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Field-enhanced sample injection for high-sensitivity analysis of peptides and proteins in capillary electrophoresis–mass spectrometry

Maria Rowena N. Monton*, Shigeru Terabe

Graduate School of Science, Himeji Institute of Technology, Kamigori, Hyogo 678-1297, Japan

Abstract

Field-enhanced sample injection (FESI) was used to improve the concentration sensitivity of a capillary electrophoresis (CE)–mass spectrometry (MS) system with sheath flow configuration. Using some bioactive peptides, more than 3000-fold improvement in signal was obtained, permitting analysis in the low nM (fmol/μl) levels. The system was further evaluated for analysis of complex peptide mixtures by using low concentration tryptic digests of standard proteins. Rapid identification of the original protein was obtained by database searching using the observed molecular masses of the peptides, and by comparison of actual MS–MS spectra of selected peptides with the predicted fragmentation patterns.

Keywords: Field-enhanced sample stacking; Peptides; Proteins

1. Introduction

Capillary electrophoresis (CE) is considered an ideal analytical method for charged species because of its unparalleled separation efficiency. The capillaries employed are typically less than 100 μm in internal diameter, thereby necessitating on-line detection, most commonly by UV absorbance [1–6] or fluorescence [7–11]. While very useful, these detection techniques provide limited structural information about the analytes. When such is warranted, like in the analysis of biomolecules, combination with mass spectrometry (MS) is an attractive alternative. For protein and peptide applications, CE–MS has drawn considerable attention, as evidenced by the wealth of publications in this area in recent years, both in the conventional capillary [12–24] and microdevice [25–27] formats. In this hyphenation of techniques, CE provides fast, efficient resolution, while MS confers high selectivity and sensitivity [28].

The most common way to interface CE with MS is by electrospray ionization (ESI), a soft ionization method which favors the formation of primary molecular ions. It is particularly suitable for peptides and proteins because the formation of multiply charged ions enables the analysis of high molecular masses even with instruments with low nominal mass limit [29]. The coupling is facilitated by one of three approaches: coaxial liquid sheath flow [12,16,17,20,21,23], sheathless [13–15,18,19,22,24], and liquid-junction [26,27].

In the sheath flow design, electrical continuity of the electrophoresis circuit is established using a sheath liquid, which mixes with the capillary effluent at the tip through a concentric stainless-steel tubing [22,30]. The sheath liquid functions as the terminal buffer reservoir in lieu of the outlet buffer vial. This is the simplest design, however, mixing tends to dilute the analyte bands resulting in reduced sensitivity [22,23]. The use of a sheathless interface, which generally employs a capillary with a tapered outlet coated with a conductive material, alleviates this problem. However, the lifetimes of coatings are limited, and the design requires the use of relatively dilute buffer systems, precluding some possible types of separation [18]. In the liquid-junction interface, a small gap filled with buffer connects the separation capillary and the ESI emitter. While it permits optimization of ESI conditions with a freely chosen make-up liquid, construction of such a junction is often difficult [30]. Hence, from the viewpoint of flexibility and ease of implementation, the sheath flow configuration is more favorable, but the sensitivity issue must be addressed. A facile way to do so is to preconcentrate the analytes directly within the capillary prior to separation.

* Corresponding author. Tel.: +81-791-58-173; fax: +81-791-58-493.
E-mail address: monton@sci.himeji-tech.ac.jp (M.R.N. Monton).

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On-line preconcentration techniques have generated considerable interest in recent years because they enable significant improvement in concentration sensitivity by simple manipulation of the composition and strength of background solutions (BGSs) and sample matrices without modification of existing instruments. In CE–MS for protein analysis, previous reports have dealt with transient isotachophoresis (transient ITP) [20] and pH-mediated stacking [23], using a sheath flow design.

In this study, field-enhanced sample injection (FESI) is used. Relative to transient ITP which requires judicious choice of electrolytes, and pH-mediated stacking which restricts the pH difference between the BGS and the sample matrix, FESI is easy to perform. The technique is based on the abrupt drop in the migration velocity of the analyte as it reaches the boundary between the low conductivity sample zone and the high conductivity BGS. With UV detection, Locke and Figeys [6] have demonstrated its potential for the analysis of low concentration samples for optimizing proteomic processes, and for evaluating protocols used for protein analysis. Taking advantage of the structure-information capability of an MS detector, we show that with nM concentrations of proteins digests, after on-line enrichment of the generated peptides by FESI, quick identification of the original protein is possible by database searching using either the molecular masses of the peptides or by comparison of the uninterpreted MS–MS spectra of selected peptides with the predicted patterns.

2. Materials and methods

2.1. Instrumentation

CE experiments were performed on a Hewlett-Packard ^{3D}CE System (Waldbronn, Germany) using uncoated fused silica capillaries (50 μm i.d. × 360 μm o.d.) from Polymicro Technologies (Phoenix, AZ, USA), which were thermostated at 25 °C. Unless specified otherwise, samples were loaded by electrokinetic injection after a 10 s injection of water at 50 mbar.

The CE instrument was coupled to an LCQ ion trap mass spectrometer (Finnigan, San Jose, CA, USA) using the standard ESI interface with a triaxial flow arrangement, in which the capillary effluent mixed with the sheath liquid at the tip and was nebulized by nitrogen gas. The sheath liquid (composed of 50% methanol, 49% water and 1% acetic acid) was delivered using flow rates of 3–5 μl/min. An ESI voltage of 4.5 kV was applied, and the heated inlet capillary was kept at 200 °C. Mass spectra were recorded in the positive ion mode using default scanning parameters (full scan MS: 50 ms maximum inject time, 3 μscans per scan; selected ion monitoring (SIM), 200 ms maximum inject time, 5 μscans per scan; full scan MS–MS, 200 ms maximum inject time, 3 μscans per scan). The instrument was automatically switched from MS to MS–MS mode when the ion current of a particular ion exceeded the predefined threshold.

2.2. Materials

Acetonitrile, acetic acid, formic acid, and morpholine were purchased from Wako (Osaka, Japan). Ammonia solution, ammonium bicarbonate, ammonium formate, and methanol were from Nacalai Tesque (Kyoto, Japan). The peptide standards, proteins and TPCK-treated trypsin were obtained from Sigma and used without further purification. Water was purified using a Milli-Q system from Millipore (Bedford, MA, USA). All solutions were passed through 0.45 μm filters (Nacalai Tesque) prior to use. For tryptic cleavages, the proteins were incubated overnight at 37 °C, at the protein-to-enzyme ratio of 50:1 (w/w), in 50 mM NH_4HCO_3 at pH 8.2. The digestion was terminated by adding acetic acid to the reaction mixture to adjust its pH to 4.

2.3. Database searching

For protein identification, the lists of observed molecular masses were searched against a sequence database (NCBInr.01.22.2003) containing over 1 300 000 entries using MS-Fit [31]. The searches assumed that the masses were monoisotopic and cysteine residues were unmodified. The maximum number of missed cleavage was set to 1 and no limitation on the species of origin was imposed. For direct searching using the uninterpreted MS–MS spectra of some peptides, TurboSEQUEST (Thermo Finnigan, Bioworks 3.0) was used.

3. Results and discussion

3.1. FESI–CE–MS

Proteins and peptides tend to adsorb on the capillary walls. Unless coated capillaries are used, their analyses are carried out under highly acidic or highly basic conditions, where they exist as cations and anions, respectively. For FESI, a low pH condition is preferred since this does not require a polarity-switching step. Initially, the capillary is filled with the BGS, followed by a hydrodynamic injection of a short plug of water, which guarantees the presence of a sufficiently long zone of low conductivity [32]. Thereafter, the analytes, prepared in an acidic (to promote protonation), low conductivity matrix, are injected electrokinetically by application of positive voltage at the inlet for a period of time longer than usual injections. The analytes move rapidly into the capillary across the water plug and stack at its boundary with the high conductivity BGS. After substituting the sample vial with a BGS vial at the inlet, the CE and ESI voltages are turned on, and the focused analytes separate according to their charge and size. Upon reaching the tip, they mix

with the sheath liquid and the nebulizer gas, are ionized, and electrostatically propelled into the mass analyzer.

3.2. Analysis of standard peptides

For easier evaluation of the focusing efficiency of the method, a synthetic mixture of four bioactive peptides was used (sample loading, 62–83 fmol), as shown in Fig. 1A. Direct infusion experiments showed that the $[M + 2H]^{2+}$ ion dominated the mass spectrum for each peptide (inset), so they were monitored at this charge state using the SIM mode.

The electrosmotic flow (EOF) greatly affects the establishment of stable ionization conditions. When the EOF is low or suppressed, the required solvent flow is provided mainly by the sheath liquid, hence, a moderate flow of 5 μl/min was used. At this rate, efficient ionization was achieved, and current drops were not observed.

Fig. 1B was obtained using a 180 s injection of a 1000-fold diluted peptide mixture at 7 kV. As may be inferred by comparison with corresponding components in Fig. 1A, the sensitivity enhancement factors (SEFs, Table 1), in terms of peak height, obtained with FESI ranged from 1600- to 3600-fold, compared to a 1 s sample injection at 50 mbar (∼0.4 nl). The amount of sample loaded by electrokinetic injection was approximated by peak area comparison. Using [Sar^1, Ile^8]-angiotensin II as basis, the peak area of which by FESI was ∼3.5 times bigger relative to that obtained by the typical pressure injection, it was estimated that ∼1.4 μl of the 1000-fold diluted solution was used.

The objective of most on-line preconcentration techniques is to enable loading of sample volumes greater than the usual 1% of the total capillary volume used in conventional runs, followed by narrowing of analyte bands. One of the most notable strengths of sample stacking by electrokinetic injection is that very little of the sample matrix is coinjected because the net electrosmotic velocity is much lower than electrophoretic velocity [32]. It must be noted, therefore, that the estimated volume of 1.4 μl represents the volume of sample consumed in the electrokinetic injection process, and not that which is actually introduced into the capillary. In addition, with prolonged injection times, it is possible to significantly deplete the sample matrix of the analytes [6], i.e., maximize the amount that can be injected. These clearly underscore the usefulness of the technique for low concentration samples.

The peptide mixture used for evaluating FESI–CE–MS contained 154–207 nM of each component. Based on angiotensin I, which registered the lowest SEF, it is estimated that at S/N = 5, the lower detection limit is about 3 nM. This sensitivity is consistent with published values obtained for different peptide standards (low nM range) using nanoelectrospray emitters [33,34].

3.3. Application to tryptic digests

To test the viability of this method for studying complex peptide mixtures, tryptic digests were analyzed. Fig. 2 shows the base peak electropherogram (BPE; A) of the separation of the digest of horse cytochrome *c* (isoelectric point, pI 9.6), along with reconstructed ion electropherograms (RIEs; B–M) of selected singly or doubly protonated fragment ions. Evidently, some peaks are not completely resolved. This, however, only serves to highlight the advantage of coupling CE with an MS detector in that a second 'separation' mode is available, i.e., selective analyte detection is possible even for comigrating components [25]. Some of the unresolved or partially resolved fragments of the horse cytochrome *c* digest may be visualized in the RIEs.

Proteolytic agents cleave the protein at very specific points. The enzyme trypsin, for example, precisely cuts on the C-terminal side of lysine (K) and arginine (R). Such cleavages produce a mixture of peptides that is characteristic of the protein, and is often used for identification. A protein may be identified by means of the molecular masses of only 4–6 of its peptides [29], however, to distinguish between closely related ones, more peptides need to be accounted for. Hence, for the analysis of low concentration digests by CE, an on-line enrichment procedure will be very helpful to amplify the signal for less abundant components.

In this case, the molecular masses of 14 fragments were used for database searching. Not surprisingly, the top candidate protein was cytochrome *c*, from *Equus caballus* (horse), accession P00004, from which 12 tryptic peptides were

Table 1
RSDs and SEF_{height}[a] of some standard peptides

	Analyte			
	[Sar^1, Ile^8]-Ang II	Bradykinin	Angiotensin I	Angiotensin II
RSD (%, $n = 3$)				
Migration time	5.7	5.6	5.3	4.6
Ion intensity	7.8	9.7	6.4	8.6
SEF_{height}	3600	2700	1600	3200

[a] Sensitivity enhancement factor = $\frac{\text{ion intensity with conventional injection}}{\text{ion intensity with FESI}} \times \text{dilution}$.

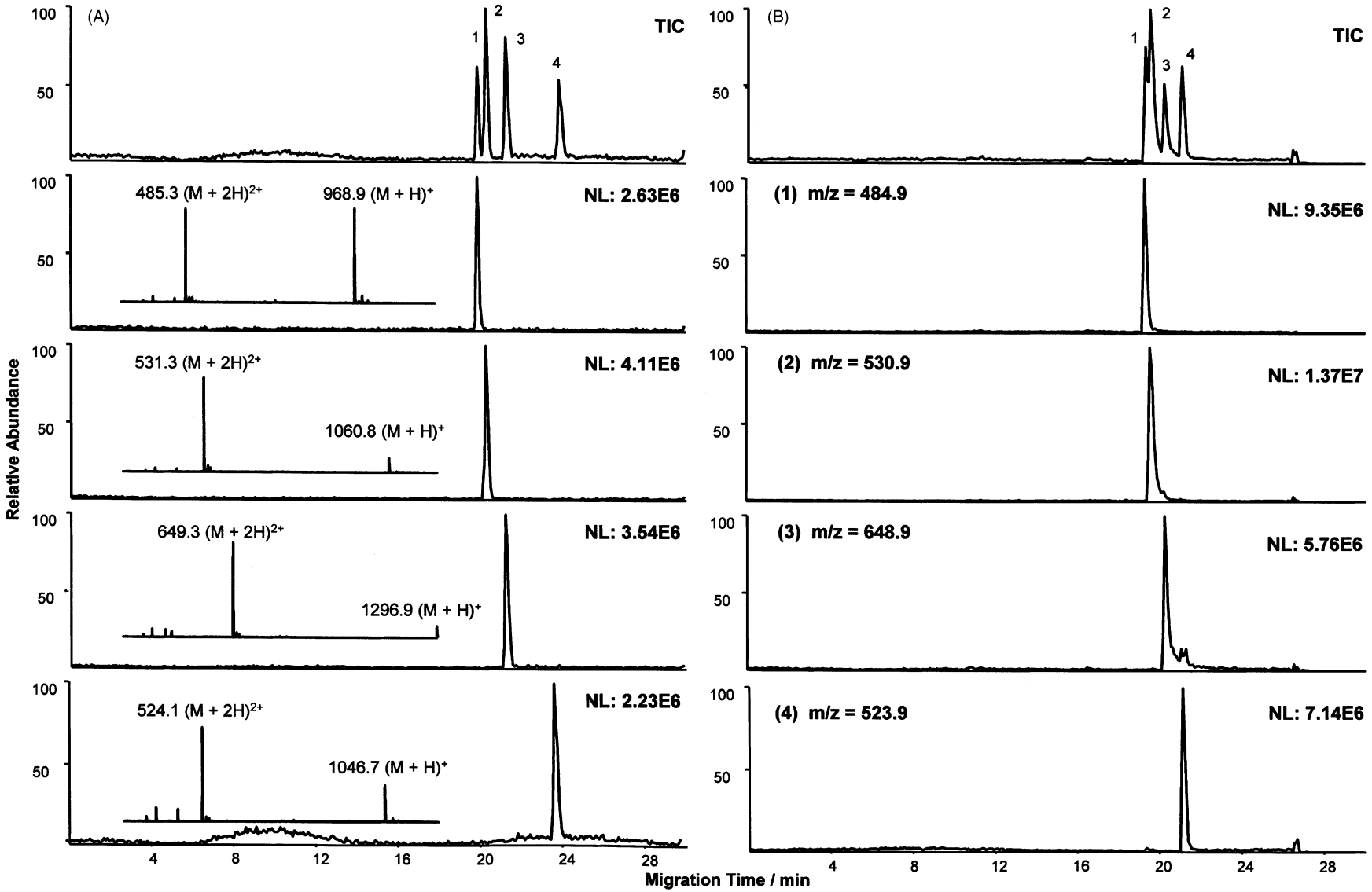

Fig. 1. CE–MS of some standard peptides. Conditions: BGS, 100 mM ammonium formate, pH 3.0; capillary, uncoated fused silica, 60 cm × 50 μm i.d.; CE voltage, +14 kV; ESI voltage, +4.5 kV; sheath liquid, 5 μl/min; injection, 1 s at 50 mbar of samples ca. 200 ppm each (A), 180 s at +7 kV of samples ca. 200 ppb each (B); peak identification, [Sar[1], Ile[8]]-angiotensin II (1), bradykinin (2), angiotensin I (3), angiotensin II (4). Selected ion monitoring mode. Samples were diluted with 0.1 mM formic acid. Other conditions are the same as given in Section 2.

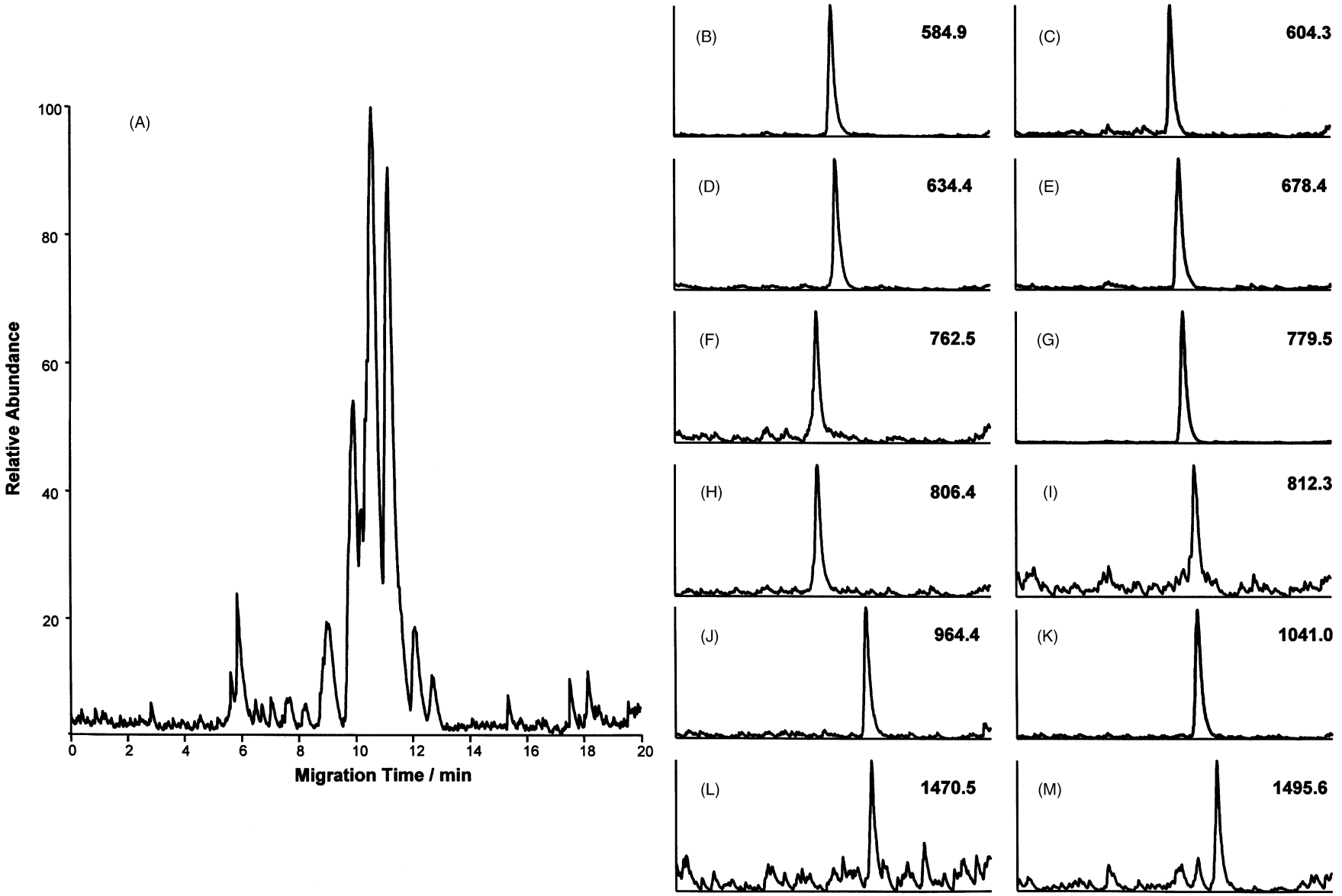

Fig. 2. FESI–CE–MS of tryptic digest of horse cytochrome *c*. Base peak electropherogram (BPE; A), reconstructed ion electropherograms of selected fragments (RIE; B–M). Conditions: BGS, 75 mM morpholine, pH 3.0; capillary, uncoated fused silica, 70 cm × 50 μm i.d.; CE voltage, +30 kV; ESI voltage, +4.5 kV; sheath liquid, 3 μl/min; injection, ca. 200 nM at +10 kV for 450 s. Full scan acquisition (570–1700 *m*/*z*). Other conditions are the same as given in Section 2.

Table 2
Tryptic peptide masses observed from the digest of horse cytochrome *c* and matched with P00004

Mass (Da)[a]		Residue	Sequence
Observed	Calculated		
604.3	604.3	56–60	(K)GITWK
634.4	634.4	9–13	(K)IFVQK
678.4	678.4	74–79	(K)YIPGTK
762.5	762.5	8–13	(K)KIFVQK
779.5	779.4	80–86	(K)MIFAGIK
806.4	806.5	73–79	(K)KYIPGTK
964.4	964.5	92–99	(R)EDLIAYLK
1168.6	*1168.6*	*28–38*	*(K)TGPNLHGLFGR*
1470.5	1470.7	40–53	(K)TGQAPGFTYTDANK
1495.6	1495.7	61–72	(K)EETLMEYLENPK
1623.6	*1623.8*	*61–73*	*(K)EETLMEYLENPKK*
2081.1	*2081.0*	*56–72*	*(K)GITWKEETLMEYLENPK*

[a] The peptides were measured as their $(M+H)^+$ ions, except those shown in italic which were measured as $(M+2H)^{2+}$.

assigned, as summarized in Table 2. Two other entries, also related to horse cytochrome *c*, registered 12 hits: reduced horse heart cytochrome *c*, accession 1GIW; and Igg1 Fab fragment complexed with horse cytochrome *c*, accession 1WEJ_F. The detection of the peptide TGQAPGFTYTDANK (m/z = 1470.5, residue 40–53, Fig. 2L) enabled the distinction from the next-ranked candidate protein, cytochrome *c*, from *Equus asinus* (ass), accession P00005, P00004 and P00005 differ only by one amino acid in their sequence: threonine 47 in the former is changed to serine in the latter.

To test the method with an even more complex peptide mixture, we used the tryptic digest of bovine serum albumin (BSA, p*I* 4.8). The BPE of the separation of the digest and RIEs of selected fragments are shown in Fig. 3. Using the molecular masses of 29 fragments for database searching, the top candidate protein encoded was serum albumin, from *Bos taurus* (cow), accession CAA41735, from which 20 tryptic peptides were assigned, as summarized in Table 3. It was followed by serum albumin precursor (Allergen Bos d 6), accession P02769, which registered 18 hits. These two proteins differ in their 607 amino acid sequence only at 214, where threonine in CAA41735 is changed to alanine in P02769. The differentiating peptides were VLTSSAR (m/z = 733.5, residue 212–218, Fig. 3E) and VLTSSARQR (m/z = 1017.7, residue 212–220, Fig. 3I).

Additionally, protein identification was carried out using data-dependent tandem mass spectrometry. Precursor ions were automatically selected when their intensities exceeded a predefined threshold. The MS–MS spectra resulting from their collisional activation with helium gas were then compared with the putative fragmentation patterns in a sequence database using TurboSEQUEST. To evaluate our method, we used the tryptic digest of horse cytochrome *c* under the conditions discussed previously, and the BPE is shown in Fig. 4. The generated MS–MS spectra were used to search the horse sequence database. The consensus table showing the top five

Table 3
Tryptic peptide masses observed from the BSA digest and matched with CAA41735

Mass (Da)[a]		Residue	Sequence
Observed	Calculated		
665.5	665.4	156–160	(K)KFWGK
689.5	689.4	236–241	(K)AWSVAR
712.5	712.4	29–34	(K)SEIAHR
733.5	733.4	212–218	(K)VLTSSAR
789.6	789.5	257–263	(K)LVTDLTK
922.6	922.5	249–256	(K)AEFVEVTK
974.6	974.5	37–44	(K)DLGEEHFK
1014.7	1014.6	549–557	(K)QTALVELLK
1017.7	1017.6	212–220	(K)VLTSSARQR
1050.6	1050.5	588–597	(K)EACFAVEGPK
1142.9	1142.7	548–557	(K)KQTALVELLK
1163.6	1163.6	66–75	(K)LVNELTEFAK
1249.8	1249.6	35–44	(R)FKDLGEEHFK
1294.9	*1294.7*	*246–256*	*(K)FPKAEFVEVTK*
1305.8	*1305.7*	*402–412*	*(K)HLVDEPQNLIK*
1439.6	*1439.8*	*360–371*	*(R)RHPEYAVSVLLR*
1567.7	1567.7	347–359	(K)DAFLGSFLYEYSR
1616.7	1616.7	118–130	(K)QEPERNECFLSHK
1633.9	1633.7	184–197	(K)YNGVFQECCQAEDK
1639.8	*1639.9*	*437–451*	*(R)KVPQVSTPTLVEVSR*

[a] The peptides were measured as their $(M+H)^+$ ions, except those shown in italic which were measured as $(M+2H)^{2+}$.

protein candidates is given as Table 4. For each MS–MS spectrum, the search program TurboSEQUEST determines the best peptide matches and the proteins corresponding to the match. The composite score ('Score') is determined by multiplying the number of times ('Hits') a protein appears as the top-ranked ('1') candidate by 10, second-ranked ('2') by 8, third-ranked ('3') by 6, fourth-ranked ('4') by 4, and fifth-ranked ('5') by 2, and summing the products [35]. Finally, the program outputs a list of candidate proteins ranked according to the 'Score'. In this case, horse cytochrome *c* was unambiguously identified. Eight MS–MS spectra were found to match best with P00004, and no other protein registered even one top-rank position.

These results indicate that, with on-line focusing of the component peptides, conclusive identification of the protein is possible at low concentration levels of the digest. Even better reliability may be obtained by using several different digestions [29].

Table 4
Top five candidate proteins from database searching using MS–MS spectra from peptides of a horse cytochrome *c* tryptic digest

Reference	Score	Hits				
		1	2	3	4	5
Horse cytochrome *c*	88.3	8	1	0	0	0
RNA-directed RNA polymerase	15.1	0	0	0	3	1
Follistatin precursor	14.6	0	1	1	0	0
Peroxidase C2 precursor	12.6	0	0	2	0	0
Horse alcohol dehydrogenase	11.1	0	0	1	1	0

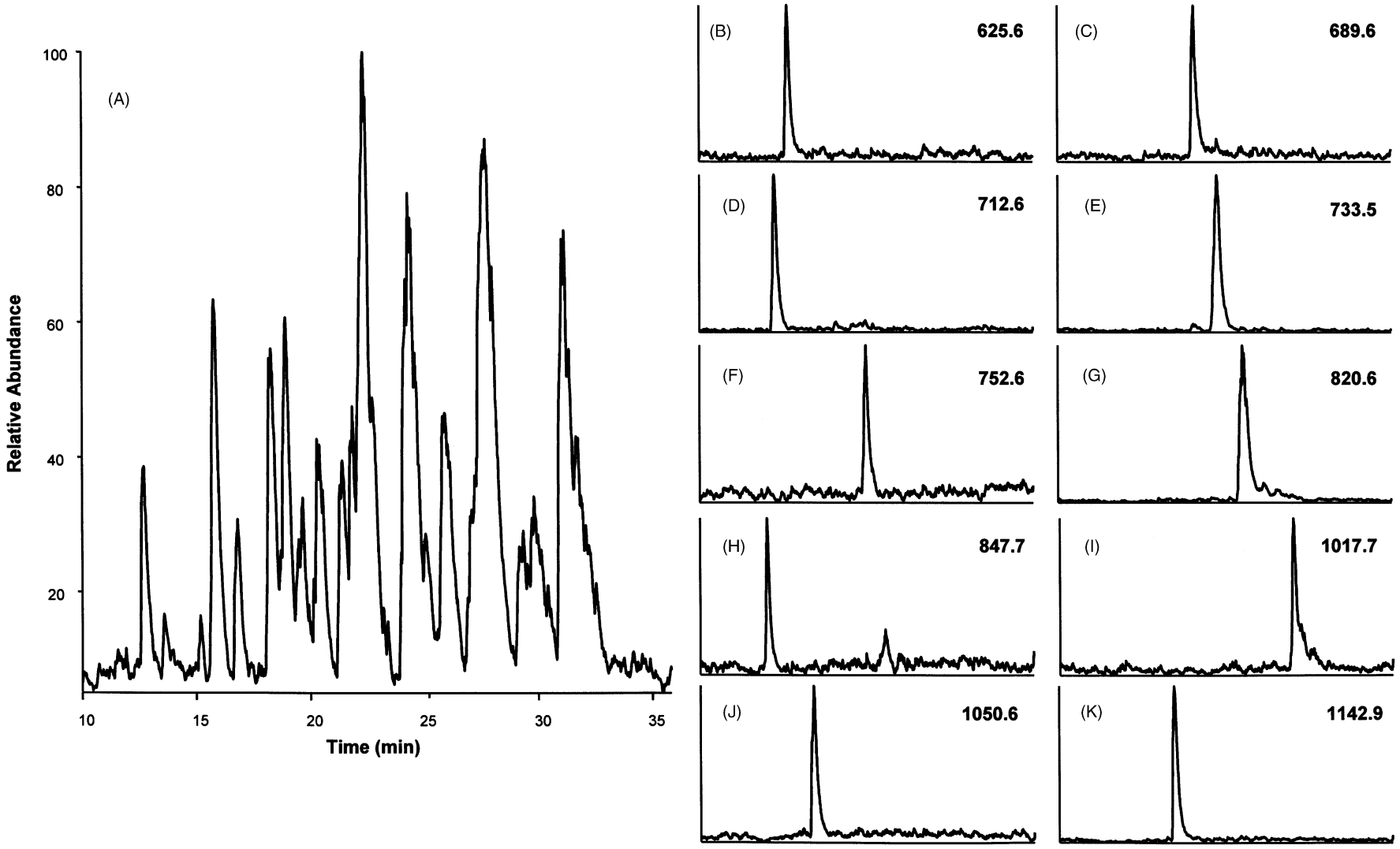

Fig. 3. FESI–CE–MS of tryptic digest of BSA. Injection, ca. 72 nM. Other conditions are the same as given in Fig. 2.

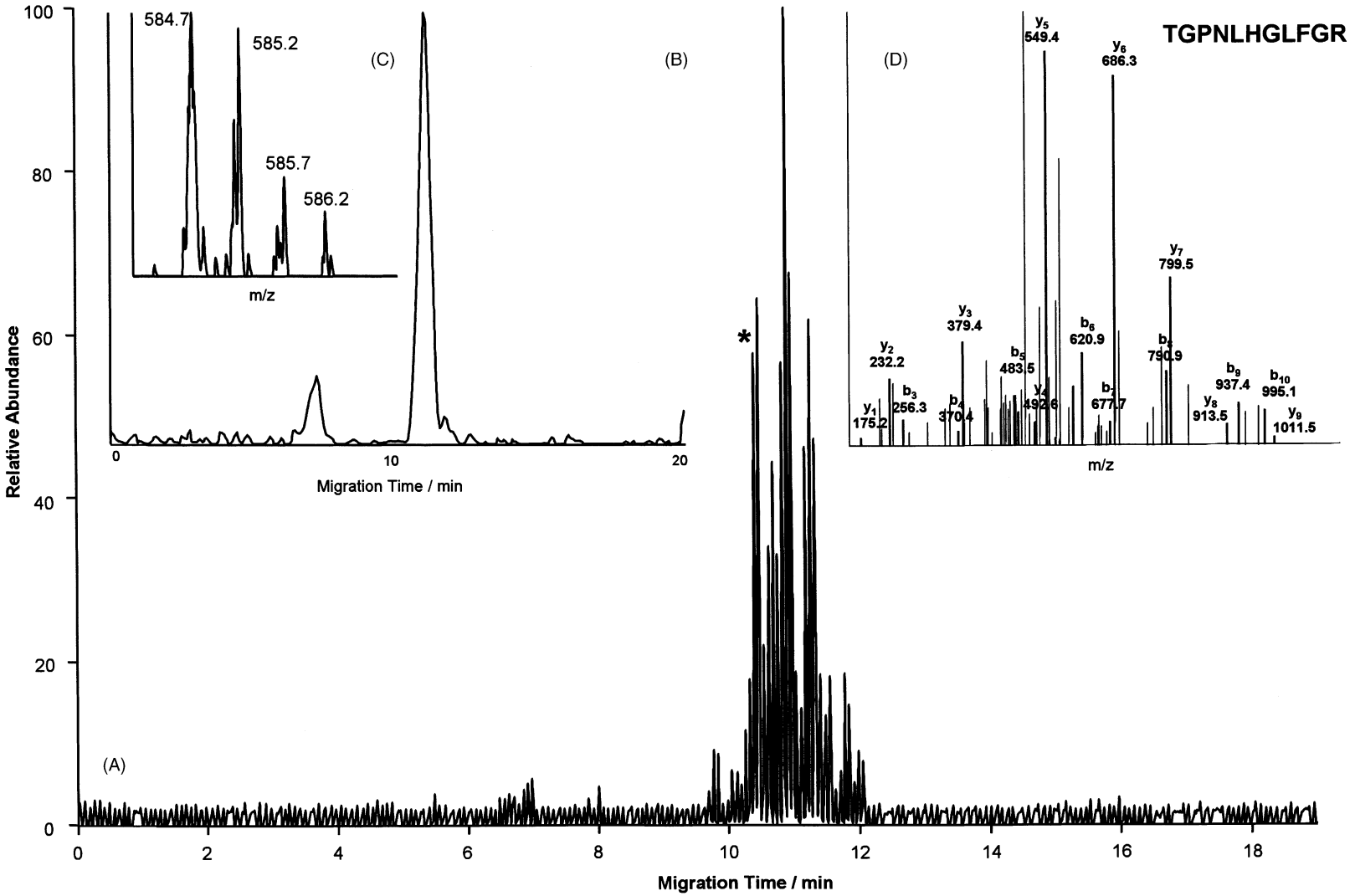

Fig. 4. FESI–CE–MS–MS of tryptic digest of horse cytochrome *c*. BPE (A), RIE of peptide marked with asterisks in A (corresponding to the doublet with $m/z = 584.8$) to show a particular example (B), zoom scan of asterisks to show its charge state (C), MS–MS spectrum of asterisks. Normalized collision energy, 35%. Other conditions are the same as given in Fig. 2.

4. Concluding remarks

We have shown a facile way of improving the sensitivity of a CE–MS system by on-line preconcentration. With three orders of magnitude enhancement in signal, proteolytic digests in the nM concentration levels could be analyzed. The structure-information capability of the MS detector, in conjunction with database searching, was exploited for identification of the original protein. This underscores the potential of such a system for performing other types of studies on low abundance proteins (e.g., detection of mutation, detection and localization of post-translational modifications) and biomolecules. Future work will be directed at developing selective electrokinetic procedures to widen the dynamic concentration range.

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References

[1] D. Josić, K. Zeilinger, W. Reutter, J. Chromatogr. 516 (1990) 89.
[2] W. Ding, J.S. Fritz, J. High Resolut. Chromatogr. 20 (1997) 575.
[3] E.M. Ban, D. Kim, E.A. Yoo, Y.S. Yoo, Anal. Sci. 13 (Suppl.) (1997) 489.
[4] I. Hamrníková, I. Mikšík, M. Uhrová, Z. Deyl, Anal. Chim. Acta 372 (1998) 257.
[5] J.E. Melanson, N.E. Baryla, C.A. Lucy, Anal. Chem. 72 (2000) 410.
[6] S. Locke, D. Figeys, Anal. Chem. 72 (2000) 2684.
[7] W. Tong, E.S. Yeung, J. Chromatogr. B 685 (1996) 35.
[8] D.M. Pinto, E.A. Arriaga, D. Craig, J. Angelova, N. Sharma, H. Ahmadzadeh, N.J. Dovichi, C.A. Boulet, Anal. Chem. 69 (1997) 3015.
[9] I.H. Lee, D. Pinto, E.A. Arriaga, Z.R. Zhang, N.J. Dovichi, Anal. Chem. 70 (1998) 4546.
[10] P.G. Coble, A.T. Timperman, J. Chromatogr. A 829 (1998) 309.
[11] M.J. Baars, G. Patonay, Anal. Chem. 71 (1999) 667.
[12] J.A. Loo, H.K. Jones, H.R. Udseth, R.D. Smith, J. Microcol. Sep. 1 (1989) 223.
[13] L. Fang, R. Zhang, E.R. Williams, R.N. Zare, Anal. Chem. 6 (1994) 3696.
[14] J.H. Wahl, D.C. Gale, R.D. Smith, J. Chromatogr. A 659 (1994) 217.
[15] D. Figeys, I. van Oostveen, A. Ducret, R. Aebersold, Anal. Chem. 68 (1996) 1822.
[16] B. Yeung, T.J. Porter, J.E. Vath, Anal. Chem. 69 (1997) 2510.
[17] E. Rohde, A.J. Tomlimson, D.H. Johnson, S. Naylor, Electrophoresis 19 (1998) 2361.
[18] M.E. McComb, A.N. Krutchinsky, W. Ens, K.G. Standing, H. Perreault, J. Chromatogr. A 800 (1998) 1.
[19] M.E. McComb, H. Perreault, Electrophoresis 21 (2000) 1354.
[20] M. Larsson, E.S.M. Lutz, Electrophoresis 21 (2000) 2859.
[21] C. Aguilar, A.J.P. Hofte, U.R. Tjaden, J. van der Greef, J. Chromatogr. A 926 (2001) 57.
[22] M. Moini, Anal. Chem. 73 (2001) 3497.
[23] C. Neuseüß, M. Pelzing, M. Macht, Electrophoresis 23 (2002) 3149.
[24] G.M. Janini, T.P. Conrads, K.L. Wilkens, H.J. Issaq, T.D. Veenstra, Anal. Chem. 75 (2003) 1615.
[25] J. Li, P. Thibault, N.H. Bings, C.D. Skinner, C. Wang, C. Colyer, J. Harrison, Anal. Chem. 71 (1999) 3036.
[26] B. Zhang, H. Liu, B.L. Karger, Anal. Chem. 71 (1999) 3258.
[27] B. Zhang, F. Foret, B.L. Karger, Anal. Chem. 72 (2000) 1015.
[28] T. Soga, Y. Ueno, H. Naraoka, Y. Ohashi, M. Tomita, T. Nishioka, Anal. Chem. 74 (2002) 2233.
[29] E. De Hoffmann, J. Charette, V. Stroobant, Mass Spectrometry: Principles and Applications, Wiley, New York, 1996.
[30] A. von Brocke, G. Nicholson, E. Bayer, Electrophoresis 22 (2001) 1251.
[31] P.R. Baker, K.R. Clauser, http://prospector.ucsf.edu.
[32] C.-X. Zhang, W. Thormann, Anal. Chem. 68 (1996) 2523.
[33] J. Li, C. Wang, J.F. Kelly, D.J. Harrisson, P. Thibault, Electrophoresis 21 (2000) 198.
[34] J. Li, J.F. Kelly, I. Chernushevich, D.J. Harrison, P. Thibault, Anal. Chem. 72 (2000) 599.
[35] Bioworks 3.0 Online Help.