RESEARCH ARTICLE

Boundary-Activated Dissociation in a Low Pressure Linear Quadrupole Ion Trap in the Presence of Nonlinear DC Fields

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Abstract

Boundary-Activated Dissociation (BAD) of multiple charge ions has been investigated in a low pressure linear ion trap (LIT) in the presence of nonlinear DC fields. Nonlinear DC fields allowed ions to be stored for a long duration at working points beyond the $\beta_y=0$ stability boundary of the regular quadrupole fields. The ions reached large stable radial amplitude trajectories gaining high kinetic energies from the drive RF field. This led to collision activation and the formation of fragments. Experimental and simulation data showed that the degree of fragmentation was strongly dependent on the q value, Mathieu stability parameter, and the strengths of nonlinear fields. In the absence of the nonlinear fields the fragmentation efficiency was 0 % at $q=0.23$ and 17 % at $q=0.4$. In the presence of nonlinear fields BAD efficiency increased to up to 94 % at $q=0.23$ and 84 % at $q=0.4$. The broadening of the stability diagram at the $\beta_y=0$ boundary also enabled the observation of fragment ions with higher mass-to-charge ratios (m/z) than the m/z of the precursor ions thus overcoming a major drawback of BAD of multiple-charged ions.

Key words: Boundary-activated dissociation, Linear ion trap, Nonlinear fields

Introduction

The influence of higher order radio-frequency (RF) field imperfections on ion motion near the boundary of the first stable region in ion traps was previously studied in conjunction with mass selective instability scan in 3D quadrupole ion traps [1]. The presence of negative even multipole RF fields or odd higher multipole RF fields of either sign showed a delay in ion ejection during the instability scan. This effect was explained by the shift of the secular oscillation frequency that occurred with increasing oscillation amplitude. This further allowed a controlled energy take-up while the ions experienced a beat motion.

When the ions were stored near the stability boundary, they absorbed power from the RF drive potential and underwent energetic collisions with the bath gas. This technique has been referred to as Boundary-Activation Dissociation [2–5].

Similarly when ion trap collision-activated dissociation (CAD) was induced using dipole resonance excitation, an ion could be fragmented more efficiently with reduced losses of the precursor ion in the presence of higher order RF fields in both 3-D and linear ion traps [6, 7]. Beating motion was also observed in a linear ion trap in the presence of higher order DC-only fields, which allowed for higher fragmentation efficiencies to be observed while maintaining relatively short resonant dipole excitation periods [8]. The higher order RF fields have also recently been reported to play a role in fragmentation efficiency when dipolar DC collision-induced dissociation (DDC CID) was performed in a 3-D commercial ion trap [9].

Earlier reports showed that the MS/MS efficiency from BAD in high pressure (4×10^{-3} Torr) 3-D and linear quadrupole ion traps ranged from 10 % to 90 % with a drop at lower q values where, due to small confinement potentials, ion ejection predominated over ion dissociation [2–5, 10]. Use of pulsed heavier gases was reported to increase the efficiency of BAD especially at q values below 0.2 [11].

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One significant disadvantage of the BAD technique was reported to be the loss of the high mass cut-off fragment ions from the dissociation of multiply-charged ions. Typically, ions were brought to the edge of the $\beta_y=0$ stability boundary by the application of a DC voltage, to either the ring electrode in the 3-D ion trap [11] or in quadrupolar fashion to the rods of a linear RF quadrupole ion trap [10]. At nonzero α -Mathieu parameter, the trajectories of the fragment ions having higher m/z than the m/z of the precursor ion, became unstable and ions hit the rods. This resulted in a loss of product ion information.

This report focuses on the characteristics of BAD in a low pressure ($<4 \times 10^{-5}$ Torr) linear ion trap in the presence of higher order DC fields. It was observed that for regular quadrupole fields, ion dissociation efficiency was less than 20 %. However, the presence of the nonlinear DC fields changed the ion dynamics preventing ions to be lost on the rods at working points at or beyond the $\beta_y=0$ stability boundary. High energies were transferred from the RF field into the kinetic energy of the ions and BAD efficiency increased to 94 %. The broadening of the stability diagram at the $\beta_y=0$ boundary also enabled the observation of fragment ions of higher m/z than the m/z of the multiple-charged precursor ions.

Experimental

All experiments were performed on a research-grade version of the QTRAP 5500 system mass spectrometer (AB SCIEX, Concord, ON, Canada) [12]. The ion path was a triple quadrupole mass spectrometer with the last quadrupole rod array (Q3) configured to operate either as a conventional RF/DC mass filter or as a linear ion trap (LIT) with mass-selective axial ejection (MSAE) [13, 14]. The short 2.5 cm-long Q3L linac electrodes from the extraction region of the Q3 LIT were replaced with 5 cm-long T-electrodes. The auxiliary electrodes each had a 6 mm-long stem along the length of the electrode that was positioned 6 mm from the central axis, Z, of the quadrupole as shown in Figure 1. A DC voltage (Q3T) ranging from -300 V to 0 V was applied to the auxiliary electrodes.

Figure 1. A cross section of the Q3 LIT

Ions were mass selected in the first mass analyzer Q1 and transferred at low energies (i.e. 10 eV), through a nitrogen-filled collision cell (Q2), into the Q3 linear ion trap. The main RF drive frequency Ω was 940 kHz allowing for the upper mass limit to be extended to 2000 Th. The operating pressure in the low-pressure trap (Q3) region was 3.0×10^{-5} Torr when the Q2 cell was filled with buffer gas at 10 mTorr. Using a micro-valve, nitrogen was pulsed into the Q3 trapping region [15] to increase the steady-state pressure to about 4.0×10^{-5} Torr. During the fill period, Q3T was 65 V attractive relative to the rod offset voltage RO3, forcing the ions to accumulate in the extraction region of the LIT.

After a cooling period, ions were subject to a quadrupole resolving DC pulse U that was applied for 1 to 40 ms. During this activation time the rod offset voltage (RO3) applied to the rods was 0 V while the exit lens was set at 10 V. During both the cooling period and the activation time the Q3T voltage was set at values ranging from 0 to -300 V. After the DC pulse U was applied, both the precursor and BAD fragment ions were cooled for another 40 ms. The voltages along the Q3 LIT were then set to the optimum scanning values and the ions were mass-selective axially ejected at a scan rate of 1 kTh/s. During the MSAE scan the Q3T voltage was set at the same voltage as the RO3 offset voltage in order to preserve mass calibration and resolving power.

The insulin chain B (oxidized, 80 % purity, Sigma Aldrich Co.) was prepared as a 20 pmol/ μ L stock solution using dilution buffer of 10 % ACN/water with 0.1 % formic acid, and then serially diluted to 1 pmol/ μ L solution using a working buffer of 50 % ACN/water with 0.1 % formic acid. The solution was infused at a flow rate of 5 μ L/min and the multiple (3+) charged ions were generated by ESI(+).

Electric Field and Ion Trajectory Simulations

SIMION 7.0 (Idaho National Laboratory) ion trajectory simulator was used to determine the potential along the axes of the linear quadrupole constructed of round rods, in the presence of the Q3 auxiliary electrodes as well as to study the ions dynamics when ions were brought at operating points near or beyond the $\beta_y=0$ boundary of the stability diagram. The electric fields were extracted from the SIMION PA files using ExSimEck 1.4 b (Rainer Umbach). The rod offset voltage RO3 was set at 0 V and the exit lens voltage was set to 10 V. The voltage on the metal collar stripes was at 750 V [12]. No collisions with the ambient gas were considered. The potential array (PA) file was updated every 0.2 μ s.

Results and Discussion

Experimental Data, Boundary Activation Dissociation

Fragmentation efficiency was found to be strongly dependant on both the Q3T voltage and the operating Mathieu stability parameter q . Figure 2a shows the BAD fragmenta-

Figure 2. (a) BAD fragmentation yield of the 1166.1 Th (3+) insulin ions as a function of the DC voltage U when activation time was 10 ms. Fill time was 5 ms. Black, red, and blue traces show the extracted ion chromatogram (XIC) of 2 individual co-added LIT spectra (MCA) for the total number of ions, precursor ion and the fragment ions (all fragments), respectively. (b) BAD fragmentation spectra of the 1166.1 Th (3+) insulin ions. The spectra were the result of 50 MCA scans

tion yield of the 1166.1 Th (3+) insulin ions as a function of the DC voltage U , at q 0.2 and Q3T -150 V. Figure 2b shows the actual BAD spectra at U 33.2 V. An almost complete y -ion series was observed with the exceptions of the y_{11} and y_{10} ions as well as the y -ions of m/z higher than 1800 Th. The b ion peaks appearing above the m/z of the precursor ion (m/z 1166.1 Th) were more intense in comparison to b -ion peaks below the m/z of the precursor ion. Another prominent feature of the spectrum was the appearance of enhanced y_6 fragment ion.

At q 0.2 it was observed that the DC voltage U , at which the depletion of the precursor ion occurred, increased linearly from 18 V at Q3T 0 V to 37 V at -175 V and dropped quadratically beyond -175 V, Figure 3a. BAD maximum efficiency increased from 0 % at Q3T 0 V to 80 % at Q3T -125 V and then dropped again to 0 % at Q3T -225 V, Figure 3b. The BAD fragmentation efficiency at

each Q3T was calculated as the ratio of the XIC of all the fragments, at a specific DC voltage U , to the XIC of the precursor ion at U 0 V.

At Q3T between 0 V and -150 V, the U voltage range over which the fragmentation occurred (i.e., the width of the distributions in Figure 3b) also increased at more negative Q3T voltages. This indicated that ions had a stable trajectory at working points deeper into the forbidden (a, q) space (i.e., outside the theoretical stability diagram) [16].

Table 1 shows the variation of maximum BAD efficiency as a function of Q3T for three different q values. The maximum BAD efficiency observed was 94 % at q 0.23 and Q3T -175 V. At q 0.4, the stronger confinement provided by the higher RF fields allowed observation of some BAD fragments even at Q3T 0 V. At Q3T -225 V the maximum

Figure 3. (a) the XIC of 2 MCA LIT spectra for the precursor ion 1166.1 Th as a function of U and Q3T. (b) the XIC of 2 MCA LIT spectra for the all-fragment ions as a function of U and Q3T. Activation q was 0.2

efficiency showed a big jump from 0 %, at q 0.2, to 80 %, very close in q -space at q 0.23. The distribution of the fragment ions across the m/z space did not change as the function of Q3T and U . For values of the activation q closer to 0.706 (the tip of the stability diagram) the efficiency dropped due to the losses of the low-mass cut-off fragment ions. Fragmentation efficiency at q values higher than 0.7 was less than 1 %.

Similar BAD efficiency profiles were observed for fragment ions of both lower and higher m/z than the m/z of the precursor ion. The drop in fragmentation was mainly due to the precursor ions hitting the rods/electrodes instead of suffering fragmentation.

Table 1. BAD Efficiency as a Function of Q3T and q

Q3T	0	-25	-50	-75	-100	-125	-150	-175	-200	-225	-250	-275	-300
q													
0.2	0	25	58	69	67	79	78	42	9	0	0	0	0
0.23	0	54	72	72	87	92	87	94	82	78	62	39	20
0.4	17	56	55	64	58	61	80	61	74	68	84	73	82

Figure 4. First mass stability diagram at various Q3T voltages. The inset figure shows a zoom around the tip of the stability diagram

The nonlinear influence of Q3T on the efficiency of boundary activation triggered a more detailed investigation of the influence of Q3T on the first stability region [16].

Experimental Mapping of the Stability Diagram as a Function of the Q3T DC Voltage

Ions were brought at working points closer and closer to the stability boundary by sequentially increasing the resolving quadrupolar DC voltage U , at q values from 0.16 to 0.9. The coordinate a corresponding to each point on the β -lines was chosen for the quadrupolar DC U voltage at which the total-ion-count of the precursor ion would drop to less than 0.1 %. The U voltage was applied for 1 ms only, in order to prevent ion fragmentation. Thus ion losses were due to their collisions with electrodes. Figure 4 shows the experimentally determined stability diagram for Q3T values from 0 to -300 V.

At 0 V the stability boundary resembled the Mathieu stability boundary for the pure quadrupole fields. At large negative voltages on the Q3T electrodes the boundaries looked rather different than the regular boundary and could be divided into four distinct regions.

The first region (I) was at $q < 0.25$. Here the stability boundary was extended toward higher a -values and had a steeper increase in a than the regular boundary in the same q space. The degree of displacement from the regular boundary depended on the Q3T voltage. The edge of this region moved to higher q at higher Q3T.

Table 2. Upper Mass Limit of the Ions Stable in the Trap at q 0.2 for 1166.1 Precursor Ions and DC 33 V

Q3T	0	-25	-50	-75	-100	-125	-150	-175	-200	-225	-250	-275	-300
m/z	638	695	762	874	1037	1284	1600	1182	1182	1166	1160	1107	1086

In the second region (II) characterized by q values from 0.25 to 0.7, the stability boundary had a similar shape to the regular boundary and it was displaced toward higher a values. The more negative the Q3T the higher the displacement.

The third region (III) was at the tip of the stability diagram at q values from 0.7 to 0.71. Ions showed a bi-stable behavior with their trajectory being stable within the “normal” boundary and in a stability island outside the regular stability boundary. The area of this island and the distance, in (a, q) space, from the regular boundary increased at large negative Q3T.

The fourth (IV) region was at q values from 0.71 to 0.9. In this region the boundary was basically identical to the

boundary for the regular quadrupole fields. This behavior was expected since the instability of the ion motion in this q space was determined only by the RF voltage and the DC Q3T did not have a strong influence.

The broadening of the stability diagram at low q values was also confirmed by an alternate set of experiments. In those experiments, the 1166.1 Th precursor ions were fragmented using beam-type CID in the Q2 collision cell. Both the fragments and the precursor ions were then trapped in the Q3. The RF voltage on the Q3 array corresponded to a $q=0.2$, for the 1166.1 Th ions. After a cooling period, a 1 ms duration DC pulse of 33 V was applied at different values of Q3T. Table 2 shows the upper limit of the observed

Figure 5. (a) The y-component of the electrostatic and RF fields (E_y) and ($-E_y$), respectively, versus distance from the Z-axis, on the Y-axis, in the XY plane 25 mm from the exit lens. (b) The x-component of the electrostatic field (E_x) versus distance from the Z-axis, on the Y-axis, in the XY plane 25 mm from the exit lens. U was set at 25 V. When E_x was opposite in sign to the x displacement, ion motion in X direction was stable; however, when E_x had the same sign as the x displacement ion motion became unstable in the X direction

fragments, as a function of Q3T. The upper mass limit showed a similar nonlinear dependence on the Q3T as the (a, 0.2) stability boundary in region I, Figure 4.

The highest upper limit was 1600 Th at Q3T -150 V. This value was lower than the upper mass limit observed in the BAD experiments at similar a , q , and Q3T values, Figure 2b. It can be speculated that this discrepancy could be due to one of the following reasons: BAD fragments had high energies and radial amplitude positions when were formed and the stability boundary in that case became even more distorted and/or the fragmentation time was longer than the activation time, in other words they formed after the quadrupolar DC pulse U was turned off.

Electric Fields and Ion Trajectory Simulations

Ion trajectory and electric field simulations were carried out to examine the ion dynamics at the stability boundary. Since significant fragmentation was observed only for $q < 0.7$ most of the simulations were focused on the $\beta_y = 0$ boundary. In this region, the negative voltage applied on the Y rods would have a destabilizing role [16].

Figure 5a shows the y component of electrostatic field on the Y axis (E_y), at different U and Q3T values at $q = 0.2$. Figure 5a shows also a plot of the theoretical values of the y component ($-E_y$) of the electric field due to the pseudo-potential RF field, corresponding to $q = 0.2$:

$E_y = -\frac{\partial D(x,y)}{\partial y} = -\frac{2V}{r_0^2} D_0$, where $D_0(x,y) = qV/4$ is the quadrupole pseudo-potential [17], $V = 366.6$ V (zero-to-peak) and $r_0 = 4.17$ mm. E_y opposed the increase of the amplitude in y direction hence E_y was negative at positive y and positive at negative y . However, for ease of comparison to the electrostatic field the (E_y), Figure 5a shows the plot of ($-E_y$).

All U and Q3T values were chosen similar to the values determined experimentally for the stability boundary at $q = 0.2$. Those points were for Q3T 0 V, -150 V, and -225 V at (a, q): (0.0203, 0.2), (0.0416, 0.2), and (0.0375, 0.2) corresponding to U 18.6, 38.15, and 34.4 V respectively.

At Q3T = 0 V E_y increased linearly with y . At negative Q3T values the field became nonlinear. At low y E_y was larger in absolute value than E_y , causing ions to accelerate towards higher oscillatory amplitudes. However, once the ions reached higher y amplitudes, E_y became weaker than E_y and the resultant electric field became repulsive, forcing the ions to move towards the axis and preventing them to hit the rods, hence the broadening of the stability boundary. The larger the y values at which the E_y and E_y curves intersected, the larger the ion trajectory amplitude in the y direction, see curves (Q3T -150 V, U 30 V) versus (Q3T -150 V, U 38 V), Figure 5a. When the electrostatic field E_y was larger than the electric field E_y generated by the non-uniform RF field, which was the case for the curve (Q3T -150 V, U 44 V), Figure 5a, the ions had an unbound trajectory.

At Q3T -225 V E_y did not exceed E_y , even at U 34 V, the loci at the experimental stability boundary, and one would expect the ions to be stable in the YZ plane.

Figure 5b reveals that the destabilizing field in this case became the E_x component of the DC field. At high amplitudes of the ion motion, in the presence of large negative Q3T voltages, the electrostatic field changed sign and pulled ions away from the YZ plane. The farther the ions moved from the axis, the closer they got to the Q3T electrodes and the stronger the destabilizing field they experienced. This would explain the change in slope of the stability boundary in the region I, Figure 4.

Ion trajectory simulations at $q = 0.2$ confirmed that the electric field generated by the Q3T potential had a stabilizing effect in the y direction, however at voltages lower than -150 V the field had a destabilizing effect in the diagonal direction defined by the Q3T electrodes.

Figure 6 shows the color-coded trajectories for the 1166.1 Th ions at different auxiliary and quadrupolar DC voltages (Q3T, U , color): (0, 18, red), (-150 , 20, blue), (-150 , 28, green), and (-225 , 28, black).

At Q3T = 0 V the ion trajectory was unstable at 18 V quadrupolar DC and the transition from a stable trajectory to an unstable trajectory occurred over a very narrow range. At Q3T -150 V the ion trajectory was stable at voltages up to 30 V. Ions reached kinetic energies of 9 eV at U 20 V and up to 65 eV at U 30 V. At Q3T -225 V the ions did not experience stable higher amplitude trajectories and were forced to hit the Q3T auxiliary electrodes at 28 V. These results explain the lack of fragmentation in region I of the stability boundary, Figure 4, as well as the different slopes of the boundary in the regions I and II. While for Q3T 0 V the experimental, theoretical and

Figure 6. The red, blue, green, and black trajectories of the 1166.1 Th ($3+$) ions were recorded using the (Q3T, U) pairs (0, 18), (-150 , 20), (-150 , 28), and (-225 , 28), respectively. The q value was 0.2

simulation values for U at the boundary were similar, at larger Q3T the boundary values for U determined from ion trajectories were slightly lower. It is believed that this might be due to the broadening of the stability boundary due to collisions with the bath gas molecules and the choice of the loci of the experimental boundary points (i.e., working points corresponding to 0.1 % of TIC of the precursor still stable in the trap after the U pulse, as well as the rather poor PA resolution (i.e., 0.2 mm per grid unit).

Presently more work is underway to better characterize the energetics of the low pressure BAD using protonated leucine enkephalin as a 'thermometer ion' of the fragmentation process.

Conclusions

It has been shown that the addition of nonlinear DC fields allowed BAD to be performed on a low pressure linear quadrupole ion trap. The higher order DC fields modified the boundary of the stability diagram of the ion trajectory within the RF quadrupole array and caused the ions to move at high amplitude stable trajectories when placed through a combination of RF and DC voltages outside of the normal boundary of the stability diagram. Ions gained high energies from the RF field and suffered high efficient fragmentation with reduced loss of the ions on the quadrupole rods. The broadening of the stability diagram at the $\beta_y=0$ boundary also enabled the observation of fragment ions of higher m/z than the m/z of the multiple-charged precursor ions.

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