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Matrix-assisted laser desorption/ionization—boundary-activated dissociation of peptide ions in a quadrupole ion trap

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Abstract

The nonresonant excitation technique of boundary-activated dissociation (BAD) has been used to obtain tandem mass (MS/MS) spectra for peptide ions generated by matrix-assisted laser desorption/ionization (MALDI) in a quadrupole ion trap. BAD MS/MS spectra for proctolin, des-Arg^9-bradykinin, and substance P are qualitatively similar to those for which resonant excitation has been used and can be obtained with the same activation time. The conditions for optimal product ion formation are easily established when BAD is used because of its dependence upon a single activation parameter. Consequently, MS/MS spectra of MALDI-generated ions are easier to obtain than when single-frequency resonant excitation is used. These advantages, in conjunction with the simpler electronic equipment required for the implementation of BAD, provide an alternative to broadband excitation when MS/MS data for MALDI-generated ions are desired. (Int J Mass Spectrom 222 (2003) 75–83)

Keywords: Quadrupole ion trap; MS/MS; MALDI; Boundary activation; Peptides

1. Introduction

The analysis of large molecules, both natural and manmade, by mass spectrometry in recent years has been advanced significantly by the development of electrospray (ESI) [1–3] and matrix-assisted laser desorption ionization (MALDI) [4–7]. Of the two, MALDI is significantly less likely to produce multiply charged ion species [4–6], making the interpretation of the mass spectra of polydisperse polymers, biological digests, and other complex mixtures more facile than when ESI is used. Additionally, MALDI is more tolerant of buffers and contaminants than ESI [8,9], making analysis of solid tissue samples possible with relatively little sample cleanup [10].

The technique of tandem mass spectrometry [11] (MS/MS) augments mass spectrometric experiments by adding structural information to the molecular weight information through the dissociation of a selected parent ion species. For example, MS/MS is capable of providing the information necessary to elucidate the sequence of amino acids in a peptide.

Among ion trapping instruments, the quadrupole ion trap has assumed a prominent role in MS/MS experiments because of its low cost and small space requirements relative to other mass spectrometers. By far the most common MS/MS experiment using a quadrupole ion trap involves collision-induced dissociation (CID). Because CID in the ion trap typically

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is achieved by resonant excitation of the parent ion species of interest, a potential problem exists if the expected resonant frequency varies over the course of an experiment. One factor affecting the secular frequencies of the trapped ions is the total number of ions [12–16]. Commercial quadrupole ion trap mass spectrometers address this issue by attempting to control the number of ions injected into the ion trap. This is not too difficult with continuous ionization sources such as ESI, but is problematic with pulsed ionization sources such as MALDI. The wide variations in the number of ions generated per laser shot in a MALDI experiment can make determination of the optimal frequency to apply difficult [17,18]. This variation in the number of ions produced can arise from shot-to-shot variations in laser intensity [19], heterogeneity of the crystalline sample across the surface of the probe tip [17,18,20], and depletion of the sample over the course of the analysis [21]. Determining the optimal resonant excitation voltage parameters, frequency and amplitude, can be a complicated task. If the amplitude of the applied voltage is too large, ejection of the parent ion species prior to dissociation can occur, while too low an amplitude results in poor MS/MS efficiency and limited product ion abundances. With increased interest in combining MALDI sources with quadrupole ion trap mass spectrometers, alternatives to the conventional resonant excitation method for implementing CID are important [22–24].

Several methods have been employed to overcome the obstacle of uncertain secular frequencies in a quadrupole ion trap with MALDI-generated ion species. The first approach involved using elevated static buffer gas pressures of either helium or argon [19]. However, degradation of some spectra in terms of resolution and sensitivity was noted. Pulsing a heavy gas into the quadrupole ion trap only during the CID step avoids the problems of spectral degradation [25,26] and broadens the acceptable range of frequencies which provide maximum MS/MS efficiency [26]; however, it adds significantly to the total time to obtain a spectrum. Stored waveform inverse Fourier transform (SWIFT) broadband excitation [27,28] has also been used to excite multiple frequencies simultaneously, avoiding some of the problems of indeterminate secular frequencies associated with MALDI-MS/MS when using single-frequency resonant excitation [29]. An alternative approach, which has been termed red-shifted off-resonance large-amplitude excitation (RSORLAE), does not involve resonant excitation and has been shown to deposit greater amounts of energy into a parent ion species than conventional resonant excitation by minimizing the probability of ion ejection [30]. However, the excitation event using this approach may require a duration of up to 1 s for optimal product ion generation during a single stage of MS/MS, a significant length of time in quadrupole ion trap MS/MS experiments. Common to all of these approaches is the specification of the expected secular frequency or range of secular frequencies for the parent ion species of interest.

The technique of boundary-activated dissociation (BAD) [31–33] provides a means of dissociating a parent ion independent of its secular frequency. In this approach, a DC voltage is applied to the ring electrode (or endcaps) to bring the working point (q_z, a_z) of the desired parent ion species to a boundary (usually $\beta_z = 0$) of the stability diagram defined by the solutions to the Mathieu equation. In the presence of a DC potential applied to the ring electrode, ions acquire translational kinetic energy from the RF trapping field as their excursions from the center of the trap increase. Collisions with buffer gas molecules present in the trap promote dissociation of the parent ion species to product ions. Thus, BAD is an alternative method to achieve CID in a quadrupole ion trap. Removal of the DC voltage after dissociation of the parent ion moves the working points of the product ions to the $a_z = 0$ axis for acquisition of the mass spectrum by resonant ejection. It should be noted that multiply charged ions are not amenable to analysis by BAD because the product ions may have greater *m/z* values than the parent ion species. In such cases the product ions would lie outside the boundaries of the stability diagram and would not be trapped for subsequent mass analysis [34].

In this study, BAD in a quadrupole ion trap has been used to analyze MALDI-generated peptide ions.

Compared to previous studies using BAD, the *m/z* values explored in this study are significantly larger. MALDI-MS/MS spectra obtained using BAD are very similar to those obtained using resonant excitation, and therefore, no loss of spectral information is suffered when BAD is used. Because boundary activation occurs in approximately the same amount of time as resonant excitation, no disadvantage in analysis time accrues. Optimization of the conditions for collisional activation is easier with BAD than with resonant excitation because only one parameter, the applied DC voltage, must be specified. Resonant excitation, by contrast, requires that both an appropriate frequency and optimal amplitude for the voltage must be determined. Furthermore, the electronics required for the implementation of BAD are considerably less complicated than those for broadband excitation, making BAD a cost-effective option for quadrupole ion trap instruments.

2. Experimental

2.1. Instrumental

All experiments were performed on a Finnigan-MAT ITMS quadrupole ion trap, extensively modified for laser desorption within the trapping volume as described previously [21]. The helium buffer gas pressure was elevated to between 3.4 and 3.9 mTorr to facilitate trapping of the desorbed ions. The conductance limiting Teflon inserts have been removed so that this is the He pressure throughout the vacuum system. The DC voltage required for BAD was provided by the ion trap electronics module and was controllable through the data system software. SWIFT waveforms [27], generated by software developed in-house using LabVIEW (National Instruments, Austin, TX), were used to isolate the desired parent ion species. (Note that while this specific instrument has a waveform generator, other methods of parent ion isolation are possible. So, a waveform generator is not necessary for the experiments described.) The waveforms were transferred to an arbitrary waveform generator (Sony/Tektronix, Beaverton, Oregon; model AWG2020, upgraded through firmware to partial AWG2021 functionality) across a GPIB interface. Triggers for the AWG were provided by the ITMS electronics module. The waveforms passed through an amplifier with a gain of approximately 5.7 and were summed with the single-frequency voltage from the ITMS electronics module used for resonant excitation. The waveforms were applied to the endcap electrodes in a dipolar manner through the use of a balun provided with the ITMS.

A nitrogen laser (Laser Sciences, Inc., Newton, Massachusetts; model VSL-337-NDS, $\lambda = 337$ nm) was used for desorption. A high-power UV beam attenuator (Newport Corporation, Irvine, California; model 935-10) was used to control the intensity of the beam irradiating the sample, as necessary. The beam was focused with a quartz lens of 1 m focal length into the vacuum chamber through a quartz window and then passed through a hole of 3.2 mm diameter drilled radially in the ring electrode onto a quartz probe tip positioned at the opposite surface of the ring electrode through a similar hole [21].

For a typical experiment the RF trapping voltage was first set to trap ions with $m/z \geq 100$ Da. The laser was then triggered by the ion trap electronics. Following the laser pulse, the trapping voltage was then increased to eject ions of $m/z < 600$ Da from the trap and then returned to the 100 Da trapping limit. Ions from five laser shots were accumulated in the trap in this manner at a triggering rate of approximately 29 Hz during this segment of the scan sequence, allowing 35 ms between successive laser shots to trap the ions of interest. A SWIFT waveform was then applied three times to isolate the isotopic cluster of the desired parent ion. The trapping voltage was then adjusted to set the q_z value of the parent ion of interest to the desired value for dissociation. Ion activation was then effected, either by boundary activation or by resonant excitation, typically for 50 ms, followed by spectrum acquisition using resonant ejection. All data shown are averaged from 100 or 250 spectra, representing 500 or 1250 laser shots.

2.2. Samples

The matrix used in these studies was 2,5-dihydroxybenzoic acid (DHB; Aldrich, Milwaukee, Wisconsin), dissolved in a 70:30 (v/v) mixture of acetonitrile:water with 0.1% trifluoroacetic acid. The analytes proctolin (RYLPT, MW 648 Da), des-Arg^9-bradykinin (RPPGFSPF, MW 903 Da), and substance P (RPKPQQFFGLM-NH_2, MW 1347 Da) were obtained from Sigma (St. Louis, Missouri). All chemicals were used as obtained without further purification. Samples were dissolved in HPLC grade water and diluted to a concentration of 100 μM. Because the goal of these experiments was to compare ion activation methods, large sample sizes were used to ensure the ability to generate ions for a large number of laser pulses. The typical peptide loading for these experiments was 40 pmol. Matrix:analyte ratios were between 5450:1 and 6975:1.

3. Results and discussion

3.1. Comparison to resonant excitation

The MS/MS spectrum of proctolin acquired using BAD at $q_z = 0.419$, $a_z = -0.099$ is shown in Fig. 1a. This working point was chosen based on experiments below and the desire to cover a similar mass range as the resonant excitation CID experiment. BAD was implemented in this instance by applying a DC voltage of 397.7 V to the ring electrode for a period of 50 ms. Considering the diversity of product ion types in the spectrum of proctolin acquired by resonant excitation in Fig. 1b, the spectrum acquired using BAD is remarkably similar. The similarity between the spectra suggests that the collisional activation process using BAD is comparable to that of the resonant excitation in terms of the rate of activation and the amount of internal energy acquired by the ion. Prior experiments

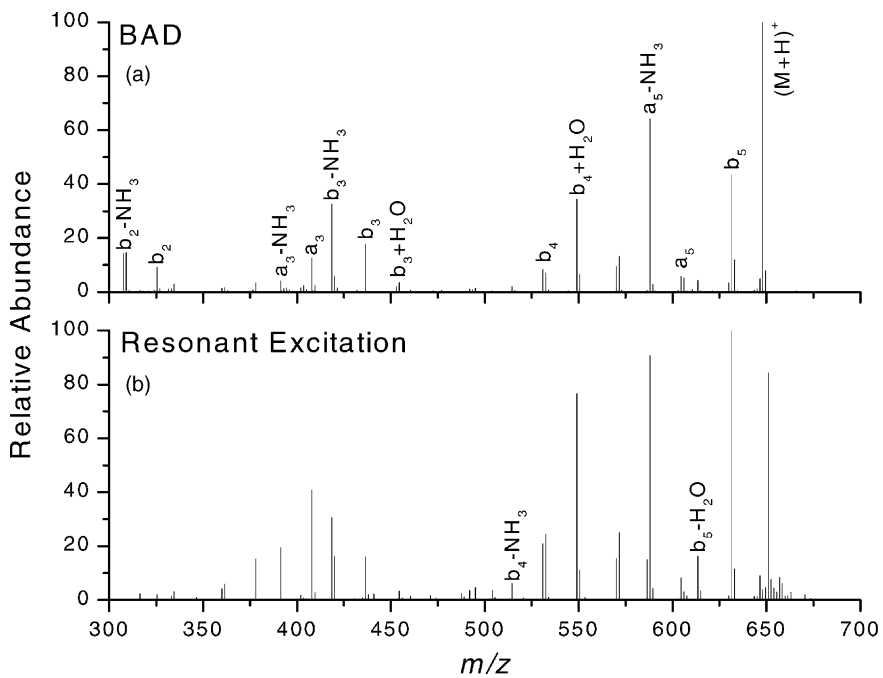

Fig. 1. Comparison of MS/MS spectra of proctolin acquired using BAD at $a_z = -0.099$ (397.7 V DC) (a) and with resonant excitation at an applied frequency of 172.2 kHz at 0.8 V (b). The large $(M + 1 + H)^+$ peak is due to the selection of the entire isotopic envelope by the SWIFT waveform, some of which remains after the selective dissociation of only the $(M + H)^+$ peak by single-frequency resonant excitation. Activation for both spectra was for 50 ms duration at $q_z = 0.419$.

using BAD on small organic molecules have been contradictory, suggesting in one case that the energy deposited can be higher [35], and in another lower [33], than that deposited during resonant excitation.

However, there are some subtle differences between the two spectra: *b* ions are more intense than *a* ions at higher *m/z* values in the spectrum acquired with resonant excitation, but the trend is reversed at lower *m/z* values. Note, for instance, the greater relative abundances of the b_5, b_5-H_2O, $b_4 + H_2O$, and b_4 ions to the a_5 and a_5-NH_3 ions in the spectrum acquired using resonant excitation when contrasted to the spectrum acquired using BAD. At lower *m/z* (below about 450 Da), however, *b* ions are more prominent than *a* ions in the spectrum acquired using BAD, as the greater relative intensities of the b_3 and b_3-NH_3 compared to the a_3 and a_3-NH_3 ions show. In fact, b_2 and b_2-NH_3 ions appear only in the BAD spectrum. Despite these slight differences, the spectrum acquired using BAD matches well with the spectrum acquired using resonant excitation and demonstrates that BAD can generate structural information comparable to that obtained by resonant excitation.

3.2. Effect of activation time

Prior studies of BAD have used activation times ranging from 30 to 100 ms. To determine the optimal length of time for the activation event, a series of experiments was performed in which the abundance of the parent ion was monitored as a function of the duration of the activation event. The results of these experiments are shown in Fig. 2 for proctolin, obtained with a DC voltage of 397.7 V ($q_z = 0.419$, $a_z = -0.099$), the point at which maximum product ion abundances were observed in the BAD experiment described above. The abundance of the parent ions decreased rapidly as the activation period was lengthened, with complete loss of parent ion occurring in the 30–50 ms range. This time is very similar to the

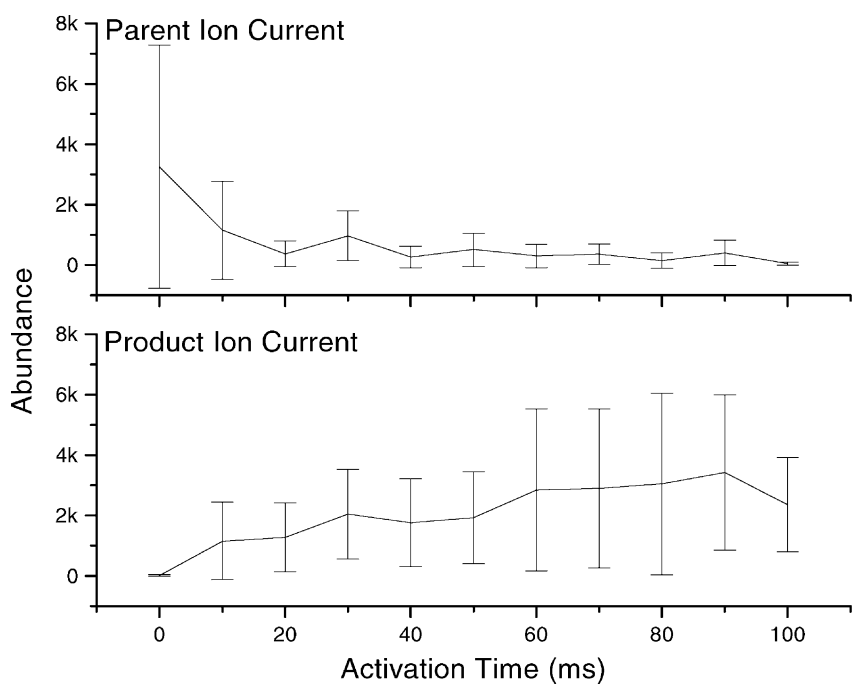

Fig. 2. Variation in parent and total product ion absolute abundances for proctolin at $(q_z, a_z) = (0.419, -0.099)$ (397.7 V DC) as the activation time is increased. The error bars are an indication of the shot-to-shot variation of the number of ions formed by MALDI.

20–40 ms typically allocated to ion activation in resonant excitation experiments. Thus, ion activation in BAD does not cause any temporal disadvantage, although slight increases in product ion intensity were observed at longer activation times. It should be emphasized that the large error bars in Fig. 2 are a result of the shot-to-shot variation in the number of ions generated by MALDI.

3.3. Sensitivity to applied voltage

The a_z values at which total loss of ion signal occurred lie very close to the values associated with maximum dissociation efficiency, suggesting that very fine control over the DC voltage is necessary to obtain acceptable quality MS/MS spectra. For this reason, the product ion abundances were monitored as a function of the applied DC voltage to determine the effective voltage range over which dissociation was observed. Fig. 3 shows that when incrementing the DC voltage 1 V at a time, there is a relatively wide range of DC voltages acceptable for dissociation. Determination of the optimal DC voltage is simplified by the fact that total loss of all ion signal places an upper limit on the magnitude of the DC voltage that may be used. In contrast, when resonant excitation is employed, both an optimal frequency and an optimal amplitude for the applied voltage must be determined. Because an ion will absorb energy from an applied voltage at or near its secular frequency, a range of frequencies will exist over which dissociation or ejection is observed. This range will be small when a low-amplitude voltage typical for an optimized resonant excitation experiment (typically <0.6 V) is applied. If the secular frequency of the parent ion species changes from spectrum to spectrum due to variations in the number of ions trapped from each desorption event, a low-amplitude voltage can result in inefficient dissociation because the amplitude may be too low for the effective secular frequency of the parent ion species for a particular scan. Larger amplitudes may be used to increase the probability of effective dissociation, but the probability of ejection of the parent ion species increases also unless the frequency of the applied waveform is adjusted. Consequently, in a situation where the parent ion intensity fluctuates significantly (as is the case with MALDI), establishing suitable dissociation conditions is much easier with BAD than with

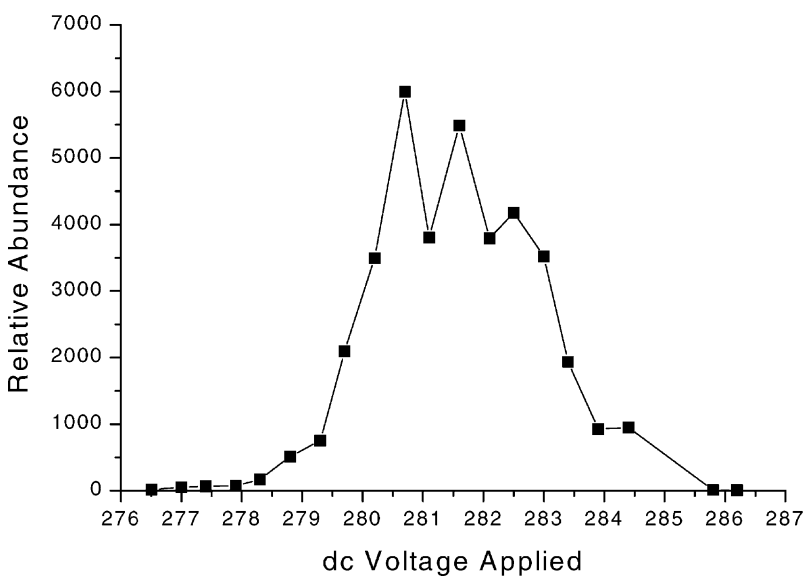

Fig. 3. Total product ion abundance for proctolin at $q_z = 0.350$ and 50 ms activation time as the DC voltage applied to the ring is varied.

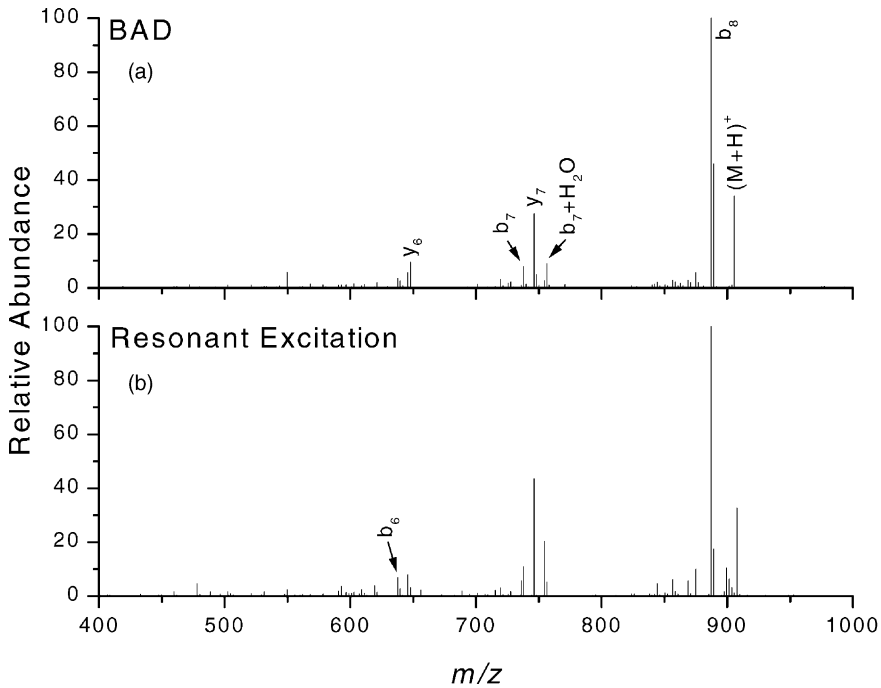

Fig. 4. Comparison of MS/MS spectra of des-Arg^9-bradykinin acquired using BAD at $a_z = -0.051$ (287.6 V DC) (a) and with resonant excitation at an applied frequency of 120.2 at 0.8 V (b). Activation for both spectra was for 50 ms duration at $q_z = 0.301$.

single-frequency resonant excitation because a single parameter with an easily observed upper limit is used.

3.4. Larger peptides

Comparisons of boundary activation to resonant excitation are shown for des-Arg^9-bradykinin and substance P in Figs. 4 and 5, respectively. The dissociation pathways for these peptides result in few product ions of significant intensity, with either activation method, but the same product ions are observed for both when boundary activation is used as when resonant excitation is used. Contrary to previous experiments [34], it was found that des-Arg^9-bradykinin can be dissociated via BAD in the presence of a helium-only buffer gas. It is possible that the ions had different conformations because different ionization techniques were used and thus required different amounts of internal energy to dissociate [36]. Additionally, substance P, which has not been analyzed using ESI–BAD because of its propensity for double charging, was also easily dissociated in a helium-only environment via BAD.

In some cases, additional steps will be required to obtain adequate structural information, as the small number of structurally informative product ion peaks in Figs. 4 and 5 suggests. For example, a product ion formed during the initial dissociation step could be selected as a parent ion for a subsequent stage of MS/MS using BAD. In the case of the peptides dissociated in Figs. 4 and 5, the $(M + \mathrm{H–H_2O})^+$ peaks (b_8 and b_{11}, respectively) would be the most likely candidates for this approach. Instead of using two discrete BAD segments in a single scan, MS^3 may also be effected by varying the RF and DC voltages simultaneously or just scanning the amplitude of the RF voltage to build up a population of more structurally informative product ions than those shown in Figs. 4 and 5 [37]. If MS^3 is not possible because of low MS/MS efficiency, the use of heavy buffer gases should induce a greater diversity of product ion types and therefore

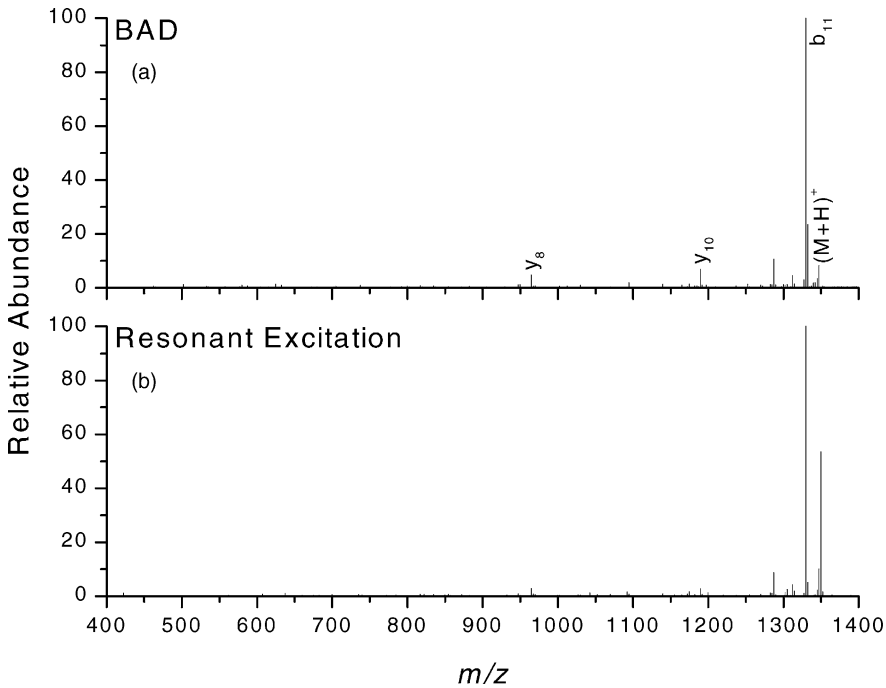

Fig. 5. Comparison of MS/MS spectra of substance P acquired using BAD at $a_z = -0.052$ (431.9 V DC) (a) and with resonant excitation at an applied frequency of 120.9 kHz at 0.8 V (b). Activation for both spectra was for 50 ms duration at $q_z = 0.303$.

provide more spectral information in a single activation event [19,25,26,34]. Although the variation in the MALDI signal prevented an accurate assessment of MS/MS efficiency, MS/MS efficiency is expected to increase as the q_z value for the parent ion species increases [32,34].

4. Conclusion

BAD is a viable alternative to resonant excitation for MALDI-generated ions, producing the same types of ions in approximately the same abundances for the peptide ions analyzed in these experiments. Therefore, spectra obtained using either dissociation method are comparable. The single parameter of DC voltage magnitude used for BAD is easily established from the stability diagram, in contrast to the two parameters of ac voltage amplitude and frequency for resonant excitation that must be determined empirically if the number of trapped ions cannot be controlled. Furthermore, the timescale of the boundary activation event is comparable to that of resonant excitation, so that no disadvantage in analysis time accrues when BAD is used. Finally, the equipment required to implement BAD, a DC power supply, is less expensive and easier to incorporate into an existing instrument than the arbitrary waveform generators typically used to provide single frequency or broadband voltages for resonant excitation.

Acknowledgements

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