

# On-Line Capillary Electrophoresis/ Microelectrospray Ionization-Tandem Mass Spectrometry Using an Ion Trap Storage/ Time-of-Flight Mass Spectrometer with SWIFT Technology

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**The development of a system capable of the speed required for on-line capillary electrophoresis-tandem mass spectrometry (CE-MS/MS) of tryptic digests is described. The ion trap storage/reflectron time-of-flight (IT/reTOF) mass spectrometer is used as a non-scanning detector for rapid CE separation, where the peptides are ionized on-line using electrospray ionization (ESI). The ESI produced ions are stored in the ion trap and dc pulse injected into the reTOF-MS at a rate sufficient to maintain the separation achieved by CE. Using methodology generated by software and hardware developed in our lab, we can produce SWIFT (Stored Waveform Inverse Fourier Transform) ion isolation and TICKLE activation/fragmentation voltage waveforms to generate MS/MS at a rate as high as 10 Hz so that the MS/MS spectra can be optimized on even a 1–2 s eluting peak. In CE separations performed on tryptic digests of dogfish myelin basic protein (MBP) where eluting peaks 4–8 s wide are observed, it is demonstrated that an acquisition rate of 4 Hz provides >20 spectra/peak and is more than sufficient to provide optimized MS/MS spectra of each of the eluting peaks in the electropherogram. The detailed structural analysis of dogfish MBP including several posttranslational modifications using CE-MS and CE-MS/MS is demonstrated using this method with <10 fmol of material consumed.**

Capillary electrophoresis-mass spectrometry (CE-MS) provides distinct advantages as a method for the separation and analysis of complex peptide mixtures resulting from protein digestion.<sup>1–22</sup>

Capillary electrophoresis is able to provide rapid separations of complex mixtures with high resolution in which more than 30 peptides can be separated in less than 15 min.<sup>16</sup> The separation is based on the electrophoretic mobility which depends on the mass and charge of the peptide, so that even modifications such as phosphorylation and deamidations can be readily detected and observed in the separation.<sup>22</sup> Also, a very small amount of often-limited sample from biological sources is consumed in analysis. Moreover, the use of CE is particularly well-suited to a mass spectrometer detector because of the sharp, highly concentrated bands that result since the mass spectrometer is a concentration-dependent detector. The result is that sensitivity limits for peptide separations in the low femtomole and into the attomole region can be obtained. The CE-MS system, in principle, can provide

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high-resolution separations of complex mixtures with mass analysis at very low levels.

CE-MS has been used successfully for analysis of protein digests in previous work generally using quadrupole,<sup>1-7</sup> magnetic sector,<sup>8,9</sup> or ion trap mass spectrometers.<sup>10,11</sup> The scanning nature of these mass spectrometers, though, is often inadequate to maintain peak shape in these rapid separations in which peaks may be 1–5 s wide. The result is often loss of resolution of peaks in complex mixtures in which the scan rate of the mass spectrometer does not allow a sufficient number of points for resolution of closely eluting peaks. In recent work, several groups have used time-of-flight mass spectrometers to detect the eluent of CE separations; the nonscanning nature of the TOF device provides the speed necessary to maintain peak shape and the resolution provided by the separation. In one configuration, an orthogonal extraction time-of-flight method operated at high repetition rates provided the speed required to respond to rapid CE separations of proteins or protein digests.<sup>12–15,17,19,20</sup> Alternatively, in recent work from our group, we have demonstrated the use of the ion trap storage/reTOF device as a detector for CE separations,<sup>16,18,21,22</sup> in which the ion trap serves to store ions before a pulsed dc injection into a reTOF detector. The IT/reTOF has been shown to operate at a sufficiently high repetition rate to maintain the resolution of the CE separation while providing high sensitivity in detection due to the integration/storage properties of the trap.

In this work, we demonstrate the IT/reTOF as a device with the capability for on-line CE-MS/MS with sufficient speed to maintain the CE separation and provide optimized MS/MS spectra, on-line, of each peak. The CE-MS/MS is performed in the ion trap using ion isolation accomplished by stored waveform inverse Fourier transform (SWIFT) methodology. This is followed by ion activation and fragmentation by a resonant TICKLE voltage. Finally, the ions are ejected into a reflectron TOF for analysis by a dc pulse. The TICKLE voltage amplitude controlling fragmentation is adjusted on-line for each eluted peak by feedback from previous MS/MS spectra. The development of the methodology for this on-line MS/MS is presented herein, including the software and hardware involved in this experiment. It is shown that for a complex protein-digest mixture that a data acquisition rate of 4 Hz provides a sufficient number of samples to optimize the on-line MS/MS fragmentation obtained for each peak while maintaining high sensitivity. The ability to perform on-line CE-MS/MS on each isolated peak becomes particularly difficult in scanning mass spectrometers because of the limited elution time of each peak caused by high resolution in the separation and the limited time for optimization of the MS/MS spectrum of each peak. The on-line CE-MS/MS and detailed sequence analysis of dogfish myelin basic protein (MBP) is demonstrated, showing that even minor posttranslational modifications in the sequence can be pinpointed.

## EXPERIMENTAL SECTION

**Sample Preparation.** Dogfish MBP was isolated and purified according to the procedure of Deibler et al.<sup>23</sup> Charge isomer components (C1, C3) were separated on a carboxy methyl cellulose (CMC) column according to the procedure described

in the literature.<sup>24,25</sup> The purified fractions were exhaustively dialyzed against distilled water and lyophilized. For each fraction, an amount equal to 20  $\mu\text{g}$  of MBP was digested overnight at 37 °C with trypsin (protein/enzyme ratio is 50:1) in an ammonium bicarbonate buffer, pH = 8.2. The digests were dried under speed vacuum and reconstituted in deionized water with a charge isomer concentration of  $\sim 1 \times 10^{-5}$  M.

**On-Line Sheathless CE/MS System.** The tryptic digests were separated using an in-house-constructed capillary electrophoresis apparatus including a 30 kV high voltage power supply (model CZE 1000R, Spellman High Voltage Electronics Corp., Plainview, NY) and a Polybrene (positive) coated capillary column produced according to the procedure given by Li.<sup>16</sup> The detector used for on-line CE-MS is an ion trap storage reflectron time-of-flight mass spectrometer (IT/reTOF-MS) described in previous work.<sup>16,18,21,22,26</sup> The apparatus consists of a quadrupole ion trap storage device (model 1251, R. M. Jordan Co., Grass Valley, CA) interfaced to a reflectron TOF mass analyzer (model D1450). Helium buffer gas (1 m Torr) is introduced into the ion trap to enhance the trapping. Detection was achieved with a 40-mm triple microchannel plate (MCP) detector (model C-2501, R. M. Jordan Co.).

To interface CE to ESI-MS, the anodic end of the CE capillary was used as the sheathless microelectrospray tip. To achieve an electrical connection, the capillary tip was coated with silver.<sup>27</sup> The power supply of the capillary electrospray was set at  $\sim -12$  kV, while the microelectrospray needle was set at a value of  $\sim 3$  kV, so that the total separation was performed with  $-15$  kV across the capillary.

**Methodology for CE-MS/MS.** The structural elucidation of peaks eluting from the electrophoretic separation is based upon their fragmentation by MS/MS in the ion trap. Isolation of ions for MS/MS requires application of a SWIFT waveform containing frequencies that remove unwanted ions from the trap prior to activation of the parent ion. The remaining desired target ions are then fragmented by applying a TICKLE voltage with resonant frequency of appropriate amplitude to cause their collision with a buffer gas. Fragment ions can be detected with a high signal-to-noise ratio since interfering ions have been removed by the SWIFT waveform.

The SWIFT waveform is constructed from a series of sine waves with frequencies corresponding to the range of possible  $m/z$  values in the trap (200–2000 u). There is a notch of excluded frequencies ( $\pm 10$   $m/z$  units typically) surrounding the secular frequency of the parent ion which allows isolation of this ion in the ion trap. A narrower notch is usually not necessary because of the excellent separation of peaks by the CE. Individual frequencies are calculated from the standard equations for the secular frequency of the ion.<sup>28</sup> The time-domain SWIFT waveform

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is computed by fast Fourier transform of a notched frequency spectrum. Quadratic phase modulation<sup>29–32</sup> is applied to reduce the amplitude range in the resulting SWIFT waveform. The TICKLE waveform is produced by simple summation of three frequencies with quadratic phase modulation. The parent ion secular frequency  $\pm$  a small frequency shift (typically 20  $m/z$  units) are used.

To perform on-line MS/MS, two sample injections are used in this work. This strategy makes sense since the CE separations are fast, i.e., typically 10 min, so that two injections take significantly less time than one LC separation. The first separation is performed without SWIFT or TICKLE waveforms and produces an integrated total ion electropherogram (TIE). The electrophoretic separation typically results in only one ion of interest being present in each eluted peak. Determination of its identity is aided by custom software that displays the entire data set as a 2D false color image with  $m/z$  spectra as one axis, TIE elution time as the other axis, and  $m/z$  intensity as image color.<sup>33</sup> As a cursor on this image map (or on the TIE plot) is moved, the corresponding individual  $m/z$  spectrum is displayed. Integration of multiple spectra over an elution-time range for an increased signal-to-noise ratio is possible. For each eluted peak in the TIE a parent-ion  $m/z$  is selected to monitor in the subsequent second injection. This list is entered into the acquisition program along with parameters such as waveform voltages and durations.

As the separation proceeds and each peak elutes, its corresponding waveforms must be applied. Because of variations in elution time, this parameter cannot be used to determine when a given peak will appear and the waveforms should be switched on. In this work, the selected parent ion list is operated upon sequentially. A small  $m/z$  range around the parent ion is monitored for changes in integrated ion intensity (selected ion count (SIC)) with no SWIFT or TICKLE waveform applied. When that value changes by a specified amount (typically 200%) above the average of some previous number of samples (typically 10), that parent ion is determined to have begun eluting. When the peak elutes, the waveforms are applied for a duration corresponding to the peak width. After that time, this detection cycle is begun again using the next parent ion in the list that corresponds to the next expected eluted peak. The waveforms are applied at preselected initial amplitudes, typically  $\sim 5V$  0-pk for SWIFT and  $\sim 2V$  0-pk for TICKLE. The SWIFT amplitude has approximately a 1 V range below which it does not remove a sufficient amount of the undesired ions and above which it removes the desired parent ion. The TICKLE voltage range is more critical and narrow and controls the extent of fragmentation. An optimum value occurs when most (about 90%) of the parent ion fragments into detectable daughter ions, useful for compound identification. It is desirable to have some parent ion remain for confirmation of its value.

The TICKLE voltage is optimized in real time from spectrum to spectrum. For each spectrum, an internal ratio of the parent to

daughter ion  $m/z$  SIC range is calculated. If this ratio is too low, the TICKLE voltage will be decreased (on the next spectrum) which causes less fragmentation. It is decreased by an amount equal to one-half the difference between the current value and a predefined lower limit. That previous value then becomes the new upper limit since it had just been shown to be too high. Corresponding changes take place if the ratio is too high. For CE with narrow peak widths, it is desirable to optimize the TICKLE voltage in as few spectra as possible so that the remaining spectra will be acquired using a TICKLE voltage which produces spectra useful for identification. There is a tradeoff in peak resolution, signal intensity available for optimization, and acquisition rate.

The sequence of events for acquiring a single MS/MS spectrum on an ion trap instrument using SWIFT and TICKLE waveforms and the corresponding waveform time scales for each event at a 4 Hz rate, for example, as used in this work, include: (1) Accumulate ions in the trap (136 ms). (2) Stop ions from entering the trap using an ion gating voltage. (3) Apply the SWIFT isolation waveform (32 ms). (4) Cool (25 ms). (5) Apply the TICKLE activation waveform (32 ms). (6) Cool (25 ms). (7) Eject ions from the trap. (8) Digitize the mass spectra. (9) Permit ions to begin accumulating in the trap again. (10) Transfer digitized data to the computer. (11) Save mass spectra data in the computer. (12) Analyze mass spectra to determine appropriate TICKLE voltage to apply on the next cycle.

**Hardware.** The SWIFT and TICKLE waveforms are produced from an arbitrary waveform generator board (AWGB) (model WSB-100-10, Quatech, Akron, OH) with analogue output module WSB-A12M. While it is capable of storing multiple individual waveforms (SWIFT, TICKLE, and cooling) simultaneously in separate memory locations on-board, looping multiple times on any one, stepping through a waveform in a forward or reverse direction, and accessing them in any order, it cannot automatically link them together to create the continuous waveform required for the complete cycle described above. Instead, software must be used to detect the end of one waveform type and then initiate the next waveform type in the cycle. This was accomplished by writing a software interrupt service routine called at the end of the last cycle in each waveform type. This routine then took the appropriate action necessary at that point in the cycle. This would include setting the waveforms output level and starting the next waveform type in the sequence. The routine also produced the output pulses for ion trapping and extraction. The AWGBs 32K bytes of memory is sufficient to only hold waveforms describing one separated eluting peak. When a new peak is to be operated on, new waveforms had to be loaded into the board. Waveforms for all the expected peaks were pregenerated (from the list selected by analysis of the first injection) before the acquisition started and stored in a RAM drive in the computer's memory for faster retrieval. To change the output amplitude of many arbitrary waveform generator boards it is necessary to rescale and reload the digital values. This approach would be too slow and would reduce resolution for this application. The board used here is capable of accepting an external voltage reference that can be used to vary its output amplitude. A separate 16 bit digital-to-analog converter (DAC) card was used for this purpose (model CIO-DAC02/16, Computer Boards, Middleboro, MA).

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The mass spectra were digitized by an 8 bit 250 MHz analogue bandwidth transient digitizer (model PI9846, Precision Instruments, Knoxville, TN) capable of 2 ns/point single shot digitizing rates with a model 905 external amplifier.

An important issue in these experiments is that ions must be prevented from entering the ion trap during the MS/MS process. This is performed using an ion gate to deflect away ions during this time period. The ion gating voltage control used an optically isolated solid-state relay (NEC no. PS7360-1A) to switch between 400 V and ground in approximately 1 ms.

**Software.** The existing software was developed in Borland Pascal for DOS. The arbitrary waveform generator, DAC waveform voltage control, and transient digitizer boards were controlled with register level software calls. Assembly language was used where advantageous for speed improvement.

Chromatographic separation methods that may yield peaks as narrow as 1–2 s require cycling through the events at a minimum of a 10 Hz rate to yield a sufficient number of spectra for TICKLE voltage optimization and peak resolution. A combination of software development, computer speed, and interface card features were used to attain these rates. We previously demonstrated a 10:1 reduction in data volume by only processing values in the mass spectra that exceed a selected threshold. Fewer points need to be processed, stored, and graphed, leading to acquisition rates exceeding 20 Hz, in non-SWIFT applications.<sup>34</sup> A typical total eluted chromatographic data set is reduced from several tens of megabytes to several megabytes. For the faster rates used here, the acquired data was initially stored to RAM drive in the computer's memory and then transferred to hard disk when acquisition was complete. In the limited testing we performed, this was faster than using a PCI bus caching hard drive controller. A Microsoft Windows 32 bit version presently being developed will permit the entire data set to be held in memory during acquisition, thereby increasing the acquisition rate by eliminating the need for any form of real-time storage.

This system is capable of sustained SWIFT and TICKLE operation at 10 Hz rates with real-time storage and display of the TIC, SIC, and complete mass spectra for each acquired individual mass spectrum. Data analysis is accomplished using the custom  $m/z$  vs elution time 2D false color image software described above<sup>33</sup> for choosing the parameters from the first non-SWIFT injection. In particular, its integration capabilities allow weak fragment ions to be identified.

## RESULTS AND DISCUSSION

In Figure 1 a capillary electrophoretic separation of a tryptic digest of dogfish MBP in which on-line mass spectrometric detection was performed using the IT/reTOF detector is shown. The tryptic digestion of dogfish MBP should yield 28 peptide fragments. Capillary HPLC using a 130  $\mu\text{m}$  C-18 column and a water/acetonitrile gradient was used to separate this digest, but only a limited number of peaks were detected in which many of the peptides coeluted. The capillary electrophoretic separation provides much enhanced separation of the peptides in which at least 18 of the peptides can be distinctly separated by CE. The use of the IT/reTOF-MS detector provided the detection of almost

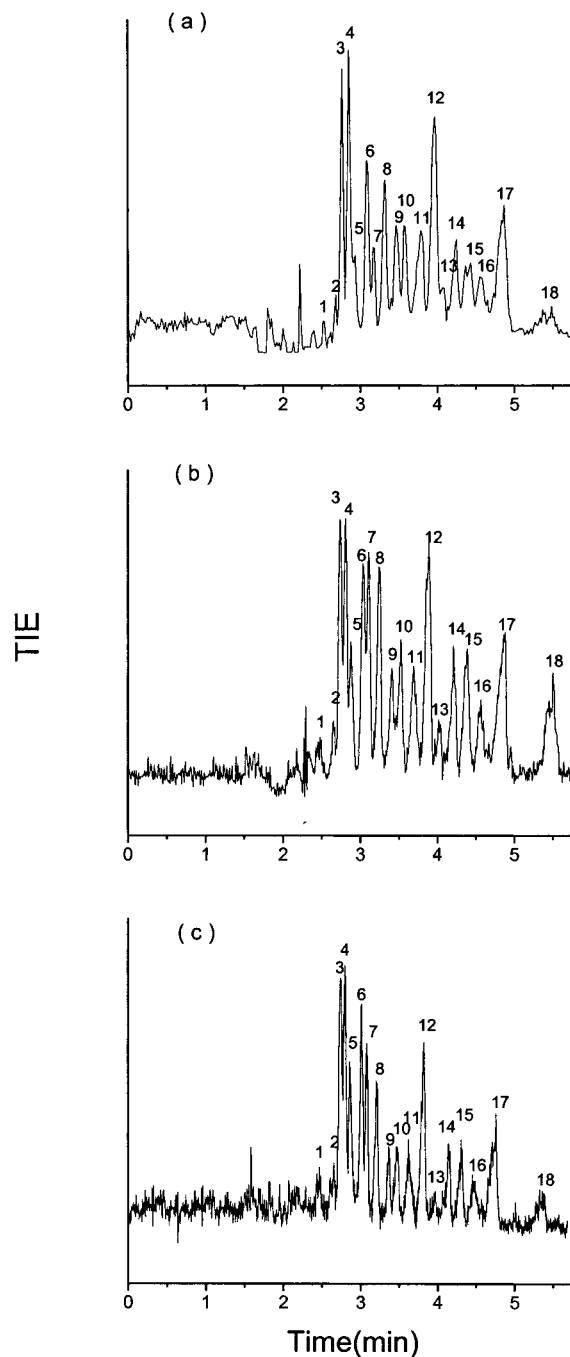


Figure 1. Electropherograms of myelin tryptic digests at different sampling rates with a mass spectrometer and a UV detector. (a) CE/TIE at 2 Hz ejection rate, 2 times average. (b) CE/TIE at 2 Hz ejection rate, no averaging. (c) CE/TIE at 4 Hz ejection rate, no averaging.

every peptide-digest product for which the theoretical and experimental  $m/z$  of the MBP tryptic digest products is tabulated in Table 1.

The nonscanning capabilities of the IT/reTOF-MS are essential in these experiments to maintain the quality of the CE separation and to provide the speed to optimize the MS/MS spectrum on-line for each peak. The peak widths resulting from the CE separation are typically 4–8 s in the spectra of Figure 1. At a rate of 2 mass spectra/s only 8 points are available to define the peak if the peak width is 4 s, while at 4 mass spectra/s 16 points are available per peak. In Figure 1 the TIC of the separation of the

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Table 1.

fragment	sequence	theoretical mass (Da)	charge	theoretical m/z (Da)	actual m/z (Da)
T1(M)	ASATTS DHAK	1030.48	d	515.8	515.8
			s	1030.5	1030.6
T2 <sup>a</sup>	QAGGAHSR	783.8	s	783.3	undetected
T3	QR	303.18	s	303.2	303.4
T4	DSGLLDQLGQLFGQEGSR	1920	t	640.6	undetected
T5	K	147.2	s	147.2	147.7
T6	VPEK	472.3	s	472.3	473.1
T7	GK	204.1	s	204.8	204.8
T8	EPATR	573.6	s	573.6	573.7
T9	SVLMAPTTHK	1084.6	d	542.8	543.1
			s	1084.6	1084.8
T10	AHQGAR	639.3	s	639.3	639.3
			d	320.2	320.5
T11	R	175.1	s	175.1	175.6
T12	QTDDSPVVHFFK	1419.7	d	710.8	710.3
			t	473.9	473.5
T13	NMMSPK	707.3	s	707.3	707.4
			d	354.2	354.6
T14	K	147.2	s	147.2	147.7
T15	APVQQK	670.4	s	670.4	670.5
			d	335.7	335.7
T16	AK	217.3	s	217.3	217
T17	SGASR	477.2	s	477.2	477.4
T18	AITK	432.3	s	432.3	432.3
T19	FIWGTGQQR	1079.5	s	1079.5	1079.6
			d	540.3	540.6
T20	AHYGAAGSSK	948.4	s	948.4	949.3
			d	474.2	475
T21	SK	234.3	s	234.3	234.4
T22	DGFR	494.2	s	494.2	494.4
T23	GR	232.3	s	232.3	232.5
T24	R	175.1	s	175.1	175.6
T25	DGSGTLSSFFK	1145.6	s	1145.6	1145.7
			d	573.3	573.4
T26	MGK	335.2	s	335.2	335.6
T27	K	147.2	s	147.7	147.7
T28	GEGSPAR	673.4	s	673.4	672.8
T29	R	175.1	s	175.1	175.6

<sup>a</sup> This peak is detected as the modified deamidated form only.

tryptic digest products of dogfish MBP obtained using the IT/reTOF-MS detector with a mass spec rate of 2 Hz (Figure 1a and b) and 4 Hz (Figure 1c) is shown. In Figure 1a the spectra are signal-averaged for 2 spectra, whereas in Figure 1b no signal averaging is used. In Figure 1, as the sampling rate increases, the quality of the separation improves since the number of points that define the resolution of the peak increases. The 4 Hz sampling rate improves separation of peak pairs 4 and 5, 9 and 10, 12 and 13, and 15 and 16 relative to the 2 Hz rate. We also repeated this separation at 6 and 8 Hz, respectively, (data not shown) but no significant improvement in the separation was observed. The use of the higher sampling rate, though, required more computer memory and a larger file with little advantage in separation. In addition, the signal intensity decreases with increased sampling rate.

In Figure 2 the on-line CE-MS/MS of several selected peaks from Figure 1c is shown. The IT/reTOF-MS sampling rate was run at 4 Hz for acquisition of the CE-MS/MS shown in Figure 2. The sampling rate becomes critical in the CE-MS/MS experiments in which a sufficient number of spectra need to be sampled to optimize the TICKLE voltage for MS/MS fragmentation in the ion trap. Although MS/MS spectra can be obtained at 2 Hz with 10 spectra taken over a 5-s-wide peak, the use of 4 Hz with 20 spectra/peak allows much improved optimization.

An important consideration for the data acquisition rate is the trade-off between MS/MS optimization and the S/N ratio. In the CE-IT/reTOF-MS system, the duty cycle for detection is close to 100%. However, in the CE-IT/reTOF-MS/MS procedure, ~77% of the time is available for storage and the remainder of the cycle is used for the MS/MS process at a 2 Hz sampling rate. As the repetition rate is increased, the percentage of time available for storage decreases in a nonlinear fashion, and the S/N ratio continues to decrease. A data acquisition rate of 4 Hz was used as a compromise between optimization of the MS/MS and that needed to obtain a reasonable S/N ratio. In the present procedure at 4 Hz, only 136 ms of the 250 ms/cycle is used to accumulate signals. The accumulation time could be increased by decreasing the time the SWIFT and TICKLE voltages were applied. This was not necessary since the S/N level was still adequate for low femtomole detection. However, at 8 Hz only 11 ms would be available for storage using the present waveform time parameters for MS/MS activation. We were able to decrease the time the SWIFT and TICKLE voltages were applied from 32 ms to 24 and 8 ms, respectively, and the cooling times could be reduced to 15 ms so that the MS/MS could be optimized and comparable results obtained for 8 Hz. The accumulation time could be increased to 61 ms of the available 125 ms/cycle using these parameters without a significant effect on the performance.

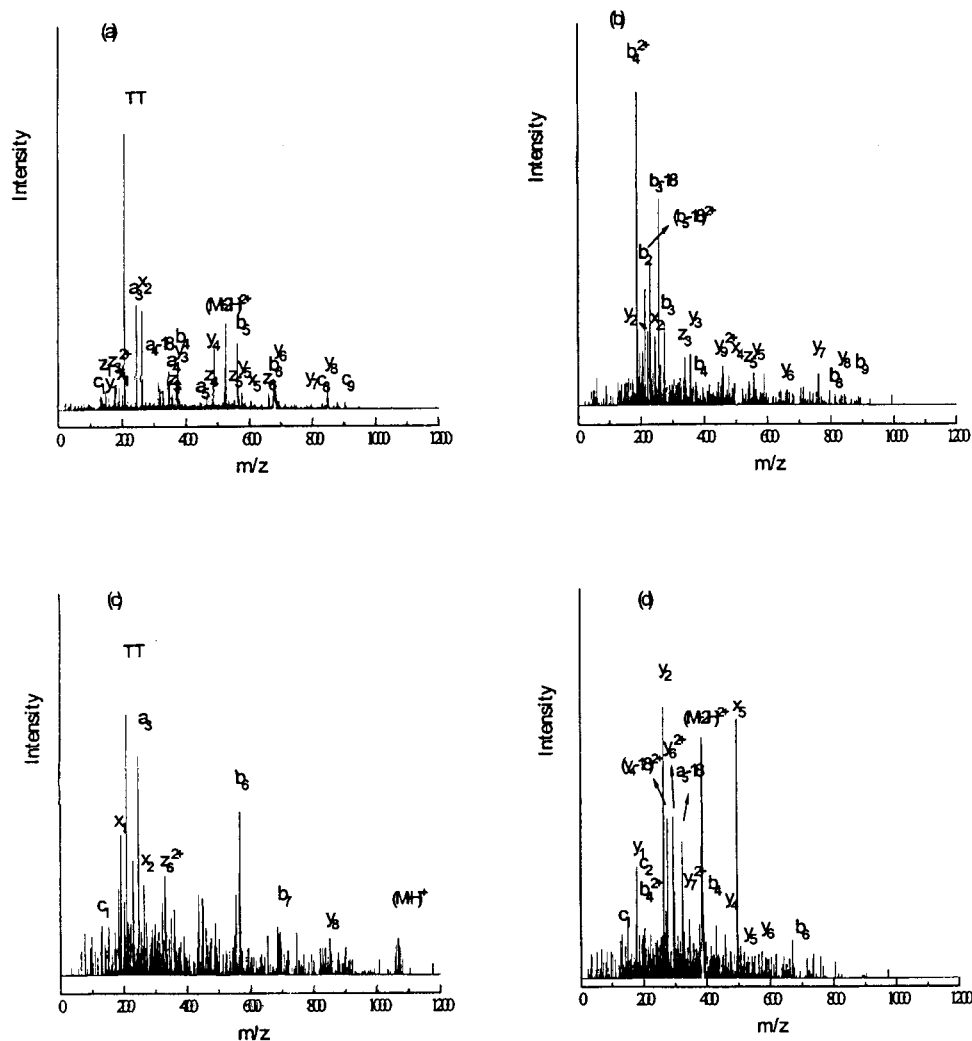


Figure 2. CE-MS/MS spectra of several peaks from the TIE of Figure 1: (a) TIE position 3, parent ion  $m/z$  523.85, hydroxylated T1, (b) TIE position 6,  $m/z$  515.6, unmodified T1, (c) TIE position 1,  $m/z$  523.85, hydroxylated T1, (d) TIE position 9,  $m/z$  384, deamidated T2.

The CE-MS/MS procedure developed in this work has been used to perform detailed site modification determination for posttranslational modification of dogfish MBP. In addition to the expected peaks observed in the peptide map (Table 1) there are other peaks detected. These peaks may result from posttranslational modifications or trypsin autodigests. The on-line CE-MS/MS was run in order to interpret the structure of these peaks. In Figure 1 in the TIE, peaks 1 and 3 are both doubly charged ion species with  $m/z$  523.85. The MS/MS spectra (Figure 2a,c) of these two peaks show that they have the same structure. The two peaks observed in the TIE are apparently the result of running the CE at  $pH = 3$  where the  $pK_a$  of the carboxyl group is close to the  $pH$ , resulting in an equilibrium between the ionized and nonionized forms.<sup>35</sup> These two forms have different migrating speeds under the applied voltage in CE. One possible identification for this peptide could be the trypsin autodigestion, for which the  $m/z$  1045 peak corresponds to the sequence LSSPATLNSR. The CE-MS/MS spectrum of this peak which is shown in Figure 2a excludes this possibility. Another possibility in the trypsin digest of MBP is the acetylated T<sub>1</sub> at  $m/z$  1030 with the sequence

ASATTSDHAK. There is a 16-Da difference between the observed peaks and the expected sequence, which could be hydroxylation on a lysine residue or oxohistidine. The MS/MS spectra of acetylated T<sub>1</sub> without modification is shown in Figure 2b. By comparison of the fragment ions, it can be confirmed that the 523.85 peak has a modified lysine residue, in which the y-series fragment ions in the CID spectrum of peak 515.8 Da are 16 Da less than the y-series fragment ions in the CID spectrum of peak 523.85 Da.

A second modification that can be detected by MS/MS is the deamidation of T<sub>2</sub>. In the CE/MS, T<sub>2</sub> is not observed at exactly 783.4 Da as in the unmodified form. One peak at  $m/z$  384 is close to the doubly charged ion (392.2 Da) expected of T<sub>2</sub> but has a mass difference of  $-8.1$  Da. This could be deamidation of a glutamine residue in QAGGAHRSR or methylation of an arginine residue in GKESATR (772/386.9 Da). The MS/MS spectrum of this peak shows that deamidation is probably the modification in the fragment QAGGAHRSR as shown in Figure 2d.

In conclusion, a system capable of the speed required for on-line capillary electrophoresis-tandem mass spectrometry of tryptic digests is described. The IT/reTOF-MS is used as a nonscanning detector for CE separations, in which the peptides are ionized

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on-line using an ESI source. Using methodology generated by software and hardware developed in our lab, we produce SWIFT isolation and TICKLE activation/fragmentation voltage waveforms to generate MS/MS at a rate sufficient for optimizing the fragmentation produced even for peaks only several seconds wide. This system has been used to analyze CE separations of tryptic digests of dogfish MBP in which a rate of 4 Hz provides >20 spectra/peak for eluting peaks 4–8 s wide. Using the on-line CE-MS/MS methodology, the detailed structure of dogfish MBP, including several posttranslational modifications, could be achieved.

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