Characterization of a High-Pressure Quadrupole Collision Cell for Low-Energy Collision-Induced Dissociation*

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A RF-only quadrupole collision cell of new design has been evaluated for use in tandem mass spectrometry experiments as a component of a triple quadrupole mass spectrometer. The new design permits operation at values of collision gas thickness higher by 1 order of magnitude than those used in most cells of this type. When operated at sufficiently high collision gas pressures, the transmission efficiency for precursor ions increases with increasing pressure, often to values greater than those observed in the absence of collision gas. Simultaneously, the attainable resolving power for fragment ions across the entire mass-to-charge ratio range, even for multiply charged precursors, also increases to the point where isomers of a quadruply charged fragment are resolved. The performance of the cell, judged in terms of yields and resolution of fragment ions, has been investigated as a function of the nature and pressure of collision gas, the kinetic energy of the precursor ions that enter the cell, and of the size and charge state of the precursors. The enhanced performance is explicable in terms of a marked deceleration of all ions that emerge from the cell to very low energies, probably a few tens of millielectronvolts, so that the cell effectively acts as an ion source for the second mass filter (fragment ion analyzer) to provide a spectrum of ions of fixed axial energy. The high transmission efficiency appears to arise from a collisional focusing effect analogous to that exploited in three-dimensional RF ion traps. The low axial energies imply that ion transit times through the cell are sufficiently long (several milliseconds) that, in precursor ion experiments where the first mass filter is scanned, a hysteresis effect is observed. This implies that in this operating mode compromises must be sought between scan speed and quality of peak shape. Examples are given of spectra obtained under realistic operating conditions that employ flow injection of samples. (*J Am Soc Mass Spectrom* 1994, 5, 1042–1063)

The use of tandem mass spectrometry [1, 2] for structural determinations requires that the mass-selected precursor ion be fragmented to structurally informative fragment ions. The precursor ions must be sufficiently excited that they subsequently decompose on the time scale of the apparatus [3]. A number of ion activation methods have been examined in recent years, including collisional activation (CA) by gaseous targets that leads to collision-induced dissociation (CID) [4, 5], surface-induced dissociation (SID) that arises from collisions with solid [6–8] or liquid [9] targets, electron capture-induced dissociation of multiply charged cations [10, 11], electron collision-induced dissociation [12–14], and photodissociation [15–18]. The most widespread method of ion activation is that of CA via gaseous targets, because it is the simplest and cheapest method to implement and has been shown to be widely applica-

ble. The use of sector instrumentation allows the study of collisions up to 100 keV [19], and in one case [20] a specially designed collision cell permitted study of CID reactions over the (laboratory frame) energy range from 0 to 4 keV. The development of the triple-quadrupole tandem mass spectrometer [21] [$Q_1q_2Q_3$, where Q_1 and Q_3 are quadrupole mass filters and q_2 is a RF-only quadrupole collision cell] and the observation of high-efficiency CID in q_2 [22] extended the range of collision energies that could be studied routinely to the 1–100-eV regime.

Many studies have been undertaken to examine the mechanisms of CA in both energy regimes [5, 23–25]. As emphasized by Shukla [26] the time scale of the ion–target interaction, defined by the velocity of the precursor ion, is probably as important as the center-of-mass energy of the ion–target collision pair. The interaction time for the collision is a defining parameter for the type of excitation that can result, for example, slow collisions tend to be electronically adiabatic and thus are restricted to rotational–vibrational excitation. The dependence of electronic transition probab-

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ity on interaction time is qualitatively described by the Massey adiabatic criterion [27], which has been discussed in the present context [28]. Thus, for reasons of both energy and interaction time, the CA processes that occur in the low-energy experiments reported here involve exclusively rotational–vibrational excitation via internuclear momentum exchange, that is, with appreciable scattering. The velocity of the precursor ions, together with the physical dimensions of the apparatus, also determines the extent of the reaction time scale accessed and thus the relative importance of various reaction types, for example, longer reaction times tend to favor fragmentations that involve rearrangements over simple bond cleavages.

The translational energies (both mean values and spreads) of the fragment ions that emerge from a collision cell present a problem for all mass spectrometers that are “tandem in space,” for both high-energy CID in sector instruments and low-energy CID in quadrupole collision cells. This problem in the latter context is a major focus of the present work and requires further discussion. In the special case that the dissociation reactions proceed without CA, the mean translational energy T_f of a fragment of mass m_f , formed from a precursor of mass m_p and translational energy T_0 , is given by [3]

$$T_f = (m_f/m_p) \cdot T_0 \quad (1)$$

(For simplicity, only singly charged precursor and fragment ions are considered here). The transformation of precursor ion internal energy to relative translational energy of the fragment ions results [3] in a distribution of energies around T_f . The width of this distribution, for the energy component along the original precursor ion trajectory, is subject to an amplification effect [3] whose magnitude varies as $T_0^{1/2}$. These energy widths lead to poor fragment ion resolution in fragment ion spectra obtained by translational energy spectroscopy [3] and are the cause of other problems for sector-based tandem mass spectrometers, for example, interference peaks that limit the precursor ion mass resolution for linked scans at constant B/E [29]. However, kinetic energy release is generally of much less consequence for quadrupole-based tandem instruments that operate at considerably lower values of T_0 .

The fact that the mean value of T_f varies with m_f (eq 1) is not a problem for sector-based instruments (and indeed forms the basis of the measurement of m_f), but is of practical concern for instruments in which the collision cell and fragment ion analyzer are quadrupoles (q_2 and Q_3 , respectively). This problem arises because practical realization of the theoretical resolving power of a quadrupole mass filter requires that the ion experience a minimum number of RF cycles in order that stable trajectories may be distinguished clearly from unstable ones [30]. If the offset potential V_3 of the quadrupole fragment ion analyzer

Q_3 is set at a value that slows down the precursor ions sufficiently that good resolution is obtained, the lower-mass fragment ions will have insufficiently large values of T_f (eq 1) to efficiently traverse the fringe fields. This well-known problem is usually circumvented, for fragmentations that occur in the absence of CA, by scanning V_3 synchronously with m_f in a linear relationship derived from eq 1. This strategy alleviates the problem of transmission of fragment ions of varying masses m_f and, provided that V_3 is adjusted appropriately, the velocities of all ions through the fragment ion analyzer are also sufficiently low that adequate resolution is obtained for all m_f values. For fragment ions with mass-to-charge ratio values greater than that of a multiply charged precursor ion, it becomes increasingly difficult in practice to find suitable compromises for functional relationships between m_f and V_3 that satisfy both the requirements of good transmission efficiency and ensure sufficiently low ion velocities for adequate resolution. In extreme cases of high ion velocities the peaks are sufficiently broad and asymmetric that uncertainties in mass-to-charge ratio assignments become significant.

The problem, in the analysis of fragment ions formed without CA are greatly magnified for CID reactions as a result of conversion of part of T_0 into internal energy. In the case of high-energy (kiloelectronvolt range) CID, exploited in sector instruments, these energy shifts can be surprisingly large especially for low-mass targets such as helium [31–33]. The corresponding shifts in T_f (via eq 1) give rise to misassignments of m_f if only translational energy spectroscopy [3] is used, but not in the case of double-focusing analyzers as a result of the velocity focusing properties. However, these energy losses can cause problems for fragment ion intensities for four-sector tandem instruments [34]. A similar situation exists in the case of low-energy (few tens of electronvolts) CID in triple quadrupole or sector-quadrupole instruments, because the appropriate linkage of V_3 to m_f is now even more uncertain than for the case of metastable ion fragmentations (no CA), discussed previously. Shushan et al. [35] showed that under carefully controlled CID conditions the values of T_f for some small precursor ions ($m_p < 200$) were given by $[T_0 \cdot (m_f/mp)^2]$ rather than by eq 1. By linking V_3 to m_f in accord with this experimentally determined energy variation, large improvements in peak width and symmetry were achieved [35]. However, the conclusions for this special case are not applicable to the wide variety of experimental conditions used in analytical practice, especially the range of multiple collision conditions used to optimize structurally informative fragmentations for different precursor ions of widely varying masses and structural types. As a result it is particularly difficult to optimize peak widths and shapes concurrently with transmission efficiency for quadrupole analyzers used to record fragment ion spectra under CID conditions. For example, a partly arbitrary scan function with two tuning param-

ters $V_{\min}$ and k (potential per unit mass) has been used to this end [36]:

$$V_3 = (m_t/m_p) \cdot (V_s - V_2) + V_2 - (V_{\min} + k \cdot m_t) \quad (2)$$

where V_s is the potential of the region from which the precursor ions are accelerated into the mass spectrometer. This region is the ion source if the ions are created in the vacuum chamber, but in the case of atmospheric pressure ionization (API) sources, V_s is the potential of the region (q_0 in the present apparatus; see succeeding text) maintained at sufficiently low pressure that the mean free path is long enough that the ions can be accelerated by the applied field with minimal perturbation by collisions. V_2 is the offset potential of the collision cell q_2 . If the slope parameter k is zero and if no energy losses occur as a result of CA in q_2 , $eV_{\min}$ is the operator-selected translational energy of all fragment ions within the fragment ion analyzer. In practice $V_{\min}$ and k were adjusted empirically [36] to optimize transmission efficiency and peak shape over the desired range by using a standard precursor ion under collision conditions estimated to be appropriate for the unknown. Unfortunately, the optimum tuning conditions (values of k and $V_{\min}$) can vary significantly between different precursor ions, even those with similar masses.

These operational problems of tandem mass spectrometers incorporating q_2Q_3 quadrupole assemblies could be avoided if the extreme condition could be achieved where all the ions in the collision cell are reduced to very low axial translational energies, independent of mass. Such a condition would require operation at much higher collision gas pressures than are usually employed and would result in the collision cell acting much as an ion source for the fragment ion analyzer. The collision cell q_2 in the triple quadrupole mass spectrometer used in the present work is of a novel design [37] that permits operation at pressures up to approximately 10^{-2} torr—an order of magnitude higher than that used in most conventional cells of this type and sufficient for the energies of the emerging ions to be low and approximately constant. We recently reported initial experiments designed to characterize the behavior of this cell in which reactive collisions were observed when ammonia was used as the collision gas [38]. An unexpected phenomenon of increased ion transmission at higher collision gas pressures was noted [38]. Along with the increase in precursor ion transmission, the attainable resolution of the fragment ions by the fragment ion quadrupole analyzer was also improved, consistent with a slowing of the ions within the collision cell to low translational energies. It is believed that the increased transmission was caused by collisional focusing of the ions, including strongly scattered fragment ions, toward the main axis of the linear quadrupole cell, akin to the effect of the buffer gas used in quadrupole ion trap instruments [39-41]. Such damping in quadrupole ion traps is

optimized if the mass of the buffer gas is as small as possible (the reason for the choice of ammonia in the preliminary report [38]) so that many elastic or near-elastic collisions, each of which alters the momentum of the ion only slightly, combine to alter the ion trajectories within the trap. This effect of low-mass background gas can be modeled [42] as that of a continuous viscous medium that results in damping of the ion trajectories until they collapse into the center of the trap. (Heavier gases have been used in quadrupole ion traps primarily to promote CID via inelastic collisions [43, 44].) Such collisional focusing also has been demonstrated in linear RF-only quadrupoles [45] used to funnel ions from an API source to a mass spectrometer vacuum chamber. Such a device (denoted q_0 here) corresponds to the source of precursor ions whose mean potential was denoted V_s in eq 2.

The choice of collision gas for CA has also been a point of contention. The larger the mass of the target, the larger is the center-of-mass energy and thus the larger is the fraction of the laboratory-frame collision energy that can be converted to internal energy while satisfying conditions of momentum conservation. This requirement for higher-mass targets, to promote CID, runs counter to that for collisional focusing within an RF-only quadrupole cell as previously discussed. In early tandem mass spectrometry experiments the effects of target gas on charge exchange and ion scattering for kiloelectronvolt precursor ions were reported [46]. Other high-energy experiments in multisector instruments [47-51] and low-energy experiments in quadrupole-based instruments [52-54] have permitted conclusions as to the best gas to use for CID in a number of different energy regimes. In an investigation by Schey et al. [54], it was concluded that the use of heavy mono- or polyatomic target species in low-energy CID of polyatomic ions may not always be advantageous, and that at least some of these unexpected findings could be attributed to increased scattering of the fragment ions by the more massive targets.

In the present work we report the effects of different collision gases and of other operating parameters on the CID of several precursor ions within the new collision cell [37, 38]. Of particular interest are compromises to simultaneously optimize the transmission efficiency of ions, the extent of fragmentation, and the resolution obtained for the fragment ions.

EXPERIMENTAL

All peptides used, except for the Lys₆, were obtained from Sigma Chemical Co. (St. Louis, MO) and were used as received. Lys₆ was a kind gift from Dr. D. Henning of the Biotechnology Research Institute, Montreal, Canada. Solutions were prepared at a concentration of 1 mg mL⁻¹ in 15% acetic acid in aqueous acetonitrile (1:1 v/v). Injections of 2 μ L were made into a solvent stream [50% acetonitrile in water con-

taining 0.1% (v/v) trifluoroacetic acid, 10 $\mu\text{L min}^{-1}$, via a Hewlett-Packard (Palo Alto, CA) 1090L high-performance liquid chromatography system. The liquid stream was delivered directly, with no split, to the Ionspray[®] source of the Sciex (Thornhill, Ontario) API III + triple quadrupole mass spectrometer $q_0q_1q_2q_3$. Here q_1 and q_3 are mass-filter quadrupoles, q_0 is a RF-only quadrupole used [45] to funnel ions from the API source aperture into q_1 , and q_2 was either a high-pressure RF-only quadrupole collision cell [37] or a cell of open design into which the collision gas was admitted as a free-jet expansion with a well-defined density profile [55]. The potential on the spraying needle was 5 kV. The bandpass windows of the mass filters q_1 and q_3 were set for each experiment to minimum values consistent with obtaining adequate signal-to-noise ratios. Fragment ion spectra were acquired by mass-to-charge ratio selection of the precursor ion in q_1 and scanning q_3 stepwise. For cases where higher resolution was desired, the step size used was 0.1 Th (thomsons) [56] with a dwell time of 1 ms.

Argon and nitrogen (Canadian Liquid Air, Montreal, Canada) were used at > 99% purity. All other collision gases used in these experiments were obtained at 99% purity from Matheson (Toronto, Ontario). Gases were admitted to the system by using a custom-built inlet manifold that could be evacuated and flushed repeatedly to minimize carryover from the previous gas. The pressure of the gas in the collision cell was monitored by using a Baratron[®] capacitance manometer (MKS Instruments Inc., Burlington, MA) upstream of the cell and connected to the cell by a calibrated flow restrictor (Lee Viscojet[®] Lee Co., Westbrook, CT). Although this arrangement does not provide an absolute value of the collision gas pressure (or equivalently the collision gas thickness) in the cell, it serves as an adequate guide for comparison purposes. The manometer-flow restrictor combination was initially calibrated in terms of collision gas thickness (CGT) by using argon, and corrections were made to account for the relative responses of the various gases used according to calculations based on information supplied by the manufacturers of the calibrated flow restrictor [57].

RESULTS AND DISCUSSION

Fragment Ion Scanning with a Fixed Offset Potential for Q_3

Figure 1 shows fragment ion spectra of the $[M + 2H]^{2+}$ ions of Tyr⁸-substance P (RPKPQQFYGLM-NH₂; molecular mass 1362.7 u). The original q_2 cell was an open design [55], to which the collision gas was admitted as a free-jet expansion with a well-defined density profile that permitted values of collision gas thickness (CGT) up to 50×10^{13} molecules cm^{-2} . In this range, attenuation of precursor ion intensity was observed as

the CGT was increased, and the problems [35] of matching V_3 to m/z (see discussion of eq 2) were evident. However, no attempt was made to link V_3 with m/z in the experiments used to obtain the fragment ion spectra shown in Figure 1a and b. Rather, for the purposes of comparison with the new cell, V_3 was maintained approximately 2 V below V_2 . For a low-collision energy [$2e(V_0 - V_2)$], of 10 eV (Figure 1a) only the fragment ions of highest translational energies (high mass-to-charge ratio) were transmitted, but their peak shapes and widths are acceptable as a result of their low velocities through Q_3 . At a collision energy of 200 eV, under otherwise identical conditions, fragment ions of even low mass-to-charge ratio had sufficient translational energy to be transmitted into Q_3 , but the widths and shapes of the peaks are unacceptable (Figure 1b) which reflects their high velocities through Q_3 . The differences between Figure 1a and b with respect to relative intensities of precursor and fragment ions reflect transmission efficiencies much more than differences in fragmentation efficiency. When V_3 was ramped with m/z in a linear fashion (data not shown) the spectra corresponding to Figure 1a and b showed increased transmission at low mass-to-charge ratio, but it was not possible to obtain good transmission and peak shape across the entire spectrum even at low collision energies. In contrast the spectra obtained by using the new high-pressure cell [37], at CGT values approximately 10 times higher than for the old cell but with $(V_2 - V_3)$ again held constant at 2 V show excellent signal-to-noise ratios and peak shapes across the entire range (Figure 1c and d). At 60 eV (Figure 1d) the collision conditions were such that extensive secondary fragmentation to smaller ions had occurred, but the peaks at higher mass-to-charge ratio values are still well defined.

The question of sensitivity is addressed in the comparison made in Figure 2 of fragment ion spectra of the $[M + 2H]^{2+}$ ions of Glu-fibrinopeptide B (EGVNDNEEGFFSAR, molecular mass 1569.6 u). At the 13-pmol level the data obtained with the old q_2 cell under its own optimum conditions provides no structural information (Figure 2a). At the 130-pmol level (Figure 2b) some fragment ion peaks are sufficiently well defined that mass measurements are possible, but the peak shapes at higher mass-to-charge ratio values are quite poor which results in considerable ambiguity in the assigned mass-to-charge ratio values. [It is essential for peptide sequencing work that masses be reliable to within 0.3 u or so. The seeming discrepancy between the apparently acceptable signal-to-noise ratios in the full range spectral plots (Figure 2a and b) and the same peaks clearly limited by ion statistics in the enlarged insets is a consequence of the plotting software used to generate greatly condensed analog versions of detailed digitized data.] In contrast, the spectrum obtained with the new q_2 cell at the 13-pmol level (Figure 2c) shows a noise level of approximately 5% of the intensity of the surviving pre-

cursor ion and in fact a sufficient number of well-defined peaks are present in this spectrum that almost the complete sequence could be deduced. At the 130-pmol level (Figure 2d) the new cell provided data that established the sequence with certainty via redundant information from the **y** and **b** type fragment ions. [Note that in this paper the Roepstorff and Fohlmann notation [58] for peptide fragment ions, which incorporates primes to denote additional hydrogen atoms and/or protons, is not used despite its ready extension to multiply charged ions. Instead the more compact notation proposed by Biemann [59] is used, with the understanding that the extra charges in, for example, species denoted as **y**ⁿ⁺ and **b**ⁿ⁺ correspond to (n - 1) additional protons.]

Figures 1 and 2 exemplify a much larger body of comparison data, obtained both immediately before and after the replacement of the old q_2 cell by that of the new design [37]. The choice of these particular examples was made to illustrate the problem of axial energies of ions entering Q_3 . The remainder of the present paper is devoted to a more detailed investigation of the performance characteristics of the new cell.

Transmission of Precursor Ions: Effect of Charge

The transmission efficiency of the high-pressure collision cell was investigated for multiply protonated protein species as a function of collision energy, collision gas thickness, and collision gas type. The most extensive series of experiments involved the eight most abundant $[M + nH]^{n+}$ ions formed by ion spray ionization of ubiquitin (molecular mass 8564 u). Figures 3 and 4 summarize the results obtained by using fixed collision potentials [differences ($V_0 - V_2$) between offset potentials of q_0 and q_2] of 5 and 30 V, respectively. At any value of the collision potential the collision energy increased in proportion to the charge on the precursor ion, but the former parameter is that directly selected by the operator while the collision energy is not necessarily known ahead of time for an unknown sample. (Note that the pressure within the cell was sufficiently high over most of the CGT range that this initial collision energy dropped to a low value after several collisions [60].) The transmission efficiencies were measured relative to the respective values with no gas in the collision cell as a function of collision gas thickness for argon. Also measured were the corresponding data (not shown) for collision potentials of 10 and 20 V. Before discussing these data it is appropriate to note that, in the absence of collision gas, transmission efficiency through the entire $q_0Q_1Q_2Q_3$ train increased with increasing values of ($V_0 - V_2$), which presumably reflects the effects of fringe fields. The extent to which this effect influenced the results obtained with collision gas present is not known.

The transmission data obtained at 5-V collision potential (Figure 3) exemplify well the collisional focusing effect in the absence of significant CID losses. Note that the range of CGT values studied here is much

larger than that used (up to approximately 50×10^{13} atoms cm^{-2}) in a previous study of transmission losses in a similar instrument [60]. The present work (Figures 3 and 4) did not investigate this range in detail because these lower CGT values were not reproducible or stable. However, it was confirmed that in this low range the precursor ion intensity did indeed decrease as the CGT was raised. The lowest CGT value studied in detail (other than zero) was 150×10^{13} atoms cm^{-2} , and in all cases the transmission efficiency for the $[M + nH]^{n+}$ ions was slightly higher at this CGT value than at zero. However, at approximately 170×10^{13} atoms cm^{-2} a rapid increase of transmission with larger CGT occurred, to a well-defined maximum at $220\text{--}260 \times 10^{13}$ atoms cm^{-2} , which presumably reflects the collisional focusing effect. Thereafter the transmission decreased with increasing CGT as a result of scattering and other loss mechanisms. The ratio of the maximum transmission to that at zero CGT increased slightly with increasing charge, from a factor of approximately 1.6 for the $n = 5, 6$ ions to approximately 3 for the species with $n = 11, 12$. It is of interest that the collisional loss cross sections, measured previously [60] in the lower CGT range, increased significantly with charge in a fashion similar to the qualitative trend observed here for the collisional focusing effect. Although a detailed understanding of the latter effect in the present apparatus is lacking, the qualitative parallel between the two different phenomena does support the suggestion that similar near-elastic collisional processes are at the root of both.

At 5 V collision potential, CID played little role in the observations for $[M + nH]^{n+}$ ions of ubiquitin (spectral data not shown). The same was true of the results obtained (not shown) for a collision potential of 10 V, which were very similar to those shown in Figure 3 except that the transmission maxima were shifted to CGT values higher than those shown in Figure 3 by $20\text{--}30 \times 10^{13}$ atoms cm^{-2} . It was possible to make comparisons between the transmission curves for the $n = 5, 6$ ions at 10-V collision potential with those for the $n = 10, 12$ ions at 5 V (i.e., comparisons at fixed initial collision energies). The corresponding curves were very different, for example, that for $[M + 6H]^{6+}$ at 10 V was much more like that for the same ion at 5 V than that for $[M + 12H]^{12+}$ at 5 V. This suggests that when dissociation is not a significant factor, the initial collision energies of the ions as they enter the cell (controlled by the collision potential) soon are reduced to low values with a narrow dispersion [60], and the observed collisional effects then mainly reflect conformational differences amongst the ions. The larger collision effects for higher charge states, observed here and previously [60], could reflect larger ion-induced dipole attractive forces via the Langevin model cross sections and/or more open conformations for the more highly charged species.

At higher collision potentials exemplified by Figure 4 (30 V), CID was a major fate of the ubiquitin ions particularly for the more highly charged species, for

Figure 1. Fragment ion spectra of the doubly protonated peptide RPKPQQFYGLM-NH₂ (monoisotopic mass of peptide 1362.7 u) obtained by flow injection of 2 μ L of a 1-mg mL⁻¹ solution (50:50 aqueous acetonitrile with 15% acetic acid) into a 10- μ L min⁻¹ solvent stream of 50:50 aqueous acetonitrile that contained 0.1% trifluoroacetic acid. The precursor ion analyzer Q₁ was operated with a 3-Th window to transmit the most intense isomers of the [M + 2H]²⁺ species, and the fragment ion analyzer Q₃ was operated with resolving power to give peaks approximately 1 Th wide near the base for precursor ions in the absence of collision gas. Argon was used as collision gas. In all cases the potential V₃ of Q₃ was maintained fixed at 2 V below that of the collision cell q₂. Conventional collision cells with collision gas thickness 38×10^{13} atoms cm⁻² at (a) 10- and (b) 200-eV collision energy. High-pressure collision cells 40 eV collision energy, with collision gas thickness 350×10^{13} atoms cm⁻² at (c) 40- and (d) 60-eV collision energy.

which the initial collision energies for ions entering the cell were as high as 360 eV. For the ions of lower charge some vestige of the collisional focusing effect is still apparent in the transmission curves. The results for a collision potential of 20 V (not shown) repre-

sented a similar compromise, but with the collisional focusing more competitive with CID, as expected.

A series of experiments also was undertaken in which the initial translational energy of the various [M + nH]ⁿ⁺ ions was maintained constant at 100 eV

Figure 2. Fragment ion spectra of the doubly protonated peptide EGVNDNEEGFFSAR (monoisotopic mass of peptide 1569.6 u), obtained by flow injection of 2 μL of a 1-mg mL^{-1} solution (50:50 aqueous acetonitrile with 15% acetic acid) into a 10- $\mu\text{L min}^{-1}$ solvent stream of 50:50 aqueous acetonitrile that contained 0.1% trifluoroacetic acid. The precursor ion analyzer Q_1 was operated with a 3-Th window to transmit the most intense isomers of the $[M+2H]^{2+}$ species, and the fragment ion analyzer Q_3 was operated with resolving power to give peaks approximately 1 Th wide near the base for precursor ions in the absence of collision gas. Argon was used as collision gas. Conventional collision cells with 140-eV collision energy, collision gas thickness 38×10^{13} atoms cm^{-2} , and (a) 13 and (b) 130 pmol injected. High-pressure collision cells with 60-eV collision energy, collision gas thickness 350×10^{13} atoms cm^{-2} , and (c) 13 and (d) 130 pmol injected.

by appropriate manipulation of the collision potential. This value of collision energy was chosen as representative of the range in which CID was not important for these ubiquitin ions under the present conditions. The transmission curves are presented in Figure 5. The

effect of charge on the magnitude of the collisional focusing effect is still apparent and is accompanied by a decrease in the optimum CGT value and a sharpening of the maximum with increasing charge. This behavior is qualitatively consistent with the previously

Figure 3. Transmission efficiencies for the $[M + nH]^{n+}$ ions of ubiquitin ($n = 5-12$) as a function of CGT for argon for a collision potential of 5 V (initial collision energies proportional to charge on precursor ion). The experiments were conducted by using flow injection under the conditions described for Figure 1. The relative magnitudes of the intensity scales for the eight ions are meaningful.

discussed idea, that the ions with higher charges have higher cross sections for the near-elastic collisions believed to be responsible for the phenomenon.

Transmission of Precursor Ions: Effect of Initial Collision Energy

The effect of initial collision energy on the transmission of the precursor ions can be extracted from the extensive experiments on multiply protonated ubiquitin that are summarized in Figures 2-5. Figure 6 shows

results of a study on the transmission curves of a much smaller precursor ion, doubly protonated gramicidin S (1141.1 u). At 60-eV initial collision energy CID was the dominant effect (Figure 6), whereas at 20 eV the collisional focusing effect dominated. The transmission curve obtained at 40 eV is clearly a superposition of these two effects that, under these conditions, competed on approximately equal terms. It is worthwhile to emphasize again that the initial collision energy, defined by the potential difference that accelerated the precursor ions into the collision cell, only applies to the

Figure 4. Transmission efficiencies for the $[M + nH]^{n+}$ ions of ubiquitin ($n = 5-12$) as a function of CGT for argon for a collision potential of 30 V (initial collision energies proportional to charge on precursor ion). The experiments were conducted by using flow injection under the conditions described for Figure 1. The relative magnitudes of the intensity scales for the eight ions are meaningful.

lower CGT values and to those collisions that occur near the cell entrance. At higher CGT values the ions are subsequently slowed down by the multiple collisions [60].

Transmission of Precursor Ions: Effect of Collision Gas

Previously the transmission characteristics of the triply protonated bovine insulin B chain molecule were reported as a function of ammonia CGT at a variety of

collision energies [38]. The transmission curves for the same species at 90-eV initial collision energy with a variety of different collision gases are shown in Figures 7 and 8. Note that bovine insulin B chain is a considerably smaller molecule (3496 u) than ubiquitin (8564 u), and thus would be expected to be more susceptible to CID. For the noble gas collision targets (Figure 7) the principal loss mechanisms for the precursor ions are likely to be scattering and CID. At higher target masses the CID efficiency is anticipated to become greater, as is the extent of scattering losses,

Figure 5. Transmission efficiencies for the $[M + nH]^{n+}$ ions of ubiquitin ($n = 5-12$) as a function of CGT for argon for an initial collision energy of 100 eV for the ions entering the collision cell. The experiments were conducted by using flow injection under the conditions described for Figure 1. The relative magnitudes of the intensity scales for the eight ions are meaningful.

whereas that of the collisional focusing effect should be smaller [39-42]. The effects of the compromises between these opposing trends are evident in the comparison summarized in Figure 7. Because neon is not efficiently pumped by the cryopump system in the instrument used for these experiments, only a limited range of CGT values could be investigated.

In the case of polyatomic targets, such as those for which the transmission data are summarized in Figure 8, an additional potential loss mechanism for the precursor ion is adduct formation with one or more collision gas molecules. This process was shown previously

[38] to be a major loss mechanism for ammonia as collision gas, and this is reflected here in the steep drop in transmission of the $[M + 3H]^{3+}$ ion from bovine insulin B chain for ammonia CGT values above approximately 380×10^{13} molecules cm^{-2} (Figure 8). Nitrogen was expected to behave in a fashion somewhat similar to that of argon (nonreactive gases of low mass), and this expectation is borne out reasonably well by a comparison of Figures 7 and 8, bearing in mind that nitrogen has a significantly lower mass than argon. The transmission behavior of the triply protonated bovine insulin B chain with SF_6 collision gas

Figure 6. Transmission efficiencies for the $[M + 2H]^{2+}$ ion of gramicidin S as a function of CGT for argon collision gas at initial collision energies of 20, 40, and 60 eV. The experiments were conducted by using flow injection under the conditions described for Figure 1. The intensity scales are normalized to the maximum (shown in the upper right of each trace) for each case.

(Figure 8) resembles that for xenon (Figure 7), although the drop to zero transmission occurs at even lower CGT values than for xenon. This was at first thought to reflect clustering to SF_6 as a significant loss mechanism in addition to scattering and CID, but full scan mass spectra revealed no evidence for this. The strong tendency for ammonia to form cluster adducts with protein ions [38] most likely reflects its ability to form hydrogen bonds, rather than purely van der Waals interactions. (It should be noted that the correc-

tion of the CGT indicated values from the argon calibration is particularly uncertain for large polyatomic molecules, so the apparently higher collision cross sections for SF_6 than for xenon may not be valid.) The results obtained for bromomethane as collision gas (Figure 8) are puzzling. At low CGT values the behavior resembles that of the targets of comparable mass (krypton, xenon, and SF_6), but above approximately 250×10^{13} molecules cm^{-2} the transmission curve resembles that for argon or nitrogen. No evidence for

Figure 7. Transmission efficiencies for the $[M + 3H]^{3+}$ ion of bovine insulin B chain as a function of CGT for noble gas targets for an initial collision energy of 90 eV for the ions entering the collision cell. The relative magnitudes of the intensity scales for the four gases are meaningful.

adduct formation was found. We have no convincing explanation for this behavior.

In summary the opposing requirements for efficiency in collisional focusing and for collisional activation for CID in the new high-pressure cell demand that a compromise be struck with respect to the mass of the target gas. In our experience argon is an effective choice for those ions sufficiently small that they realistically can be expected to yield informative fragment ion spectra under low-energy CID conditions.

Yields and Resolution of Fragment Ions

The nature of the problem of fragment ion resolution is illustrated in Figure 9, which compares two fragment ion spectra of a doubly protonated octadecapeptide (morphine modulating peptide AGEGLSPFWSLAAPQRF, molecular mass 1920.0 u),

obtained by using the same instrument except that in one case the conventional low-pressure collision cell [55] q_2 was used, whereas for the other the high-pressure cell [37] was installed. These spectra were obtained five weeks apart, under nominally identical conditions of flow injection (2.5 nmol injected) and electrospray ionization, as well as Q_1 and Q_3 settings (4- and 1.5-Th windows, respectively) and Q_3 scan speed. However, the collision conditions were separately optimized for each of the two cells (140 eV and argon CGT 38×10^{13} atoms cm^{-2} for the conventional cell and 60-eV and argon CGT 350×10^{13} atoms cm^{-2} for the high-pressure cell). In the case of the conventional cell it was not possible to empirically adjust the relationship between m_f and V_3 such that good peak shapes were obtained across the entire mass-to-charge ratio range from 100 to 1800. The spectrum shown (Figure 9a) represents the particular compromise, with

Figure 8. Transmission efficiencies for the $[M + 3H]^{3+}$ ion of bovine insulin B chain as a function of CGT for polyatomic gas targets for an initial collision energy of 90 eV for the ions entering the collision cell. The relative magnitudes of the intensity scales for the four gases are meaningful.

respect to both transmission and resolution, chosen to provide the performance indicated in the detail shown for the y_{13} fragment ion (m/z 1494) for the high mass-to-charge ratio range above the precursor ion at m/z 961, while maintaining some degree of performance at the low end (exemplified by the a_5 fragment ion at m/z 400). No such problems were encountered with the new cell (Figure 9b), which requires simply that V_3 be maintained at some constant value (2 V or so) below V_2 (the relative values are reversed for negative ions). The markedly superior performance with respect to peak shape (unit mass resolution at half-height) across the entire range was thus obtained with far less effort than was involved in optimizing the conditions for the conventional collision cell. This feature reflects the fact that all ions (precursors and fragments over the entire mass-to-charge ratio range) that emerge from

the new cell [37] operated at CGT values in the range where the collisional focusing effect was operative ($350\text{--}400 \times 10^{13}$ atoms cm^{-2}) possess a common low axial energy. Crude stopping curve experiments, conducted by manipulating the offset potential V_3 of Q_3 , could not distinguish this common energy from zero. Under the high CGT conditions the q_2 cell effectively behaves like an ion source for Q_3 , as discussed in the Introduction. In addition to the greatly improved peak shapes, the overall signal level in Figure 9b was more than 1 order of magnitude higher than that in Figure 9a.

The effect of operating parameters for the collision cell on the attainable resolution at useable sensitivity was investigated for the $[M + 2H]^{2+}$ ion from Lys₆ (molecular mass 786.6 u). Figure 10 shows three regions extracted from the full-scan (m/z 50–800) frag-

Figure 9. Fragment ion spectra of the doubly-protonated peptide AGEGLSPFWSLAAPQRF (monoisotopic mass of peptide 1920.0 u) obtained by flow injection of 2 μL of a 1-mg mL^{-1} solution (50:50 aqueous acetonitrile with 15% acetic acid) into a 10- $\mu\text{L min}^{-1}$ solvent stream of 50:50 aqueous acetonitrile that contained 0.1% trifluoroacetic acid. The precursor ion analyzer Q_1 was operated with a 4-Th window to transmit the most intense isomers of the $[M + 2H]^{2+}$ species, and the fragment ion analyzer Q_3 was operated with resolving power to give peaks approximately 1.5 Th wide near the base. Q_3 was scanned in steps of 0.1 Th, with a dwell time of 1 ms per step. Argon was used as collision gas. (a) Conventional collision cell, 140-eV collision energy, and collision gas thickness 38×10^{13} atoms cm^{-2} . (b) High-pressure collision cell, 60-eV collision energy, and collision gas thickness 350×10^{13} atoms cm^{-2} .

ment ion spectra obtained by using the new cell with a fixed initial collision energy of 30 eV and varying the argon CGT values. In this instance Q_1 and Q_3 were operated with 3- and 1-Th windows, respectively. The spectra show the $[M + 2H]^{2+}$ and $[M + 2H - H_2O]^{2+}$ ions, as well as a singly charged fragment ion at m/z 403. The resolution of the 0.5-Th separations of the isomers of the doubly charged ions improves with

increasing CGT values. The spectrum obtained with a CGT of 400×10^{13} atoms cm^{-2} (not shown) was comparable with that shown for 450×10^{13} atoms cm^{-2} . The variation of these spectra with initial collision energy at a fixed CGT of 400×10^{13} atoms cm^{-2} is shown in Figure 11. Higher initial collision energies also improve the resolution, but this effect appears to reach a limit, because the performance at a collision

Figure 10. Fragment ion spectra of the doubly protonated peptide Lys₆ (monoisotopic mass of peptide 786.6 u), obtained by flow injection of 2 μ L of a 1-mg mL⁻¹ solution (50:50 aqueous acetonitrile with 15% acetic acid) into a 10- μ L min⁻¹ solvent stream of 50:50 aqueous acetonitrile that contained 0.1% trifluoroacetic acid. The precursor ion analyzer Q₁ was operated with a 3-Th window to transmit the most intense isomers of the [M + 2H]²⁺ species, and the fragment ion analyzer Q₃ was operated with resolving power to give peaks 1 Th wide near the base. Q₃ was scanned in steps of 0.1 Th, with a dwell time of 1 ms per step. Argon was used as collision gas. The initial collision energy was 30 eV in all cases. Collision gas thicknesses (a) 250, (b) collision gas thickness 350, and (c) 450 $\times 10^{13}$ atoms cm⁻².

energy of 35 eV (not shown) was comparable with that obtained at 30 and 40 eV (Figure 11). The overall intensities were comparable in all cases illustrated in Figures 10 and 11, so the variations in resolution are not merely reflections of improved ion statistics. Higher CGT values ensure that all ions that emerge from q₂ had a fixed low axial energy, but it is not apparent

why increasing the initial collision energy should have improved the resolution (Figure 11).

Figure 12 shows fragment ion spectra of two multiply protonated forms of ubiquitin, obtained by using flow injection and a wider scan range than used for the Lys₆ case. Data were acquired at a collision potential of 40 V and argon CGT of 380 $\times 10^{13}$ atoms cm⁻². The

Figure 11. Fragment ion spectra of the doubly protonated peptide Lys_6 (monoisotopic mass of peptide 786.6 u) obtained by flow injection of 2 μL of a 1-mg mL^{-1} solution (50:50 aqueous acetonitrile with 15% acetic acid) into a 10- $\mu\text{L min}^{-1}$ solvent stream of 50:50 aqueous acetonitrile that contained 0.1% trifluoroacetic acid. The precursor ion analyzer Q_1 was operated with a 3-Th window to transmit the most intense isomers of the $[\text{M} + 2\text{H}]^{2+}$ species, and the fragment ion analyzer Q_3 was operated with resolving power to give peaks 1 Th wide near the base. Q_3 was scanned in steps of 0.1 Th, with a dwell time of 1 ms per step. Argon was used as collision gas, at a CGT of 400×10^{13} atoms cm^{-2} . Initial collision energies (a) 10, (b) 20, (c) 30, and (d) 40 eV.

spectra illustrate the expected increase in degree of fragmentation with increasing charge, though it is not realistic to expect that such a large precursor ion could be sequenced in this way. However, as illustrated by the expanded inset in Figure 12a, even under these scan conditions it was possible to partly resolve the 0.5-Th separations of the isomers of the b_{18}^{2+} fragment ion. The resolution obtained in the experiments reported here, all of which employed flow injection anal-

ysis, was limited in practice by ion statistics at the scan speeds required. By using continuous infusion of samples and multiple scan accumulation, resolution of the isotopomers of the b_{14}^{4+} fragment of the $[\text{M} + 4\text{H}]^{4+}$ ion from renin substrate (DRVYIHPFHLVYS) has been demonstrated [61] using a similar apparatus.

A systematic study of variations in fragment ion yields as a function of collision cell operating parameters was undertaken for the $[\text{M} + 3\text{H}]^{3+}$ species of

Figure 12. Fragment ion spectra of the multiply protonated forms of ubiquitin (molecular mass 8564 u), obtained by flow injection of 2- μ L of a 1-mg mL⁻¹ solution (50:50 aqueous acetonitrile with 15% acetic acid) into a 10- μ L min⁻¹ solvent stream of 50:50 aqueous acetonitrile that contained 0.1% trifluoroacetic acid. The precursor ion analyzer Q₁ was operated with a 3-Th window to transmit the most intense isomers of the $[M + nH]^{n+}$ species, and the fragment ion analyzer Q₃ was operated with resolving power to give peaks 1 Th wide near the base. Q₃ was scanned in steps of 0.1 Th with a dwell time of 1 ms per step. Argon was used as collision gas, at a CGT of 380×10^{13} atoms cm⁻², and the initial collision potential was 40 V. (a) $[M + 7H]^{7+}$ precursor; (b) $[M + 8H]^{8+}$ precursor.

bovine insulin B chain. The results obtained are summarized in Figure 13, where the total intensity of the fragment ions is simply the total ion current (uncorrected for the mass-to-charge ratio dependence of Q₃ transmission) minus the intensity of the isotopomer cluster of the precursor ion. As the initial collision energy was increased from 30 to 120 eV (10–40-V collision potential), the intensity of the surviving precursor ions decreased dramatically. The yields of fragment ions increased as expected with increasing collision energy (though the maximum yield at 120 eV was slightly less than at 90 eV) and maximized at CGT values, which also showed an increase with increasing collision energy. Not shown in the summary provided by Figure 13 is the distribution of fragment ions across the mass-to-charge ratio range. As expected, the relative contributions at low mass-to-charge ratio values increased at higher values of CGT and of collision energy. Because the transmission efficiency of any linear quadrupole falls off with increasing mass-to-charge

ratio, these varying distributions distorted the total yield curves shown in Figure 13, which therefore should be regarded as only a demonstration of trends.

The sensitivity question may be less ambiguously addressed in the context of quantitative analyses by multiple reaction monitoring (MRM). A comparison of the two q₂ cells in this context was conducted by using standard solutions of erythromycin A. Analytical methods for this antibiotic in salmon flesh that employ liquid chromatography mass spectrometry and tandem mass spectrometry were developed previously in this laboratory [62]. The three most intense fragmentations of the $[M + H]^+$ ions (m/z 734.4) were monitored for triplicate injections of a dilution series of standard solutions by using both cells. In both cases excellent reproducibility (average < 5% relative standard deviation for the triplicate injections) was obtained, with linear response over 2 orders of magnitude and zero intercepts to within experimental uncertainty. However, the limits of detection and of quantitation ob-

Figure 13. Fragment ion yields (triangles) from the $[M + 3H]^{3+}$ ions of bovine insulin B chain, and intensities of surviving precursor ions (circles) obtained by flow injection of $2 \mu\text{L}$ of a 1-mg mL^{-1} solution (50:50 aqueous acetonitrile with 15% acetic acid) into a $15 \mu\text{L min}^{-1}$ solvent stream of 50:50 aqueous acetonitrile that contained 0.1% trifluoroacetic acid. The precursor ion analyzer Q_1 was operated with a 3-Th window to transmit the most intense isomers of the $[M + 3H]^{3+}$ species and the fragment ion analyzer Q_3 was operated with resolving power to give peaks approximately 2 Th wide near the base. Q_3 was scanned in steps of 0.5 Th, with a dwell time of 1 ms per step. Argon was used as collision gas. Note the scale factors for the precursor ion intensities at collision energies of 30 and 40 eV [(c) and (d), respectively]. The relative intensity scales in (a)–(d) are meaningful.

tained for pure standards with the new q_2 cell were lower, by at least an order of magnitude, than those determined previously [62] with the conventional cell.

Hysteresis Effect in Precursor and Neutral-Loss Scans

A peculiar effect observed when precursor ion scans were conducted with the new cell is illustrated in

Figure 14. In this case, precursor ions of the singly charged fragment ion at m/z 269 from ion sprayed human angiotensin I (DRVYIHPFHL) were sought, and the precursor ion region around m/z 433 (triply protonated angiotensin I) is shown. For faster scans (Figure 14a) the peak shape was unresolved and tailed toward higher mass-to-charge ratios. Incremental increases in the dwell time per 0.1-Th step to 30 ms (Figure 14e) progressively improved the resolution ob-

Figure 14. Partial precursor ion spectra for the singly charged fragment ion at m/z 269 from angiotensin I (DRVYIHPFHL) in the region of the triply charged precursor ion at m/z 433 (triply protonated angiotensin I). Q_3 was set at m/z 269 with a 1-Th window, whereas Q_1 was set to have a 1-Th window (measured at the peak base) and was scanned in steps of 0.1 Th with variable dwell times as shown and no interstep pause. Argon was used as collision gas at CGT of 350×10^{13} atoms cm^{-2} , and the potential difference ($V_0 - V_2$) that controlled the collision energy was 5 V.

tained for this triply charged precursor ion (0.3-Th spacings) to the point where further slowing of the scan had no effect. A similar improvement in resolution was obtained (not shown) by keeping the dwell time per step fixed at 1 ms, but increasing the pause time between steps (no recording of ion intensity during the pauses). The effects on constant neutral-loss scanning were different; they involved a significant

loss of sensitivity but with resolution still controlled by the bandpass settings of Q_1 and Q_3 .

These observations are consistent with the proposal that all ions that emerge from the high-pressure q_2 cell possess very low axial kinetic energies. It seems surprising that ions slowed down to this extent by collisions are so efficiently transmitted by q_2 , and the collisional focusing effect [39-42] must be invoked to

account for this, although the present work did not attempt to clarify this point further. In turn, these low exit energies are consistent with long transit times through this cell (on the order of 10 ms for the example shown in Figure 14). Although it was not possible to measure the exit energies by stopping curve experiments in the present work, other than to specify that they are appreciably less than 1 eV, it is possible to estimate them from the approximate transit times. By assuming an exponential falloff in axial kinetic energy along the cell, it is readily shown that a transit time of 10 ms for an ion of mass 1000 u with 30-eV initial energy implies an exit energy of only some 20 meV or so. Although this property is the key to the ease with which good peak shapes across the entire mass-to-charge range are easily obtained, the implication of long transit times does have the drawback that whenever Q_1 is scanned (or stepped as in multiple reaction monitoring experiments), hysteresis delays on the time scale of a few milliseconds are inevitable. Thus, when the median of the mass-to-charge ratio bandpass of Q_1 has been scanned away from the mass-to-charge ratio where precursor ions actually exist, it is possible that straggler fragment ions from q_2 can still be transmitted through Q_3 to the detector and be interpreted as higher mass-to-charge ratio precursor ions (Figure 14a) by the scan time-to-mass conversion algorithm for Q_1 . Some compromise between scan speed and peak shape must therefore be sought. No deleterious effects of this phenomenon are observed in fragment ion scanning, because the collision cell q_2 is then under effectively constant illumination by the selected precursor ions that emerge from a fixed Q_1 . In the case of constant neutral-loss scanning (not shown), because Q_3 is now scanned synchronously with Q_1 , the straggler ions from q_2 are not passed by Q_3 , which accounts for the sensitivity loss without degradation of resolution.

CONCLUSIONS

On a purely phenomenological level, the new high-pressure collision cell [37] generally affords greatly improved performance. The original q_2 cell, in the instrument used in the present work, was slightly unusual in that it had an open construction and the collision gas was introduced as a controlled free-jet expansion [55] that permitted the calculation of collision gas thickness by using well-established theories of gas dynamics. The CGT values thus achieved in the original cell fell within the usual operating range for more conventional low-pressure cells. However, this feature is irrelevant to the problem of obtaining adequate peak shapes and widths, which in turn arises from difficulties in matching translational energies of the ions that emerge from q_2 to the offset potential of the quadrupole analyzer Q_3 . The new cell has been in operation in this laboratory for one year and has greatly improved the sensitivity and quality of the tandem

mass spectrometry data obtainable. An additional important feature, apparent in the results presented here but not mentioned explicitly thus far, is the fact that optimum conditions exist over a reasonably broad range, that is, the optimum collision conditions do not require a narrowly defined set of parameters (CGT and collision potential). This greatly facilitates the acquisition of good quality data for unknowns—a significant operational advantage. Another advantage of the new cell is that the very high collision rate completely removes from consideration the directional focusing properties of quadrupole assemblies, which manifest themselves as maxima and minima in the transmission of precursor ions as a function of collision energy, RF amplitude, and so forth [63–65].

As for all experimental devices, the new cell has limitations. One important limitation is the restriction to low-energy collisions, so that it is not possible, for example, to distinguish between Ile and Leu via side-chain cleavages. Although the cell appears to be efficient in inducing CID, it does so via a large number of low-energy collisions of both precursor and primary fragment ions. The fact that over the most useful range of collision gas pressure the precursor ion intensity can increase with increasing pressure implies that the widely used criterion of fractional precursor ion attenuation cannot be used as a basis of comparison between different instruments.

The only real disadvantage of the high-pressure cell, relative to conventional cells that operate at CGT values lower by an order of magnitude, arises from the hysteresis effect observed when Q_1 is scanned or stepped (Figure 14). This effect reflects the ion flight times of a few milliseconds through the gas in q_2 , an inevitable consequence of the otherwise desirable characteristic of very low axial energies of the emerging ions. The extent to which this feature is regarded as a substantial disadvantage depends on the importance attached to precursor and neutral-loss scanning experiments. Over the past year in this laboratory only 1–2% of all tandem mass spectrometry experiments conducted on this instrument have been of this type. Furthermore, most MRM experiments have used a fixed precursor ion (Q_1 setting) for each of a number of liquid chromatography retention time windows, so that fast stepping of Q_1 is seldom required in our experience. In any event, it is usually possible to find an acceptable compromise between scan rate, peak width, and peak shape that produces data no worse than that obtainable with conventional q_2 cells operated at collision gas pressures lower by about an order of magnitude. The latter cells are not subject to the noticeable hysteresis effects exemplified by Figure 14, but on the other hand do require that V_3 be linked to m_p (instantaneous Q_1 setting) in the same ill-defined way that V_3 is linked to m_f for fragment ion scanning (e.g. eq 2).

Although the present work did not set out to investigate directly the physical phenomena underlying the

operation of this new cell [37], the greatly improved fragment ion resolution is explicable by a qualitative picture in which all ions are slowed down by elastic or near-elastic collisions to the point where they emerge with extremely low axial energies. The same elastic collisions also are responsible for the collisional focusing effect, which in turn accounts for the high transmission efficiency for these slow precursor ions and for refocusing the more strongly scattered fragment ions that result from inelastic collisions.

Some previous reports [66, 67] of experiments with triple quadrupole instruments in which collision gas pressures up to $2\text{--}5 \times 10^{-3}$ torr were employed, made no mention of the effects noted here. Indeed, the peak shapes in some of the spectra showed signs of severe mismatches between ion energy and V_3 at higher mass-to-charge ratios but a much better match at lower mass-to-charge ratios [66, 67]. The reasons for this different behavior are unclear at present.

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