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Collisional Cooling of Large Ions in Electrospray Mass Spectrometry

Igor V. Chernushevich* and Bruce A. Thomson

MDS SCIEX, 71 Four Valley Drive, Concord, Ontario L4K 4V8 Canada

Collisional cooling of ions in the rf-only multipole guides has become a method of choice for coupling electrospray sources to various mass analyzers. Normally parameters of such ion guides (length, pressure) provide enough thermalization and focusing for ions in a wide mass range. Noncovalent complexes, however, have more compact conformations than denatured biomolecules of similar mass and, therefore may not be transmitted efficiently through standard ion guides, as demonstrated by theoretical analysis, simulations, and experiments. Several methods of improving collisional cooling for large compact ions have been developed on a quadrupole time-of-flight instrument, which include operating the ion guides at higher pressure and trapping ions to increase the cooling time. Improved transmission of heavy ions obtained with those methods is studied in experiments with proteasome 20S, an oligomeric protein noncovalent complex with molecular weight around 692 000, and a few other compounds.

Over the past decade, electrospray ionization mass spectrometry (ESI-MS) has emerged as a powerful tool for studies of supramolecular complexes held together by noncovalent interactions.[1−4] The success of ESI is based on the fact that it is a soft ionization method and allows weakly bound complexes to be detected. Several studies have convincingly demonstrated that specific noncovalent complexes involving protein/protein, protein/ligand, or protein/DNA interaction can survive the electrospray process.[4−7] On the other hand, this area of mass spectrometry is far from a routine technique, since a careful optimization of the operating conditions is needed.

From the very first mass spectrometric studies of noncovalent complexes performed at near physiological conditions[5] it became evident that they appear with lower net charge compared to ions produced from denatured proteins of similar size. This may be due to the fact that basic amino acids are buried in the native conformation and do not become protonated or due to strong electrostatic repulsion of closely located charges. In either case, lower charge is an indication of a more compact conformation, which, as will become clear, is a primary reason for the investigation presented here.

The low charge of complexes translates into high m/z values at which their peaks appear in the mass spectra. For heavy complexes, these m/z values are often outside of the limited range of most quadrupole analyzers, and the majority of such studies are therefore now performed on time-of-flight (TOF) mass spectrometers with orthogonal injection, which became commercially available in the past few years. The m/z range of this type of instrument is limited by ion detection only, which makes it possible to measure ions in the megadalton mass range.[8−10]

Despite the theoretically unlimited m/z range of the TOF instruments, several groups have reported problems with recording ions of complexes starting with molecular weight around 100 000 unless the pressures in the first one or two vacuum chambers are increased. Heck et al. noted that when "...using standard pressure settings no ions could be detected" on a quadrupole time-of-flight instrument.[11] Several other groups have confirmed that careful control of pressure gradients in the different pumping stages of the instrument was important for the detection of macromolecular particles.[12−14] In these previous studies, the benefits of increased pressure were generally attributed to providing a pressure regime that allowed gentle declustering without fragmentation.

In this paper, we show strong evidence that, at least in our instrument, increased pressure provides improved collisional cooling and focusing of large ions in the quadrupole guides and, therefore, better transmission through the quadrupoles and TOF. Several methods to improve cooling are suggested and characterized.

* Corresponding author. Phone: (905)660−9006, × 2470. Fax: (905)660−2623. E-mail: chernuiv@sciex.com.

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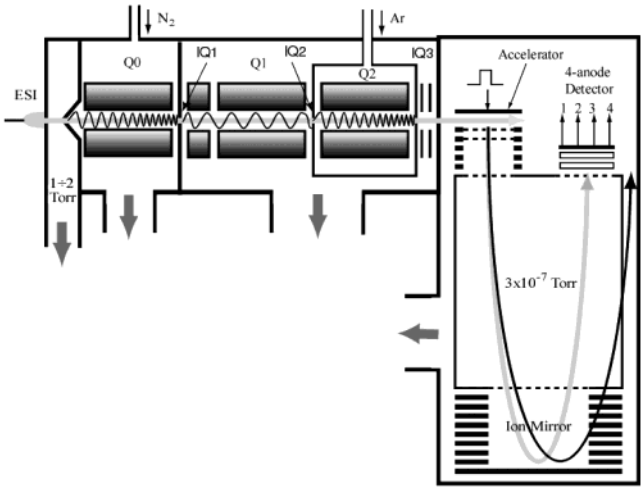

Figure 1. Schematic diagram of the QqTOF mass spectrometer.

EXPERIMENTAL SECTION

Reagents. Proteasome 20S was purchased from Calbiochem. Alcohol dehydrogenase (ADH, from baker's yeast), concavalin A, ammonium acetate (99.999% purity), cesium iodide, cesium hydrogen carbonate, and tridecafluoroheptanoic acid were purchased from Sigma-Aldrich. All noncovalent complexes were filtered with Amicon Ultra centrifugal filters (Millipore) with molecular weight cutoff 10 000 and diluted to micromolar concentrations (calculated with the MW of the multimeric complex) with 10−30 mM aqueous ammonium acetate (pH ∼6.0).

Mass Spectrometry. All experiments were performed on two similar quadrupole time-of-flight (QqTOF) prototype systems shown schematically in Figure 1 and described in detail in previous publications.[15,16] The instrument consists of three quadrupoles (ion guide Q0, mass analyzer Q1, collision cell Q2) and a reflecting TOF analyzer with orthogonal injection of ions. Several modifications have been made in the instruments in relation to the present investigation. A throttling valve and a thermocouple gauge (both Leybold, Germany) were added into the interface pumping line. A needle valve (Swagelok) connected to a nitrogen line and Pirani gauge (Varian) were added to the vacuum chamber to be able to control and measure the pressure in the Q0 area (as discussed later, we were therefore able to control the pressure in the interface region without affecting the pressure in the Q0 region, to separate the effects). By using a baffle at the pump entrance to limit the conductance, pressures of up to 40 mTorr could be achieved in Q0 by adding nitrogen through the needle valve, without overloading the turbopump. For separate experiments, a flow-restricting "sleeve" in a form of a metal tube (32 mm i.d., 100 mm long) was installed around the front part of the Q0 rods to increase pressure locally. The diameter of the inlet and exit apertures of the collision cell Q2 was decreased from 2.4 to 1.6 mm, thus increasing the pressure range in Q2 from normal 2−8 to 4−16 mTorr. TOF mass spectra were recorded with a four-channel time-to-digital converter (TDC, IonWerks, Houston, TX) connected to a G3 Macintosh computer.

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RESULTS AND DISCUSSION

Collisional Cooling and Stopping Path. Electrospray ions are generated at atmospheric pressure and introduced into the vacuum chamber of the mass spectrometer in a flowing gas stream (jet), usually through a nozzle and a skimmer, with subsequent stages of pressure reduction. Fluid dynamical modeling of the gas and ion motion (unpublished work) has confirmed that all ions are accelerated in the free jet to velocities of several hundred meters per second. Even very large ions experience relatively little velocity slip from the supersonic neutral gas velocity. While these speeds may not present a problem for small ions, larger ions (mass >500 Da) may acquire energies in the electronvolt range, comparable to those obtained from their acceleration in electrostatic fields within a mass analyzer, since energy is proportional to mass for ions having the same velocity. The energy acquired from the gas jet may have both axial and radial components and may result in various types of transmission losses depending on the particular analyzer. For example, in quadrupole mass filters radial oscillations of ions lead to reduced transmission through interquadrupole apertures, while excessive axial energy will result in reduced resolution. In trapping mass analyzers (ion traps, FTICR MS), energy variation with mass may lead to mass discrimination effects during trapping. Finally, in TOF instruments with orthogonal injection (apart from similar problems of poor transmission through apertures or slits), ions having excessive axial energy ("orthogonal" with respect to TOF axis) may partially or completely miss the detector, as discussed below.

Douglas and French proposed use of quadrupole ion guides at elevated pressures (milliTorr range) in order to thermalize ions in collisions with gas molecules (usually nitrogen).[17] "Collisional cooling" results in significant reduction of both axial and radial kinetic energy components. Loss of radial component causes the ions to move to the minimum of the effective potential at the center of the quadrupole ("collisional focusing"). Ions are therefore more efficiently transmitted through the exit or interquadrupole apertures and into the downstream mass analyzing quadrupole. Although originally developed for quadrupole mass filters, collisional cooling was later found to be extremely useful for ion introduction into TOF,[18] ion trap, and FTICR mass analyzers. The recently developed collisional cooling of MALDI ions[19] has been shown to provide similar instrumental benefits, as well as others that are beyond the scope of the present discussion.

The parameters of the quadrupole or multipole ion guides (such as length and pressure) are normally chosen to provide enough thermalization and focusing for ions in a relatively wide mass range. To estimate the requirements for collisional cooling, let us consider a simplified model of ion motion in the gas, assuming that a large ion with mass M and initial velocity v_0 moves through a stagnant gas of molecules with mass m. The ion velocity changes in time as

$$v(t) = v_0 \exp(-t/\tau), \qquad \tau = 3M/4mn\sigma v_{kT} \qquad (1)$$

where n is the gas density, σ is collision cross section, and v_{kT} is

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Table 1. Stopping Path for Proteins at $p = 7$ mTorr

protein	M, Da	σ, Å^2	S, cm	ρ, g/cm^3
denatured:				
motillin	2699	850[21a]	3.5	
ubiquitin	8564	1460[21a]	6.4	
cytochrome	12231	2370[21a]	5.6	
myoglobin	16951	2520[21a]	7.3	
BSA	66430	11400[21a]	6.4	
native:				
cytochrome	12231	900[23]	14.8	1.0
carb. anhydrase	29180	1660[23]	19	0.96
proteasome 20S	692000	19400[27]	39	0.78

[a] σ from ref 21 taken for the smallest charge state Z.

the thermal velocity of gas.[20] Ions that enter the vacuum from atmospheric pressure are accelerated to sonic velocity (v_s) at the orifice by the gas expansion, corresponding to a kinetic energy of tens to hundreds of electronvolts for very large ions. Once the ion enters the stagnant background gas in the interface or Q0 region, the stopping distance S of this ion is

$$S = \int_0^\infty v_s \exp(-t/\tau)\, dt = v_s\tau = \frac{3}{4}\frac{Mv_s}{mn\sigma v_{kT}} \sim M/\sigma \quad (2)$$

Under denaturing conditions, proteins in solution typically have a loose conformation and they acquire higher charge states in ESI. The ions then have larger cross sections, and the majority of them have the ratio "M/σ" in a narrow range centered ~5 Da/Å^2,[21] thus keeping the stopping distance S almost constant across a wide range of masses (see Table 1). This is not the case for biomolecular ions in their native (usually more compact) conformation, which tend to have a constant density close to that of water ($\rho = 1$ g/mL).[22,23] For such ions

$$M = \frac{4}{3}\pi R^3 \rho N_A \quad (3)$$

where N_A is the Avogadro constant, and

$$M/\sigma = \sqrt[3]{M}\left(\frac{4\rho NA}{3\sqrt{\pi}}\right)^{2/3}, \qquad S \sim \sqrt[3]{M} \quad (4)$$

indicating that their stopping distance increases with the cube root of mass. A megadalton ion would therefore have a 10 times longer stopping path than a kilodalton ion.

Ion Transmission versus Q0 Pressure. If an ion is not "cooled" well enough in a collisional quadrupole (either Q0 or Q2), it has a high probability of being lost. Insufficient radial cooling results in poor transmission through apertures IQ1, IQ2, and IQ3 connecting the quadrupoles (Figure 1), while insufficient axial cooling makes ions miss the TOF detector. One of the simplest solutions to this problem is to increase the pressure in Q0 to fit the stopping path within the existing quadrupole length. Figure 2 shows the results of ion trajectory simulations for a 20-cm-long Q0 for three different cases: myoglobin ion ($M = 16\,951$ Da) at 8 mTorr and proteasome 20S ion (noncovalently bound 28-mer with $M \sim 692$ kDa) at the same pressure (panel B) and at the elevated pressure of 30 mTorr. The results of individual collisions were calculated with a stochastic hard-sphere collision model (developed in-house[24]), using neutral target molecules moving at thermal velocities (ion−neutral cross sections are shown in Table 1). Simulations were performed under the simplifying assumptions that all ions are accelerated to a velocity of 400 m/s between the nozzle (sampling orifice) and skimmer prior to entering the Q0 area where the gas is stagnant. Under these conditions, myoglobin ions can be focused into a beam of <0.1 mm in diameter at the usual operating pressure of 8 mTorr (panel A), while the characteristic beam size for the proteasome ions at the Q0 exit (panel B) is comparable to a 1.3-mm-diameter exit aperture IQ1, thus causing reduction in ion signal. Note that those ions which pass IQ1 successfully have some probability to be lost at the collision cell entrance aperture IQ2, since there is no further cooling in the collisionless Q1 quadrupole. Provided there is enough gas in Q2, the "lucky survivors" may be cooled to a level appropriate for passing the IQ3 exit aperture and being recorded on the TOF detector. However, to avoid ion losses on all apertures, full thermalization can be achieved in Q0 by increasing pressure to 30 mTorr (panel C).

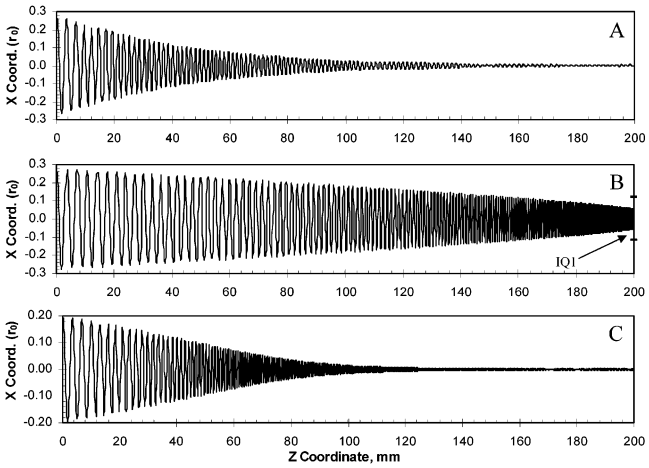

Figure 2. Simulations of ion trajectories in Q0 (XZ plane). (A) Myoglobin ion ($M = 16\,951$ Da, $\sigma = 2{,}520$ Å^2) at 8 mTorr; (B) proteasome 20S ion ($M = 692$ kDa, $\sigma = 19\,400$ Å^2) at 8 mTorr; (C) proteasome 20S ion at 30 mTorr.

Interestingly, the four-channel detection system used in this instrument allows the "quality" of the overall collisional cooling (in both Q0 and Q2) to be monitored by observing the ion distribution over the four detector anodes. The anodes are arranged as 8-mm-wide stripes perpendicular to the direction of the ion trajectories in the quadrupoles. Normally the ion acceleration along two perpendicular axes is arranged in such a way that the ions arrive at the detector naturally, without any further deflection, and spread evenly across the anodes. However, if ions have any extra energy left from acceleration either by the gas jet or by voltages before Q2, they will arrive mostly on anode 4 or

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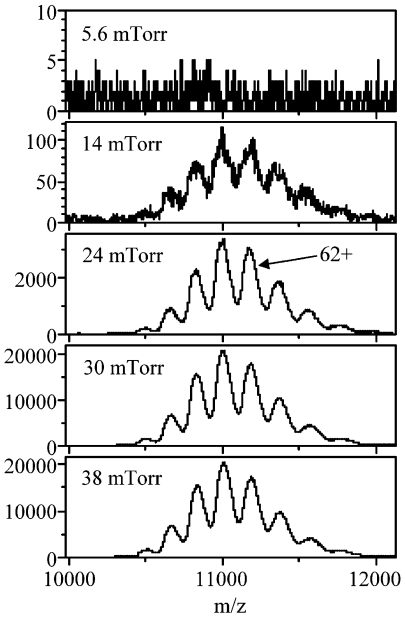

Figure 3. Segments of the nanospray spectra of proteasome at different Q0 pressures.

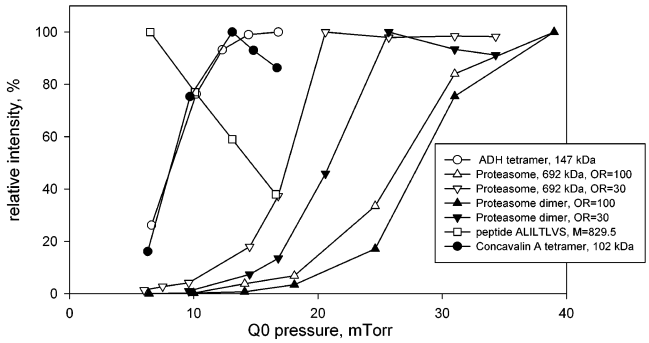

Figure 4. Intensities of several noncovalent complexes vs gas (N_2) pressure in Q0. Two plots for proteasome ($z = 58-66$) and its dimer ($z = 85-95$) correspond to different declustering voltage applied between the sampling orifice (OR) and skimmer, which was maintained at 30 V throughout these measurements. ADH tetramer was measured with $z = 24-30$, concavalin A tetramer with $z = 21-24$, and the peptide was singly charged.

miss the detector completely, as shown in Figure 1 (black parabolic line).

Ion transmission as a function of Q0 pressure was studied experimentally, and the results are illustrated by spectra in Figure 3 and plots in Figures 4 and 5. Segments of spectra of the proteasome 20S multimeric complex each recorded in 40 s at five different pressures are shown in Figure 3. No proteasome ions were recorded at the lowest pressure of 5.6 mTorr when no extra nitrogen was added into Q0; i.e., all gas was supplied through skimmer. Ion signal increases rapidly (exponentially) with pressure, until a plateau is reached at ~30 mTorr. The same trend is clearly visible in Figure 4 where relative signal intensity is plotted as a function of the Q0 pressure for several compounds. A few other conclusions can be drawn from this graph. First, ions with $M > 100$ kDa (concavalin A, ADH) cannot be recorded efficiently unless pressure is increased to ~15 mTorr. Second, the pressure required for thermalization depends not only on mass but also on the declustering voltages, i.e., the orifice and skimmer potentials, indicating that large ions may acquire significant energy

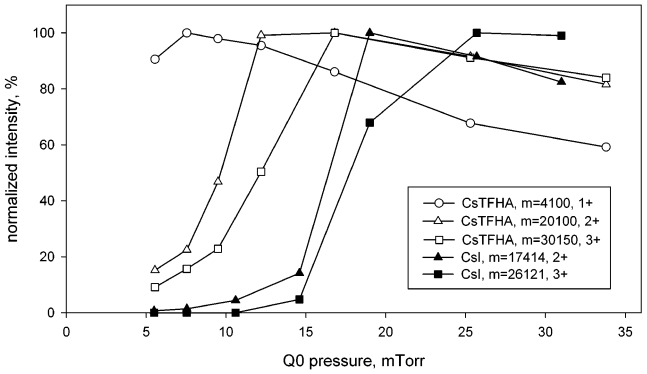

Figure 5. Intensities of several CsI and CsTFHA (cesium salt of tridecafluoroheptanoic acid) clusters vs gas (N_2) pressure in Q0. Positively charged clusters $Cs_m^{m+}(CsI)_n$ and $Cs_m^{m+}(CsTFHA)_n$ were monitored with charge $m = 1-3$.

both from the gas jet and from the electric field applied in the interface area. Finally, the intensity of small ions such as a peptide ALILTLVS ($M = 829.54$ Da) from our calibrating mixture decreases with increasing pressure, suggesting that pressure requirements may be contradictory for small and large ions. Obviously, high pressure leads to premature thermalization of small ions somewhere in the first half of the Q0, but the mechanism of the subsequent ion losses is still unclear.

Although the main subject of this investigation is large noncovalent complexes, it is worth mentioning that there are other classes of compounds that may also have compact conformations and therefore those ions may not be thermalized and efficiently recorded under standard pressure conditions. Salt clusters are one of them. Clusters were produced from concentrated solutions of two salts: 10 mM cesium iodide and 5 mM cesium salt of tridecafluoroheptanoic acid (CsTFHA), which is a convenient calibrant for mass analyzers having a wide m/z range.[25] Cluster ions up to m/z ~25 000 Th were detected from those solutions, and relative transmission of a few of them is plotted versus Q0 pressure in Figure 5. Again, only a small fraction of heavy clusters with $M > 15\,000$ Da is recorded at normal Q0 pressures, and the problem intensifies with mass. Clusters of CsI and of CsTFHA having similar masses (17 414 and 20 100 Da, 26 121 and 30 150 Da) behave differently, presumably due to a more compact structure of CsI. A CsI cluster with $M = 17\,414$ Da has a hard-sphere cross section of ~450 Å^2, assuming the bulk density of 4.21 g/cm^3. Given an initial velocity of 400 m/s from the gas jet expansion, simulations show that a pressure of nearly 20 mTorr is required for thermalization.

Collisional cooling of cluster ions was also studied by recording their "stopping curves" (Figure 6). Ions exit Q0 at 20 V and (if thermalized in Q0) should be stopped whenever Q1 rod offset (RO1) exceeds 20 V. At 9 mTorr, the heaviest ions retain tens of electronvolts energy, showing that they are not thermalized in Q0. Note that only those ions which are transmitted successfully through Q0 and IQ1 are sampled in this experiment.

Alternative Methods To Improve Collisional Cooling. Pressure increase is certainly the most straightforward but not the only practical solution to improve collisional cooling. If achieved by adding more gas into Q0, the turbopump can be

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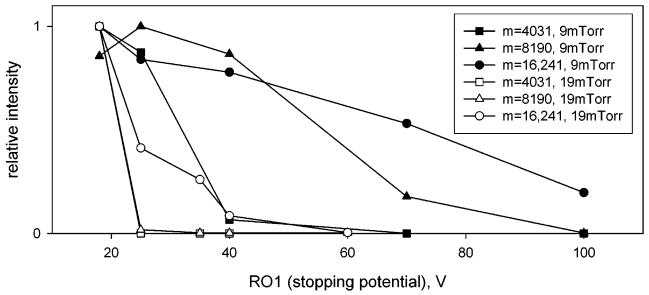

Figure 6. Q1 stopping curves for several singly charged CsI clusters at low and high Q0 pressure.

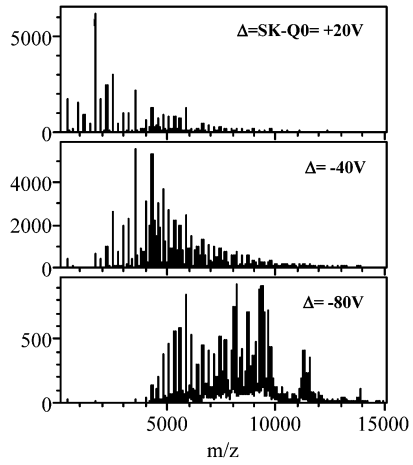

Figure 7. CsI clusters at different decelerating voltages between skimmer (SK) and Q0 rod offset. Normally this voltage is between +10 and +20 V.

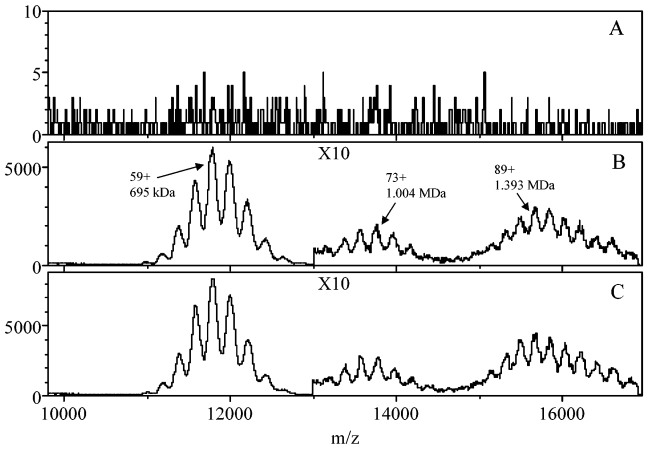

Figure 8. Spectra of proteasome 20S with (B) and without (A) trapping in Q0. Two minor components are the dimer of proteasome ($M \sim 1.39$ MDa) and an unknown impurity ($M \sim 1.0$ MDa).

overloaded. Permanent throttling of the pump eliminates this problem but reduces transmission of low-mass ions. Several other methods have been developed, which either do not have those problems or can be applied without hardware modifications.

1. Ions can be decelerated by a reversed electrostatic field between skimmer and Q0. Ions accelerated by a gas jet move against the electric field, and their axial energy component is reduced so that they have more time for thermalization in Q0. This method increases sensitivity in a limited mass range, as shown in Figure 7: higher deceleration enhances heavier clusters while rejecting the light ones.

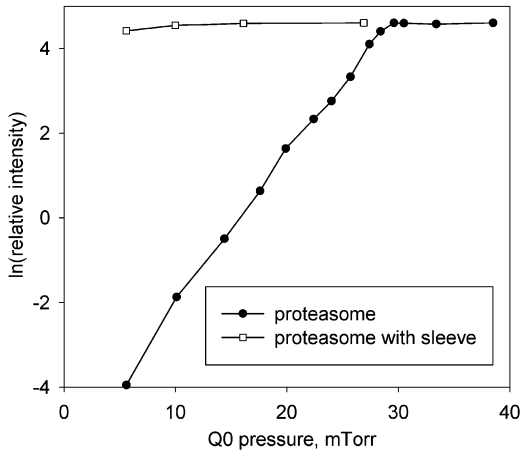

Figure 9. Intensity (log scale) of the proteasome signal ($z = 58-66$) vs Q0 pressure with and without a flow-restricting sleeve. The OR−SK voltage was 100 V.

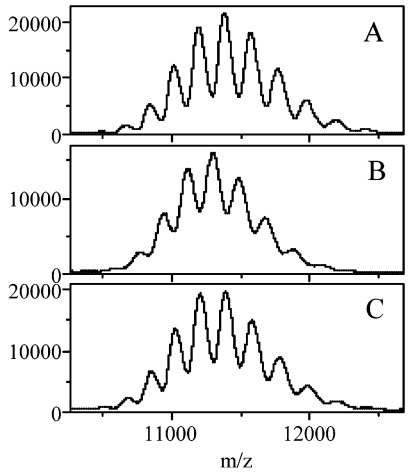

Figure 10. Nanospray spectra of proteasome 20S recorded with the sleeve in Q0 at different interface conditions: (A) interface pressure 0.71 Torr, Q0 pressure 5.6 mTorr; (B) interface pressure 1.6 Torr, Q0 pressure 18 mTorr; (C) same pressures as in (B), but the orifice voltage increased from 200 to 300 V, and skimmer voltage increased from 50 to 200 V.

2. Ions can be trapped in Q0 (by increasing the potential barriers at the ends) for as long as several seconds without significant losses. Thermalized ions are then ejected by dropping the potential of the exit aperture IQ1. Figure 8 shows nanospray spectra of proteasome 20S recorded at the regular Q0 pressure of 5.6 mTorr without (panel A) and with trapping ions (panel B), as well as the spectrum obtained at the increased pressure of 30 mTorr shown for comparison (panel C). Proteasome ions were trapped for 175 ms and then released in 25 ms; these cycles of trapping/releasing were repeated until the spectrum was recorded in 30 s. As is clear from Figure 8, the trapping method is almost as efficient in transmission as that involving the pressure increase. Since there is no axial field driving ions out of Q0 (except at the ends), some ions could stay in Q0 longer until they are released in the next few cycles.

The time of the full cycle (200 ms in this case) could be varied in a wide range from 100 ms to 5 s, but two considerations have to be taken into account. If the rf voltage of the Q0 quadrupole is stepped between two or more values in order to cover a wider m/z range, trapping/releasing cycles should be performed for

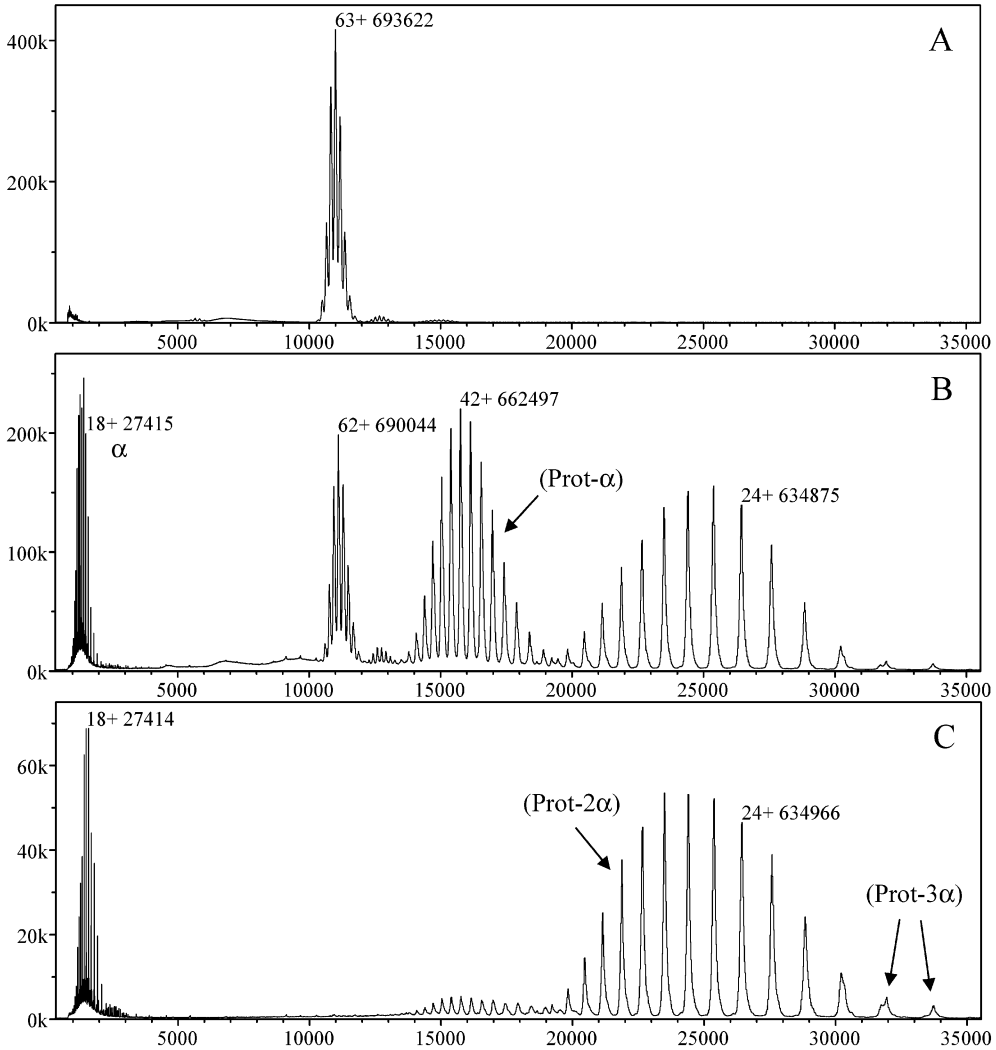

Figure 11. Nanospray spectra of proteasome 20S ($M \sim 692\,000$ Da), consisting of ($14\alpha + 14\beta$) protein subunits recorded at low, high, and very high declustering voltages. All three spectra were obtained with a sleeve around Q0, with Q0 pressure adjusted to 30 mTorr: (A) orifice at 100 V, skimmer at 40 V; (B) orifice at 300 V, skimmer at 200 V; (C) orifice at 350 V, skimmer at 250 V.

each rf voltage alternatively. Moreover, cycles should be long enough (~1 s) so that the majority of trapped ions can exit Q0 in a single release period; otherwise they may be lost during the next rf step. This effect is especially noticeable when lighter ions (e.g., monomers produced as fragments of noncovalent multimers) disappear from the spectra due to rf quadrupole cutoff. On the other hand, bunching of ions through trapping and releasing may affect ion recording by overloading the TDC if the ion current is too high.

3. By using a flow-restricting "sleeve" (described in the Experimental Section), pressure can be increased locally, without bringing extra gas into Q0 and overloading the turbo pump. Figure 9 demonstrates that transmission of the proteasome ions no longer depends on the Q0 pressure with the sleeve installed around the front section of the rods.

The sleeve allows ions of up to 1 MDa to be recorded without adding gas into Q0 (at moderate declustering potentials). If necessary, gas can be added to record even heavier ions or to use higher declustering potentials. Another advantage is that heavy ions are enhanced without simultaneous noticeable suppression of light ions.

Ion Transmission versus Interface Pressure. The first vacuum stage in our system is bounded by a 2-mm gap between the orifice and the tip of the skimmer. The expansion from atmospheric pressure forms a barrel-shaped supersonic jet, with the boundaries defined by shock waves of recompression.[26] In the usual configuration, the tip of the skimmer samples from the zone of silence upstream of the Mach disk shock wave, a region where the flow through the skimmer is unaffected by the background pressure in the surrounding area. As the background pressure is increased (by, for example, throttling the pump to reduce the pumping speed), the Mach disk moves closer to the orifice and the diameter of the Mach barrel decreases, but the pressure at the tip of the skimmer remains the same as long as it is within the zone of silence. Therefore we expect no difference in ion sampling or declustering (nor in pressure in the next vacuum stage) until the boundaries of the expansion begin to be sampled by the skimmer.

To investigate whether there was any advantage to having the ions pass through the higher pressure region of the shock wave

(26) Ashkenas, H.; Sherman, F. S. In *Rarified Gas Dynamics, Fourth Symposium*; De Leeuw, J. H., Ed.; Academic Press: New York, 1966; Vol. 2, p 84.

before entering the Q0 chamber, gas was added to Q0 (bringing the pressure to 25 mTorr) and then the valve was gradually closed to throttle the interface pumping speed. The pressure in Q0 was kept constant at 25 mTorr by reducing the extra flow into Q0. In this way we were able to vary the sampling conditions in the interface (in front of the skimmer) independently of the Q0 pressure. Up to a pressure of ~1.1 Torr, the Q0 pressure was constant; beyond 1.1 Torr, the flow into Q0 had to be decreased in order to maintain a constant pressure, indicating that the shock wave region was being sampled by the skimmer. With the CsI solution, which exhibited clusters up to greater than m/z 22 000 Th, we observed a loss in sensitivity by a factor of ~2-fold as the interface pressure was varied from 0.98 to up to 2.2 Torr.

In a separate experiment on another QqTOF instrument with a larger skimmer aperture (diameter 2.4 vs 1.5 mm), proteasome spectra were recorded with the sleeve in Q0 while the interface pressure was varied as described above. The presence of the sleeve guaranteed that proper collisional cooling was achieved even at lower interface/Q0 pressures, as demonstrated already in Figure 9. As the throttling valve was gradually closed, the interface pressure increased from 0.71 to 1.60 Torr with a simultaneous increase of Q0 pressure from 5.6 to 18 mTorr. Proteasome spectra were recorded as pressures were increased, but since the changes were small and gradual, only the spectra recorded at the smallest and largest pressures are shown in Figure 10. Ion signal decreases by ~25% and peaks get wider at the highest interface pressure (panel B) when the throttling valve is completely closed, and this region is pumped through skimmer only. However, both intensity and the peak shape can be almost restored by increasing the orifice and skimmer potentials (panel C). The above experiments strongly suggest that increasing the pressure in the interface region alone does not provide any benefit to the sampling and declustering process in this type of interface.

Applications. The described methods of improved collisional cooling developed for large ions made possible comprehensive studies of the proteasome 20S multimeric complex, which have been presented recently[27] and will be described in detail elsewhere.[28] Contrary to methods involving the increase of the interface pressure,[11−14] the above-mentioned methods do not reduce the ability to fragment large noncovalent complexes by increasing the interface potentials (declustering voltages). Figure 11 shows three spectra of proteasome 20S where by varying the orifice and skimmer potentials the degree of fragmentation was changed from negligible (panel A) to extensive (panel C), thus providing valuable structural information.

CONCLUSIONS

Heavy ions (M >50 000 Da or m/z >4000 Th) that are also compact appear to need additional collisions in order to be fully collisionally cooled and focused in an rf ion guide under standard conditions. The addition of a sleeve to Q0 offers the simplest method of accomplishing this. Although all experiments presented here were performed on the QqTOF instruments, most of the conclusions and methods are applicable to other types of mass spectrometers that require ion thermalization and focusing.

In a related investigation, we found that increasing the pressure in Q2 by decreasing the inlet and exit apertures improved the MS/MS sensitivity for large precursor ions at high collision energy, presumably for the same reasons of improved collisional cooling.

In our "*in-line*" interface configuration (vs "Z-spray"), no benefits of elevated interface pressure, independent of the increased pressure in Q0, were discovered.

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