SHORT COMMUNICATION

Phase-Enhanced Selective Ion Ejection in an Orbitrap Mass Spectrometer

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The mass resolution achieved in selective ion isolation using resonance excitation is usually limited by the frequency resolution of the ac waveform and by unintended off-resonance excitation. A new method of phase-enhanced selective ion ejection based on broadband dipolar excitation and ion ejection applicable to the Orbitrap is described and shown to allow an isolation resolution of 28,400. The method is calculated to be able to provide a mass resolution for ion ejection of up to 100,000. (J Am Soc Mass Spectrom 2007, 18, 980–983) © 2007 American Society for Mass Spectrometry

Ion isolation is the primary event in tandem mass spectrometry. High mass resolution in the ion selection step provides high-quality tandem mass spectrometry and, therefore, benefits analyses of complex samples [1]. For example, only when a single isotopomer of a multiply charged protein ion is mass selected will the fragment ions be isotopically pure, in turn allowing their masses to be assigned unambiguously.

The Orbitrap, a new mass analyzer, provides high mass resolution (up to 100,000), high mass accuracy (2 ppm) [2], a mass/charge ratio range of at least 6000 [3], and a linear dynamic range of >5000 within a mass spectrum [4]. In the Orbitrap, the mass-to-charge ratio is obtained from the frequency of harmonic ion oscillations of the orbitally trapped ions along the axis of the electric field. This axial frequency is independent of the angular and radial motion of the ions and is determined using image current detection and fast Fourier transforms. The frequency is related to the mass-to-charge ratio (m/z) by the relation $\omega = (kz/m)^{1/2}$ [5].

Ion motion control experiments involving mass-selective ion excitation and de-excitation of trapped ions in the Orbitrap using ac dipolar waveforms have been reported recently [6, 7]. These experiments demonstrate that ion axial motion (i.e., in the z-direction) can be controlled by applying ac waveforms to the two outer electrodes of the Orbitrap. Excitation of an ion's axial motion occurs when the ac electric field is resonant with the ion's characteristic axial frequency. If the ac waveform is applied 180° out of phase with respect to ion motion, the ion can be de-excited to the center plane ($z = 0$) of the Orbitrap, re-excited to re-gain full amplitude of its harmonic motion, and finally ejected from the Orbitrap if sufficient excitation is provided. Ions can also be ejected from the Orbitrap directly if the excitation waveform is applied in phase with (i.e., 0°) the ion motion.

The capability to control ion axial motion in the Orbitrap is important because it provides the possibility of improving the performance of the Orbitrap and adding new functions to this device. For example, ac excitation and ejection can be used to explore inter-packet space charge effects in the Orbitrap. The evaluation of space charge (ion–ion repulsion) effects either in the Orbitrap or in the ion injection optics can be conducted by performing selective ion ejection of either one of two populations and then comparing the results obtained before and after ion ejection. Selective ion ejection is particularly intriguing because it might enhance exact mass measurement of ions of interest, especially of multiply charged ions, by ejecting adjacent ion species. Because ions with similar mass/charge ratios have similar rotational radii in the Orbitrap, as well as very similar axial frequencies, they spend more time together in closer proximity and thus maximize space charge effects.

Ac de-excitation and re-excitation may be useful for improving the peak shape, resolution, and mass measurement accuracy when the ion signal is distorted by space charge effects in the Orbitrap or in the injection optics. It might also be useful in studying ion packet dephasing processes in the Orbitrap and, therefore, in developing a method to extend the transient time and improve performance. Axial de-excitation, "storage" of ions at $z = 0$ for up to 4 s, and subsequent re-excitation were recently demonstrated [8]. This capability is useful if long trapping of ions is desired and offers the opportunity to achieve tandem mass spectrometry in the Orbitrap.

Selective ion ejection in the Orbitrap was previously demonstrated using resonant dipolar ac excitation [6, 7] but mass resolution of ion ejection was limited by the frequency resolution of the excitation waveform and

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off-resonance excitation, the same situation as in FTICR [9]. In the Orbitrap, the highest mass resolution for ion ejection reported was 1960 by using an excitation waveform with a very low amplitude of 0.16 V [6]. In this paper, we report on a new strategy—phase-enhanced selective ion ejection—for improving mass resolution in ion ejection while using broadband ac waveforms.

Experimental

Reagents

All chemicals used were purchased from Sigma (St. Louis, MO, USA) and dissolved in water/methanol (50:50 vol %:vol %) with 1% (vol %) formic acid. The sample solution contained 0.03 mg/mL [Val[5]]-angiotensin II and 0.1 mg/ml Lys-des-Arg[9]-bradykinin. The sample was introduced into the electrospray source at a flow rate of 0.2 μL/min using a syringe pump (Harvard, South Natick, MA). Typically, the ESI capillary was held at 3000 V and room temperature.

Orbitrap Mass Spectrometer

The Orbitrap mass spectrometer used in these experiments is a prototype of the commercial Thermo Orbitrap and is described in detail elsewhere [3]. An electrospray source creates ions, which are accumulated (100–400 ms), energized (1300 V), and bunched into a small ion packet with a spatial spread of a few millimeters. The ion packet is then accelerated and deflected into the Orbitrap for mass analysis.

Resonant ac waveforms are applied on the split outer electrode before ion detection. A digital delay generator (DDG, Stanford Research Systems Model DG535) triggers the waveform generator (model AFG320, Sony Tektronix); the DDG itself is activated by the ion injection gate. Therefore, the relative phase angle of the ac with respect to the ion axial motion is simply controlled by the delay of the DDG [6]. Typically, axial excitation was performed after an initial delay (60–80 ms) relative to ion injection and ion detection started 20 ms after ac application. The initial delay is needed for radial and angular dephasing of the ion packets [3, 5, 10]. Signal is acquired as an image current and the transient is recorded for 800 ms at a sampling rate of 5000 kHz. The transient is Fourier transformed using the MIDAS data analysis program [11] into a mass spectrum, with one level of zero filling and no apodization.

Results and Discussion

Mass resolution achieved in ion selection in the Orbitrap can overcome the constraints imposed by the limited frequency resolution of the applied ac waveforms. The reason for this is that the two processes of ion de-excitation/re-excitation and ion ejection (that is, ion excitation from large axial amplitude) are imple-
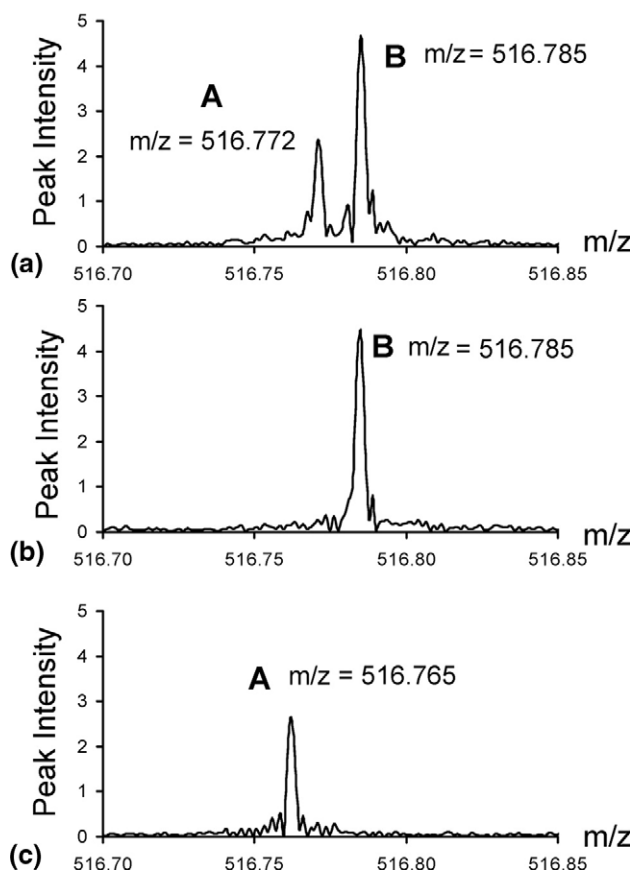
Figure 1. (**a**) Mass spectrum of two ions, with theoretical mass-to-charge ratios of [M+2H]$^{2+}$ = 516.76,671 Th (ion A) and [M+2H]$^{2+}$ = 516.78,490 Th (ion B). (**b**) Mass spectrum obtained by re-exciting ion B while ejecting ion A. (**c**) Mass spectrum obtained by re-exciting ion A while ejecting ion B.

mented using different phase relationships relative to ion axial motion. Figure 1 shows an example of selective ejection for ion isolation in the Orbitrap. Figure 1a is a mass spectrum acquired by electrospraying a solution containing both [Val[5]]-angiotensin II and Lys-des-Arg[9]-bradykinin. The two doubly charged ions generated from electrospray ionization (ESI) were [M+2H]$^{2+}$ = 516.76,671 Th (Thomson, ion A) and [M+2H]$^{2+}$ = 516.78,490 Th (ion B). The theoretical mass/charge ratio difference between these two ions is 18.2 mTh and the minimum mass resolution of ion selection required here is 28,400.

The corresponding frequency spectrum of these two ions is shown in Figure 2a. The measured frequencies of these two ions in the Orbitrap were 485.159 and 485.168 kHz. The ac signal used for this experiment, shown in Figure 2b, had an amplitude of 3.8 V$_{pp}$. In all, 150 cycles were needed to eject ions when the waveform was applied in phase with the ion motion and 470 cycles were needed to obtain fully re-excited signals while the ac was applied 180° out of phase with respect to the ion motion. Centered at a frequency of 485.16 kHz, the bandwidth of this excitation waveform is 2 kHz (width of the main lobe at baseline) and thus can be used to
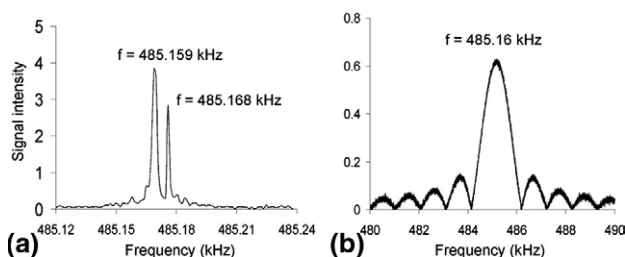
Figure 2. Two frequency spectra. (**a**) Frequency spectrum of two ions A and B from the Orbitrap. (**b**) Bandwidth of 470 cycles of ac waveform with an amplitude of 3.8 V, which is wide enough to cover the resonant frequency range of both ions A and B. Note the different frequency scales of the two plots.

excite ions at both mass-to-charge ratios. Note that Figure 2a and Figure 2b have different frequency scales.

This ac waveform was applied after specifying a delay relative to ion injection. The delay of the excitation waveform was adjusted for two different cases. In case (i), the delay was adjusted so that after the application of the ac waveform, only ion B was observed and ion A signal disappeared as shown in Figure 1b. In case (ii), the excitation ac waveform was applied with a delay of 1.05 μs longer than the delay in case (i). For case (ii), only ion A signal was detected while ion B was gone, as shown in Figure 1c.

In case (i), ion B was de-excited and re-excited by applying the ac 180° out of phase with respect to the ion motion of B. In contrast to case (i), ion A was de-excited and re-excited in case (ii) by using the ac at a phase angle of 180° with respect to the ion motion of A. Note that the period of harmonic motion for both ions was 2.1 μs and the difference between the two excitation delays was 1.05 μs—the half-period of their harmonic motion. This means that these two excitation waveforms were applied 180° out of phase with each other. Therefore, the excitation ac waveform in case (i) was applied in phase with ion A while it was 180° out of phase with ion population B; and the excitation waveform in case (ii) was in phase with ion B but was 180° out of phase with ion A. Because the initial ion axial amplitude in the Orbitrap is sufficiently great that excitation and ejection occur more quickly than de-excitation [6], 470 cycles of this ac signal are more than enough to eject the ion if this ion is in phase with the ac excitation signal. Therefore ion A was ejected in case (i) with ion B fully re-excited and ion B was ejected in case (ii) with ion A re-excited. The mass resolution of ion isolation for these two cases is 28,400.

Finding this particular phase point was accomplished by simply estimating from the frequencies of the two ions. These two ions used had similar mass/charge ratios and thus they arrived at the entrance of the Orbitrap and started their oscillations in the Orbitrap at essentially the same time. Therefore, they must have oscillated nearly in phase with each other at the beginning of the experiment. Figure 3a illustrates the relative phase relationship of these two ions right after

ion injection. The beat frequency of their oscillations was about 6.9 Hz, which is the difference of their two frequencies. This beat frequency indicates that these two groups of ions would repeatedly come into phase with each other with a period of about 144.6 ms. Therefore, about 72.3 ms after ion injection, these two groups of ions would be 180° out of phase with each other, as shown in Figure 3b. It is precisely this phase difference between the two mass-to-charge ratios—and spatial separation—that underlies the success of this method. If the ac excitation waveform was applied after an initial delay of about 72.3 ms after ion injection and also fine-tuned into phase with one of these two ions, then the excitation waveform would be 180° out of phase with the other ion. Therefore, one ion would be ejected from the Orbitrap while the other one would be de-excited and re-excited and, thus, retained in the Orbitrap.

Interestingly, the maximum mass resolution of ion selection by this method in the Orbitrap may be estimated. In practice, the maximum mass resolution of ion selection using our method of phase-enhanced selective ion ejection is limited by the exponential decay of the time-domain transient signal obtained from the Orbitrap. The application of the ac waveform should be performed before the transient has significantly decayed; selective ejection may be achieved if the two groups of ions become 180° out of phase with each other more quickly than the signal transient decays. For example, if the ion signal decays with lifetime $\tau \simeq 200$ ms (reasonable because the transient normally lasts 1–1.5 s) [5, 10], the longest delay in applying the waveform would also be 200 ms. This is equal to the time when the two ions, close in mass, are 180° out of phase with each other, or one-half of a beat period has elapsed. Therefore, the beat period of these two ions could be 2×200 ms and the beat frequency 2.5 Hz. For ions having mass/charge ratios around 517 Th, the maximum frequency resolution would be $R_f = 485,000/2.5 = 194,000$, and thereby the maximum mass resolution $R_m = R_f/2 = 97,000$ [5].

Another interesting aspect of this experiment is that the two peaks in Figure 1a have an observed mass/charge ratio difference of 13 mTh, almost 33% less than
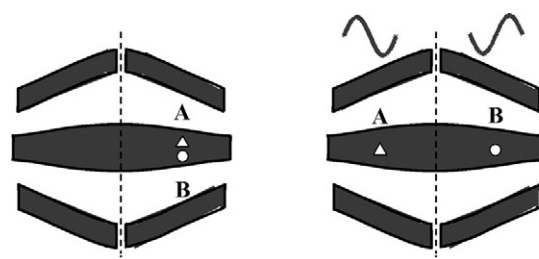
(a) 0 ms after ion injection (b) 72.3 ms after ion injection

Figure 3. Relative phase relationship of two ions A and B. (**a**) These two ions are in phase with each other right after ion injection. (**b**) These two ions are 180° out of phase with each other 72.3 ms after ion injection.

the theoretical value of 18.2 mTh. After ion A was ejected (as shown in Figure 1b) and after ion B was ejected (as shown in Figure 1c), the measured mass/charge ratio difference of these two ions is 20 mTh, a more accurate value, even though this number is from two different mass spectra (these two spectra were collected one immediately after the other). The standard deviation in the observed frequency for either peak was <0.6 Hz, roughly 1 part per million. This corresponds to a 2 part per million standard deviation in the mass, 1 mTh.

The reason for the observed mass shift in Figure 1a might be space charge operating in the Orbitrap. First, these ions are *both* doubly charged, increasing the strength of the Coulomb interaction fourfold over the interaction of two singly charged species. Second, the two ion populations spend considerable time in close proximity. Because they have nearly identical axial frequencies, the ions will stay in phase much longer than, say, two ions separated by even one mass unit. Additionally, because the two ions have very similar mass/charge ratios, they will have almost the same rotational radius, placing the angularly and radially dephased "rings" of ions in close contact. After the ejection of either one of the two ions, the space charge effect is reduced. More experiments will be conducted; for example, an internal mass (frequency) standard can be added to provide solid evidence for the mass shift of the two ions in the normal mass spectrum and then measure the mass accuracy after the ejection of either ion.

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