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Atmospheric pressure matrix-assisted laser desorption/ionization (AP MALDI) on a quadrupole ion trap mass spectrometer

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Abstract

A new atmospheric pressure ionization technique, atmospheric pressure matrix-assisted laser desorption/ionization (AP MALDI), was recently introduced by Laiko et al. [U.S. Patent 5,965,884; Abstracts of the 4th International Symposium on Mass Spectrometry in the Health and Life Sciences, San Francisco, CA, 25–29 August 1998; Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry, Proceedings of the 47th ASMS Conference on Mass Spectrometry and Allied Topics, Dallas, TX, 1999; Anal. Chem. 72 (2000) 652]. This source has been coupled to an orthogonal time-of-flight mass spectrometer [U.S. Patent 5,965,884; Abstracts of the 4th International Symposium on Mass Spectrometry in the Health and Life Sciences, San Francisco, CA, 25–29 August 1998; Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry, Proceedings of the 47th ASMS Conference on Mass Spectrometry and Allied Topics, Dallas, TX, 1999; Anal. Chem. 72 (2000) 652] and to an ion trap mass spectrometer (ITMS) [Anal. Chem. 72 (2000) 5239; Atmospheric Pressure MALDI/Ion Trap Mass Spectrometry, Proceedings of the 48th ASMS Conference on Mass Spectrometry and Allied Topics, Long Beach, CA, June 2000; An Atmospheric Pressure MALDI Probe for Use with ESI Source Interfaces, Proceedings of the 48th ASMS Conference on Mass Spectrometry and Allied Topics, Long Beach, CA, June 2000; Atmospheric Pressure Matrix-Assisted Laser Desorption Ionization, Proceedings of the 48th ASMS Conference on Mass Spectrometry and Allied Topics, Long Beach, CA, June 2000]. Here, we present the current status of the work involving the development of an AP MALDI source for an ITMS. In addition, we present recent work from our own laboratory to demonstrate the utility of this novel configuration.

Keywords: AP MALDI; Time-of-flight; ITMS; Tandem mass spectrometry; Proteins; Protein digests; Acetylation; Oligosaccharides; Sulfation; Phosphorylation

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1. Introduction

1.1. Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry

Since its introduction a little over a decade ago [9–14], MALDI has established itself as a powerful technique for the analysis of biomolecules by mass spectrometry. In 1987, Karas and Hillenkamp [9] introduced a matrix-assisted technique utilizing a frequency quadrupled (266 nm) Nd:YAG laser to desorb intact molecular ions of proteins that were co-crystallized in a nicotinic acid matrix solution. In 1988, Karas and Hillenkamp [12] published a spectrum of the molecular ions of bovine serum albumin at *m*/*z* 66,750. The incorporation of analyte into an organic matrix solution and desorbing intact molecular ions from the matrix–analyte crystal using an ultraviolet laser has become one of the most widespread techniques in the mass spectral analysis of biological samples [15].

The TOF mass spectrometer has been the detector of choice for MALDI sources. The pulsed nature of the MALDI source as well as the high initial kinetic energy distributions of MALDI-generated ions is amenable to TOF detectors. Additionally, the TOF analyzer has the ability to record ions of all masses over a wide mass range (Fellget advantage), which makes it a suitable detector for the analysis of the high *m*/*z* of biological ions generated by MALDI [16]. However, a major disadvantage of TOF is its limited capability to perform tandem MS (MS^n) experiments, which is useful in structural determination of biological molecules. Since MS^n in TOF instrumentation is tandem in space, each degree of MS^n would require an additional phase of TOF [17], rendering this sort of analysis impractical.

1.2. MALDI ion trap mass spectrometry (ITMS)

There has been much interest in coupling a MALDI source to mass analyzers with the ability to perform

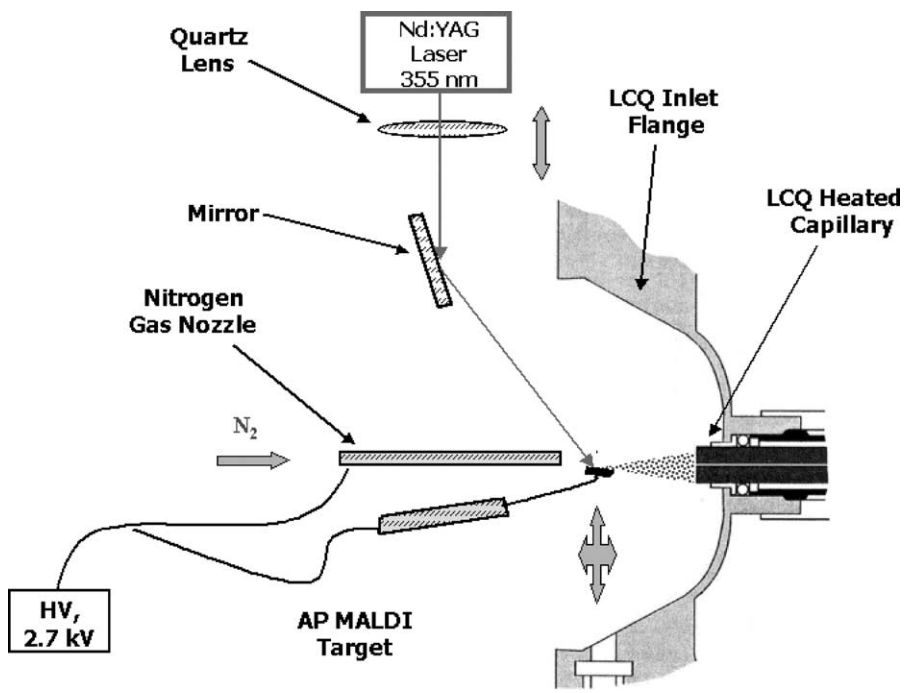

Fig. 1. Schematic of AP MALDI source introduced by Laiko and coworkers coupled to a Finnigan LCQ ITMS.

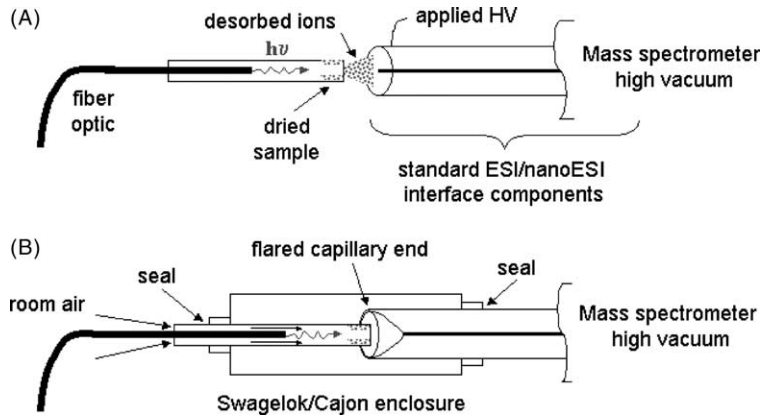

Fig. 2. Schematic of the AP MALDI source introduced by Danell and Glish (figure courtesy of Professor Gary L. Glish, University of North Carolina Chapel Hill). The configuration described in (A) was coupled to a Bruker Esquire ITMS. The configuration in (B) was coupled to an R.M. Jordan TOF instrument.

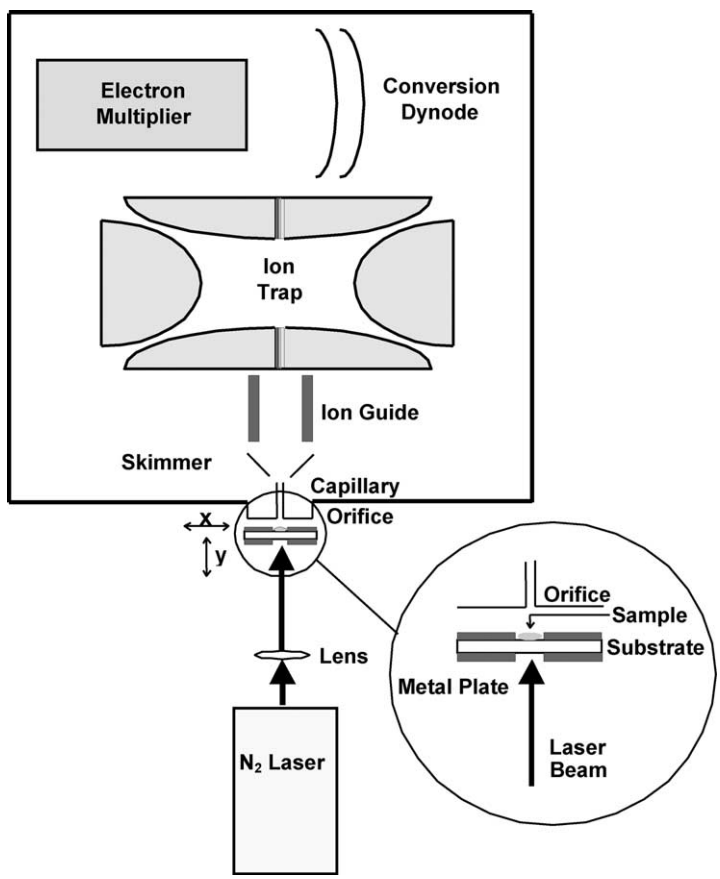

Fig. 3. Schematic of a transmission geometry AP MALDI source coupled to a Finnigan LCQ ITMS as introduced by Callahan and coworkers (figure courtesy of Dr. John H. Callahan).

MS/MS and MS^n, such as Fourier transform mass spectrometry (FTMS) and quadrupole ITMS. The ability to perform multiple levels of tandem-in-time mass spectrometry [18], high sensitivity [19], a theoretical mass range above *m*/*z* 70,000 [20], and high mass resolution [21] make the ITMS an appealing detector for MALDI-generated ions. In addition, the use of milliTorr pressures of helium buffer gas in the ion trap assists in cooling MALDI ions, thereby reducing their kinetic energy distributions.

Cox et al. performed MALDI experiments with an ITMS with ionization occurring external to the ion trap. They reported a full scan spectrum of bovine insulin B-chain at *m*/*z* 3496 in an experiment utilizing a Nd:YAG laser operating in the fourth harmonic and a nicotinic acid matrix [22]. Chambers et al. [17] described a configuration where they directly introduced MALDI ions into the ITMS. They showed a full scan spectrum of the molecular ion (12,360 Da) of horse cytochrome *c* and demonstrated MS^4 of the peptide, bradykinin. Doroshenko et al. [16] modified an ion trap detector (ITD) to accommodate a MALDI probe within the ion trap and reported an extended mass range of 3300 u utilizing the resonant-ejection mode of operation. They introduced a novel method for trapping MALDI ions within the ion trap by implementing controlled gating of the trapping field [23,24]. This method differs from other reports [17,22,25,26] in that it does not require collisions of ions with buffer gas for trapping. Rather, the collisions are utilized for obtaining high sensitivity and improved mass resolution, with a reported mass resolution of 12,000 for the single shot spectrum of gramicidin S [24]. Jonscher et al. [25] reported a MALDI/ITMS configuration with an external ionization source. They demonstrated analysis of the 26-kDa protein, porcine elastase, and observed the 34-kDa protein dimer of myoglobin. Schwartz and Bier [26] utilized a configuration

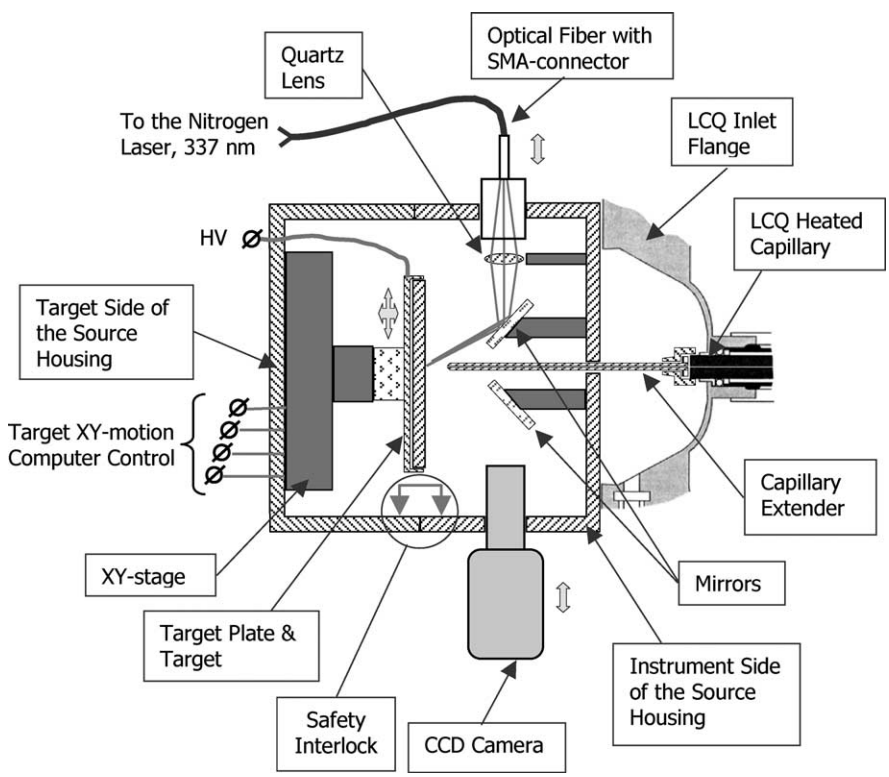

Fig. 4. Schematic of the commercially available AP MALDI source from Mass Technologies.

similar to that of Jonscher and coworkers, and were able to observe the molecular ion of chicken egg albumin (molecular weight (MW) = 43,300 Da). Further advances to the MALDI/ITMS configuration include the introduction of pulsed heavy gases for increased CID efficiency [27] and improvements to the trapping efficiency of externally generated ions [28,29].

1.3. AP MALDI/ITMS

A new atmospheric pressure ionization technique, AP MALDI, has recently been invented and coupled to an orthogonal acceleration TOF mass spectrometer by Laiko et al. [1–4]. The advantages of this technique include: sample handling under normal atmospheric pressure conditions, softer analyte ionization compared with conventional vacuum MALDI, and increased resolution of individual components of complex analyte mixtures.

Another attractive feature of AP MALDI is that it is an external ion source with respect to the mass analyzer. As a result, any type of MS instrument capable of analyzing atmospheric pressure ions may be coupled with this source with minor modifications. AP MALDI offers the advantages typically associated with a MALDI source such as minimum sample cleanup, ease of sample preparation, multiple analyses from a single spot as well as simplified spectra from complex mixtures, that are easily interpreted [1–4]. At the same time, AP MALDI does not require a vacuum region and is readily coupled to instruments possessing

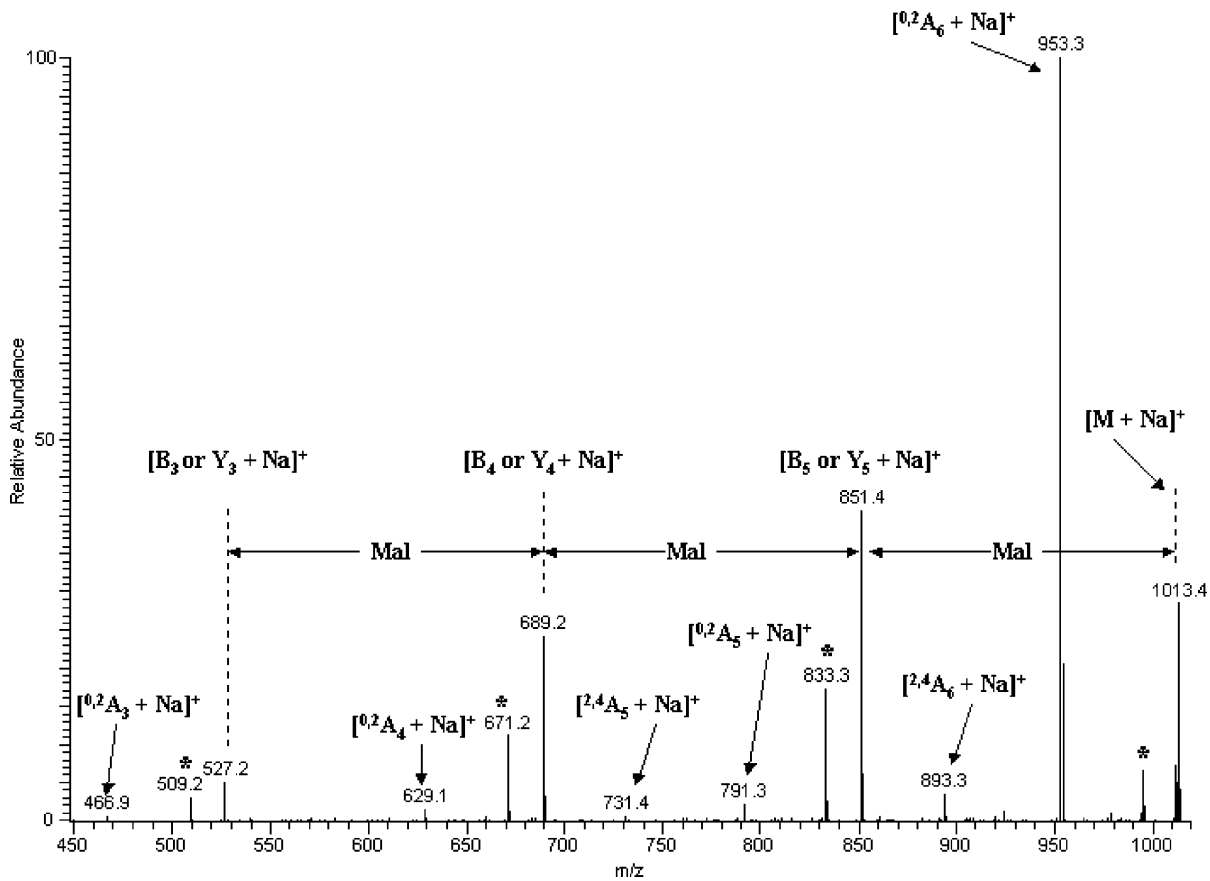

Fig. 5. Positive ion mode MS/MS spectrum of maltohexaose using arabinosazone matrix.

an atmospheric pressure interface, which makes it easily interchangeable with other atmospheric pressure sources, such as electrospray ionization (ESI).

The introduction of the AP MALDI technique generated much interest in coupling this source to an ITMS. Commercially available ITMS instruments, such as the Finnigan LCQ (ThermoFinnigan, San Jose, CA), are currently interfaced with atmospheric pressure ionization sources: ESI and atmospheric pressure chemical ionization (APCI). In these configurations, injection of ions into the ITMS is accomplished using two rf-only octopoles in series, which results in narrow spatial and energy distributions of ions entering the ion trap. These features make the commercially available ITMS instruments amenable to AP MALDI sources.

Recently, a number of groups have combined AP MALDI with commercial ion trap mass spectrometers [5–8]. Coupling the AP MALDI source with an ion trap mass analyzer combines the benefits of MALDI sample preparation and simplicity of spectral analysis resulting from the production of predominantly singly charged ions, with the MS^n capabilities of the quadrupole ITMS. This configuration has proven to be useful in obtaining structural information from peptides and protein digests [30–33] as well as for the identification and characterization of posttranslational modifications [32].

1.3.1. AP MALDI/ITMS configuration

In our laboratory, a prototype AP MALDI source was built and coupled to a Finnigan LCQ “Classic”

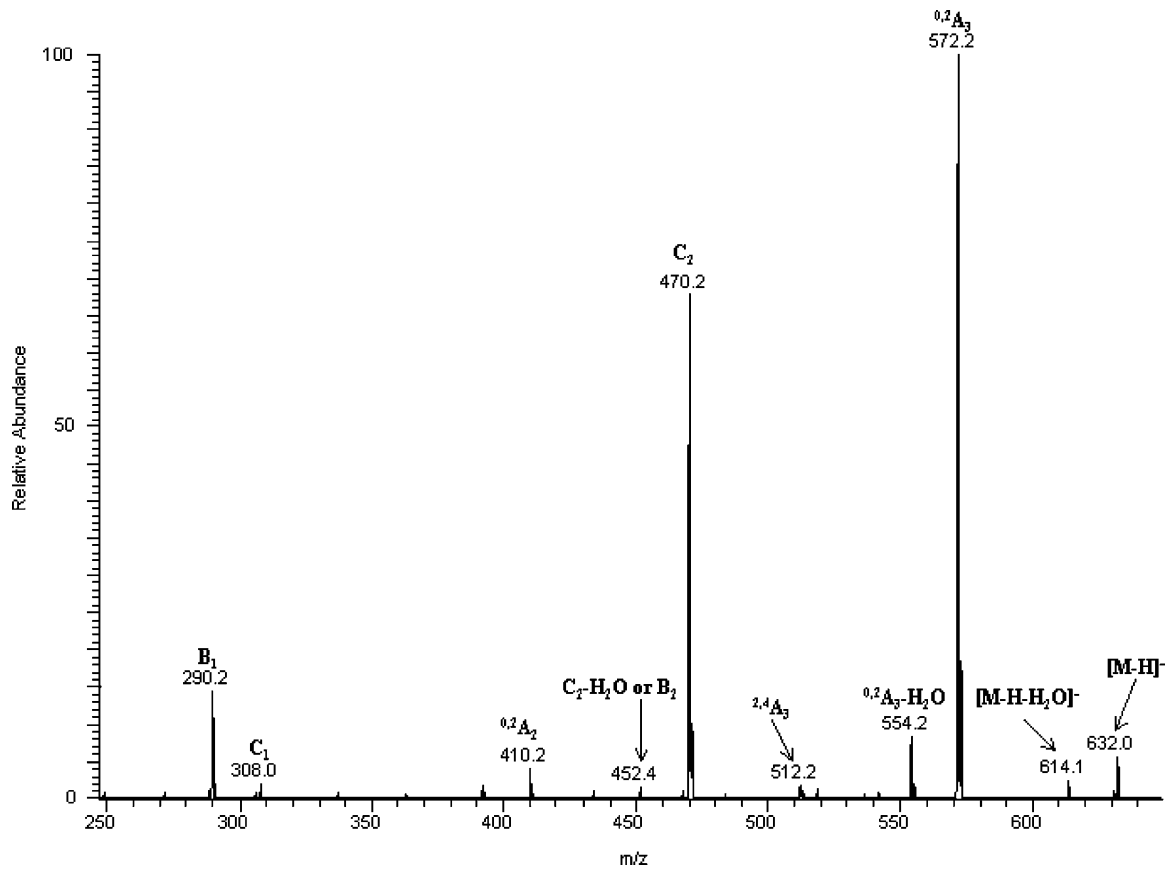

Fig. 6. Negative ion mode MS/MS spectrum of 6′-SL using arabinosazone matrix. The sialic acid portion of this oligosaccharide remains intact, yielding structural information.

ITMS (Fig. 1) [5,6]. A Nd:YAG laser was tuned to generate 355 nm pulses at 10 Hz with ∼0.3 mJ of energy and was focused by a system of two mirrors and a quartz lens ($F = 500$ mm) onto the surface of the target plate. The laser was operated asynchronously with the trapping cycle of the ITMS.

A stream of dry nitrogen was applied through a stainless steel capillary to the area surrounding the AP MALDI target. A potential of 2.5 kV was applied between the target tip and LCQ inlet transport capillary and the temperature of the transport capillary was 180 °C.

Enhanced sensitivity of the AP MALDI/ITMS configuration was achieved by turning off the automatic gain control (AGC) option on the Finnigan LCQ. The ion trapping time was set manually to 200–400 ms. Times above 400 ms decrease the spectrum resolution if analyte deposition exceeds the 500–800 fmol level [5]. This observation may be due to the space charge effect inside the ion trap. The ion accumulation period covers several laser pulses and the instrument scan time is short compared with the ion accumulation phase. As a result, the duty cycle of the AP MALDI/ion trap instrument is close to 100% even without synchronization of laser pulses with the trapping cycle.

The detection limit of our AP MALDI/ion trap is 10–50 fmol of analyte deposited on the target surface for a four-component mixture of peptides with 800–1700 Da MW. This source is easily interchangeable with the commercially available ESI source and was demonstrated to be useful in the analysis of peptide structure by MS/MS and MS^3 experiments [5,6].

1.3.2. AP MALDI probe for use with ESI source interfaces

Danell and Glish [7] introduced an AP MALDI probe consisting of a glass capillary sample holder

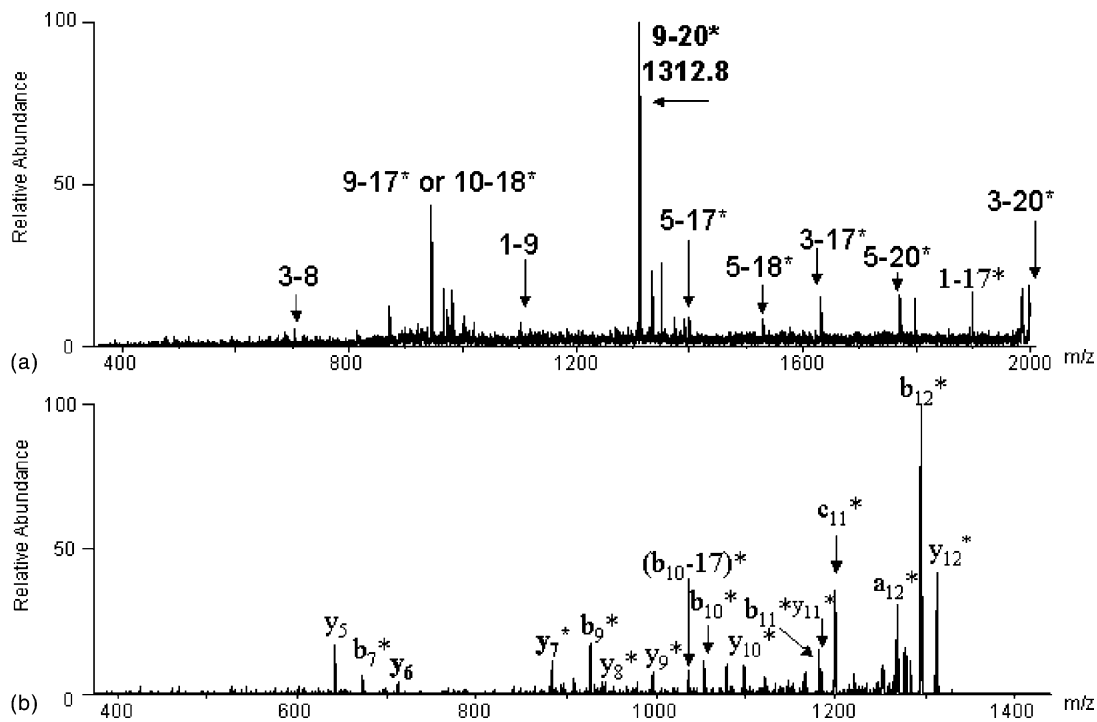

Fig. 7. (a) Full scan of the acetylated histone H3 peptide tryptic digest mixture. The asterisks (∗) denote fragments that display an increase in mass (42 Da) consistent with one acetylation site. (b) MS/MS of the acetylated tryptic fragment 9–20*. The MS/MS data of this tryptic fragment confirms the position of the acetylation site to be lysine 14.

employing fiber optic laser illumination. Fig. 2 shows the schematics of two variations of this configuration. The orientation of the laser radiation with respect to the MALDI sample in this setup is similar to that employed in transmission MALDI probes. The source depicted in Fig. 2A was interfaced with a Bruker Esquire ITMS. The source shown in Fig. 2B was coupled to an R.M. Jordan linear TOF in order to compare the performance of this configuration under both atmospheric pressure and vacuum conditions. A Nd:YAG laser operating in the third harmonic was coupled to an optical fiber in order to deliver laser radiation to the sample.

Approximately 5–10 μL of a mixture of matrix and analyte were introduced into the glass tubes via capillary action and allowed to dry. Analysis by this method yielded detection limits in the mid-picomole range. This source also produced spectra with intense matrix, matrix–analyte clusters, and doubly charged analyte ion peaks.

Further advances to this source included the addition of a heating coil to the glass transfer capillary to assist in desolvation of AP MALDI-generated ions [34]. Heating the source resulted in production of mostly singly charged analyte peaks, elimination of cluster ions, improved reproducibility and lowering of the detection limit by three orders of magnitude. The optimal temperature for AP MALDI experiments was reported to be 225 °C. Any further increase in

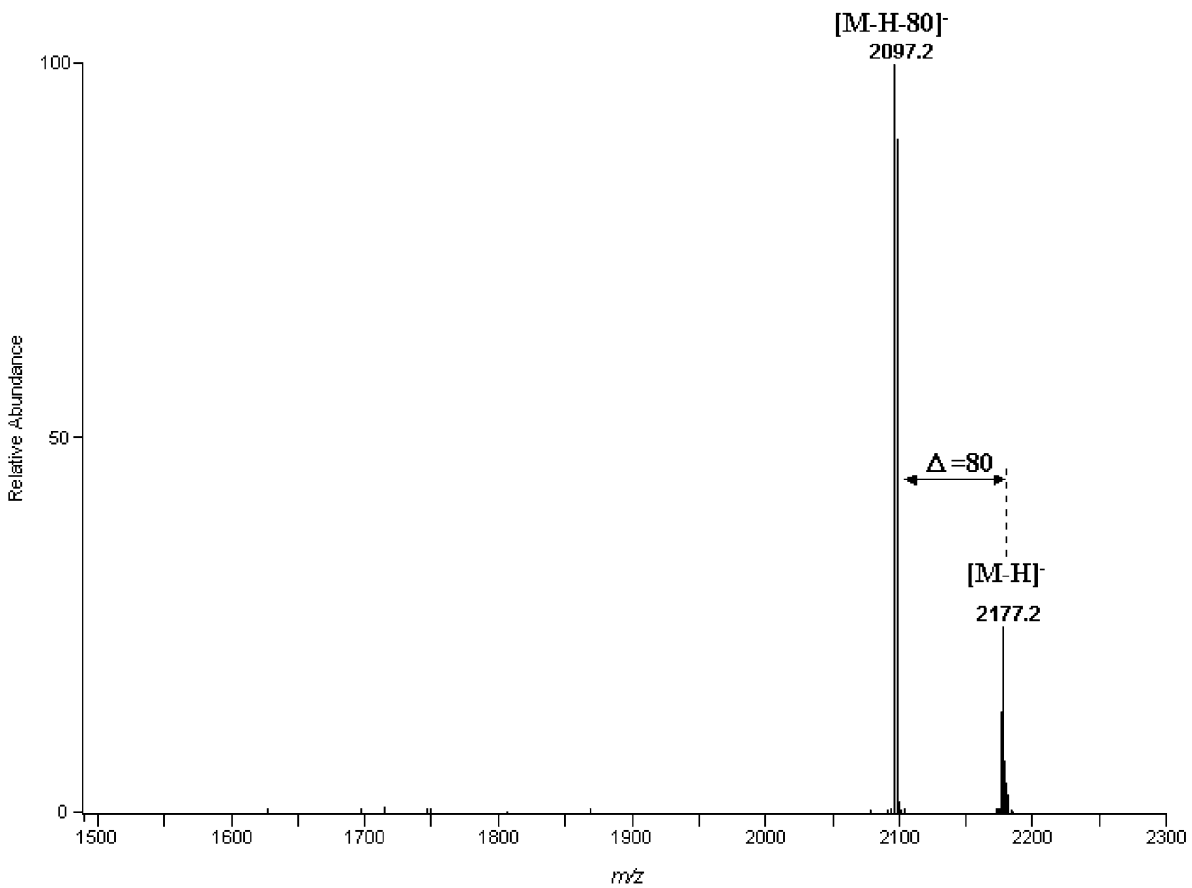

Fig. 8. Negative ion mode MS/MS of sulfated gastrin II at *m*/*z* 2177.2. Detection of the molecular ion of this sulfated peptide is possible in the negative mode, where only the desulfated ion is observed in the positive mode (data not shown).

temperature resulted in thermal fragmentation of the AP MALDI ions.

1.3.3. Transmission geometry for AP MALDI

Callahan et al. [35] described a setup that incorporates transmission geometry for an AP MALDI source. In a typical MALDI experiment, the laser irradiates the sample on the front side of an opaque surface, in a configuration known as reflection geometry. Fig. 3 illustrates this AP MALDI transmission geometry setup, coupled to a Finnigan LCQ ITMS. Transparent materials with either conducting or insulating properties were evaluated in these experiments, however, these properties seemed to have little effect on the performance of the ion source. The sample slides were placed between two metal plates with holes through the center in order to apply high voltage and to allow laser illumination of the sample plate from behind. A nitrogen laser was focused onto the sample slide resulting in desorption and ionization of the analyte.

Since Callahan et al. [8] had previously constructed an AP MALDI source utilizing reflection geometry, they were able to perform a direct comparison of transmission vs. reflection geometry AP MALDI configurations coupled to an ITMS. The transmission geometry of AP MALDI required higher laser power than that needed for reflection geometry: 150–190 μJ/pulse for transmission geometry as opposed to the 25 μJ/pulse

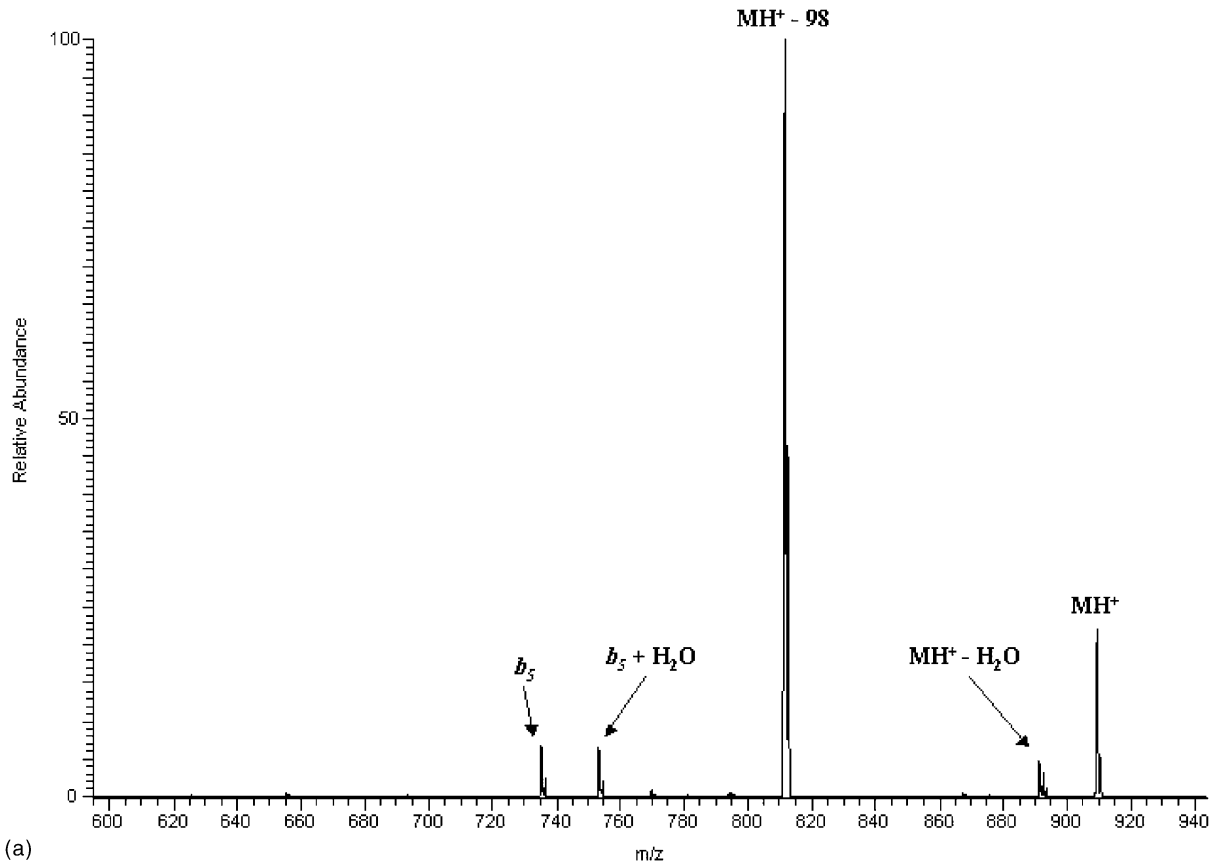

Fig. 9. (a) MS/MS of the $[M+H]^+$ ion of KR(pT)IRR. Loss of 98 Da from the precursor ion is observed. (b) MS/MS of the $[M+H]^+$ ion of KRP(pS)QRHGSKY. Loss of 98 Da from the precursor ion is observed. (c) MS/MS of the $[M+H]^+$ ion of KKLIEAAE(pY)AAKG. Loss of 98 Da from the precursor ion is observed.

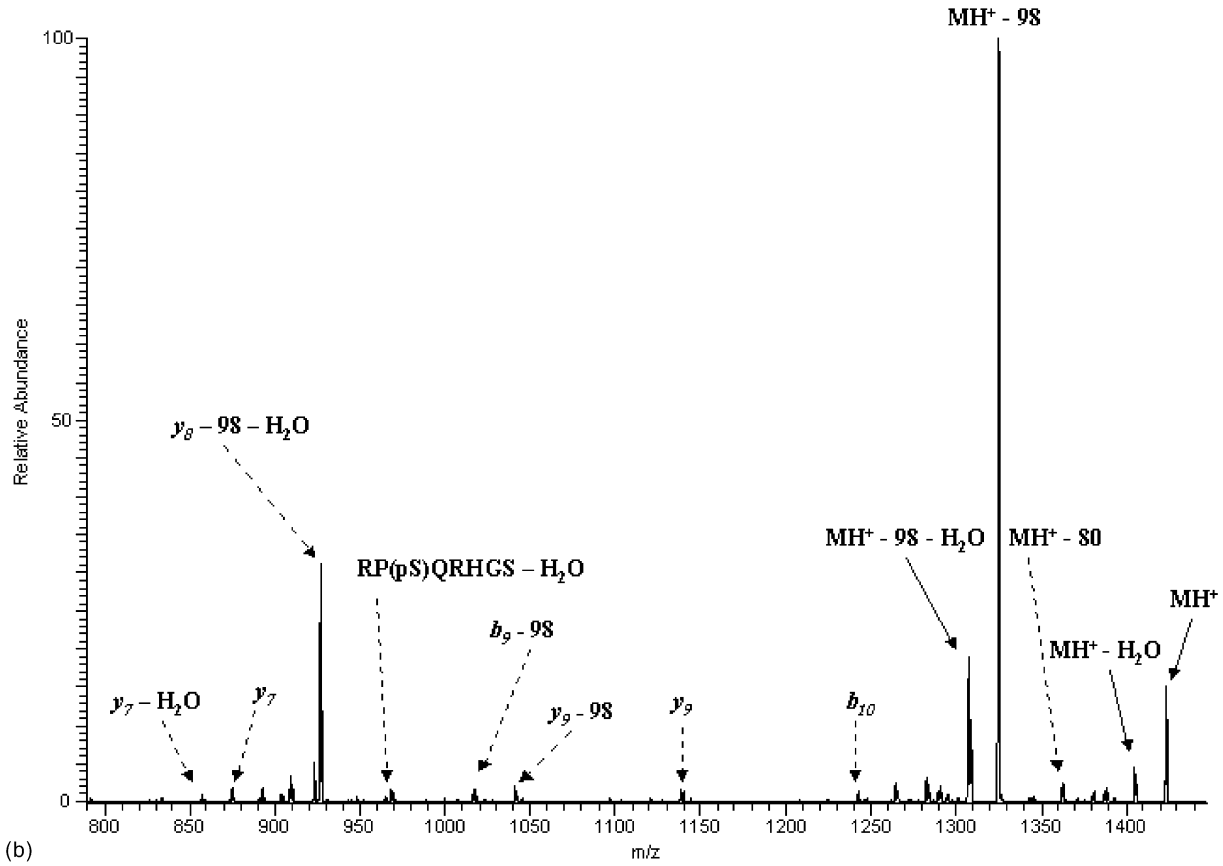

Fig. 9. (*Continued*).

used in reflection geometry. The transmission geometry results in signal intensity about one order of magnitude less than that of reflection geometry, however, spectra obtained from the transmission geometry did not possess the analyte–matrix clusters observed in the reflection geometry.

2. Experimental

2.1. AP MALDI sources

AP MALDI experiments were carried out using two different sources: the Laiko and coworkers configuration described earlier and a commercially available source from Mass Technologies. The Mass Technologies source (shown in Fig. 4) incorporates a fiber optic laser delivery system and a computer-controlled XY sample stage. For our experiments, we utilized a Nd:YAG laser operating in the third harmonic (355 nm) (Minilite, Continuum, Santa Clara, CA) rather than the nitrogen laser described in Fig. 4.

2.2. Materials

Maltohexaose, sulfated gastrin II, bovine β-casein, mini gastrin (MG), dynorphin 1–7 (Dyn 1–7), and dynorphin 1–13 (Dyn 1–13) were obtained from Sigma Chemical Company (St. Louis, MO). α-Cyano-4-hydroxycinnamic acid (HCCA), sinapinic acid (SA), and 6-aza-thiothymine (ATT) were purchased from Aldrich (Milwaukee, WI). 6-Sialyllactose (6′-SL)

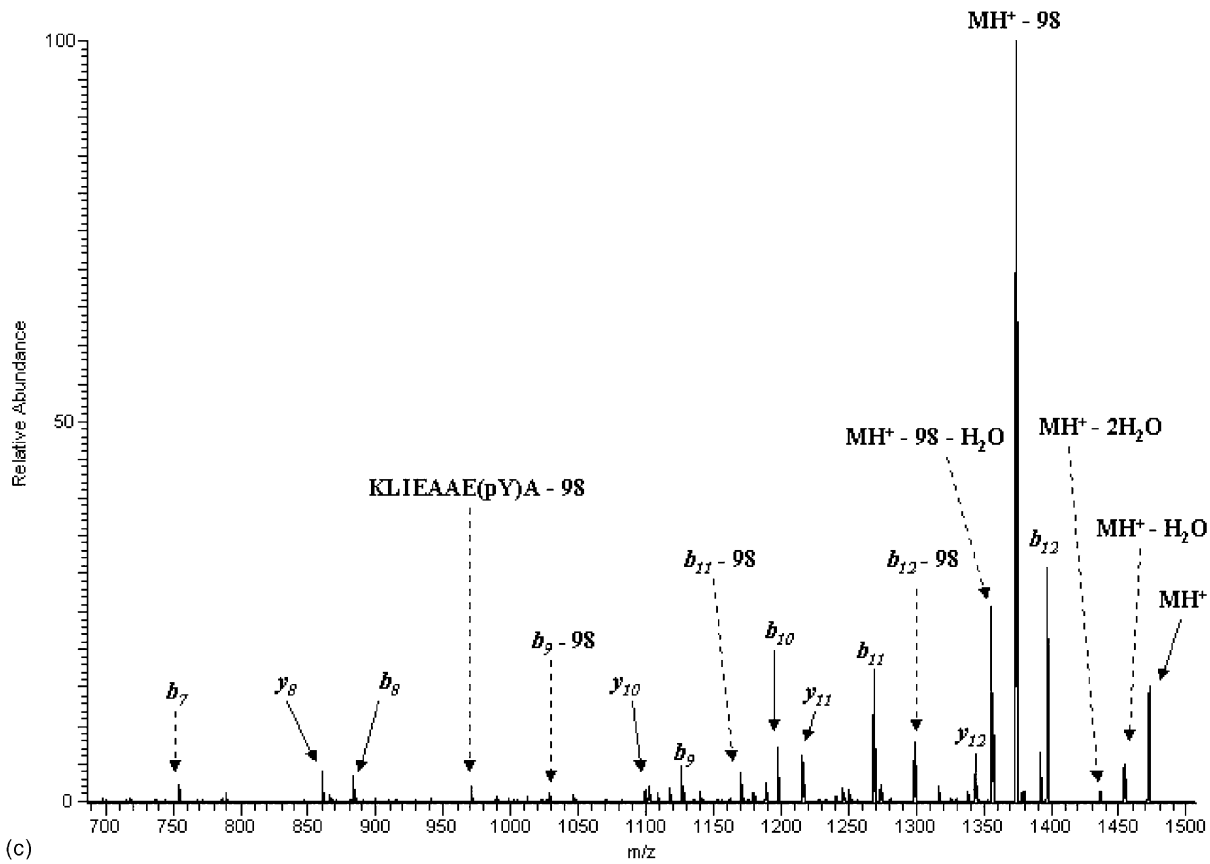

Fig. 9. (*Continued*).

was acquired from Glyko (Novato, CA). Unmodified and acetylated histone H3 peptide was provided by Dr. Philip Cole (Johns Hopkins University School of Medicine, Baltimore, MD). Sequencing-grade trypsin and Lys-C were acquired from Roche Molecular Biochemicals (Indianapolis, IN). The phosphopeptides, KR(pT)IRR and KKLIEAAE(pY)AAKG were synthesized at the Peptide Synthesis Core Facility at Johns Hopkins University School of Medicine (Baltimore, MD). The phosphopeptide, KRP(pS)QRHGSKY, was obtained from the University of Michigan Protein and Carbohydrate Facility (Ann Arbor, MI). Glycerol was obtained from J.T. Baker (Phillipsburg, NJ) and graphite was purchased as dry graphite lubricant (Sprayon, Sherwin Williams, Solon, OH).

2.3. Matrices

D-Arabinosazone was synthesized as per the method described by Chen et al. [36] and used as a matrix solution at a 10 mg/mL concentration in ethanol. Glycerol/graphite matrix was prepared and used as described by Dale et al. [37]. HCCA, SA, and ATT matrices were prepared as saturated solutions in 50:50 (v/v) ethanol/water.

2.4. Methods

2.4.1. Acetylated peptide digest

Tryptic digests of both the unmodified histone H3 peptide and the acetylated histone H3 peptide were

examined. Tryptic digests were performed by combining 2 μL of trypsin (1.0 μg/μL), 2 μL of the peptide solution (control peptide, 100 pmol/μL; acetylated peptide, 25 μL of distilled water was added to dry sample), and 2 μL of buffer solution (25 mM ammonium bicarbonate, pH 8). The reaction mixture was allowed to incubate for various time points up to 2 h at 37 °C. A 0.5 μL aliquot of the tryptic digest was added to the sample probe and the reaction quenched by the addition of 0.5 μL HCCA matrix.

2.4.2. Bovine β-casein digest

A Lys-C digest of bovine β-casein was performed. Bovine β-casein was dissolved in acetonitrile and then dried down. The protein was then reconstituted in water to a concentration of 0.1 μmol/μL. Six microliters of the protein solution was combined with 12 μL of 25 mM NH_4HCO_3 (pH 8) and 6 μL of Lys-C (0.1 μg/μL). The reaction mixture was allowed to incubate between 2–4 h at 37 °C. A 0.5 μL aliquot of the Lys-C digest was added to the sample probe and the reaction quenched by the addition of 0.5 μL HCCA matrix.

2.4.3. Peptide–peptide interactions

Methanol and glycerol were combined to form a 70:30 (v/v) solution. An equal volume of graphite was added to the methanol/glycerol solution and sonicated for 1 h. A 1 μL aliquot of the solution was added to the target, allowing the methanol to dry. A 0.5 μL of peptide solution was added to the target and the liquid was analyzed.

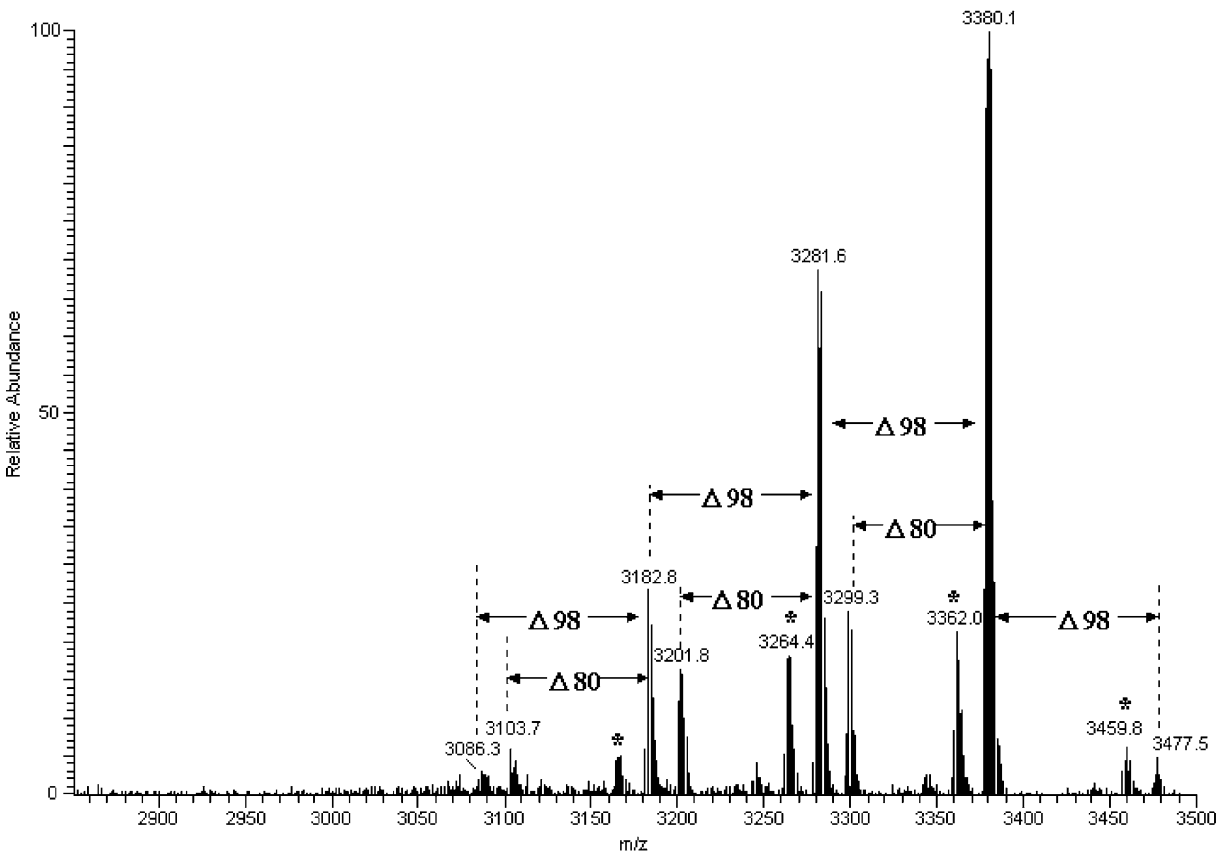

Fig. 10. MS/MS at *m/z* 3477.5 of β-casein Lys-C fragment 1–28, RELEELNVPGEIVE(pS)L(pS)(pS)(pS)(pS)EESITRINK. The peaks marked with an asterisks (∗) denote loss of water.

MG, Dyn 1–7, and Dyn 1–13 were dissolved in HPLC-grade water to a concentration of 1 nmol/μL. The acidic peptide (MG) was combined with a basic peptide (Dyn 1–7 or Dyn 1–13) in a 1:1 (v/v) ratio. A 0.5 μL aliquot of the peptide–peptide mixture was applied to the probe and 0.5 μL of matrix solution was added. The matrices utilized in the study of peptide–peptide interactions were: glycerol/graphite (pH 7), SA (pH 3.5), and ATT (pH 5).

3. Results and discussion

3.1. Oligosaccharide analysis

Carbohydrates are highly abundant in nature and display a wide array of functionality in biological systems. For instance, carbohydrates have been shown to play a role in conformation and stability of proteins, modification of protein function, mediation of cell–cell and cell–matrix interactions, as well as a wide variety of other physiological tasks [38]. The AP MALDI/ITMS configuration was evaluated for its usefulness in obtaining structural information for oligosaccharide samples. The analysis of underivitized carbohydrate samples by MALDI has always been a challenge, since neutral sugars do not readily protonate. In addition, carbohydrates containing sialic acids do have inherent negative charge, but these groups are readily lost, resulting in poor signal. Chen et al. [36] reported the utility of the arabinosazone matrix in the positive mode analysis of carbohydrates. They demonstrated that the use of arabinosazone matrix resulted in improvements in resolution, signal-to-noise ratio

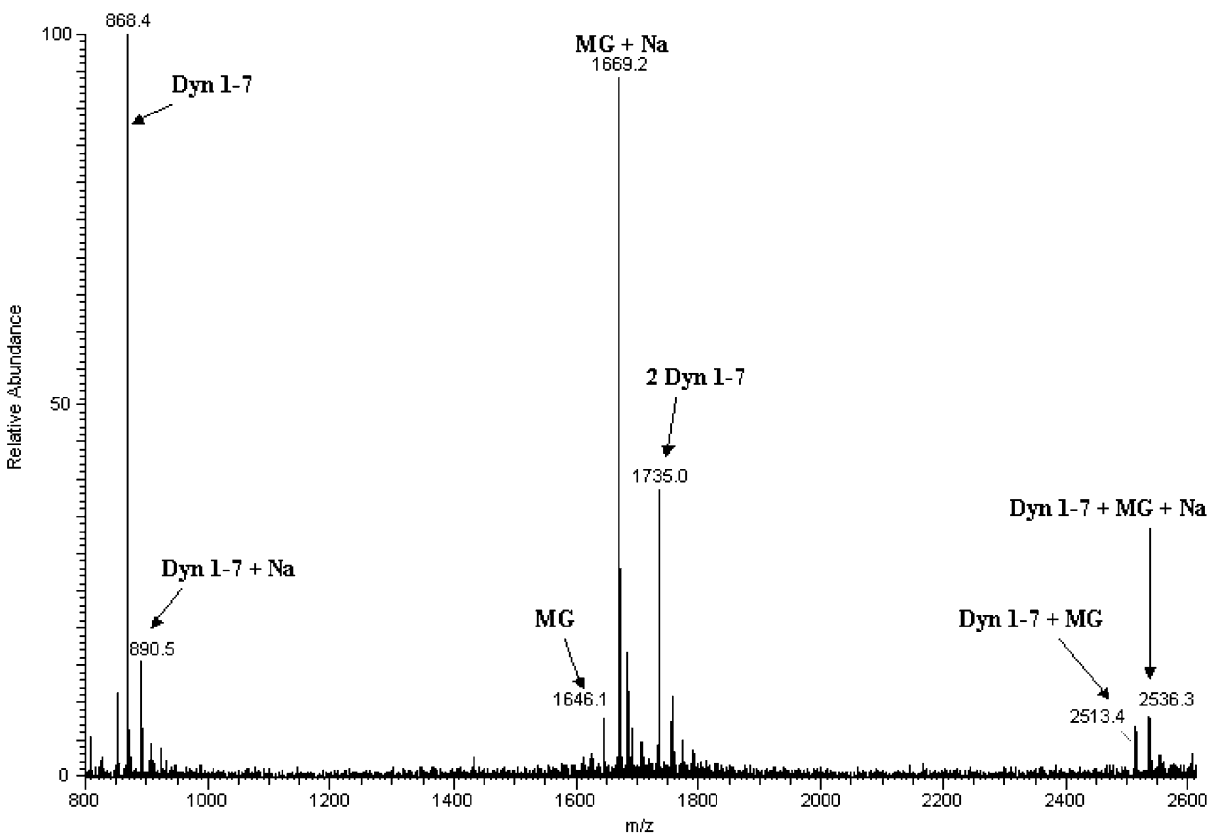

Fig. 11. Dyn 1–7 + MG in glycerol/graphite matrix, pH 7.

and detection limits for neutral sugar analysis over other matrices, such as 2,5-dihydroxybenzoic acid. Fig. 5 shows the positive ion mode MS/MS spectrum of maltohexaose. The nomenclature used to describe the fragmentation patterns of carbohydrates is that established by Domon and Costello [39]. As seen in Fig. 5, the arabinosazone matrix produces abundant sodiated ions from the neutral oligosaccharide, maltohexaose. Abundant sodiated A, B, and Y ions are present in this spectrum, with B and Y ions depicting losses of maltose units and A ions describing cross-ring fragmentation.

Fig. 6 is the negative mode MS/MS spectrum of the sialated oligosaccharide, 6′-SL. As shown previously by Wheeler and Harvey [40], the arabinosazone matrix is useful for obtaining negative ion spectra of small sialic acid containing glycans. With intact sialic acids it is possible to observe the negative ions of these types of oligosaccharides. The spectrum in Fig. 6 displays A, B, and C ions. The A ions are indicative of cross-ring fragmentation across the lactose rings. The C_2 ion represents cleavage between the two lactose units. The B_1 ion at *m*/*z* 290.2 is sialic acid.

3.2. Acetylation site determination

Acetylation of the histone proteins is believed to alter histone–DNA interactions, resulting in transcriptional regulation. Identification of the acetylation sites in histone proteins is essential in elucidating the role of these proteins in transcription. The histone acyltransferase, p300/CBP-associated factor, has been shown to

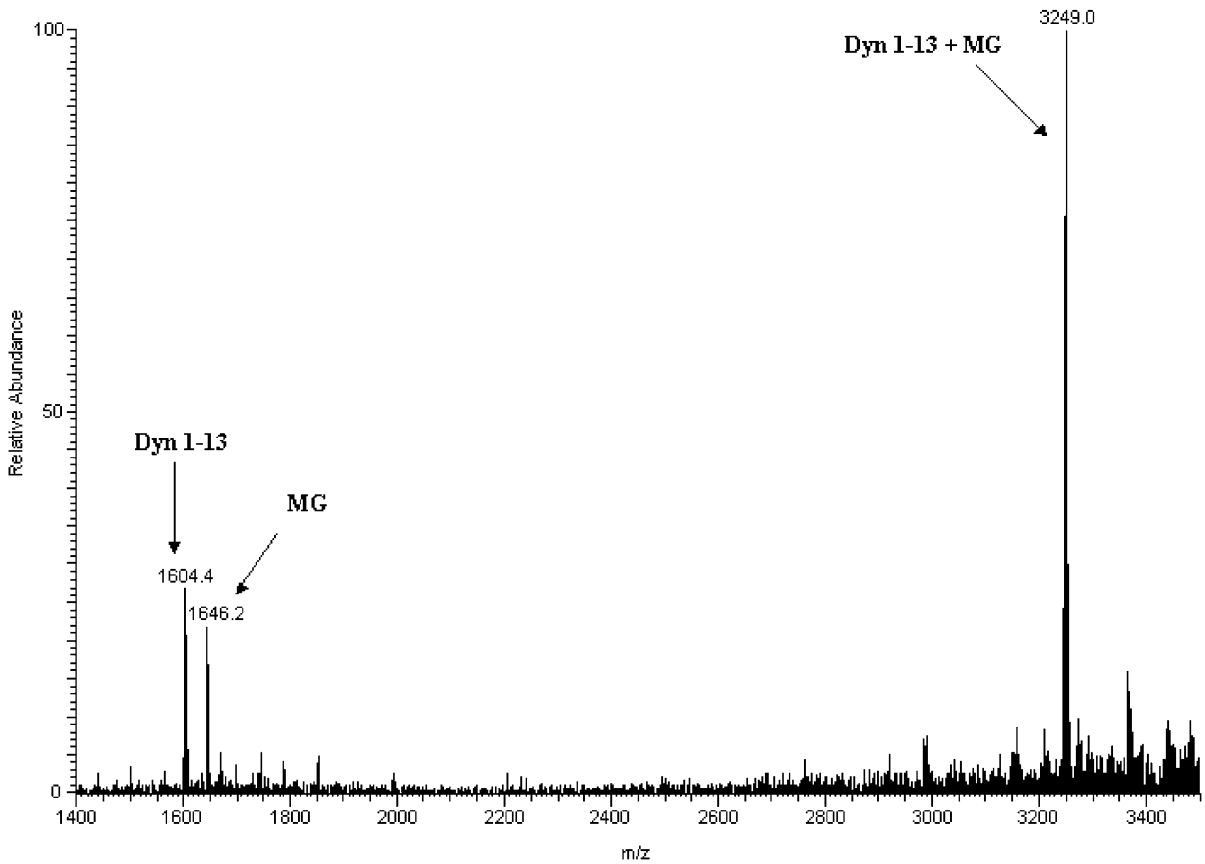

Fig. 12. Full scan spectrum of Dyn 1–13 + MG in ATT matrix, pH 5.

preferentially catalyze the acetylation of the ε-amino group on lysine 14 in the histone H3 protein [41]. Because this peptide consisting of the first 20 residues of the acetylated histone H3 protein contains four lysine residues, a tryptic digest and subsequent MS/MS by AP MALDI/ITMS of the peptide was conducted in order to confirm the position of the acetylation site.

Fig. 7a shows the full scan spectrum of the tryptic digest of the acetylated histone H3 peptide, Ac-ARTKQTARKSTGG(Ac-K)APRKQL. This spectrum was compared with the spectrum of the tryptic digest of the unmodified histone H3 peptide, Ac-ARTKQTARKSTGGKAPRKQL (data not shown), in order to determine which fragments contain the mass shift of 42 Da that is consistent with acetylation.

Fig. 7b is the MS/MS spectrum of the modified tryptic fragment 9–20 at *m*/*z* 1312.8. This spectrum clearly shows a series of b and y ions. In particular, the y_7–y_{12}, b_7, and b_9–b_{12} ions all contain the modification consistent with a 42 Da mass shift from the unmodified peptide tryptic digest. The y_6 and y_5 ions do not contain the modification. This fragmentation data was sufficient to confirm the site of the acetylation modification to be on lysine 14.

3.3. Sulfation

Detection of a sulfate modification in positive mode MS may be difficult due to the labile nature of the sulfate group. Therefore, negative mode analysis of sulfated peptides is necessary in order to detect this modification. Fig. 8 is the negative mode MS/MS spectrum of sulfated gastrin II. As shown in this spectrum, CID of this peptide resulted in a major loss of 80 Da from the molecular ion at *m*/*z* 2177.2; a loss consistent with a sulfate modification. However, another important posttranslational modification, phosphorylation, results in a mass increase of 80 Da from the unmodified protein. Detection of a phosphate moiety is readily accomplished in positive mode MS/MS. However, as seen in the following discussion, the fragmentation of a phosphopeptide in AP MALDI/ITMS is characterized by a loss of 98 Da, not 80 Da. Therefore, MS/MS analysis of samples by AP MALDI/ITMS allows for the differentiation of sulfated and phosphorylated peptides and proteins.

3.4. Phosphorylation

The presence of a phosphorylation site on a peptide was also investigated. Three different peptides, each containing a phosphoserine, phosphothreonine or phosphotyrosine, were studied (Fig. 9). Fragmentation of these phosphorylated peptides revealed a significant loss of 98 Da, corresponding either to H_3PO_4 or to a sequential loss of HPO_3 followed by elimination of water.

A Lys-C digest of bovine β-casein was performed. Fig. 10 shows the positive mode MS/MS of the Lys-C digest fragment 1–28. This enzymatic fragment contains four phosphorylated serines. CID of β-casein 1–28 produced fragments with consecutive losses of 80 and 98 Da. The data points to a mechanism of dephosphorylation from phosphoserine that involves the consecutive losses of HPO_3 and H_2O, rather than a concerted loss of H_3PO_4.

3.5. Peptide–peptide interactions

ESI is considered to be the method of choice for the study of noncovalent complexes by mass spectrometry. Since ESI is a soft ionization technique, resulting in little or no molecular fragmentation, weakly bound complexes can be detected. However, several laboratories have pursued the possibility of detection of noncovalent complexes by MALDI. Previous work by Woods and Huestis [42] demonstrated that peptide–peptide interactions could be observed by MALDI mass spectrometry if the pH of the matrix was raised. It was shown that this increase in pH preserved the ionic interactions between the peptides. AP MALDI presents the possibility of observing noncovalent complexes in samples prepared using conventional MALDI techniques that undergo soft ionization resulting from collisional cooling in the atmospheric pressure region.

Figs. 11–13 provide examples of peptide–peptide interactions analyzed by the AP MALDI/ITMS

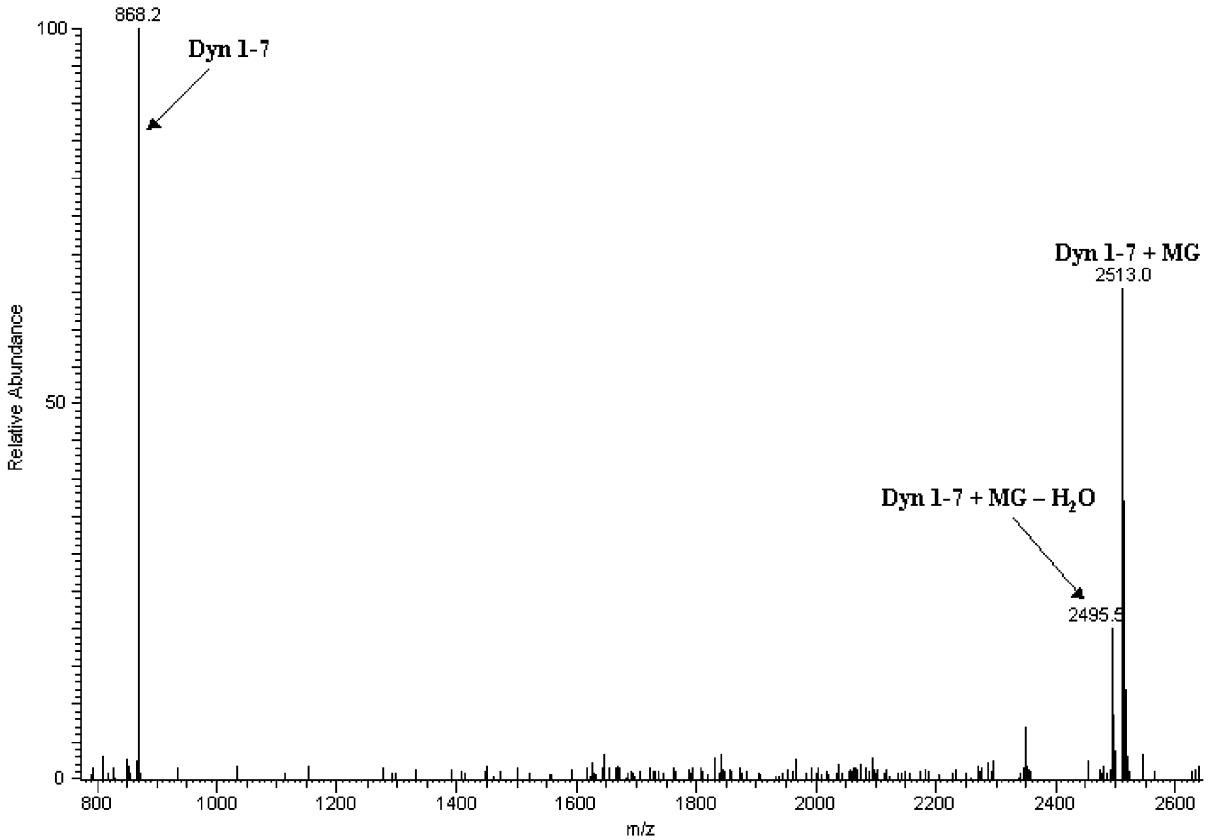

Fig. 13. MS/MS spectrum of Dyn 1–7 + MG complex at *m*/*z* 2513.0 using SA matrix, pH 3.5. The positively charged portion of this complex, Dys 1–7, is observed at *m*/*z* 868.2.

configuration. Fig. 11 shows the full scan spectrum of the peptide–peptide complex formation between the basic peptide, Dyn 1–7, and the acidic peptide, MG, using a glycerol/graphite liquid matrix. The glycerol/graphite matrix is neutral and the peptide–peptide complex is evident at *m*/*z* 2536.3.

Fig. 12 depicts the full scan spectrum of the peptide–peptide complex formation between Dyn 1–13 and MG using an ATT matrix at pH 5. An abundant peptide–peptide peak is present at *m*/*z* 3249.0.

Fig. 13 shows the positive mode MS/MS spectrum of the peptide–peptide complex of Dyn 1–7 and MG at *m*/*z* 2513.0 using SA, pH 3.5. Fragmentation of the molecular ion of this complex resulted in the detection of the positively charged basic portion of this complex, Dyn 1–7, at *m*/*z* 868.2.

The combination of increased matrix pH and collisional cooling of ions in the AP MALDI process may be important in the preservation of ionic interactions between peptides. In addition, the data shows that noncovalent complexes can be observed even after irradiating a crystal with multiple laser shots, which may suggest that sample preparation and ionization conditions are critical in the observation of noncovalent complexes by MALDI.

4. Conclusion

The development of new mass spectrometric techniques in the past decade have allowed for the unprecedented investigation of biological samples that are only

available to investigators in minute quantities. However, until recently, one needed an extensive technical and instrumental knowledge to perform these analyses. New strides in instrumentation, such as the development of AP MALDI will make such tools readily available to the experimental biochemist. A number of different AP MALDI configurations have recently been developed in the last few years. These sources coupled to an ion trap (or other mass analyzer) have only just begun to show their potential for analyzing a myriad of biological species.

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