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Identification of proteins by capillary electrophoresis–tandem mass spectrometry
Evaluation of an on-line solid-phase extraction device

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Abstract

Capillary electrophoresis–tandem mass spectrometry has been used successfully for the analysis of complex peptide mixtures. The method is limited by a relatively high concentration limit of detection and by matrix effects. Here we describe on-line coupling of a solid-phase microextraction device to a capillary electrophoresis–tandem mass spectrometry system. The performance of the integrated instrument was evaluated for the identification of proteins by their amino acid sequence. We report that the concentration limit of detection was improved at least 1000 fold to the low attomole/μl range and that matrix effects were minimized by extensive sample clean-up during solid-phase extraction. We demonstrate that the implementation of a solid-phase extraction device significantly enhances capillary electrophoresis–tandem mass spectrometry as a method for the identification of low abundance proteins isolated from high-resolution two-dimensional polyacrylamide gels.

Keywords: Extraction methods; Detection, electrophoresis; Proteins; Albumin

1. Introduction

The comprehensive investigation of biological processes and systems is usually complicated by the fact that numerous proteins participate in a particular function, either in an executive or a regulatory capacity. Their identification by amino acid sequence and the determination of their respective states of modification represent key steps in understanding biological processes. The increasing number of DNA sequences available through rapidly expanding databases provides a fast way of identifying proteins by correlation of the information contained in the amino acid sequence with that on the databases. Different algorithms have been developed that quickly identify proteins by either their amino acid compositions, partial amino acid sequences or the data obtained by a variety of mass spectrometric experiments [1].

Recent developments in the field of mass spectrometry have greatly increased the sensitivity achievable for protein and peptide analysis. For electrospray ionization mass spectrometry (ESI-MS), major improvements were obtained by the introduction of microspray and nanospray interfaces [2–9]. We and others have previously demonstrated the power of capillary electrophoresis (CE), combined with micro electrospray–tandem mass spectrometry (ESI-MS–MS) [6–9], in particular for the rapid identification of proteins [9]. In this approach, the protein of interest was digested with trypsin and the peptide components of the digest were separated by CE and subjected to collision-induced dissociation (CID) in a triple quadrupole mass spectrometer. The uninterpreted tandem mass spectra are then used by

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the SEQUEST program [10,11] to search sequence databases. Using this technique, we achieved a mass detection limit (LOD) at the attomole (amol) level [9]. However, as is typical for CE, the concentration LOD was too low for the analysis of proteins present in minute quantities and matrix effects were found to affect both the separation efficiency of CE and peptide detection by MS.

Here, we report on improvements of the technique described above. By coupling a solid-phase extraction (SPE) device, for the concentration of analytes from dilute samples on-line, with the CE–MS–MS system, we achieved a concentration LOD in the low amol/μl range and demonstrated on-line sample clean-up for the reduction of matrix effects. We believe that the described improvements now make the technique compatible with the analysis of low abundance proteins separated by gel electrophoresis.

2. Experimental

2.1. Materials

Acetic acid, hydrofluoric acid, acetonitrile and methanol were from J.T. Baker (Phillipsburg, NJ, USA). The peptide standards were obtained from Sigma (St. Louis, MO, USA). Ultra-pure, carrier-grade helium was from Air Products (Allentown, PA, USA). The capillary tubing was from Polymicro Technologies (Phoenix, AZ, USA). PTFE sleeves were from Upchurch Scientific (Oak Harbor, WA, USA; 250 μm I.D.) and from Cole-Parmer (Niles, IL, USA; 150 μm I.D.). Rapidly hardening epoxy glue was from Devcon (Danvers, MA, USA). The C_{18} column material was from Phase Separations (Norwalk, CT, USA). The PTFE membrane was purchased from Applied Biosystems (Foster City, CA, USA) and the water was distilled and deionized (18 MΩ) using a Milli-Q system from Millipore (Bedford, MA, USA).

2.2. Fabrication of the SPE–CE device

The solid-phase extraction (SPE) device was built in a manner similar to the ones reported previously [12–15]. All of the capillaries used in this system were bare fused-silica. In our design (Fig. 1), a piece of capillary (5 cm×50 μm I.D.×150 μm O.D.) was inserted into a PTFE sleeve (250 μm I.D. or 150 μm I.D.) and glued in place using "5 min" epoxy glue. From the other end of the sleeve, a piece of PTFE membrane was inserted and pushed against the capillary. Then, a suspension of C_{18} derivatized silica material (5 μm beads with 300 Å pores) in methanol was inserted into the sleeve by applying a weak vacuum at the capillary end. The beads were prevented from entering the capillary by the PTFE membrane and therefore accumulated in the sleeve. After approximately 1 mm of the sleeve had been filled with the C_{18} material, the column was washed with methanol and another piece of PTFE membrane was inserted on top of the column. Finally, one end of the CE capillary (40–60 cm×50 μm I.D.×150 μm O.D.) was inserted into the sleeve and glued in place.

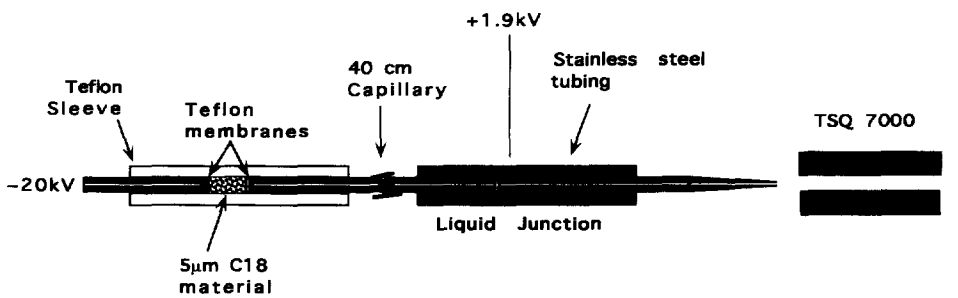

Fig. 1. Schematic diagram of the SPE–CE–microelectrospray-MS–MS system. All of the experiments were performed using 50 μm I.D.×150 μm O.D. capillaries. CE was performed at −20 kV and a pressure of 0.5 p.s.i. at the injection end. The microelectrospray was generated by applying a potential of +1.7 to +1.9 kV at the liquid junction.

2.3. Coupling of the SPE–CE device to a microsprayer

A schematic representation of the connection of the SPE–CE device to the microsprayer is shown in Fig. 1. The free end of the CE capillary was inserted into a clean piece of 180 μm I.D. stainless steel tubing (2–3 cm long) and glued in place. At the other end of the stainless steel tubing, approximately 3 cm of 50 μm I.D. fused-silica capillary (microsprayer) was inserted up to the CE capillary and glued in place. The small gap between the two capillaries provided a liquid junction to establish electrical contact with the solvent. The polyimine coating of the distal 1–2 cm of the microsprayer had been removed previously in a gentle flame. The exposed silica was either etched using hydrofluoric acid or pulled in a flame to reduce the outer diameter. Both procedures were suitable for the generation of satisfactory microsprayer tips. The stainless steel tube was mounted on a laboratory-made holder, with the microspray tip towards the orifice of the mass spectrometer. The mass spectrometer high-voltage power supply was connected through a series of five 5 MΩ high-voltage resistors to the stainless steel tube. The purpose of the resistors was to limit the maximal current generated by the mass spectrometer high-voltage power supply to less than 10 μA in order to protect circuit boards in the mass spectrometer from damage. Using an XYZ translation stage, the tip of the microspray end of the capillary was placed approx. 5 mm away from the capillary entrance of the mass spectrometer.

2.4. Protocol for the SPE–CE–microspray experiment

The SPE column was thoroughly washed with acetonitrile and CE running buffer (10 m*M* acetic acid, pH 3.3 in 10% methanol). The sample was forced through the column by applying a pressure of 15 p.s.i. at the injection end of the set-up (1 p.s.i.= 6894.76 Pa). This pressure generated a flow of approximately 1.5 μl/min. Once the sample was completely applied, the resin was washed extensively with the CE running buffer for 5 to 10 min at a pressure of 15 p.s.i. Elution was achieved by injecting a plug of 63% acetonitrile in 3 m*M* acetic acid at 6 p.s.i. for 6 s. The CE buffer was installed at the injection end of the capillary and the CE experiment was started by applying −20 kV at the injection end and +1.8 kV at the microsprayer end, while maintaining a pressure of 0.5 p.s.i. The CE system with controllable pressure was laboratory-made.

2.5. Mass spectrometry

MS was performed on a model TSQ 7000 triple quadrupole mass spectrometer (Finnigan Mat, San Jose, CA, USA), as previously described [9,16]. All of the experiments were performed at unit resolution. The mass spectrometer was first used in the full scan mode using the third quadrupole to detect the analytes eluting from the capillary. The second quadrupole was filled with 3.5–4.0 mTorr of argon (1 Torr=133.322 Pa), but the energy supplied to the ions was not sufficient to induce CID. Consequently, the masses of the intact peptide ions were detected. Once the ion current for a particular peptide ion exceeded a pre-set threshold, the mass spectrometer automatically switched to the product ion scan mode. The first quadrupole selected the ion of interest and the energy in the second quadrupole was increased to induce fragmentation of the precursor ions by CID. The masses of the product ions were recorded by scanning the third quadrupole. Four full scans at different energies were obtained for each selected ion before the mass spectrometer automatically returned to the full scan mode. Specific instructions in the instrument control language protocol prevented repeated selection of the same precursor ion for CID within a pre-determined time window. This precaution was chosen to avoid the possibility that a high intensity matrix ion could obscure peptide ions of lower intensity and enabled the acquisition of CID spectra from closely eluting peptides.

2.6. Sequence database search strategy

Sequence databases were searched using the SEQUEST search program [10,11]. For each set of CID spectra, SEQUEST extracted the ten best peptide matches from the sequence database and ranked them according to their correlation coefficients. The correlation coefficient indicated the quality of the match between the experimental CID spectrum and

the predicted CID spectra of any isobaric peptide from the sequence database. The Δ correlation factor, which represented the difference between the top scored peptide and the next best matches, was also calculated and displayed. From experience, we know that a correlation coefficient of two or higher indicates a highly significant match, while a Δ correlation factor higher than 0.1 indicates a good distinction between the top and the next best match.

In cases in which a homogeneous protein was analyzed, the CID spectrum of each peptide was analyzed and matched individually to the sequence database. Then, the matches of all of the peptides derived from one protein were collectively scored for the identification of the protein. The composite score was obtained by summing up the individual scores from each peptide using an arbitrarily chosen score of ten for a first position peptide identification, eight for the second position, six for the third, four for the fourth and two for the fifth position.

2.7. Tryptic digest

Bovine serum albumin (BSA) was digested in solution at a concentration of 8 pmol/μl. A 2-μl volume of trypsin was mixed with 3 μl of 100 m*M* ammonium bicarbonate, pH 7.8–acetonitrile (90:10, v/v) and added to 5 μl (1 μg/μl) of the protein solution in the same solvent, to give a total volume of 10 μl. The mixture was incubated at 37°C for 2–3 h. A 1.5-μl volume of 10% TFA was added to the solution to stop the reaction.

Yeast proteins were separated by high-resolution two-dimensional polyacrylamide gel electrophoresis (2DE), transferred to nitrocellulose by electroblotting and fragmented on the membrane as previously described [16–18].

3. Results and discussion

3.1. CE–MS–MS of a protein digest

In a previous study we evaluated the potential of CE–MS–MS for the analysis of complex peptide mixtures [9]. Using standardized samples, we achieved a 300-amol mass detection limit for the detection of peptides in the full scan operating mode and a 660-amol mass detection limit for detection in the MS–MS mode of operation. To test the suitability of CE–MS–MS peptide mapping for the identification of proteins in a realistic experimental setting, we applied the method to the analysis of a tryptic digest of BSA that had been subjected to gel electrophoresis and isolated by electroblotting onto nitrocellulose [16,17]. Results are shown in Fig. 2. It became apparent that the main peaks in the electropherogram (e.g. the peaks eluting around 10.5 and 11 min) mainly contained non-peptidic contaminants. While some of peptide ions derived from BSA were identified, others were obscured by matrix ions. Furthermore, the concentration LOD required for the identification of BSA was in the range of 100–200 fmol/μl, which is in good agreement with the concentration LOD required for successful protein identification using the nanospray technology [2,3]. These results indicate that the routine analysis of low abundance proteins separated by gel electrophoresis requires improvements in both the concentration LOD and the influence of matrix effects on the analysis.

3.2. Construction of a SPE–CE–MS–MS system

To overcome these limitations, we evaluated the use of a SPE device coupled on-line with a CE–MS–MS system equipped with a microelectrospray interface. The system we built is schematically shown in Fig. 1. This system is similar in construction to others [12–15,19,20]. However, in our system, C_{18} beads are used for the extraction instead of C_{18} membrane [19,20], microelectrospray is used instead or regular electrospray with sheath liquid and the identification of the protein is done automatically using the SEQUEST program. Our experience with the C_{18} beads indicates that once the parameters affecting the system are well understood, it performs well. Many different types of beads are available (reversed phase and affinity), in the case of membrane, the choices are limited. For the operation of the SPE device, it was critical to avoid a high back pressure, which would impede solvent flow through the capillary. We identified two principal causes for high back pressure. The first was the resin packed in the extraction column and the second was a misalignment of the capillaries in the liquid junction. We

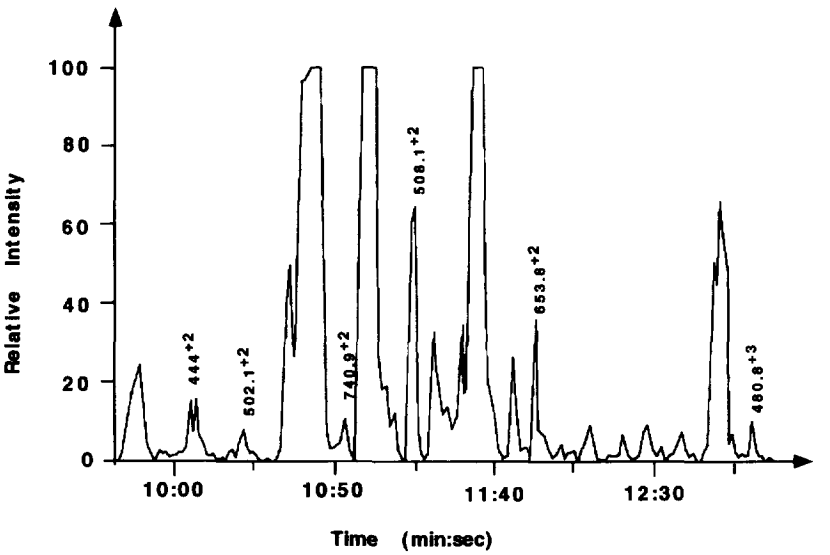

Fig. 2. Analysis of a BSA tryptic digest by CE–MS. BSA was subjected to gel electrophoresis and isolated by electroblotting onto nitrocellulose. Tryptic fragments were separated in a 100-cm long 3-aminopropylsilanated capillary (−20 kV at the injection end and +2.2 kV at the microspray end). Sample injection was for 20 s at −20 kV at the injection end and ground at the microspray end. The ions identified from BSA and their respective charge states are indicated on the spectrum.

found that it was important to pack the C_{18} material relatively loosely in the SPE device and to keep the amount of column material to a minimum. Excess material also resulted in band broadening. Misalignment of the capillaries in the stainless steel needle of the liquid junction could completely block the solvent flow. This mainly occurred if the spacing between the sprayer and the separation capillary was so small that the CE capillary blocked the entrance to the microsprayer. Blockage in the liquid junction could be detected easily before using the system.

Provided that these parameters were understood, the system could be built reproducibly in less than 30 min. Each device could be used for numerous analyses over several months with good separation. The cost for the fabrication of the device is negligible and the system is simple to operate.

3.3. Limit of detection

Using a tryptic digest of a standardized BSA sample, we attempted to evaluate the effect of the added SPE device on the mass detection limit and the concentration LOD. The mass detection limit was defined as the number of moles of sample necessary to generate a signal that was equal to three times the standard deviation of the background signal. The concentration LOD was defined as the concentration of sample necessary to obtain a signal equal to three times the standard deviation of the background signal, if 20 μl of sample were applied. We arbitrarily evaluated the concentration LOD using sample volumes of 20 μl because this volume is typical for the proteolysis of electroblotted proteins. It is obvious that by increasing the sample volume, even lower concentration LODs could be achieved. Each of the LODs was first evaluated using the mass spectrometer operated in the single MS mode for the detection of peptides only. In a second experiment MS–MS spectra of selected peptide ions were generated by CID and analyzed by the SEQUEST program. This experiment measured the LOD for the identification of proteins by searching a composite bovine database using CID spectra.

Fig. 3 shows the electropherogram obtained at a concentration of 420 amol/μl with the detector operated in the single MS mode. The labeled peaks were identified as being derived from BSA. The peaks eluting before 7 min 30 s were due to the change in buffer composition caused by the acetoni-

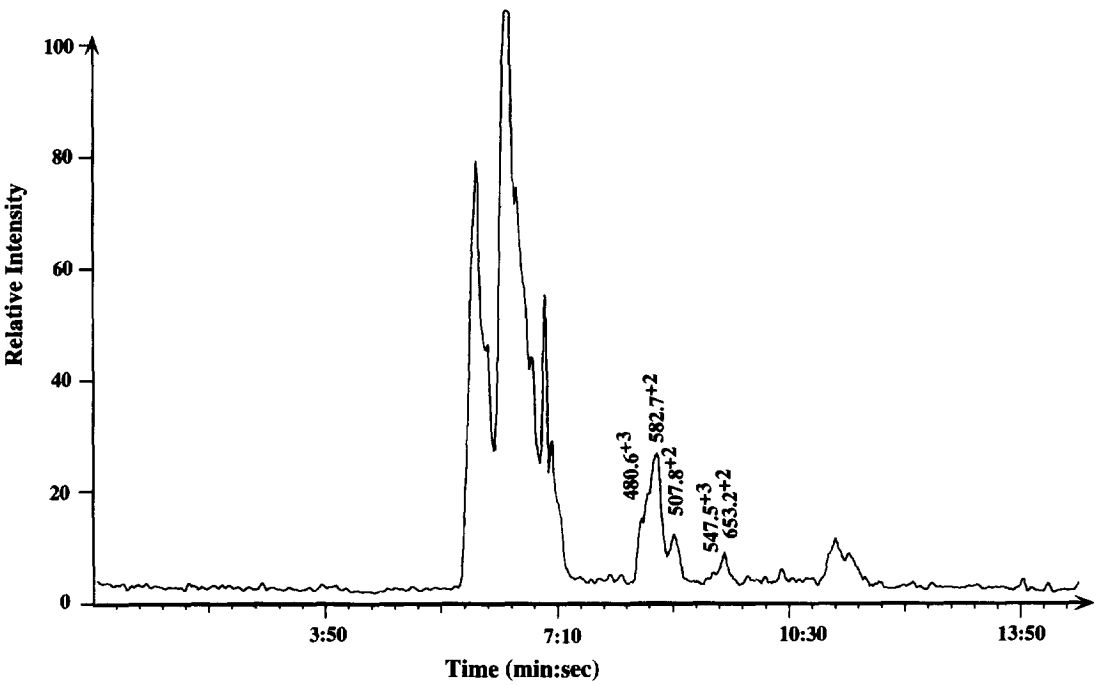

Fig. 3. Analysis of a BSA tryptic digest by SPE–CE–MS. The sample was applied at 420 amol/μl to the SPE–CE–microelectrospray-MS system. The CE capillary was 47 cm long. An 18-μl volume of the digest was applied to the system at 12 p.s.i. for 16 min. Other conditions were as indicated in Fig. 1. The total separation time was 13 min.

trile plug that was used to elute the peptides from the SPE device. From the intensity of the peak and the standard deviation of the background signal, we calculated a mass detection limit of 660 amol and a concentration LOD at 33 amol/μl.

A similar set of experiments was performed to determine the LOD in the MS–MS mode. The CID spectra obtained were searched against a bovine protein database using the SEQUEST program. Fig. 4 shows the electropherogram obtained at a sample concentration of 2.5 fmol/μl and Table 1 indicates the values of the correlation coefficient and the Δ correlation factors obtained for the peptides derived from BSA. The peaks eluting at less than 6 min were due to a change in buffer composition. From these data, we calculated that in MS–MS mode, the mass detection limit was 6 fmol and the concentration LOD was 300 amol/μl. This value for the mass detection limit is in good agreement with results we obtained by CE–microspray MS–MS [9], except that the concentration LOD obtained in the present experiment is at least three orders of magnitude lower than that achieved using CE–microspray MS–MS without a SPE device.

3.4. Identification of yeast proteins separated by 2DE

The data shown in Fig. 2 indicate that the sample preparation of proteins separated by gel electrophoresis can introduce significant amounts of contaminants which can obscure the detection of low abundance peptide peaks. Therefore, we next evaluated the effect of the addition of a SPE device on our ability to identify proteins separated by 2DE using the CE–MS–MS system. Proteins contained in a total cell lysate from yeast were separated by 2DE and transferred to nitrocellulose by electroblotting. In Fig. 5, the two protein spots selected for further analysis are marked on a silver-stained replica of the

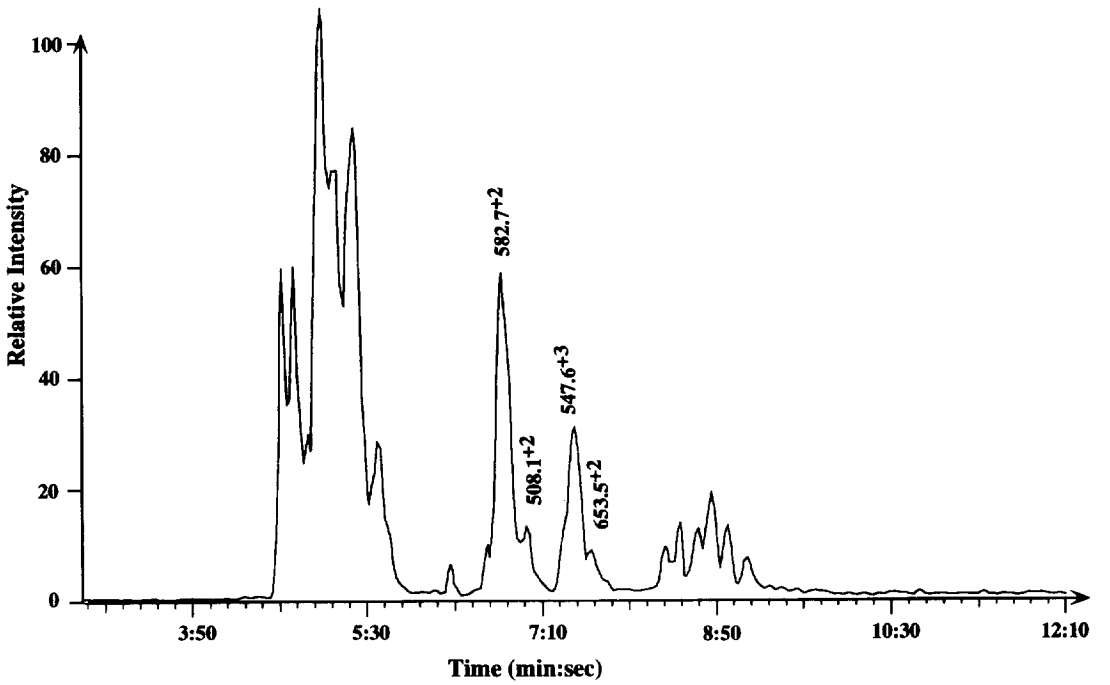

Fig. 4. Analysis of a BSA tryptic digest by SPE–CE–MS–MS. Sample was applied at 2.5 fmol/μl to the SPE–CE–microelectrospray-MS–MS system. A 9-μl volume of the digest was applied at 11 p.s.i. for 10 min. Other conditions were as described in Fig. 3. The switch to the selection of a second ion migrating in a peak or the switch to acquire a second set of MS–MS spectra within the same peak for the same ion is detectable by a discontinuity or shoulder on the peak displayed. The total separation time was 11 min.

blotted gel. Fig. 6 displays the electropherogram obtained from the analysis by SPE–CE–microspray–MS–MS of the protein contained in spot 1 as an example of a high intensity spot and Table 2 summarizes the database search results obtained from that sample. A total of thirteen CID spectra were individually matched to the sequence database entry ENO2-YEAST with good correlation coefficients (Table 2A) and the composite matching score unambiguously identified the protein contained in spot 1 as enolase 2 (Table 2B). Although the scaling used in this scoring system is arbitrary, it is sufficient to quickly identify proteins using CID spectra generated by the experiment. Enolase is a predominant protein in yeast and it generated intense signals in the mass spectrometer. Even though we only used 5 μl of the total sample volume of 30 μl, the peptides saturated the extraction material and caused displace-

Table 1
Results of a bovine sequence database search with CID spectra of BSA-derived tryptic peptides

Mass (MH^{+1})	Correlation coefficient	Δ Correlation	Sequence	Position
1164.3	2.4	0.2	(K)LVNELTEFAK	66→75
1306.5	3.6	0.6	(K)HLVDEPQNLIK	402→412
1640.9	3.7	0.4	(R)KVPQVSTPTLVEVSR	437→451
1015.2	2.5	0.3	(K)QTALVELLK	549→557

The sequence position of the peptides within the BSA sequence is indicated.
All the masses are presented by their MH^{+1} value.

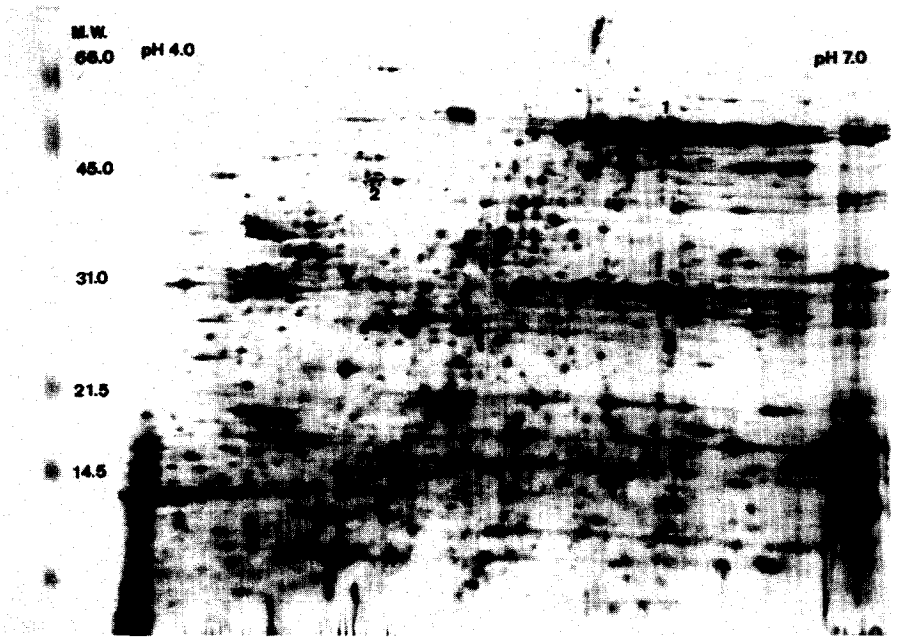

Fig. 5. A two-dimensional gel of proteins in yeast total cell lysate. Separated proteins were detected by silver staining. Proteins were separated in the first dimension by isoelectric focusing using immobilized pH gradients. The p*I* range of 4 to 7 is displayed. The second dimension separation was by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. A 200-μg amount of total protein was applied to the gel. The spots that were further analyzed are numbered. Spot 1 was identified as yeast enolase 2 and spot 2 as the product of gene code YJ75-YEAST in the Swiss Prot sequence database.

ment chromatography effects. With increasing saturation of the column material, the ions with the highest partition coefficient started replacing the ions with lower partition coefficients and eventually this led to the complete elimination of the weakly interacting peptides. This clearly indicates that the small bed of packing has a limited capacity and can be easily saturated with tryptic peptides from the most intense spots. When dealing with intense spots from a blot, better results are therefore obtained if only a fraction of the sample is applied to the system. From the intensity of the spot, we can easily decide if only a fraction of the digest should be used.

The results obtained from the analysis of the protein contained in spot 2 (Fig. 7) are representative for low intensity spots. The electropherogram indicates that numerous peptide species were detected and subjected to CID. Ten tandem mass spectra were used to identify the protein as the product of the Swiss Prot database entry YJ75-YEAST (Table 3). The amount of protein contained in spot 2 was estimated to be 10–20 ng. From the molecular weight (36 372 g/mol) of this protein and assuming 50% peptide recovery after digestion, we calculated that the sample contained 80 fmol of peptide at a concentration of 6 fmol/μl.

Both samples, in particular the sample derived from spot 2, would have been difficult to analyze by CE–MS–MS, due to their low concentrations and matrix effects. Coupling the SPE device on-line with the CE–MS–MS system permitted the analysis of a protein digest at a concentration in the low fmol/μl range and intensive clean-up of the sample loaded on the extraction device. The matrix components that were present initially in the sample (Fig. 2) were not retained on the reversed-phase resin. The only other matrix components that might affect the analysis of protein digests are those that have a high affinity for the extraction material and are positively charged in solution. The results in Fig. 7 indicate that such

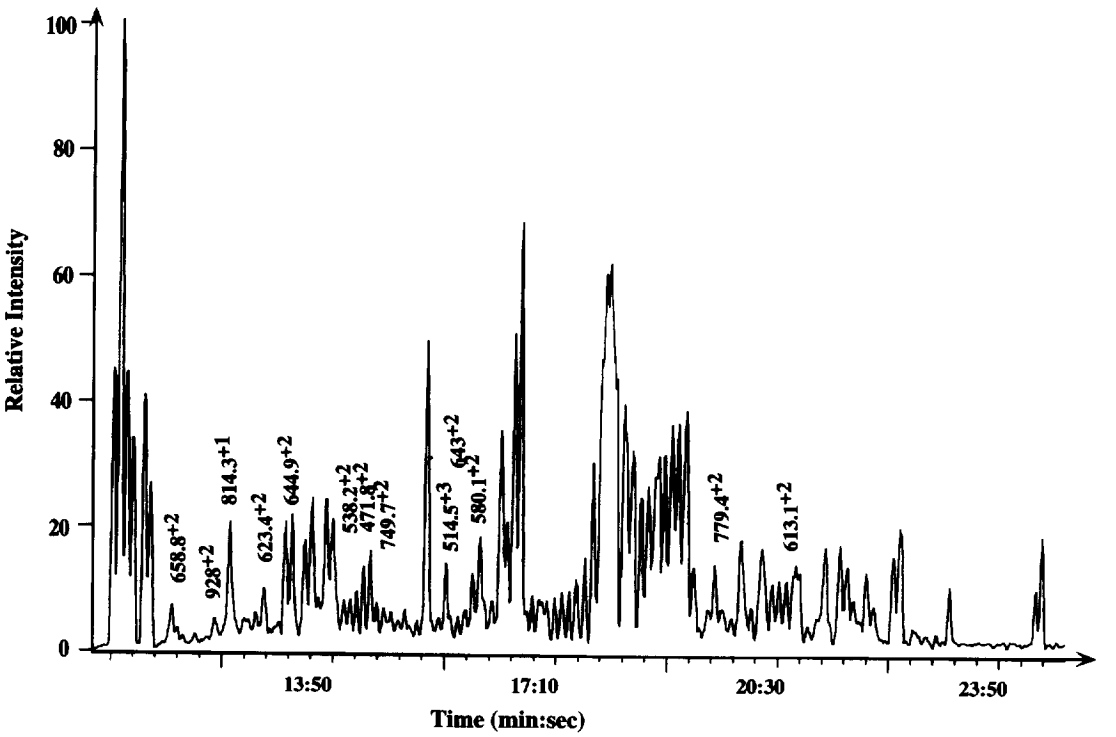

Fig. 6. SPE–CE–MS–MS analysis of the protein in spot 1. A 5-μl volume out of the total sample volume (30 μl) was added to 10 μl of water. A 13-μl volume of this solution was applied to the system at 12 p.s.i. for 10 min. Other conditions were as described in Fig. 4.

species are present at very low concentrations, if at all, in samples prepared by the protocol used.

4. Conclusions

A SPE device was coupled to a CE–MS–MS system equipped with a microspray interface. The system was used to analyze peptide mixtures derived by tryptic digestion of proteins. Specifically, we wanted to evaluate the effects of the addition of the SPE device on the concentration LOD and on the reduction of matrix effects for high sensitivity measurements. We demonstrate concentration LODs at the low amol/μl level for the system operated in the MS mode and at the mid amol/μl level for the system operated in MS–MS mode. Furthermore, we demonstrate significant reduction of matrix effects by SPE. The practical value of the system was demonstrated by the identification of yeast proteins separated by 2DE.

The on-line coupling of the SPE device therefore represents a considerable improvement to the field of CE related to the analysis of peptide mixtures. The usefulness of CE for analytical applications at high sensitivity has been limited by the small injection volume that could be applied to the capillary. The addition of the SPE device as a sample concentration step allows the application of relatively large sample volumes. The second advantage of this system over conventional CE is the fact that intensive washing of the system can be performed after loading the sample. This means that matrix effects can be dramatically reduced. Therefore, this system is compatible with samples containing high concentrations of salt and other polar contaminants found in protein digests.

This system is robust, inexpensive and user-friendly. In particular, no sheath flow is required to achieve

Table 2

(A) Results of yeast sequence database search with CID spectra of tryptic peptides derived from spot 1

Mass (MH^{+1})	Correlation coefficient	Δ Correlation	Sequence	Position
1245.8	3.9	0.24	(N)PTVEVELTTEK	17→27
1541.5	2.9	0.24	(V)PSGASTGVHEALEMR	35→49
1498.3	2.4	0.18	(K)NVPLYQHLADLSK	126→138
1285.0	2.7	0.23	(V)PLYKHLADLSK	128→138
1159.2	2.8	0.25	(R)IGSEVYHNLK	185→194
1316.5	3.1	0.20	(K)IGLDGASSEFFK	243→254
1855.0	5.4	0.32	(K)TAGIQIVADDLTVTNPAR	312→329
942.5	2.4	0.15	(K)KAADALLLK	337→345
814.3	3.1	0.10	(K)AADALLLK	338→345
1288.7	2.8	0.12	(K)VNQIGTLSESIK	346→357
1075.4	2.2	0.21	(N)QIGTLSESIK	348→355
1557.8	3.9	0.29	(K)AVYAGENFHHGDKL	423→436
1225.2	3.1	0.15	(Y)AGENFHHGDKL	426→436

(B) Total score and ranking for the proteins identified in spot 1

Protein	Total score	1	2	3	4	5
ENO2-YEAST	**130**	**13**	**0**	**0**	**0**	**0**
YJ62-YEAST	32	1	1	2	0	1
YBI8-YEAST	32	2	0	2	0	0
BFR1-YEAST	30	2	1	0	0	1
FAB1-yeast	28	0	2	2	0	0

The sequence position of the peptides within the enolase 2 sequence is indicated.
All the masses are presented by their MH^{+1} values

Table 3

(A) Results of a yeast sequence database search with CID spectra of tryptic peptides derived from spot 2

Mass (MH^{+1})	Correlation coefficient	Δ Correlation	Sequence	Position
1579.8	2.78	0.19	(K)YSLKENDAILVDAK	27→40
1844.1	5.42	0.44	(R)GAAYVLGAGQVVYFGSVGK	71→90
1053.2	2.45	0.05	(Y)VLGAGQVVYF	76→85
1481.7	4.08	0.36	(Y)VLGAGQVVYFGSVGK	76→90
2062.3	6.56	0.49	(R)SLVTDLGAANFFTPDHLDK	131→149
1376.5	2.08	0.17	(G)AANFFTPDHLDK	137→149
1069.2	3.79	0.40	(K)HWDLVEAAK	150→158
1554.8	3.2	0.15	(Y)IGGFHLTVSPDAIVK	162→176
1801.6	6.92	0.58	(K)TVIFTHGVEPTVVVSSK	254→270
1600.9	5.50	0.46	(V)IFTHGVEPTVVVSSK	256→270

(B) Total score and ranking for the proteins identified in spot 2

Protein	Total score	1	2	3	4	5
YJ75-YEAST	**100**	**10**	**0**	**0**	**0**	**0**
PAK1-YEAST	24	2	0	0	1	0
S55124	20	2	0	0	0	0
S49777	20	1	0	1	1	0
S50225	20	2	0	0	0	0

The sequence position of the peptides within the YJ75-YEAST sequence is indicated.
All of the masses are presented by their MH^{+1} values.

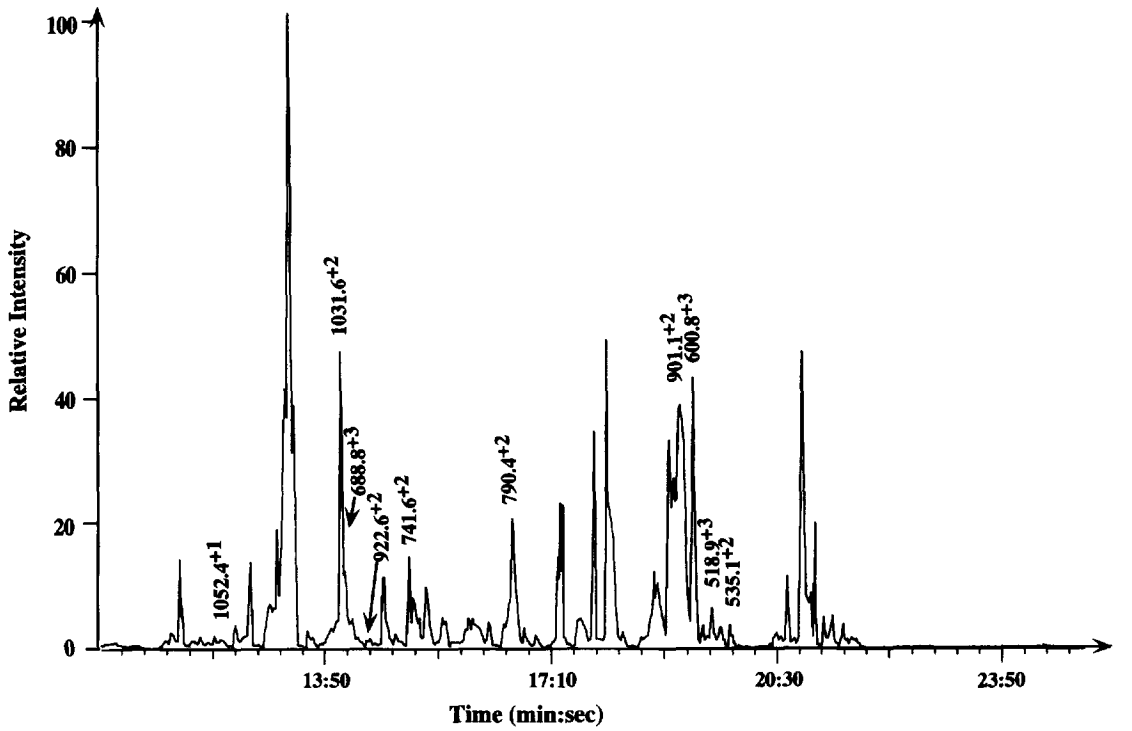

Fig. 7. SPE–CE–MS–MS analysis of the protein in spot 2. A 5-μl volume out of a total sample volume of 15 μl was added to 10 μl of water and applied to the system at 10 p.s.i. for 15 min. Other conditions were as described in Fig. 4.

a stable signal in the mass spectrometer. Finally, this system can be adapted easily for use with affinity materials of different selectivity to extract specific analytes. We therefore expect to see increasing use of this type of system and its adaptation to new types of analyses in the near future.

5. List of Abbreviations

CE	Capillary electrophoresis
ESI	Electrospray ionization
MS–MS	Tandem mass spectrometry
CID	Collision induced dissociation
SPE	Solid-phase extraction device
LOD	Limits of detection
2DE	Two-dimensional polyacrylamide gel electrophoresis
BSA	Bovine serum albumin
amol	Attomol
fmol	Femtomol

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