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High pressure MALDI-FTMS: implications for proteomics

Bogdan A. Budnik[a,b], Susanne C. Moyer[b], Jason L. Pittman[b], Vera B. Ivleva[b], Ulf Sommer[b], Catherine E. Costello[a,b], Peter B. O'Connor[a,b,*]

[a] *Cardiovascular Proteomics Center, Department of Biochemistry, School of Medicine, Boston University, 650 Albany St. X-116, Boston, MA 02118-2526, USA*
[b] *Mass Spectrometry Resource, Department of Biochemistry, School of Medicine, Boston University, 715 Albany St. R806, Boston, MA 02118-2526, USA*

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Dedicated to Professor Alan G. Marshall on the occasion of his 60th birthday.

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Abstract

Tandem mass spectra of multiply charged ions typically generate more complete fragmentation patterns and thus are desirable for peptide sequencing. However, multiply charged species are not typically observed in spectra obtained with matrix-assisted laser desorption/ionization (MALDI) Fourier transform mass spectrometers. Current efforts with high pressure (HP) MALDI-Fourier transform mass spectrometry (FTMS) have resulted in abundant production of multiply charged ions, introducing the potential for new investigations into the MALDI ion formation mechanism, and increasing the utility of tandem mass spectrometry with MALDI-FTMS. This work focuses on the use of an HP MALDI-FTMS instrument for proteomics applications. The performance of HP MALDI and the power of FTMS and MS/MS of multiply charged ions for proteomics applications are discussed.
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Keywords: MALDI; FTMS; Proteomics; Multipe charges

1. Introduction

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry is particularly well suited to the detection of biomolecules [1]. Initial results with high pressure (HP) MALDI-Fourier transform mass spectrometry (FTMS) [2,3] showed reduced fragmentation of highly labile sialylated glycolipids known as gangliosides [4] and also resulted in improved abundance of $[M + 2H]^{2+}$ ions of the insulin β-chain peptide [5]. Since it has been known for some time that infrared MALDI increases the abundance of multiply charged ions [6,7], it was speculated that the reduced observation of the higher charge states in MALDI was primarily due to metastable decay. This has been observed frequently with PSD spectra in the past [8]. In order to test this hypothesis, a number of peptides were analyzed with the previously described HP MALDI-FTMS in an attempt to increase the abundance of $[M + 2H]^{2+}$ ions, and these same peptides were fragmented by SORI-CAD to demonstrate the utility of multiply charged ions.

In addition, this instrument was tested for suitability to the analysis of tryptic digest peptide mass fingerprints [9] for proteomic analyses. The unique identification of a protein from a peptide mass fingerprint requires high sequence coverage or high mass accuracy or both [10]. Thus, high sensitivity for low abundance analyte peaks, high mass accuracy for improved database identification, and high resolution for correct identification of neighboring peptides are the three most important parameters required from a mass spectrometer for the proteomics research. These parameters, combined with a need for high throughput, make an HP MALDI-FTMS instrument promising for proteomics.

MALDI for proteomic analysis has particular advantages compared to electrospray as it is less sensitive to salt and detergents, and analytes that have been applied on the MALDI spot can be cleaned up on the plate, or can be stored and analyzed later [11]. Furthermore, large MALDI plates that can

* Corresponding author. Tel.: +1-617-638-6705; fax: +1-617-638-6761.
E-mail address: poconnor@bu.edu (P.B. O'Connor).

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hold thousands of spots are useful for automation and, therefore, result in high throughput sample analysis. Coupling liquid chromatography separation to deposition of the eluent on MALDI sample plates [12] is a logical step that allows automated screening of complex mixtures of proteins and peptides, particularly when coupled to database searches. One limitation of MALDI, even on reflectron time of flight instruments [13], is that hot MALDI ions undergo metastable decay on a sub-millisecond timeframe. Newer high pressure MALDI methods [14–16] allow the user to control metastable decay, thus solving this long-standing fundamental limitation of MALDI and allowing analysis of labile post-translational modifications.

Fourier transform ion cyclotron resonance mass spectrometry (FTICR or FTMS) [17], developed in the early 1970s by Marshall and coworkers [18–22], has consistently demonstrated superior resolution and mass accuracy compared with time-of-flight, quadrupole, magnetic sector, or Paul ion trap mass spectrometers. The highest resolution of FTMS mass spectral peaks, in which a resolving power of 8,000,000 was demonstrated and the fine structure of the isotopic peaks is clearly visible, was obtained on the 9.4 T instrument at the National High Magnetic Field Laboratory in Tallahassee, FL, USA [23]. The best mass accuracy results are routinely in the 0.1–1 ppm range with internal calibration [24–26] and in the 1–5 ppm range routinely with external calibration [27]. In addition, sub-attomole limits of detection have been demonstrated [2,28,29]. In order to carry out effective proteomics experiments, an instrument must provide superior performance in the area of resolution, sensitivity, and mass accuracy, making FTMS an obvious choice for a high performance proteomics instrument.

In this work, we discuss the performance of a new HP MALDI-FTMS instrument for high throughput proteomics research. Future prospects for automation and proteomic applications are discussed.

2. Experimental

2.1. Mass spectrometry

All data were obtained on a home built HP MALDI-Fourier transform mass spectrometer using a 7 T actively shielded magnet (Cryomagnetics Inc., Oak Ridge, TN, USA). Data were acquired using an IonSpec (Irvine, CA, USA) Omega^{TM} data station. The instrumental performance [2] and design [3] has been previously described. All spectra were generated under high gas pulse pressure (∼3 mbar) at the target surface. The MALDI plume (generated by a "multishot" procedure [30]) expanded directly into the accumulation hexapole where ions were trapped and allowed to cool vibrationally and translationally via ion–ion and ion–neutral collisions for 2 s (typically). Ions were subsequently pulsed from the hexapole to the cell by dropping the "hexapole back trap" plate voltage from +10 to −10 V, dumping the ions into a 1.2 m rf-only quadrupole ion guide which transfers the ions to the ICR cell. Ions were captured in the ICR cell by gated trapping using a gas pulse to cool the ions to the cell center, increasing sensitivity. Ions were isolated by SWIFT [31] (512 K output waveform, clocked out at 1 MSPS) with excitation typically occurring between 25 and 120 V depending upon the *m*/*z* of the ions of interest. SORI-CAD [32] was performed using a previously defined method [33]. Unless otherwise stated, all spectra are single scan spectra.

2.2. Sample preparation

Peptides and matrices were obtained from Sigma (St. Louis, MO, USA), and were used without further purification. The preferred matrix was α-cyano-4-hydroxycinnamic acid (CHCA) based on previous performance of this matrix for the analyses of peptides using the current instrument configuration [2]. Analyte and matrix were deposited on a Teflon^{TM} surface. The immunoglobulin digest was purchased from Michrom BioResources, Inc (Auburn, CA, USA) and diluted from the powder according to the company protocol. The Tv60 protein was provided by B.N. Singh, SUNY Upstate Medical University (Syracuse, NY, USA). MALDI spots were prepared by the standard dried droplet procedure [34], and were allowed to dry for several minutes in air prior to insertion into the mass spectrometer.

3. Results and discussion

The high pressure MALDI-FTMS produces increased abundance of multiply charged ions compared to traditional MALDI-FTMS under high vacuum conditions [5]. Fig. 1 demonstrates the presence of these multiply charged ions. While these ions (in this relative abundance range) are relatively common on MALDI-TOF instruments (particularly in linear mode), the only other reports of multiply charged MALDI ions on an FTMS instrument is for >8 kDa proteins [5,35]. Fig. 1A presents the HP MALDI-FTMS spectrum of the small phosphopeptide, DRVpYIHPF. The spectrum demonstrates that the ionization is cool enough to prevent loss of the phosphate as previously reported [2], but it also shows $[M + 2H]^{2+}$ ions at approximately 1:15 ratio. This $[M + 2H]^{2+}$ ion overlaps with the 2nd harmonic of the reduced cyclotron frequency, ω_+, but is shifted ∼0.5 *m*/*z* from it due to the presence of the extra hydrogen. The harmonic does not completely overlap with the 2+ ion due to the slight non-linearity of the calibration [36]. Fig. 1B shows the same thing for the negative ion mode spectrum of substance P. The second harmonic is not observed in this spectrum, due to the lower total ion signal. The ion abundance ratio, $[M - H]^-/[M - 2H]^{2-}$, is 17.5. This is slightly lower than the positive mode signal which will be discussed below.

In order to definitively prove that the signal observed at half the mass of the $[M+H]^+$ ion is, indeed, the $[M+2H]^{2+}$

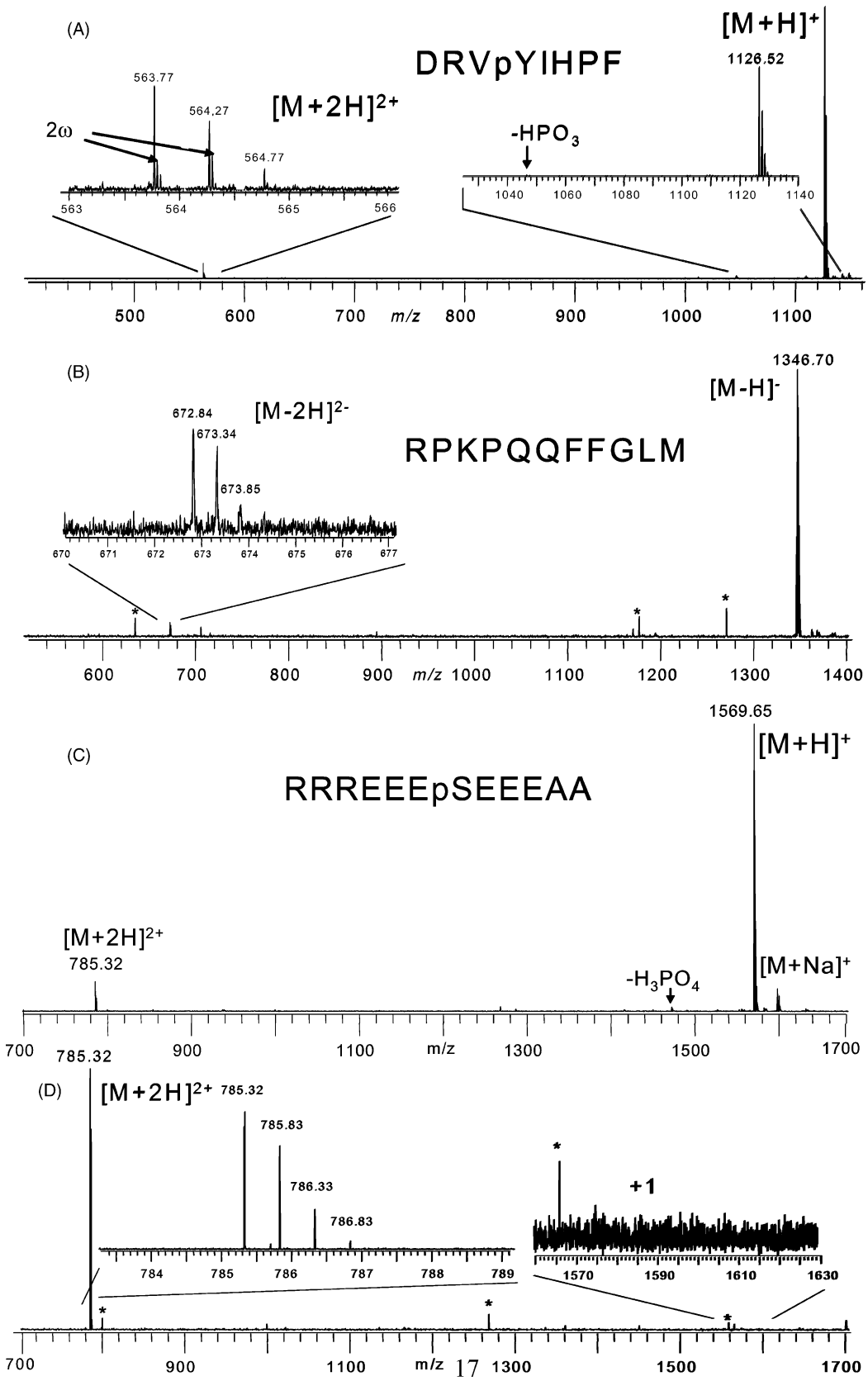


Fig. 1. (A) The HP MALDI spectrum of a phosphopeptide in the positive ion mode. Arrows indicate overtones from the singly charged ions. (B) HP MALDI spectrum of substance P in the negative ion mode. (C) Positive mode HP MALDI-FTMS spectrum of the phosphopeptide, RRREEEpSEEEAA, using CHCA matrix with the $[M+H]^+$ ion at *m/z* 1570.65 and the $[M+2H]^{2+}$ ion at *m/z* 785.83. (D) SWIFT isolation of the $[M+2H]^{2+}$ ion, indicating that the signals are not overtones or artifacts.

ion and not some unusual harmonic or artifact, the $[M + 2H]^{2+}$ ion was isolated by SWIFT. The resulting spectra are shown in Fig. 1C and D. Fig. 1C is the spectrum of the phosphopeptide, RRREEEpSEEEAA, which has a $[M + H]^{+}/[M + 2H]^{2+}$ ion abundance ratio of 9.4. The $[M + 2H]^{2+}$ ion is isolated in Fig. 1D, and the inset shows that the abundance of the $[M + H]^{+}$ ion is below the detection limit of the instrument.

In order to further understand the process of the formation of these surprisingly abundant $[M+2H]^{2+}$ and $[M-2H]^{2-}$ ions in the HP MALDI-FTMS, the ratio of the multiply charged to singly charged ions' abundances were plotted as a function of laser power and collision gas pressure. These results, plotted in Fig. 2, were acquired on a different day with the source somewhat hotter than in Fig. 1, which accounts for the reduced relative abundance of the $[M + 2H]^{2+}$ ions, however, the trend was observed on many different days and is consistent. Pressure dependence of the abundance ratio of $[M + 2H]^{2+}/[M + H]^{+}$ ions is dramatic. Under high vacuum MALDI-FTMS conditions, the $[M + 2H]^{2+}$ ion is not observable, but the ratio rises dramatically when the pressure in the MALDI source increases during desorption. In Fig. 2A, the pulse valve backing pressure varies from ~250 to ~1300 Torr. A backing pressure of ~600 Torr has previously been estimated (very crudely) to cause an increase in the pressure in the plume region to ~3 Torr [3]. However, the $[M+2H]^{2+}/[M+H]^{+}$ ion abundance ratio clearly peaks in this region, and stabilizes to a lower value at higher pressures.

With the high pressure MALDI source, multiply charged ion signal increases dramatically compared to MALDI under high vacuum conditions. The plateau observed from 800 to 1300 Torr in Fig. 2A is likely due to a competition between collisional activation or aerodynamic turbulence and

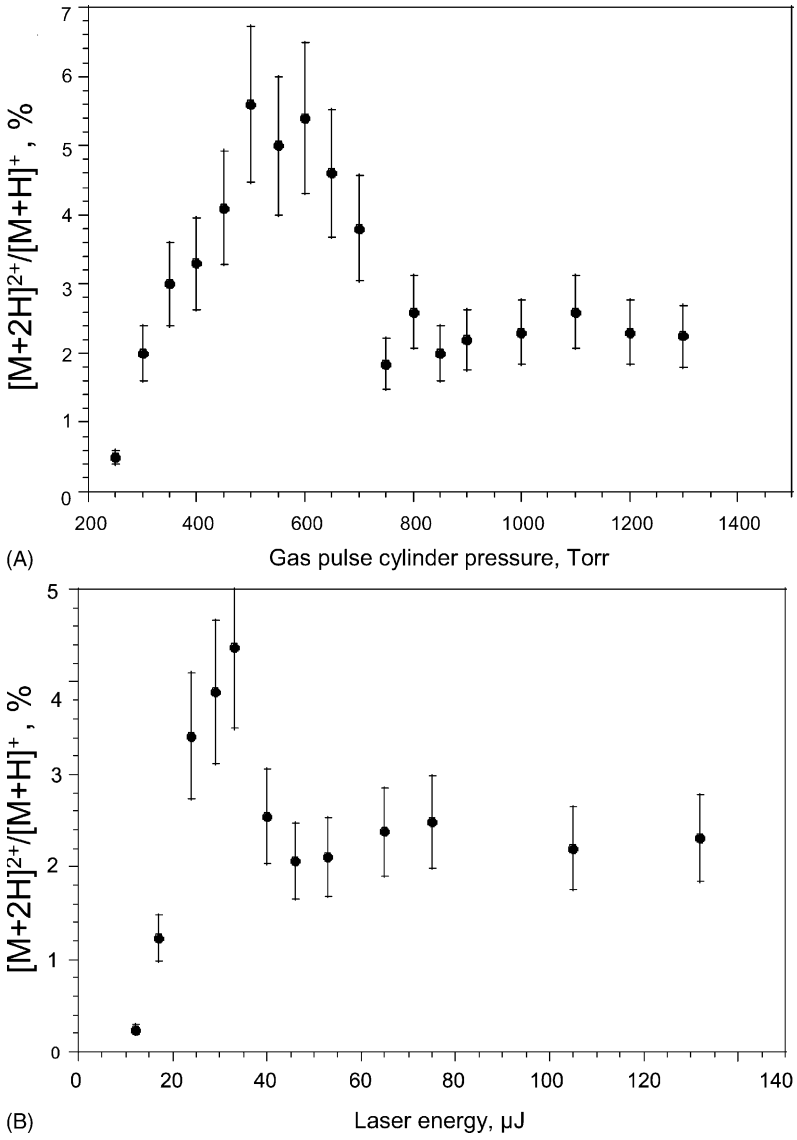


Fig. 2. (A) Collision gas pressure dependence of $[M + H]^{2+}$ ion abundance in HP MALDI-FTMS. (B) Laser energy dependence of $[M + H]^{2+}$ ion abundance in HP MALDI-FTMS.

collisional cooling in the plume itself. Spectra acquired with backing pressures below 250 Torr showed no evidence of multiply charged ions, and are comparable to high vacuum MALDI-FTMS spectra. It is important to notice that the $[M+2H]^{2+}/[M+H]^{+}$ ion abundance ratio increases in this plot from zero at high vacuum MALDI conditions, to ∼0.5% with a backing pressure of 250 Torr, to maximize at ∼5.5% (in this case) with a backing pressure of ∼500–600 Torr. Thus, there is an 11-fold increase in 2+ ion abundance from 250 to 550 Torr, and the increase is greater when compared to high vacuum conditions.

The laser power dependence of the $[M+2H]^{2+}/[M+H]^{+}$ ion abundance ratio is not as strong as the dependence on pulse gas pressure. There is an expected, strong onset at the MALDI desorption threshold, a peak at ∼37 μJ (for a ∼200 μm diameter laser spot), and a leveling off at ∼40 μJ. Thus, for production of abundant $[M+2H]^{2+}$ ions, it is important to adjust the laser fluence to just above the threshold. The cause of the leveling affect at higher fluence is not clear at this time.

These data have interesting implications on the mechanism of ion formation in MALDI. First of all, the "lucky survivor" model [37,38] generally predicts that multiply charged ions will be suppressed compared to singly charged ions due to recombination of the multiply charged species with photoelectrons in the MALDI plume. This has been corroborated in work by Frankevich et al. [39,40], who proposed desorption from non-conductive surfaces to reduce production of photoelectrons and thus increase the abundance of higher charge states. Thus, all spectra shown were generated by desorbing the ions from a thin piece of Teflon tape, and our results agree that desorption from Teflon increases the abundance of the $[M+2H]^{2+}$ ions. The big advantage of desorbing from Teflon was that spots tended to "ball up" simplifying observation of the spot and preventing cross-contamination on the sample plate as the Teflon was replaced for every spot. The improvement in $[M+2H]^{2+}$ ion abundance between desorption from Teflon versus desorption from steel was ∼10% (data not shown), while the pressure dependence results above show a much larger, >11-fold increase. Clearly, there is more than one factor controlling the multiply charged ion abundance.

The pressure dependence can be explained by vibrational cooling of ions in the plume during the laser pulse [4]. Multiply charged ions are substantially more labile than singly charged ones and are more likely to be lost to metastable decay, so that collisional cooling of these ions prior to fragmentation will increase their abundance. Furthermore, multiply charged ions are substantially lower in proton affinity than their singly charged analogs [41], thus increasing the probability that they will lose a proton to the much more abundant neutral matrix molecules always present in the MALDI plume. This interpretation correlates with the increased observance of $[M-2H]^{2-}$ ions in negative mode HP MALDI-FTMS spectra when desorbed from Teflon, which would be expected to be unaffected by photoelectrons.

The singly and doubly charged ions of renin substrate (DRVYIHPFHLVIHN) generated by HP MALDI were isolated in the ICR cell by SWIFT and were subsequently subjected to SORI-CAD fragmentation (Fig. 3A and B). Fig. 3A is the SORI-CAD MS/MS spectrum of the $[M+H]^{+}$ ion, and yields only four fragments, the subsequent loss of two H_2O molecules, the loss of a CO_2 molecule, and the $y_{13}{}^{+}$ ion. Higher SORI amplitudes merely decreased the abundance of the $[M+H]^{+}$ precursor ion and increased the abundance of these four ions without generating any other fragments. The SORI-CAD MS/MS spectrum of the $[M+2H]^{2+}$ ion, Fig. 3B, yields 17 fragments, mostly b/y type cleavages and some small molecule losses, cleaving 11 out of 13 possible bonds. The fragment at *m/z* 835.94 could possibly be an $x_{13}{}^{2+}$ ion, but was found to be 1 Da higher than the theoretically predicted mass, possibly due to deamidation. The doubly charged ion at *m/z* 827.95 has not been identified and is possibly the result of an internal cleavage, as was observed with the ion at *m/z* 707.62, which is identified as the internal fragment PFHLVI. The b_6 and y_8 ions are a complementary ion pair, and demonstrate full sequence coverage. Clearly, the $[M+2H]^{2+}$ ion is much more useful for MS/MS than the singly charged ion. While this fact is well known, it does show the utility of generating $[M+2H]^{2+}$ ions on an MALDI-FTMS instrument.

For comparison, the SORI-CAD MS/MS spectra of the electrospray ions of the same renin substrate were also generated (Fig. 3C and D). The SORI-CAD excitation parameters were identical to the parameters applied to the HP MALDI generated ions. The SORI-CAD tandem mass spectrum of the isolated $[M+H]^{+}$ ion of renin substrate is shown in Fig. 3C. The base peak in the spectrum is the dehydrated molecular ion and a second loss of a water molecule is observed in the spectrum. The next most abundant ion is the $y_{13}{}^{+}$ fragment ion that results from a cleavage C-terminal to the aspartic acid residue. An increase in the excitation energy resulted in enhanced water losses, as well as in an increased intensity of the $y_{13}{}^{+}$ ion. No further sequence information was obtained from this MS/MS spectrum. Similarly, Fig. 3D is the SORI-CAD spectrum of the isolated $[M+H]^{2+}$ ion of renin substrate. As observed above with the MALDI spectra, the $[M+2H]^{2+}$ ion generates many more fragments than the $[M+H]^{+}$ ion. Nine out of 13 total fragments were doubly charged species. All observed fragments are b- and y-type ions or ions corresponding to the loss of small molecules, such as H_2O, NH_3, or CO_2. dehydrated ions. As above, an unidentified peak in the spectrum at *m/z* 827.96, and the peak at *m/z* 707.60 resulted from internal fragmentation yielding PFHLVI. As observed above with the MALDI data, fragment ions of the $[M+2H]^{2+}$ ion result in cleavage of nearly all inter-residue bonds and provide much better sequence information compared to the singly charged MS/MS spectrum.

The comparison of the SORI-CAD spectra of $[M+2H]^{2+}$ versus $[M+H]^{+}$ ions generated from HP MALDI-FTMS and ESI-FTMS allows an interesting observation. While

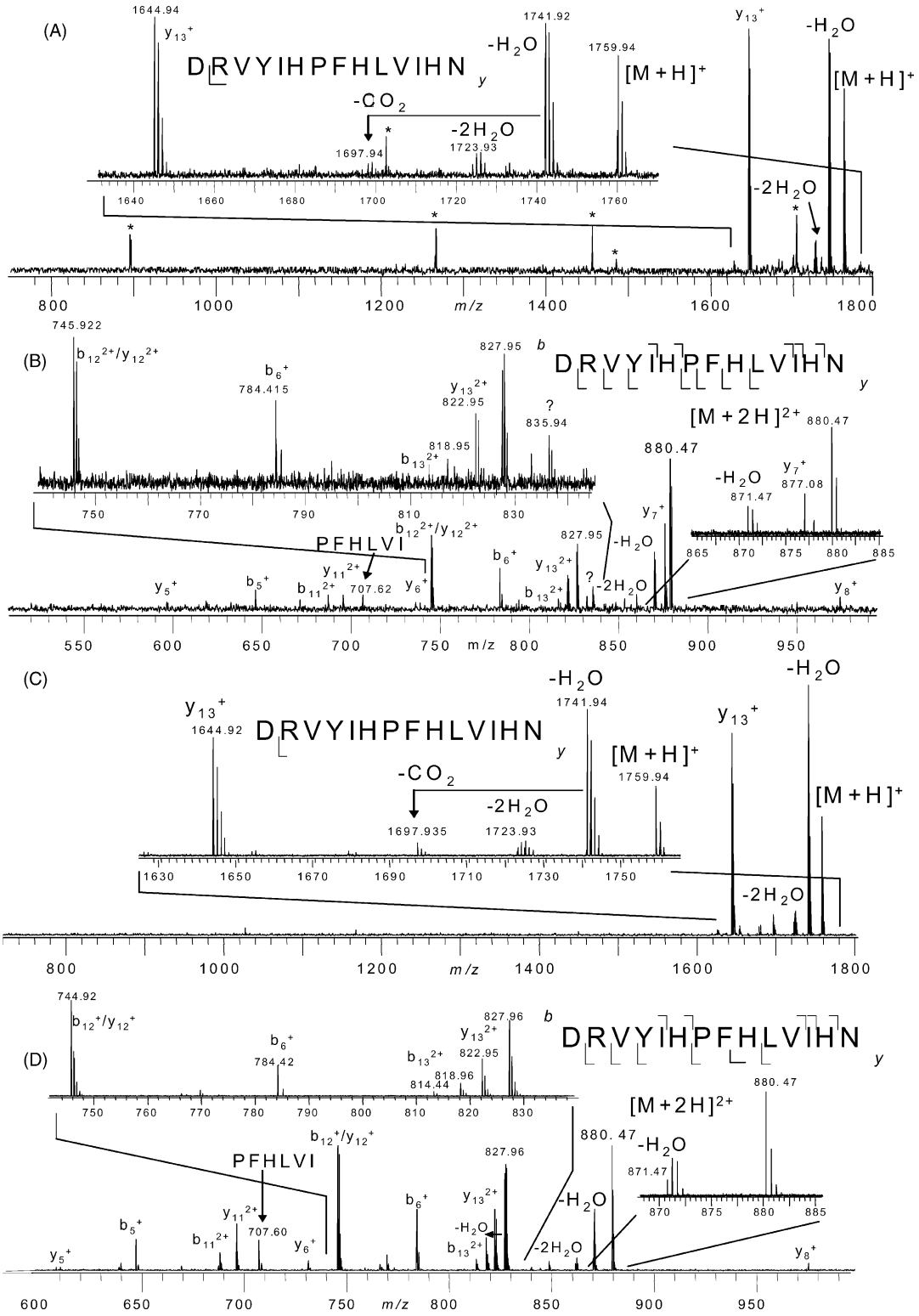


Fig. 3. (A) HP MALDI SORI-CAD of renin substrate $[M+H]^+$ ion. (B) HP MALDI SORI-CAD of renin substrate $[M+2H]^{2+}$ ion. (C) ESI SORI-CAD of renin substrate $[M+H]^+$ ion. (D) ESI SORI-CAD of renin substrate $[M+2H]^{2+}$ ion.

the abundance of the ions is different in the two cases, the cleavages observed are almost identical, implying that MALDI ions generated under pulsed collision gas at elevated pressures have similar internal vibrational energy to their ESI-FTMS counterparts. Thus, one implication of high pressure MALDI-FTMS data for proteomic studies is that these instruments provide the ability to produce vibrationally cool, multiply charged ions in a rapid, high

throughput MALDI source. Such ions are desirable for MS/MS studies.

However, "bottom up" proteomics also greatly benefits from the high mass accuracy of the FTMS. Fig. 4 demonstrates two such experiments using protein tryptic digests. Fig. 4A involves a clean, highly abundant protein, and Fig. 4B involves a protein where the concentration is reduced approximately threefold.

The protein Tv60 is a 53 kDa protein purified as a fraction of a cysteine protease preparation from *Trichomona vaginalis* and was previously characterized by peptide sequencing as an *S*-adenosyl homocysteine hydrolase [42,43]. Fig. 4A shows an HP MALDI-FTMS spectrum that represent the average of 20 scans of Tv60 tryptic digest peptides. The aqueous solution containing 100 fmol of the sample was applied to the MALDI target using the dried droplet technique. This spectrum is highly informative, containing more than 50 isotopic distributions with resolving power ranging from 45,000 to 75,000. A calculated tryptic digest of Tv60 yields 40 possible digest peptides (ignoring missed cleavages) with masses greater than *m*/*z* 450, which is the lowest mass in the recorded spectrum. In total, 18 peptides were identified from the database search [44] with accuracy better than 7.4 ppm (the calculated average error +5.1 ppm, and standard deviation ±1.4 ppm) representing more than 50% sequence coverage. With internal calibration, this accuracy improves to ∼1 ppm (average error 0.1 ppm, standard deviation 1.3 ppm). These peptides are noted in Table 1. The difference between the internal and external mass accuracies is due to the space charge frequency shifts.

Fig. 4B shows a spectrum of an immuno-γ-globulin tryptic digest. An aliquot containing 30 fmol of the sample was applied to the MALDI target by the standard dried droplet technique. The single scan spectrum contains about 30 isotopic peak distributions with resolving power raging from 38,000 to 65,000. Matching these peaks with a theoretical tryptic digest of the known protein sequence (ignoring missed cleavage sites) gives 30 possible digest peptides. The seven labeled peptides were identified with accuracy of 3–7 ppm externally calibrated (note that the ∼5 ppm systematic error is the space charge effect) is sufficient for a highly accurate identification [45–47]. Although the mascot search yielded a score of 28 for the expected variant, the four highest scoring proteins were all immunoglobulins from various species. The three highest peaks in the spectrum and many of the smaller peaks have not been identified and are likely due to a splice variant of the immunoglobulin. Even so, due

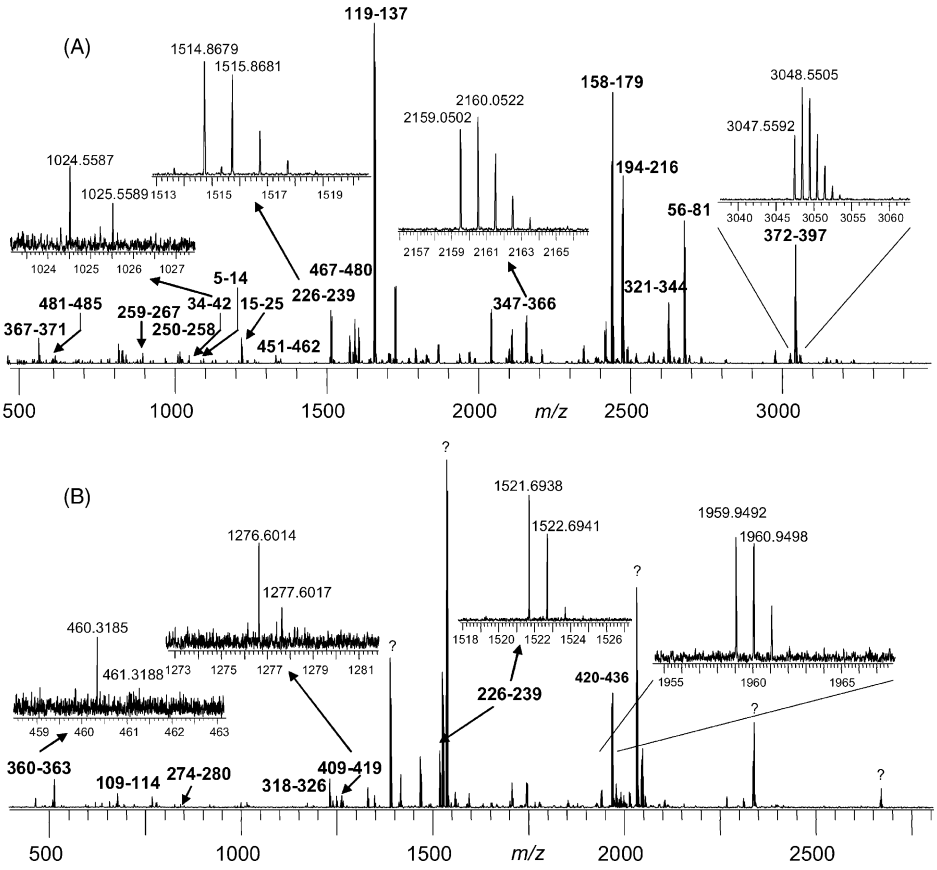
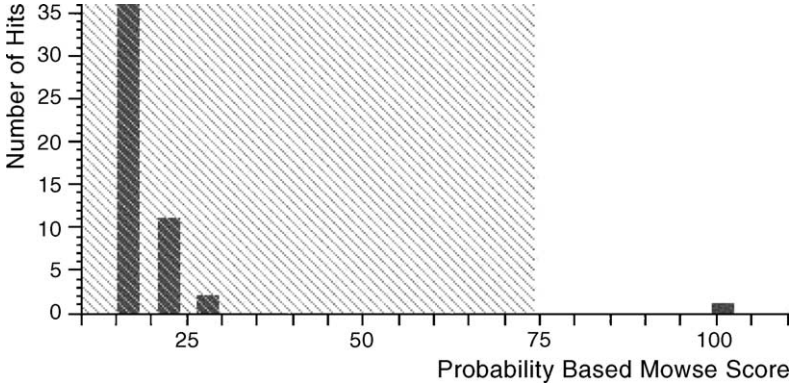


Fig. 4. (A) HP MALDI spectrum of Tv60 protein digest, average of 20 scans. 100 fmol was applied on the MALDI target. (B) HP MALDI spectrum of an immuno-γ-globulin protein digest, one scan. 30 fmol was applied onto the MALDI target.

Table 1
Tv60 protein digest, with all identified peptides ranging from *m/z* 450 to *m/z* 3000 from Fig. 3A

#	From	To	Theoretical MH^+	Measured MH^+	Externally calibrated error (ppm)	Internally calibrated error (ppm)	Digest peptide sequences
44	481	485	611.2784	611.2812	+4.5	−0.4	SDAYR
39	437	442	720.4291	720.4318	+3.8	−1.2	VYTLPK
24	259	267	877.4448	877.4501	+6.0	+1.1	ASDVMEGGK
23	250	258	1024.5534	1024.5587	+5.2	0.0*	HSLIDGINR
6	34	42	1045.5533	1045.5608	+7.2	+1.9	EMPGLMVLR
2	5	14	1094.5265	1094.5316	+4.7	−0.6	SPAGAPFEYR
25	268	279	1210.6136	1210.6179	+3.6	−1.2	TALVMGYGDVGK
3	15	25	1220.711	1220.7185	+6.1	+0.9	IADINLHVLGR
20	226	239	1514.8577	1514.8679	+6.7	+1.4	LLFPAINVNDAVTK
43	467	480	1592.7955	1592.8028	+4.6	−0.3	QADYINVPVEGPYK
12	119	131	1657.7493	1657.7605	+6.8	+1.5	GETLPEYWENTYR
33	347	366	2159.0437	2159.0502	+3.1	−1.9	AIVGNIGHFDNEIDTDGLMK
14	158	179	2442.1096	2442.1153	+3.3	−1.6	GFEFETAGAVPEPTEADNLEYR
17	194	216	2491.1531	2491.1632	+4.1	−0.7	NHWHTVAAGMNGVSEETTTGVHR
13	132	157	2681.3417	2681.3604	+6.7	+1.4	ALTWPDGQGPQQVVDDGGDATLLISK
10	56	81	2696.4651	2696.4739	+3.4	−1.6	ISGSLHMTVQTAVLIETLTALGADVR
35	372	397	3047.5448	3047.5592	+4.7	0.0*	HIPIKPEYDMWEFPDGHAILLLAEGR
				Average error	5.1	0.1	
				Standard deviation	1.4	1.3	



to high mass accuracy, identification of the protein was unambiguous (Table 2).

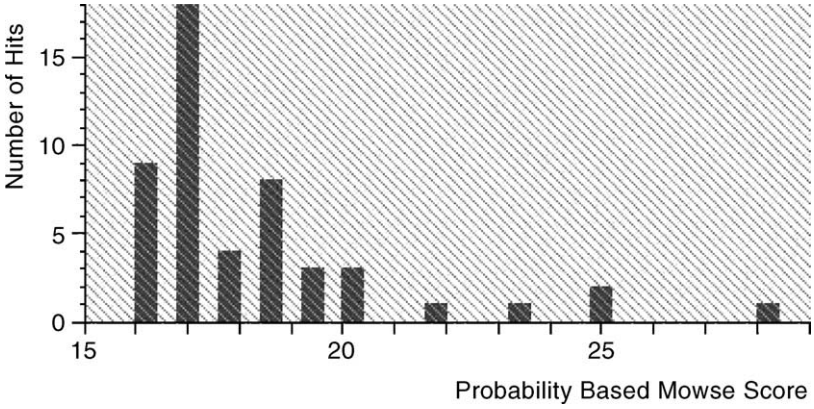
The data shown have interesting implications for the use of HP MALDI-FTMS for proteomics research. One implication is that, until the space charge effect can be either eliminated or accurately calibrated, it will be necessary to perform internal calibration (e.g., using InCAS as performed either by O'Connor and Costello [25] or Brock et al. [26]) to routinely achieve the ∼1 ppm mass accuracy that is characteristic of FTMS. Alternatively, it may be possible to improve the calibration equation to accurately account for space charge [48], increase the space charge limit by increasing the magnetic field [49], or to improve the detection limits of the instrument sufficiently that the effect of space charge is no longer the limiting factor in calibration [50]. These last two approaches are one of the primary goals of the Cryogenic FTMS development.[51] The higher magnetic field, lower base pressure, and improved sensitivity of the Cryogenic FTMS should decrease the space charge frequency shifts to the point where <1 ppm can be achieved routinely with external calibration, and perhaps <0.1 ppm can be achieved routinely with internal calibration.

Another implication of these (and other [2]) spectra are that chemical noise is a substantial problem for HP MALDI-FTMS data. This chemical noise has been described and evaluated by Krutchinsky and Chaite [52] and consists primarily of non-covalently bound matrix clusters and matrix-analyte complexes. Thus, methods to reduce or eliminate this chemical noise are needed in order to improve the detection limits.

If the spectra are internally calibrated, the most obvious implication of these spectra is that the automated generation of high throughput enzymatic digest maps of proteins with ∼1 ppm mass accuracy will greatly simplify protein identification. Even to the point of being able to identify a protein from a single, highly accurate peptide mass, as is now done routinely with shotgun proteomics methods by electrospray ionization but with high confidence on the results assignments. Brock et al. [26] recently published a demonstration of this type of high throughput analysis with MALDI.

Table 2
Immuno-γ-globulin digest, with all identified peptides from *m/z* 450 to *m/z* 3500 from Fig. 4B

#	From	To	Theoretical MH^+	Measured MH^+	Externally calibrated error (ppm)	Digest peptide sequences
24	360	363	460.3130	460.3185	+11.9	IISK
8	109	114	692.3210	692.3272	+8.9	TEDTAR
23	352	359	882.5043	882.5109	+7.4	DLPAPITR
19	318	326	1173.5171	1173.5248	+6.5	EEQFNSTYR
30	409	419	1276.5917	1276.6014	+7.5	NGQPELEGNYR
2	23	38	1525.8333	1525.8417	+5.5	LVESGGGLVQPGGSLR
31	420	436	1959.9334	1959.9492	+8.0	TTPPQQDVDGTYFLYSK
				Average error	+7.9	
				Standard deviation	2.0	



4. Conclusions and outlook

The production, transfer, and isolation of multiply charged species by HP MALDI-FTMS has been demonstrated. Collisional cooling was found to be highly important in the formation of $[M + 2H]^{2+}$ ions as these species tend to be more labile than their singly charged counterparts and thus are more easily lost to metastable fragmentation. This collisional cooling is also of particular importance in the analysis of labile post-translational modifications. As expected, the SORI-CAD fragmentation patterns of singly versus doubly charged ions are different from one another, but ions of the same charge state, whether generated by ESI or HP MALDI, produced strikingly similar fragmentation spectra. MS/MS of multiply charged ions generates many more fragments than MS/MS of singly charged ions and thus will have strong utility in proteomic applications.

The current HP MALDI system showed good fundamental performance for initial experiments in peptide mass fingerprinting. Desorption of ions at elevated pressure produces vibrationally cool molecules allowing for the analysis of peptides with labile post-translational modifications. The results presented demonstrate that the mid-femtomole range of sensitivity for protein digests is routinely available in the current instrument. Mass accuracy (with internal calibration) is in the ∼1 ppm range for peptide mass fingerprinting experiments. Methods to eliminate or reduce chemical noise, improved calibration equations that handle the space charge effect more accurately, and improved detection limits will all greatly improve the mass accuracy of the MALDI-FTMS experiment.

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