“Fast Excitation” CID in a Quadrupole Ion Trap Mass Spectrometer

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Collision-induced dissociation (CID) in a quadrupole ion trap mass spectrometer is usually performed by applying a small amplitude excitation voltage at the same secular frequency as the ion of interest. Here we disclose studies examining the use of large amplitude voltage excitations (applied for short periods of time) to cause fragmentation of the ions of interest. This process has been examined using leucine enkephalin as the model compound and the motion of the ions within the ion trap simulated using ITSIM. The resulting fragmentation information obtained is identical with that observed by conventional resonance excitation CID. “Fast excitation” CID deposits (as determined by the intensity ratio of the a4/b4 ion of leucine enkephalin) approximately the same amount of internal energy into an ion as conventional resonance excitation CID where the excitation signal is applied for much longer periods of time. The major difference between the two excitation techniques is the higher rate of excitation (gain in kinetic energy) between successive collisions with helium atoms with “fast excitation” CID as opposed to the conventional resonance excitation CID. With conventional resonance excitation CID ions fragment while the excitation voltage is still being applied whereas for “fast excitation” CID a higher proportion of the ions fragment in the ion cooling time following the excitation pulse. The fragmentation of the (M + 17H)17+ of horse heart myoglobin is also shown to illustrate the application of “fast excitation” CID to proteins. (J Am Soc Mass Spectrom 2003, 14, 785–789) © 2003 American Society for Mass Spectrometry

Collision-induced dissociation (CID) was first demonstrated in a quadrupole ion trap mass spectrometer in 1987 by Louris et al. [1] by applying to the end-cap electrodes a small supplementary RF voltage, at the same secular frequency as the ion of interest. This produces an increased amplitude of ion motion for ions of that particular mass-to-charge, which then fragment after collisions with the helium buffer gas. This has been the most popular method used for CID in the quadrupole ion trap mass spectrometer and has been used to study an extremely large range of species. Alternative methods for fragmenting ions within a quadrupole ion trap mass spectrometer have been reported and these include surface induced dissociation [2], photo induced dissociation [3, 4], boundary activated dissociation [5–7], and red shift off resonance large amplitude excitation [8]. All of these methods, to a greater or lesser extent, have sought to improve the ease of performing CID, the amount of fragmentation information obtained, and the mass range of the product ion spectrum. Here we report studies aimed at increasing the speed of performing a single CID experiment. In a conventional resonance excitation CID experiment a small voltage (e.g., 1 Volt peak-peak) is applied for a set period of time (e.g., 30 ms) followed by a short cooling time prior to analysis. Here we apply a large voltage (e.g., 20 Volt peak-peak) for a very short period of time (e.g., 90 μs) followed by a short cool time prior to analysis.

Experimental

All data were acquired on an unmodified Thermoquest LCQ quadrupole ion trap mass spectrometer (Thermo-Finnigan, San Jose, CA) controlled by version 1.1 of the Navigator software (Thermo-Finnigan, San Jose, CA). Leucine enkephalin and horse heart myoglobin (Sigma-Aldrich, Poole, Dorset, UK) were dissolved in 50:50 CH3OH:H2O 0.1% formic acid at 1 pmol/μl and directly infused into the mass spectrometer at 3 μl/min.
All lenses were optimized for optimal transmission of the base peak ion.

The MS/MS experiment consists of injecting the ions into the ion trap, isolating the ions of interest, and exciting these ions to cause fragmentation. A short cooling time follows excitation to allow the ions to be collisionally focused to the center of the ion trap before the spectrum is acquired. This is shown in Figure 1. The CID analytical segment of the MS/MS scan on an LCQ consists of a number of sections. After the excitation period at a q<sub>e</sub> value of 0.4 the RF is ramped down to a q<sub>e</sub> value of 0.25 over the course of approximately 3 ms. The ions are then stored at this q<sub>e</sub> value for approximately 3 ms before the acquisition ramp is initiated. Thus there is a period of time of approximately 6 ms after the excitation in which the ions can continue to fragment or be cooled to the center of the ion trap. The minimum excitation time set by the hardware is 89 μs.

Ion trajectory simulations were performed on ITSIM (version 4.1) developed by Cooks and co-workers for the modeling of ions within a quadrupole ion trap mass spectrometer [9, 10]. A 4th order Runge-Kutta algorithm was employed for these simulations. The effect of the buffer gas was simulated with a hard-sphere collision model with non-zero scattering angle for helium at 1 mTorr. For the purposes of this study, simulations phase of the excitation voltage for the excitation signal to drop to zero. This can be seen by examining Figure 2.

The experimental procedure for calculating the efficiency of the fragmentation process consisted of the following steps: an ion isolation experiment is performed at the q<sub>e</sub> value of interest with the standard automatic gain control (AGC) value of 2 × 10<sup>7</sup>. From this initial experiment an injection time is determined. The experiment is then repeated with this injection time set to obtain a more accurate measure of the initial ion intensity. This value is used as the initial precursor ion intensity. The collision-induced dissociation studies were then performed in order to find the excitation voltage at which the CID processes were most efficient and the sum of the fragment ions at this particular voltage was used in the calculation of the MS/MS efficiency.
were performed to provide only a relative representation of ion motion as expected from purely quadrupole ion trap mass spectrometer and not as an exact representation of the actual LCQ analyzer.

**Results and Discussion**

Shown in Figures 3 and 4 are the fast excitation CID and conventional resonance excitation CID of the pseudomolecular ion (MH⁺) of leucine enkephalin, (YGGFL, MW 555 Da) respectively under the excitation conditions indicated. As can be seen both fragmentation patterns are identical with the exception of some ions observed below \( m/z \) 240 in the fast excitation CID experiment. Leucine enkephalin has been extensively studied and the fragmentation observed are common with those previously reported [11–13]. The two ions that were observed below \( m/z \) 240 for the case of fast excitation CID are due to the excited ions continuing to fragment when the RF trapping voltage has been dropped to \( q_x = 0.25 \) prior to the acquisition. This gives an indication that the ions are still fragmenting after the excitation.

To determine how these ions were behaving within the quadrupole ion trap a number of simulation studies were performed using ITSIM. Figures 5 and 6 show the output for the simulations of fast excitation CID and the resonance excitation CID respectively performed for an ion of \( m/z \) 556 in a quadrupole ion trap mass spectrometer. With fast excitation CID, the ions, very quickly, appear to obtain very large amplitude trajectories in the z direction before slowly cooling to the center of the ion trap by collisions with the helium buffer gas. For conventional resonance excitation CID the ions slowly gain increased motion in the z direction.

The performance of the experiment is also dependent on the amplitude of the RF voltage that is applied to the ring electrode as this relates to the depth of the potential well and thus how many of the precursor ions are retained during and after excitation, as well as how efficiently the fragment ions are trapped. Shown in
Figure 7. Graph of the MS/MS efficiency of the MH$^+$ ion of leucine enkephalin for fast excitation CID and for conventional resonance excitation CID over a range of $q_z$ values. [Inverted filled triangle = fast excitation CID (89 $\mu$s), filled square = conventional resonance excitation CID (30 ms)]. The excitation voltages applied are shown in square brackets).

Figure 8. Graph of the ratio of $a_4/b_4$ fragment ions for fast excitation CID ($q_z = 0.4$, excitation time 89 $\mu$s) in a quadrupole ion trap mass spectrometer.

Figure 9. Graph of the ratio of $a_4/b_4$ fragment ions for conventional resonance excitation CID ($q_z = 0.4$, excitation time 30 ms) in a quadrupole ion trap mass spectrometer.

Figure 10. Fast excitation CID of the (M + 17H)$^{17+}$ of horse heart myoglobin at $q_z = 0.4$, excitation time of 89 $\mu$s and excitation voltage of 29 V.

Figure 11. Graph of the ratio of $a_4/b_4$ fragment ions for conventional resonance excitation CID at $q_z = 0.4$ shows two linear portions for the $a_4/b_4$ ratio between 0.6 and 2.2 V and 2.2 and 4 V. This is different from the CID data of leucine enkephalin previously reported which showed a linear relationship between the amount of excitation and the $a_4/b_4$ ratio [14, 15]. A full explanation for the observation of this non-linear plot for conventional resonance excitation CID data is beyond the scope of this paper. In the comparison between the fast excitation CID data and the conventional resonance excitation CID data the spectra obtained are identical except that the efficiency of the fast excitation process is lower than that for the conventional resonance excitation process. For example the efficiency for obtaining an $a_4/b_4$ ratio of 10 would be $\sim 10\%$ for conventional resonance excitation CID as opposed to $\sim 6\%$ for fast excitation CID.

It has been reported that the intensity ratio of the $a_4/b_4$ fragment ions for leucine enkephalin gives a gauge of the internal energy deposited onto the MH$^+$ ion of the peptide [14, 15]. Shown in Figures 8 and 9 are the $a_4/b_4$ intensity ratios for fast excitation CID with the related conventional resonance excitation CID ratios respectively. The plot for conventional resonance excitation CID at $q_z = 0.4$ shows two linear portions for the $a_4/b_4$ ratio between 0.6 and 2.2 V and 2.2 and 4 V. This is different from the CID data of leucine enkephalin previously reported which showed a linear relationship between the amount of excitation and the $a_4/b_4$ ratio [14, 15]. A full explanation for the observation of this non-linear plot for conventional resonance excitation CID data is beyond the scope of this paper. In the comparison between the fast excitation CID data and the conventional resonance excitation CID data the spectra obtained are identical except that the efficiency of the fast excitation process is lower than that for the conventional resonance excitation process. For example the efficiency for obtaining an $a_4/b_4$ ratio of 10 would be $\sim 10\%$ for conventional resonance excitation CID as opposed to $\sim 6\%$ for fast excitation CID.

Shown in Figures 10 and 11 are the fast excitation CID and conventional resonance excitation CID for the $m/z$ 998 [(M + 17H)$^{17+}$] ion of horse heart myoglobin. The fragmentation patterns observed are very similar for the two excitation methods. Loss of ammonia or water [(M+17H)$^{17+}$-NH$_3$/H$_2$O] from the precursor ion is however not observed in the conventional resonance excitation CID. Presumably, this is because the resonance excitation pulse is wide enough to excite this
More importantly fast excitation CID may provide a means of increasing the rate of the acquisition of CID data as compared with conventional resonance excitation CID and therefore the obtaining of MSⁿ data from a limited amount of sample.

References


