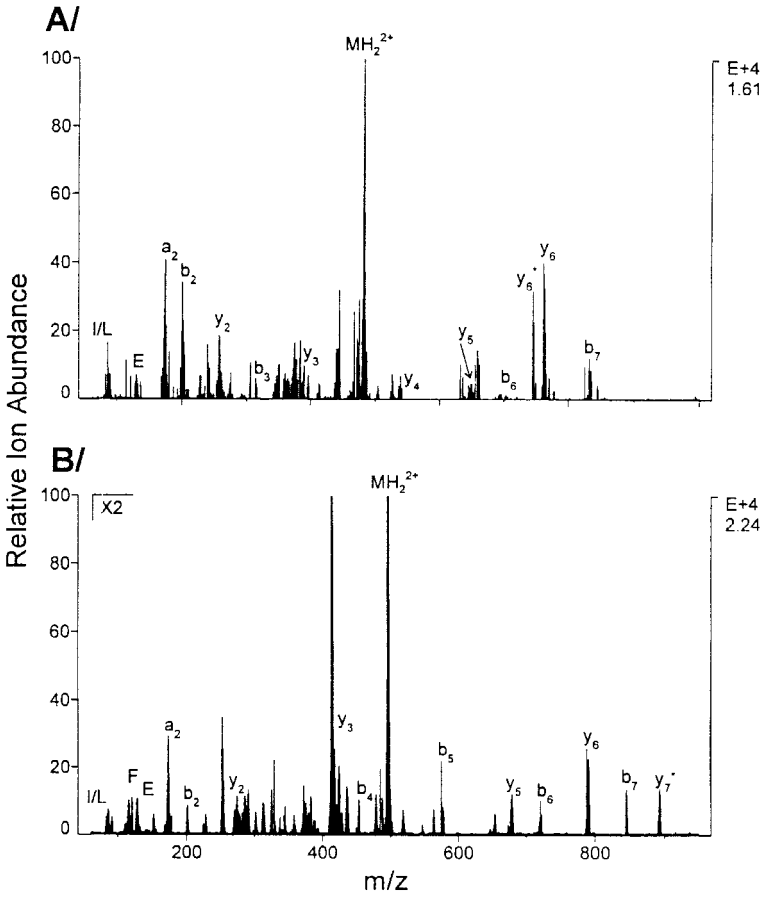

Figure 7 Effect of methylation on the product ion spectrum of SIINFEKL. Conditions as for Figure 4A except: A) ~80 femtomoles of native peptide - precursor ion MH_2^{2+}= 482. B) ~40 femtomoles of methylated peptide - precursor ion MH_2^{2+}= 510.

when the chromatographic system and mass spectrometer are considered as one. In this regard, as ESI-MS sensitivity is increased when the internal diameter of the spray needle is reduced, and it was subsequently demonstrated that a smaller bore mPC-CE capillary leads to enhance mPC-CE-MS/MS performance. While this tends to increase analysis times due to a slower hydrodynamic flow resulting in longer on-line sample injection and cleanup times, this potential limitation of mPC-CE may be overcome by off-line preparation of the mPC-CE cartridge. The mPC-CE cartridge being installed into the CE capillary only when ready for analyte elution.

Finally, in this report we have demonstrated ultimate mPC-tITP-CE-MS/MS performance was obtained when the physicochemical properties of the synthetic MHC class I peptides were considered. Hence, in this example, esterification of the C-terminus and glutamic acid residue at position 6 of these peptides yielded more complete sequence data while consuming less peptide. Thus while we continue to refine this strategy to enable sequence determination of smaller amounts of peptide, we have demonstrated the potential of on-line mPC-tITP-CE-MS/MS for sequencing low concentrations (~40 fmol) of a series of synthetic MHC class I peptides.

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References

(1) Biemann, K., Sequencing of Peptides by Tandem Mass Spectrometry and High Energy Collision Induced Dissociation, in Methods in enzymology: Mass Spectrometry Vol. 193, McCloskey, J.A. ed. Academic Press, San Diego, 1990, p.455.

(2) Biemann, K., Primary Structures of Peptides and Proteins, in Biological Mass Spectrometry: Present and Future, Matsuo, T., Caprioli, R.M., Gross, M.L. and Seyama, Y. eds. Wiley & Sons, Chichester, 1994, p.275.

(3) Janeway, Jr.,C.A., Sci. Am., 269, 72, 1993.

(4) Hunt, D. F., Henderson, R. A., Shabanowitz, J., Sakaguchi, K., Michel, H., Sevilir, N., Cox, A. L., Appella, E. and Engelhard, V. H., Science, 255, 1261, 1992.

(5) Cox, A. L., Skipper, J., Chen, Y., Henderson, R. A., Darrow, T. L., Shabanowitz, J., Engelhard, V. H., Hunt, D. F. and Slingluff, C. L.,Jr., Science, 264, 716, 1994.

(6) Tomlinson, A. J., Nevala, W. K., Wettstein, P. J. and Naylor, S., Converted no journal, 181, 1994.(Abstract)

(7) Tomlinson, A.J., Nevala, W.K., Braddock, W.D., Strausbauch, M.A., Wettstein, P.J. and Naylor, S., A strategy for isolating and sequencing MHC class I peptides by CE-ESI-MS, in Proceedings: The 42nd ASMS Conference on Mass Spectrometry and Allied Topics, 1994, p.23.

(8) Naylor, S., Braddock, W.D., Oda, R.P., Nevala, W.K., Strausbauch, M.A., Wettstein, P.J.and Tomlinson, A.J., A strategy for isolating and sequencing MHC class I peptides by preconcentration-capillary electrophoresis-ESI-MS, in Third International Symposium on Mass Spectrometry in the Health and Life Sciences, 1994, p.108.

(9) Cai, J. and El Rassi, Z., J. Liq. Chromatogr., 15(6&7), 1179, 1992.

(10) Benson, L. M., Tomlinson, A. J., Reid, J. M., Walker, D. L., Ames, M. M. and Naylor, S., J. High Resol. Chromatgr., 16, 324, 1993.

(11) Mikkers, F. E. P., Everaerts, F. M. and Verheggen, Th. P. E. M., J. Chromatogr., 169, 1, 1979.

(12) Abersold, R. and Morrison, H. D., J. Chromatogr., 516, 79, 1990.

(13) Gebauer, P., Thormann, W. and Bocek, P., J. Chromatogr., 608, 47, 1992.

(14) Schwer, C. and Lottspeich, F., J. Chromatogr., 623, 345, 1992.

(15) Beckers, J. L. and Ackermans, M. T., J. Chromatogr., 629, 371, 1993.

(16) Chien, R. L. and Burgi, D. S., Anal. Chem., 64, 489A, 1992.

(17) Foret, F., Sustacek, V. and Bocek, P., J. Microcol. Sep., 2, 229, 1990.

(18) Smith, R. D., Fields, S. M., Loo, J. A., Barinaga, C. J., Udseth, H. R. and Edmonts, C. G., Electrophoresis, 11, 709, 1990.

(19) Beckers, J. L. and Everaerts, F. M., J. Chromatogr., 508, 19, 1990.

(20) Stegehuis, D. S., Irth, H., Tjaden, U. R. and vanderGreef, J., J. Chromatogr., 538, 393, 1991.

(21) Tinke, A. P., Reinhoud, N. J., Niessen, W. M. A., Tjaden, U. R. and Van der Greef, J., Rapid Commun. Mass Spectrom., 6, 560, 1992.

(22) Foret, F., Szoko, E. and Karger, B. L., J. Chromatogr., 608, 3, 1992.

(23) Lamoree, M. H., Reinhold, N. J., Tjaden, U. R., Niessen, W. M. A. and Van der Greef, J., Biol. Mass Spectrom., 23(6), 339, 1994.

(24) Guzman, N. A., Trebilcock, M. A. and Advis, J. P., J. Liq. Chromatogr., 14, 997, 1991.

(25) Guzman, N.A., Gonzalez, C.L., Trebilcock, M.A., Hernandez, L., Berck, C.M. and Advis, J.P., The use of capillary electrophoresis in clinical diagnosis, in Capillary Electrophoresis Technology, Guzman, N.A. ed. Marcel Dekker Inc., New York, 1993, p.643.

(26) Guzman, N. A., World Patent WO 93/05390, March 18, 1993.

(27) Tomlinson, A. J., Benson, L. M. and Naylor, S., J. Cap. Elec.,1, 127, 1994.

(28) Tomlinson, A. J., Benson, L. M., Oda, R. P., Braddock, W. D., Strausbauch, M. A., Wettstein, P. J. and Naylor, S., J. High Resol. Chromatgr., 17, 669, 1994.

(29) Benson, L. M., Tomlinson, A. J. and Naylor, S., J. High Resol. Chromatgr., 17, 671, 1994.

(30) Tomlinson, A. J., Benson, L. M., Braddock, W. D., Oda, R. P. and Naylor, S., J. High Resol. Chromatgr., 17, 729, 1994.

(31) Naylor, S., Benson, L. M. and Tomlinson, A. J., J. Cap. Elec., 1, 181, 1994.

(32) Tomlinson, A. J., Braddock, W. D., Benson, L. M., Oda, R. P. and Naylor, S., J. Chromatogr., submitted,1995.

(33) Tomlinson, A. J., Benson, L. M., Oda, R. P., Braddock, W. D., Riggs, B. L., Katzmann, J. A. and Naylor, S., J. Cap. Elec., 2, 97, 1995.

(34) Tomlinson, A. J., Benson, L. M., Braddock, W. D., Oda, R. P. and Naylor, S., J. High Resol. Chromatgr., In Press,1995.

(35) Tomlinson, A. J. and Naylor, S., J. High Resol. Chromatgr., In Press,1995.

(36) Tomlinson, A.J. and Naylor, S., J. Cap. Elec., Submitted, 1995.

(37) Kasicka, V. and Prusík, Z., J. Chromatogr., 273, 117, 1983.

(38) Debets, A. J. J., Mazereeuw, M., Voogt, W. H., van Iperen, D. J., Lingeman, H., Hupe, K. -P. and Brinkman, U. A. Th., J. Chromatogr., 608, 151, 1992.

(39) Swartz, M. E. and Merion, M., J. Chromatogr., 632, 209, 1993.

(40) Morita, I. and Sawada, J., J. Chromatogr., 641, 375, 1993.

(41) Hoyt, A. M.,Jr., Beale, S. C., Larmann, J. P.,Jr. and Jorgenson, J. W., J. Microcol. Sep., 5, 325, 1993.

(42) Fishman, H. A., Shear, J. B., Colon, L. A., Zave, R. N. and Sweedler, J. V., U.S. Patent 5,318,680, June 7, 1994.

(43) Pálmarsdóttir, S. and Edholm, L. -E., J. Chromatogr. A., 693, 131, 1995.

(44) Beattie, J. H., Self, R. and Richards, M. P., Electrophoresis, 16, 322, 1995.

(45) Naylor, S., Tomlinson, A. J., Benson, L. M., Braddock, W. D. and Oda, R. P., U.S. Patent Application, 1995.

(46) Tomlinson, A. J., Benson, L. M. and Naylor, S., LC-GC, 12, 122, 1994.

(47) Tomlinson, A. J., Benson, L. M. and Naylor, S., Am. Lab., 26, 29, 1994.

(48) Tomlinson, A. J. and Naylor, S., J. Cap. Elec., Submitted,1995.

(49) Tomlinson, A. J. and Naylor, S., manuscript preparation,1995.

(50) Tomlinson, A. J., Braddock, W. D., Pesch, R. and Naylor, S., Rapid Commun. Mass Spectrom., Submitted,1995.

(51) Wahl, J. H., Gale, D. C. and Smith, R. D., J. Chromatogr., 659, 217, 1994.

(52) Kebarle, P. and Tang, L., Anal. Chem., 65, 972A, 1993.

(53) Hunt, D. F., Yates, J. R., Shabanowitz, J., Winston, S. and Haver, C. R., Proc. Natl. Acad. Sci., 83, 6233, 1986.

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