RCM

Resonance excitation and dynamic collision-induced dissociation in quadrupole ion traps using higher-order excitation frequencies

Ünige A. Laskay and Glen P. Jackson*

Center for Intelligent Chemical Instrumentation, Department of Chemistry and Biochemistry, Ohio University, Athens, OH 45701-2979, USA

Fragmentation of the pentapeptide leucine enkephalin (YGGFL) is accomplished via higher-order resonances combined with simultaneous analysis of low-mass product ions. Two methods of achieving excitation are explored: (1) 0.5 ms resonant excitation at the ω and at Ω-ω secular frequencies of ion motion (where Ω is the radio-frequency (rf) drive frequency) in a manner similar to both pulsed q collision-induced dissociation (PQD) and high amplitude short time excitation (HASTE), and (2) 0.5 ms pulse of the ω or at Ω-ω excitation frequencies when the secular frequency of the ions is quickly swept across resonance conditions (pulsed q dynamic CID, PqDCID). In both methods of excitation, the rf amplitude on the ring electrode is rapidly decreased after excitation, therefore enabling analysis of low-mass product ions. Maximum fragmentation efficiencies of ~20% can be obtained with pulsed CID with both regular and high-order frequency excitation, while pulsed DCID offers maximum efficiencies of ~12%. All the excitation methods studied offer increased internal energy depositions when compared to conventional CID, as measured by the a_4/b_4 product ion ratios of leucine enkephalin. These ratios were as high as 13:1 for pulsed CID and 8:1 for PqDCID. Successful mass analysis of the low-mass ions is observed with both pulsed CID and PqDCID. The combined benefit of high internal energy deposition and wider dynamic mass range offers the possibility of increased sequence coverage and the identification of unique internal fragments or high-energy product ions which may provide complementary information to biological applications of conventional CID. This is the first report on deliberate fragmentation of precursor ions at a higher-order component of the ion secular frequency combined with a successful mass analysis of the low-mass ions through pulsed CID and PqDCID. Copyright © 2008 John Wiley & Sons, Ltd.

The employment of the quadrupole ion trap (QIT) as a platform for tandem mass spectrometry (MS/MS) arises from the great flexibility and sensitivity of this instrument in analysis of volatile organic compounds. Through the combination of ion traps with atmospheric ionization sources such as electrospray ionization (ESI), fragmentation of biological molecules can be tackled in both the top-down[1] and bottom-up[2] approaches. Increasing the size of precursor ions of interest, such as proteins, lipids or polysaccharides, increases the number of degrees of freedom such that probing the fragmentation of such large species is problematic. Due to the low internal energy depositions in the high collision-frequency environment of ion traps, tandem mass spectra in ion traps tend to lack fragments arising from pathways with high activation barriers. While this is often not a problem, per se, fundamental chemistry and applications such as *de novo* peptide sequencing could benefit from the ability to probe higher energy pathways.

Correspondence to: G. P. Jackson, Department of Chemistry and Biochemistry, Ohio University, 136 Clippinger Laboratories, Athens, OH 45701-2979, USA.
E-mail: jacksong@ohio.edu

Increasing the collection efficiency and collision energy is a problem well known to researchers developing QITs, and one can find extensive efforts towards optimization of all stages of the mass analysis. For example, an improved injection and more efficient isolation may increase the number of ions successfully mass-analyzed;[3] investigating different scanning parameters such as scanning rate,[4,5] scan direction[6,7] or ejection q_z leads to a better sensitivity and, to a certain degree, mass resolution may be enhanced.[8] As for the aspect of fragmentation, increasing the number and energy of collisions utilizing different bath gases with variable pressures have been successfully accomplished.[9,10] A more selective fragmentation of precursor ions can be achieved by application of customized waveforms to achieve on-resonance conditions for the ions of interest.[11,12]

Ions trapped in the quadrupolar field of the ion trap have relatively stable trajectories and modest kinetic energies. Perturbation of the motion of the ions using supplementary alternating current (ac) waveforms leads to a modification of their kinetic energies and may lead to their fragmentation via collisions with the bath gas, or to ion ejection, if their excursion path exceeds the physical dimensions of the trap or if their trajectories become unstable. Mathematically, the

stability of an ion with mass m and charge e may be approximated by solving the Mathieu equation.[13,14] Accordingly, when a radio-frequency (rf) field of Ω angular frequency and $V_{0\text{-}p}$ amplitude is applied to the ring electrode, the stability parameter in the axial direction z dimension is:

$$q_z = \frac{8eV}{m(r_0^2 + 2z_0^2)\Omega^2} \qquad (1)$$

where r_0 is the radius of the ring electrode and $2z_0$ is the distance between the two end-cap electrodes. The $\omega_{n,z}$ frequency of motion of the ion trapped in the quadrupolar field for $q_z < 0.4$ is given by the following approximation:

$$\omega_{n,z} = (n + \frac{q_z}{2\sqrt{2}})\Omega \qquad (2)$$

Here, n is the order of the frequency and assumes values of $0, \pm 1, \pm 2, \pm 3$, etc. For values of q_z above 0.4 the solution to the Mathieu equation is a continued fraction expression and the formula of the frequency becomes a more complex mathematical equation.[15] When $n = 0$, ω is referred to as fundamental secular frequency[16] and this is the frequency for which axial modulation is almost exclusively achieved. For values $n \neq 0$, the axial motion has a higher-order frequency component with a lower absorptivity; in this case a larger excitation amplitude is required to affect ion motion.[17] Although these higher-order resonances have been considered undesirable side effects of the imperfections in the quadrupolar field, according to thorough simulation studies conducted by Franzen in the early 1990s on higher-order multipole resonances, these overtones can also be used for axial modulation.[18]

Commercial instruments usually achieve collision-induced dissociation (CID) by exciting precursor ions with an excitation waveform that is resonant with the fundamental secular frequency, typically between 100–300 kHz. However, higher-order resonances can be employed for resonant ejection and excitation, as several research groups have previously reported. For example, higher-order and non-linear resonances have been found to have major effects on the trapping field,[19–21] and considerable work has been accomplished to mathematically predict the presence of these resonances.[16,22,23] Splendore et al. investigated the effect of higher-order resonances to improve the mass selective ejection,[24] and Creaser and Stygall used the Ω-ω overtone frequency to achieve mass range extension, as well as to achieve resonance excitation of selected precursor ions.[25] To our knowledge, the paper by Creaser and Stygall is the only report in the literature for the deliberate fragmentation of precursor ions using higher-order resonances.

One of the major disadvantages of the QIT in MS/MS applications is the appearance of the low-mass cutoff (LMCO), which severely limits the amount of obtainable information from the mass spectrum. This limitation is severe for large precursor ions such as bimolecules, where small m/z product ions (immonium ions, internal fragmentation products, N- or C- termini fragments) would greatly enhance the sequence coverage capabilities.[26,27] It has been previously shown that fast excitation followed by a rapid decrease of the rf amplitude on the ring electrode permits low-mass product ions to be successfully trapped[27,28] and

Thermo Scientific now includes this capability through the 'Pulsed Q' feature on their line of linear ion traps (LXQ, LTQ XL, hybrid LTQ).

Excitation of ions via CID in ion traps is achieved by application of an ac or direct current (dc) waveform to one or both end-cap electrodes to achieve resonance with the secular frequency of the ion. Predicaments such as non-ideal trapping field or space charge effects can cause a change in the secular frequency of the precursor ion; therefore, the theoretical resonance frequency does not always offer true resonance conditions.[29] This, in turn, may lead to insufficient fragmentation efficiencies and/or decreased internal energy depositions. Instrument manufacturers overcome this shortcoming by applying a broad-band excitation waveform centered at the theoretical secular frequency,[11] or by gradually sweeping the rf potential on the ring electrode up and down during resonance excitation in an approach called secular frequency modulation (SFM).[30] Pulsed Q dynamic CID (PqDCID) is an extension of our previous work on dynamic CID (DCID),[4,31,32] and is similar in concept to SFM in the 'dynamic' nature of the secular frequency of the ion during excitation. However, SFM is performed by modulating the rf potential in both directions, thereby permitting multiple resonance conditions during the excitation segment of the scan. In PqDCID, there is a single rf potential sweep and the ions are on resonance with the excitation waveform only once. Another major difference between PqDCID and SFM is that the time required for SFM is on the order of tens, even hundreds of milliseconds,[33] which leads to a significantly increased total scan time, whereas PqDCID is achieved in 0.5–1 ms.

We herein report on the ability to achieve fragmentation of precursor ions using the Ω-ω higher-order resonance (where Ω is the drive frequency and ω is the fundamental secular frequency and Ω-ω is a sideband of the secular frequency in a pure quadrupolar field) using two different approaches: (1) excitation when the oscillation frequency of the precursor ions is constant (i.e. HASTE or PQD),[27,28,34] and (2) excitation when the frequency of motion of the precursor ions is dynamic (PqDCID).[4,31,32]

EXPERIMENTAL

Experiments were performed using a modified Polaris Q QIT mass spectrometer (Thermo Electron Corp., Austin, TX, USA). For application of custom waveforms, software programs were written in Visual Basic© 6.0 (Redmond, WA, USA) using the XCalibur development kit (XDK) command language provided by Thermo Electron, as described previously.[31,32] In order to analyze liquid samples, the Polaris Q mass spectrometer was interfaced with a Deca XP atmospheric sampling interface with an ESI source.[4] Leucine enkephalin solution (10 μM) in 50:50 MeOH/H$_2$O, 1% AcOH was introduced with a constant injection rate of 350 μL/h, needle voltage of 1.8 kV. Leucine enkephalin and 99.99% acetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA), and methanol was purchased from EMD (Gibbstown, NJ, USA). Water (18 MΩ) was obtained with a Milli-Q system (Millipore, Billerica, MA, USA). We have observed in our studies that the energetics

of the fragmentation is dependent on the number of ions in the trap; therefore, to ensure a consistent number of precursor ions, all experiments were performed at a fixed ion injection time of ~1 ms for a total ion count of ~1×10^7.

Fragmentation efficiencies were calculated by taking the percent ratio of the product ion intensities at a given excitation amplitude to the isolated precursor ion at zero volts excitation amplitude.[35] The precursor and product ion intensities were exported into Microsoft Office Excel in real time; to keep the data transfer speed at minimum, only the six major product ions were monitored during these experiments. Although with this approach we inadvertently underestimated the calculated fragmentation efficiencies, we considered that the quality of the data mined from each mass spectrum holds its informative purpose. Leucine enkephalin is a widely used test molecule due to its well-characterized fragmentation channels; the a_4/b_4 product ion ratios are correlated with the internal energy deposition.[36,37] All reported excitation amplitudes and widths are derived from the FFT waveforms measured from the exit end-cap electrode using a digital oscilloscope (Tektronix, TDS, 3032B, Richardson, TX, USA). When single frequency waveforms were applied, the waveform widths were less than 1 kHz at the base, centered at the reported frequencies.

To ensure consistent nomenclature throughout our discussion, application of a 0.5 ms CID waveform to achieve resonance excitation – in common with HASTE PQD and 'Fast' CID – will be hereon referred to as 'pulsed CID'; the method where the secular frequency of the precursor ions is swept through a fixed 0.5 ms excitation frequency will be referred to as 'PqDCID'.

RESULTS AND DISCUSSION

Figures 1(a), 1(b) and 1(c) show the scanning function of the ion trap in conventional CID, 'pulsed' CID and PqDCID excitation modes, respectively. In all approaches, ion injection is followed by isolation of precursor ion and a short (10 ms) cooling time to allow focusing of the precursor ions into the center of the trap. Mass acquisition times are also constant at 100 ms and are calculated for a scan from 50–600 Th at 5500 Th/s. For all experiments described henceforth, a single excitation frequency was employed. In the default conventional operation mode of the Polaris Q mass spectrometer, a resonance excitation period of 15 ms is used, whereas pulsed CID and PqDCID employ excitation times of 0.5 ms. For pulsed CID (Fig. 1(b)) the 0.5 ms excitation time represents the total time the ion spends on resonance with the excitation frequency. In PqDCID (Fig. 1(c)) the ions are not on resonance for the entire 0.5 ms, but are only on resonance for a fraction of this time. For example, in PqDCID, the secular frequency of the precursor ions is increased by ramping the rf amplitude on the ring electrode; the PqDCID pulse (fixed excitation frequency) is turned on when the secular frequency reaches 1 kHz below the frequency of excitation and turned off 1 kHz after on-resonance conditions are expected to occur. If the excitation frequency is 171 kHz (q_z 0.45), the excitation pulse is turned on when the secular frequency of the ions reaches 170 kHz, and the excitation pulse is terminated when the

secular frequency of the ions reaches 172 kHz. Thus, the effective on-resonance time for PqDCID is dependent on the width of frequencies over which the precursor ions are scanned, the scan rate and the frequency-dependent absorptivity of the ion species. It is well known that the secular frequencies of ions in an ion trap may shift due to the effect of space charge.[6] This phenomenon has detrimental effects on CID fragmentation, as only a fraction of the precursor ions will be on resonance with the resonance excitation waveform. Due to the fact that the precursor ion stability is increased and the excitation waveform is fixed during a PqDCID experiment, it is ensured that all ions of a selected mass undergo excitation, even if they have a broad kinetic energy distribution. The 2 kHz excitation window and 0.5 ms excitation time were experimentally determined to be the most beneficial in terms of the ability to obtain simultaneously efficient and energetic fragmentations, regardless of the amplitude of the excitation waveform. We have determined experimentally that, for a given excitation amplitude, shorter excitation times are generally less efficient and longer excitation times generally favor lower-energy fragmentation pathways (data not shown).

When higher-order frequency excitation is performed, the pulse time is calculated in a similar manner. However, instead of the 171 kHz frequency, the Ω-ω (1030 kHz – 171 kHz) = 859 kHz excitation frequency is applied. Following the excitation period, the low-mass cutoff (rf amplitude) is dropped to m/z 50 in 0.01 ms to ensure trapping of the low-mass ions, and a 0.5 ms cooling time is applied to allow collisional cooling of the ions before mass analysis. Longer or shorter cooling times had no effect on the product ion spectra. The mass acquisition scan was performed in a similar way for all excitation methods.

The supplemental ac waveform in Figs. 1(a)–1(c) is increased during isolation of the precursor ion to aid ejection of undesired ions present after injection. CID is achieved by applying a resonance excitation waveform during the fragmentation segment (Fig. 1(a)). Pulsed CID (Fig. 1(b)) is achieved by applying the resonance excitation waveform for a short period after isolation. In the PqDCID approach (Fig. 1(c)) the resonant excitation waveform is off during the fragmentation period; here excitation of the precursor ion is achieved by application of a fixed, custom-defined supplemental waveform to the end-cap electrodes while the amplitude of the rf frequency is increased.

The MS/MS spectrum of leucine enkephalin obtained by resonance excitation at q_z 0.45 with a 0.5 ms CID pulse is shown in Figs. 2(a) and 2(b). Figure 2(a) shows the product ion spectrum when a 171 kHz, 2.5 V_{PP} resonance excitation waveform is applied for 0.5 ms. Figure 2(b) shows the fragmentation with a Ω-ω = 859 kHz, 15.1 V_{PP} excitation waveform. The spectra are very similar, even though a much larger amplitude of excitation is required to achieve acceptable fragmentation efficiencies using higher-order excitation.

The PqDCID spectra of leucine enkephalin using 171 kHz, 4.7 V_{PP} and an 859 kHz, 20.5 V_{PP} fixed excitation frequencies are shown in Figs. 3(a) and 3(b), respectively. The Dehmelt pseudopotential well depth is dependent on the q_z of the excitation, as discussed in the introduction. Because the
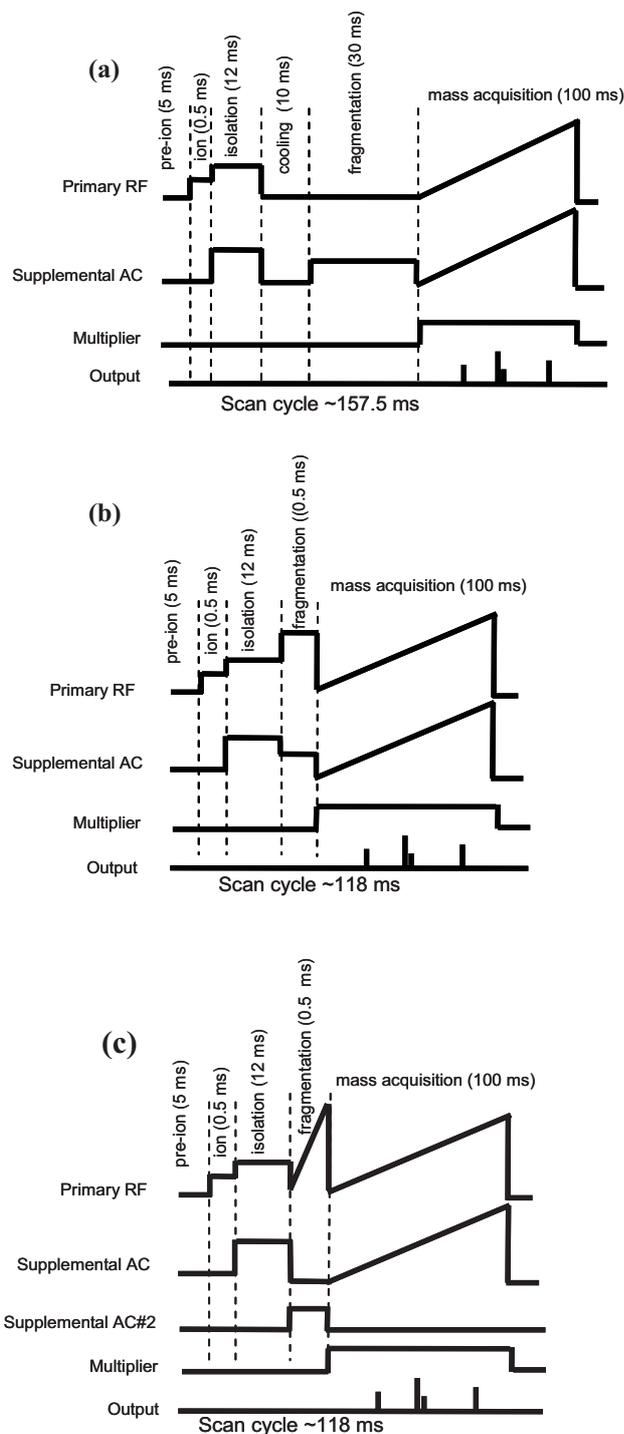
Figure 1. Scan function of the ion trap operation in (a) conventional CID; (b) pulsed CID; and (c) pulsed DCID mode.

Dehmelt pseudopotential well depth is deeper at a q_z parameter between 0.3–0.6 than at lower q_z values, larger excitation amplitudes – leading to greater internal energy depositions – are possible. In addition, at larger values of q_z the secular frequency of the ion is increased, therefore a greater number of resonant excitation cycles are possible per unit time. At smaller values of q_z and shallow Dehmelt potential well depths, the ions suffer a competition between resonance ejection and resonant excitation.[38] This may be overcome by increasing the q_z of excitation. However, in conventional CID, performing excitation at large q_z values
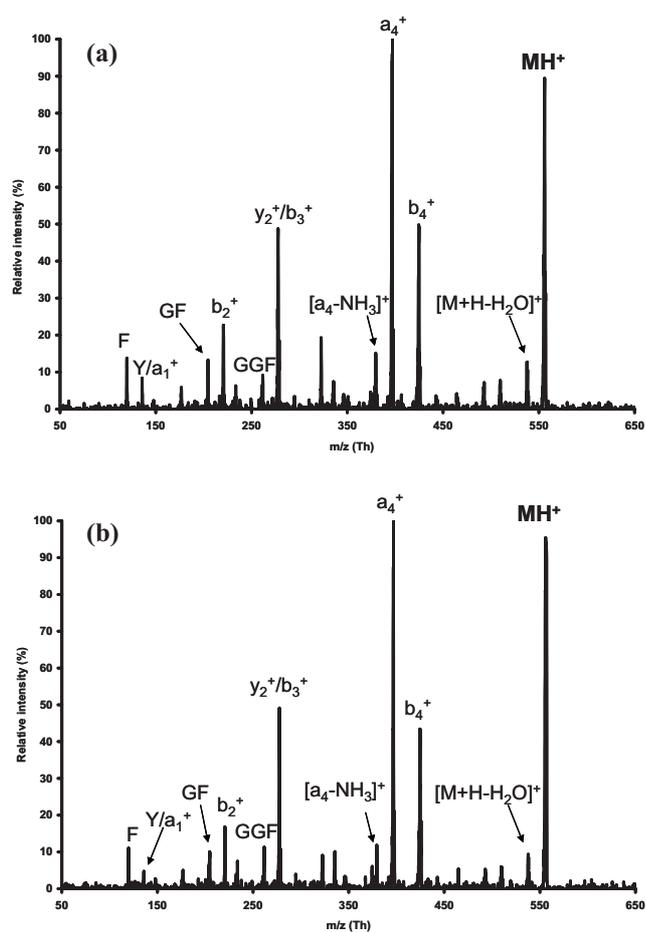
Figure 2. MS/MS product ion spectra of the m/z 556 $[M+H]^+$ molecular ion of leucine enkephalin when a 0.5 ms pulsed CID waveform of (a) 171 kHz frequency, 2.5 V_{pp} amplitude and (b) 859 kHz frequency, 15.1 V_{pp} amplitude is applied to the end-cap electrodes to achieve fragmentation.

has the disadvantage of a high LMCO, and a significant percentage of the low-mass product ions are unable to be collected before mass analysis. Thus, important structural information about the precursor ion is often missing. It is clearly visible in Figs. 2 and 3 that this phenomenon is readily evaded by lowering the rf amplitude, and thus the LMCO, immediately following a fast excitation. In this manner, the low-mass product ions y_2, b_2, internal fragments GF and GGF and immonium ions of F and Y can be successfully trapped and mass analyzed.

The LMCO at a given excitation q_z for a certain precursor ion can be calculated with the following formula:

$$\text{LMCO}(m/z) = \frac{(m/z_{\text{precursor}}) \times (q_z)}{0.908} \quad (3)$$

Accordingly, for the m/z 556 $[M+H]^+$ precursor ion of leucine enkephalin resonantly excited at a q_z of 0.4, the LMCO is:

$$\text{LMCO}(m/z) = \frac{556 \times 0.4}{0.908} \cong 245$$

It has been demonstrated by Murrell and coworkers that for conventional CID or even for pulsed CID at $q_z = 0.4$ (with lowering the LMCO to q_z 0.25 after excitation), ions smaller
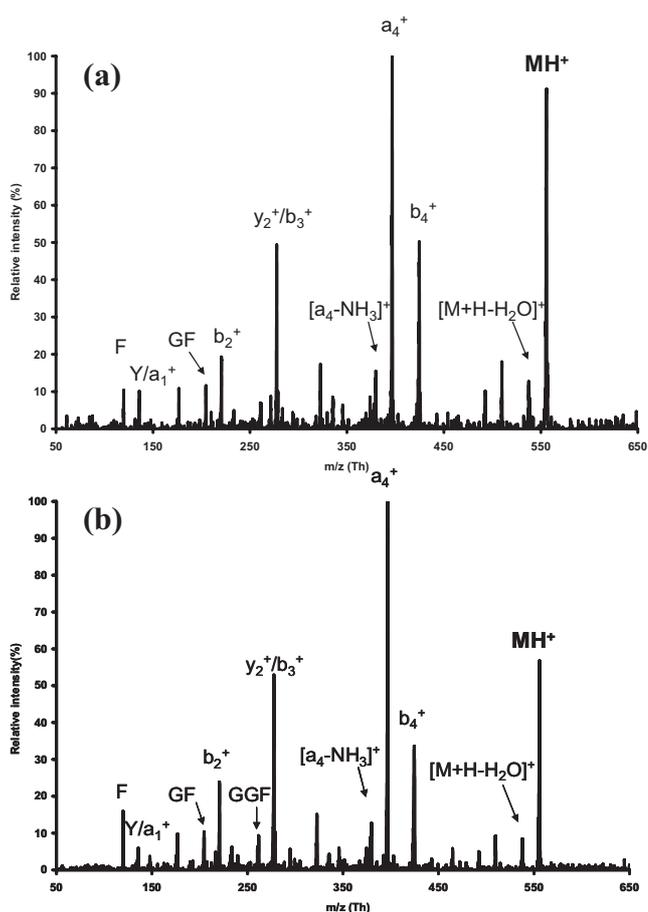
Figure 3. MS/MS product ion spectra of the m/z 556 $[M+H]^+$ molecular ion of leucine enkephalin when a 0.5 ms PqDCID waveform of (a) 171 kHz frequency, 4.7 V_{pp} amplitude and (b) 859 kHz frequency, 20.5 V_{pp} amplitude is applied to the end-cap electrodes to achieve fragmentation.

Figure 4. Fragmentation efficiencies and a_4/b_4 product ion ratios of leucine enkephalin with (a) 171 kHz and (b) 859 kHz 0.5 ms CID pulse.

than the b_2^+ fragment at 220.9 Th are not observable.[34] Note that the MS/MS product ion spectra in Figs. 2 and 3 are recorded at different, arbitrarily selected excitation frequencies and amplitudes; therefore, a direct comparison of the a_4 and b_4 product ion ratios is irrelevant. These figures are presented to demonstrate the successful analysis of low-mass product ions with pulsed CID and PqDCID using both the ω and the Ω-ω excitation frequencies.

To obtain a comparison of the fragmentation energetics and efficiencies for pulsed CID and PqDCID, a series of experiments were conducted under fixed pulse times and excitation frequencies and variable excitation amplitudes. Figures 4(a) and 4(b) show absorption curves for pulsed CID with 171 kHz and 859 kHz excitation frequency, respectively. As expected, excitation amplitudes below a certain threshold do not allow sufficient energy deposition for fragmentation; by increasing the excitation amplitude to 2 V_{pp}, 0.5 ms pulse of the 171 kHz resonance excitation frequency causes the most efficient fragmentation, approximately 20% (the reported fragmentation efficiencies are very conservative because only the six major product ion intensities were taken into consideration in the calculations). Under the most efficient fragmentation conditions the internal energy

deposition remains modest. The 20% fragmentation efficiency is obtained by probing primarily low internal energy fragmentation channels; at 2 V_{pp} the a_4/b_4 product ion ratio obtained was ~1. By further increasing the excitation amplitude an excess internal energy deposition is possible, leading to a more abundant b_4 product in the mass spectrum. However, under these conditions, the precursor ion is conferred with large kinetic energies, leading to the undesirable ejection before fragmentation, and, ultimately, to low fragmentation efficiencies. Similar behavior is observed using higher-order resonance excitation, as shown in Fig. 4(b). In accordance with previous studies, much greater excitation amplitudes are required if a higher-order eigenfrequency is employed; in our case 20% fragmentation efficiency with an 859 kHz, 0.5 ms pulse was achieved with 15 V_{pp} excitation amplitude.

Our findings indicate that a short CID pulse of 0.5 ms permits considerable internal energy depositions; at q_z 0.45 a 3 V_{pp} excitation with the first-order resonance frequency a maximum a_4/b_4 ratio of 13 was observed at 3% efficiency, whereas excitation at the Ω-ω component allows a maximum ratio of ~9 at 18 V_{pp} excitation amplitude at 5% efficiency. The observation of high internal energy deposition with 'fast excitation' described by Murrel et al.[34] reportedly permits a_4/b_4 ratios up to 10 using a much faster pulse of 0.1 ms. Collisional activation of leucine enkephalin in sector instruments, Fourier transform ion cyclotron ion resonance (FTICR) instruments and conventional ion traps usually does

(a)

(b)
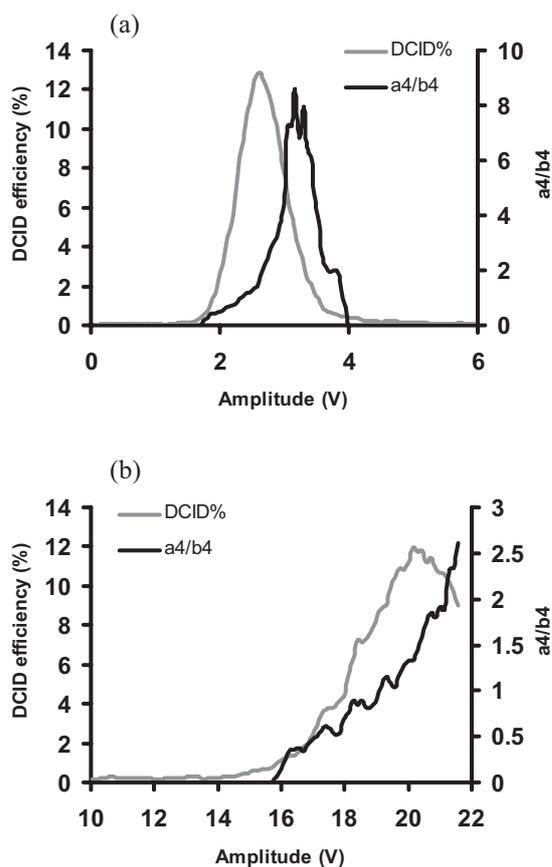
Figure 5. Fragmentation efficiencies and a_4/b_4 product ion ratios of leucine enkephalin with (a) 171 kHz and (b) 859 kHz 0.5 ms DCID pulse.

Figure 6. Fragmentation efficiencies and a_4/b_4 product ion ratios of leucine enkephalin with a 131 kHz, 0.5 ms DCID pulse.

not permit a_4/b_4 ratios greater to 4.5, even with heavier bath gases,[36,39–41] with the exception of one study reporting a_4/b_4 ratios up to 20.[34] The fast excitation methods presented in the present work are therefore amongst the most highly energetic reported to date.

In comparison to pulsed CID, PqDCID is at best 50% less efficient, in agreement with our previous studies.[4,31,32,39] As shown in Figs. 5(a) and 5(b), absolute fragmentation efficiencies up to 12% may be achieved using either the 171 kHz or 859 kHz waveform. PqDCID with a 0.5 ms pulse at q_z 0.45 imparts less internal energy than pulsed CID under the same conditions, probably because the ions are not on resonance and are not excited for the entire 0.5 ms period in PqDCID. The maximum recorded a_4/b_4 ratios were ∼8 with both the 171 kHz and the 859 kHz PqDCID waveform, at efficiencies of 5% and 9%, respectively.

We chose the q_z 0.45 working point for comparison purposes; here both pulsed CID and PqDCID offered acceptable fragmentation and internal energy depositions with excitation amplitudes lower than 20 V_{PP} with either the ω or the Ω-ω frequency component employed for excitation. As seen in Fig. 6, at a q_z of 0.3 a PqDCID pulse of 0.5 ms and 131 kHz frequency offers a_4/b_4 ratios of up to 16 with an excitation amplitude of 3 V_{PP}. In this series of experiments, the most efficient conditions were determined to be with a 2.7 V_{PP} excitation amplitude, where a_4/b_4 ratios of 10 were observed at fragmentation efficiencies of 8%. No investigations involving high excitation frequencies were con-

ducted at this q_z value because very large excitation amplitudes were required to achieve any significant fragmentation, and the maximum excitation amplitude that may be applied to the end-cap electrodes with our instrument was measured to be ∼22 V_{PP}.

CONCLUSIONS

Using the method of immediately lowering the rf amplitude after a short, high-amplitude excitation step to trap the low-mass ions proved to be successful in fragmenting the selected peptide leucine enkephalin, regardless of the order of the excitation. The q_z of excitation plays an important role in the fragmentation process for both pulsed CID and PqDCID. As is commonly observed elsewhere, the internal energy deposition, as well as the fragmentation efficiency, is highly dependent on the amplitude of excitation in an inverse relationship; excitation conditions that lead to efficient fragmentation are likely to offer small internal energy depositions, and vice versa.

The effective time spent on resonance with the excitation waveform is less for PqDCID compared to CID, PQD or HASTE; therefore, PqDCID regularly requires larger excitation amplitudes than the other methods at both low- and higher-order frequencies of excitation to achieve similar fragmentation conditions. At smaller q_z values, the internal energy deposition of a 0.5 ms PqDCID pulse is comparable to those observed with pulsed CID. Unlike CID, no pre-tuning is required for PqDCID because the latter method assures on-resonance conditions. The faster duty cycle and wider product mass range permitted through fast/pulsed CID and PqDCID coupled with a fast mass scanning method could be very beneficial for coupling with a fast high-performance liquid chromatography (HPLC) or fast gas chromatography (GC) separation; in such applications, a faster duty cycle is highly beneficial allowing for more mass spectra per chromatographic peak.[42]

RCM

REFERENCES

1. Amunugama R, Hogan JM, Newton KA, McLuckey SA. *Anal. Chem.* 2004; **76**: 720.
2. Wenner BR, Lovell MA, Lynn BC. *J. Proteome Res.* 2004; **3**: 97.
3. Doroshenko VM, Cotter RJ. *J. Mass Spectrom.* 1997; **32**: 602.
4. Laskay ÜA, Pasilis S, Duckworth DC, Hyland JJ, Jackson GP. *J. Am. Soc. Mass Spectrom.* 2007; **18**: 2017.
5. Yang CG, Bier ME. *Anal. Chem.* 2005; **77**: 1663.
6. Dobson G, Murrell J, Despeyroux D, Wind F, Tabet J-C. *J. Mass Spectrom.* 2004; **39**: 1295.
7. Doroshenko VM, Cotter RJ. *J. Am. Soc. Mass Spectrom.* 1997; **8**: 1141.
8. Goeringer DE, Whitten WB, Ramsey JM, McLuckey SA, Glish GL. *Anal. Chem.* 1992; **64**: 1434.
9. Danell RM, Danell AS, Glish GL, Vachet RW. *J. Am. Soc. Mass Spectrom.* 2003; **14**: 1099.
10. Doroshenko VM, Cotter RJ. *Anal. Chem.* 1996; **68**: 463.
11. Julian RK, Cooks RG. *Anal. Chem.* 1993; **65**: 1827.
12. Lammert SA, Cooks RG. *Rapid Commun. Mass Spectrom.* 1992; **6**: 528.
13. Mathieu EM. *J. Math. Pure Appl. 1868;* **13**: 137.
14. March RE. *Rapid Commun. Mass Spectrom.* 1998; **12**: 1543.
15. March RE, Todd FJ. *Quadrupole Ion Trap Mass Spectrometry*, (2nd edn). John Wiley: New York, 2005.
16. Sudakov M, Konenkov N, Douglas DJ, Glebova T. *J. Am. Soc. Mass Spectrom.* 2000; **11**: 10.
17. Franzen J, Gabling R-H, Schubert M, Wang Y. In *Practical Aspects of Ion Trap Mass Spectrometry*, March RE, Todd JFJ (eds). CRC: Boca Raton, 1995.
18. Franzen J. *Int. J. Mass Spectrom. Ion Processes* 1991; **106**: 63.
19. Alheit R, Hennig C, Morgenstern R, Vedel F, Werth G. *Appl. Phys. B-Lasers Opt.* 1995; **61**: 277.
20. Alheit R, Gudjons T, Kleineidam S, Werth G. *Rapid Commun. Mass Spectrom.* 1996; **10**: 583.
21. Vedel F, Vedel M. *Phys. Rev. A* 1990; **41**: 2348.
22. Franzen J. *Int. J. Mass Spectrom. Ion Processes* 1994; **130**: 15.
23. Wang Y, Franzen J, Wanczek KP. *Int. J. Mass Spectrom. Ion Processes* 1993; **124**: 125.
24. Splendore M, Marquette E, Oppenheimer J, Huston C, Wells G. *Int. J. Mass Spectrom.* 1999; **191**: 129.
25. Creaser CS, Stygall JW. *Int. J. Mass Spectrom.* 1999; **191**: 145.
26. Tabb DL, Smith LL, Breci LA, Wysocki VH, Lin D, YatesIII JR. *Anal. Chem.* 2003; **75**: 1155.
27. Schwartz JC, Syka JEP, Quarmby ST. In *Proc. 53rd ASMS Conf. Mass Spectrometry and Allied Topics*, San Antonio, TX, 2005.
28. Cunningham C, Glish GL, Burinsky DJ. *J. Am. Soc. Mass Spectrom.* 2006; **17**: 81.
29. Plomley JB, March RE, Mercer RS. *Anal. Chem.* 1996; **68**: 2345.
30. Schachterle S. In *Proc. 42nd ASMS Conf. Mass Spectrometry and Allied Topics*, Chicago, IL, 1994.
31. Jackson GP, Hyland JJ, Laskay ÜA. *Rapid Commun. Mass Spectrom.* 2005; **19**: 3555.
32. Laskay ÜA, Hyland JJ, Jackson GP. *J. Am. Soc. Mass Spectrom.* 2007; **18**: 749.
33. Lausevic M, Splendore M, March RE. *J. Mass Spectrom.* 1996; **31**: 1244.
34. Murrell J, Despeyroux D, Lammert SA, Stephenson JL, Goeringer DE. *J. Am. Soc. Mass Spectrom.* 2003; **14**: 785.
35. Yost RA, Fetterolf DD. *Mass Spectrom. Rev.* 1983; **2**: 1.
36. Thibault P, Alexander AJ, Boyd RK, Tomer KB. *J. Am. Soc. Mass Spectrom.* 1993; **4**: 845.
37. Campbell JM, Vestal ML, Blank PS, Stein SE, Epstein JA, Yergey AL. *J. Am. Soc. Mass Spectrom.* 2007; **18**: 607.
38. Charles MJ, McLuckey SA, Glish GL. *J. Am. Soc. Mass Spectrom.* 1994; **5**: 1031.
39. Collin OL, Beier M, Jackson GP. *Anal. Chem.* 2007; **79**: 5468.
40. Vachet RW, Glish GL. *J. Am. Soc. Mass Spectrom.* 1996; **7**: 1194.
41. Herrmann KA, Somogyi A, Wysocki VH, Drahos L, Vekey K. *Anal. Chem.* 2005; **77**: 7626.
42. Shen YF, Smith RD, Unger KK, Kumar D, Lubda D. *Anal. Chem.* 2005; **77**: 6692.