Origin of the Tailing Signal on the Low-Energy Side of the Main Beam in Mass-Analyzed Ion Kinetic Energy Spectra

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The tailing signal on the low-energy side of the precursor ion signal observed during fast atom bombardment (FAB) mass-analyzed ion kinetic energy spectrometric (MIKES) analyses is due largely to ions of higher m/z value than the chosen precursor. The majority of these ions are independent, unfragmented species that emerge from the ion source with less than the full amount of kinetic energy predicted by the source potential. The tailing *precursor ion* signal observed under helium collision-activated decomposition conditions is too short to account for the protracted MIKES tail (as judged from mass-to-charge ratio-deconvoluted MIKES analyses performed on a BEqQ hybrid instrument), and a tailing precursor signal is not observed under unimolecular decomposition conditions. Measurements of the mass-to-charge ratios of the ionic species comprising the MIKES tail demonstrated that ions higher in mass-to-charge ratio than the chosen precursor are present throughout the tail, with the mass-to-charge ratio increasing as kinetic energy decreases. These ions possess the same momentum as the chosen precursor, and thus were formed prior to the magnetic field. The existence of intact, source-formed $[M + H]^+$ ions with reduced kinetic energy was demonstrated through several types of tandem mass spectrometric experiments. These $[M + H]^+$ ions with reduced kinetic energy do not appear to have undergone collisional deceleration, because they do not possess increased internal energy (as judged by observation of their fragmentation patterns). The kinetic energy profiles of unfragmented FAB-desorbed ions were determined and found to exhibit a tailing character similar in appearance to that of the MIKES tail. The population of ions emerging from the source under FAB conditions thus incorporates the characteristics necessary to account for the MIKES tail, namely, the presence of ions of a mass-to-charge ratio higher than the chosen precursor (due to matrix and other background ions), which possess reduced kinetic energy such that their momentum is identical to that of the selected precursor. These ions may arise via desolvation and declustering processes in the acceleration region of the ion source, or via FAB or chemical ionization processes in regions removed from the FAB target. (*J Am Soc Mass Spectrom 1992, 3, 644–655*)

Mass-analyzed ion kinetic energy spectrometry (MIKES) analyses are frequently used to obtain kinetic energy release information for mechanistic studies of ion decomposition processes, and to generate structurally informative product ion spectra under high-energy (kiloelectronvolt) collision-activated decomposition (CAD) conditions. Such analyses are generally performed on reverse geometry (BE) instruments, where the magnet (B) precedes the electric sector (E). During these analyses a precursor ion is selected with B, and E is scanned to generate the kinetic energy spectrum of product ions formed in the field-free region between B and E (second field-free region, FFR2). In CAD experiments, the precursor is subjected to collision with an inert gas in a cell located in FFR2. Assignment of mass-to-charge ratio to the observed product ions is indirect, based upon the assumption of kinetic energy partitioning between the product ion and the corresponding neutral particle.

A pronounced tailing signal (decreasing in intensity from high to low kinetic energy) is frequently observed during MIKES analyses of fast atom bombardment (FAB) desorbed ionic species. This tail is observed under both unimolecular decomposition and high-energy CAD conditions. The tail begins at the low-energy side of the main (precursor) beam, and generally decreases in intensity toward lower kinetic energy values, often extending across 30% (or more) of the range below the kinetic energy of the precursor.

The upper portions of this tail can be as intense as some of the identifiable product ion peaks in the MIKES spectrum, and less intense product ion peaks may be obscured by the tail.

There are several possible origins for the signals that contribute to this tail. Under high-energy CAD conditions, a relatively short portion of the tail can be attributed to decelerated precursor ions, that is, ions which have undergone collision in FFR2 (thereby losing kinetic energy) but have not fragmented. Several groups have studied this phenomenon, both on BE [1–4] and on E_1BE_2 [5, 6] instruments (the latter permitting study of decompositions between B and E_2). The portion of the energy tail in MIKE spectra attributable to decelerated precursors is much more pronounced when helium is employed as a collision gas than when argon or xenon is used. It has been suggested [5] that this is due to a wider scattering angle induced by the larger target gasses, resulting in decreased collection efficiency of collided (and therefore decelerated) but unfragmented ions. Even with helium, however, the signal corresponding to decelerated precursors can only account for a small portion of the tail in MIKE spectra, because these signals extend less than 2% of the range below the kinetic energy of the precursor [1–6]. Furthermore, decelerated precursors cannot account for the tail observed under unimolecular decomposition conditions (no collision gas) unless collisions with surfaces or residual gas in the vacuum system are involved. (To avoid confusion, unless otherwise specified, all references to the "tail" or the "tailing signal" below refer to the long tailing signal observed in MIKES under both unimolecular decomposition and CAD conditions, and not to the short tailing signal corresponding to decelerated precursors.)

A well-characterized source of discrete peaks apparently unrelated to the chosen precursor in MIKE spectra involves metastable decomposition of ions higher in mass-to-charge ratio than the chosen precursor in the regions of the instrument that precede the magnet [7, 8]. If an ion, higher in m/z than the precursor chosen for MIKES analysis, fragments in either the acceleration region or the first field-free region (FFR1) to yield a product ion (also of a higher mass-to-charge ratio than the chosen precursor but lower in kinetic energy) that happens to possess the same momentum as the chosen precursor, the product ion will be transmitted by the momentum analyzer (B) along with the intended precursor. Because the product has lower kinetic energy than the selected precursor, the product will appear as a discrete peak in the MIKE spectrum, usually at a point inconsistent with that of a product ion derived from the intended precursor. The occurrence of such peaks and the explanation for their observation have been previously described [7, 8]. Processes such as these could certainly contribute to the tail observed in MIKE spectra of FAB-desorbed ions. If matrix and other background ions give rise to metastable decomposition products in the acceleration region or FFR1, some of these could have the same momentum as the chosen precursor. To appear as a tail, such processes (even those occurring across the potential continuum of the acceleration region) would have to result in many neighboring ions higher in mass-to-charge ratio than the chosen precursor (but equal in momentum), so that the metastable decomposition products would overlap in the MIKE spectrum and constitute a tail rather than individual peaks. While such processes are likely to contribute to the tail, it seems improbable that this could account for all of the signal observed in the MIKES tail.

Another possible explanation for the tail is intact, unfragmented source-formed ions that are higher in mass-to-charge ratio than the chosen precursor but possess kinetic energy less than Vez (where V is the accelerating voltage), which is the kinetic energy predicted from the ion source potential. If ions emerged from the ion source with all possible kinetic energies between zero and Vez, then for every mass-to-charge ratio value larger than the chosen precursor there would exist a corresponding kinetic energy such that the higher mass-to-charge ratio ion would possess the same momentum as the chosen precursor, and thus would be transmitted by the magnet. Because there is a background ion at every mass-to-charge ratio value with FAB, these ions would represent a virtual continuum both in increasing mass-to-charge ratio and in decreasing kinetic energy, and thus might appear as a tail in the MIKE spectrum. The present studies indicate that the majority of the ionic species present in the MIKES tail fall into this category. Here we describe these studies and discuss possible origins for unfragmented ions that leave the ion source with reduced kinetic energy.

Experimental

Materials. The unlabeled peptides leucine-enkephalin (amino acid sequence Tyr-Gly-Gly-Phe-Leu) and bradykinin (1-5) (Arg-Pro-Pro-Gly-Phe) were purchased from Bachem (Torrance, CA). [$^{18}O_2$]Leucine-enkephalin, labeled at the C-terminus, was prepared by acid catalyzed isotopic exchange as previously described [9].

Mass spectrometry. Tandem mass spectrometric analyses were performed with a VG ZAB-SEQ (VG Analytical Ltd, Manchester, UK) hybrid instrument of BEqQ configuration (B = magnetic sector, E = electric sector, q = rf-only quadrupole, and Q = quadrupole mass filter), modified as previously described [10] to permit independent control of the electric sector and ion source potentials. The ion source was operated at an accelerating voltage of 8010 V for all experiments. Ionization was by FAB using 8-keV xenon for the primary atom beam. The liquid matrix for peptide studies was a 1:1 mixture of 2,2'-dithiodiethanol and thioglycerol, saturated with oxalic acid. For studies with cesium iodide,

1–2 μL of a saturated aqueous solution of CsI was applied to the FAB probe tip (no matrix) and dried in the forevacuum lock of the instrument. All scan data were acquired using the multichannel analyzer (MCA) mode of the VG 11/250J data system, with 6 to 8 scans typically accumulated. No postacquisition smoothing was applied to the raw data. Depending on the experiment, the mass spectrometer was scanned either under data system control, or under hardware control with the data system acting purely as a recording device. The details of the various scanning experiments are presented as appropriate in the discussion below and in the figure legends. Unless otherwise specified, the tandem mass spectrometric experiments were conducted in the absence of collision gas.

Ion optical modeling. Computer modeling of the ion source for the ZAB-SEQ was performed on an 80386/80387–based AT-compatible personal computer, using the SIMION PC/PS2 Version 4.0 ion optical modeling program [11]. The potentials for the various electrodes were determined experimentally, with the instrument tuned to operating conditions typical for low resolution analyses.

Results and Discussion

To illustrate the problem addressed during these investigations, we present in Figure 1a a portion of the MIKES spectrum of the $[M + H]^+$ ion of the simple pentapeptide leucine-enkephalin (m/z 556) obtained under unimolecular decomposition conditions, with the ion source operated at 8010 V. This spectrum was obtained at high gain, and some of the observed product ions were off scale. The identities of the discrete product ions present in this spectrum are well established [12–16] and are indicated in the figure. (The nomenclature employed to identify the product ions in this and other figures is that suggested by Roepstorff and Fohlman [17].) Underlying these signals, particularly in the higher energy portion of the spectrum, is a broad tailing signal detectable as low as 5500 eV. The intensity of the tailing signal in the upper portion of the spectrum is greater than those of some of the product ions.

Decelerated Precursors

In order to determine directly whether the tailing signal in the MIKE spectrum of leucine-enkephalin represents precursor ions that have lost kinetic energy though collision with either surfaces or residual gas in the vacuum system, the peptide was analyzed by mass-to-charge ratio-deconvoluted MIKES analysis. This experiment exploits the fact that a mass analyzer quadrupole is a true mass-to-charge ratio–measuring device [18–21]. For this type of analysis on a BEqQ hybrid, the precursor ion is selected with B (as in

conventional MIKES) and the mass analyzer quadrupole is set to transmit only the ion of interest; E is then scanned over an appropriate range. Thus, the kinetic energy profile of ions of a specific mass-to-charge ratio value is obtained [18, 19, 21, 22]. Such a scan, carried out to obtain the kinetic energy profile of the precursor ions of leucine-enkephalin (m/z 556), is shown in Figure 1b. This spectrum, obtained in the absence of collision gas, shows good peak symmetry with no hint of precursor ion tailing, even at very high gain. Figure 1c shows a similar analysis obtained with sufficient helium in the collision cell between B and E to reduce the main beam intensity by 90%. Under these conditions precursor ion tailing is evident down to 30 eV (0.4% of the range) below the centroid of the main beam. Very little tailing was evident when argon was used as the collision gas. These results are in good agreement with those obtained by others using the conventional MIKES technique [1–6].
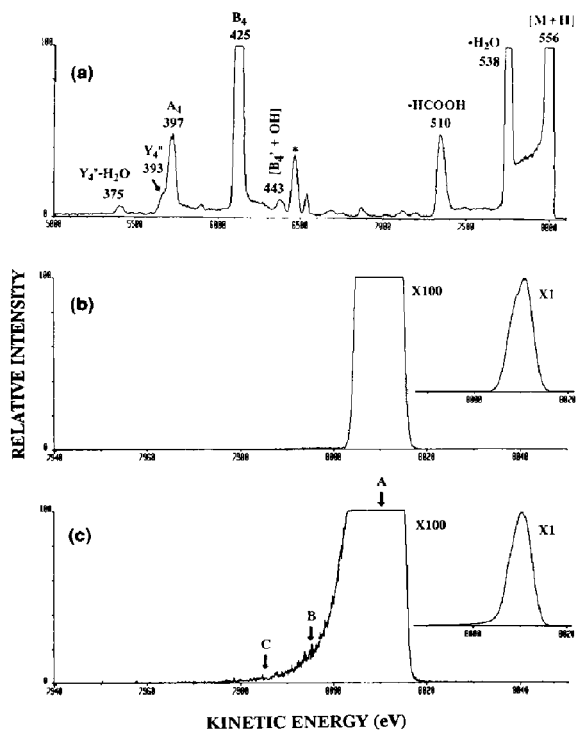

Figure 1. (a) Portion of the conventional MIKE spectrum of leucine-enkephalin obtained at high gain under unimolecular decomposition conditions. (The peak indicated with an asterisk is due to decomposition of the background matrix.) (b) Mass-to-charge ratio–deconvoluted MIKE spectrum of leucine-enkephalin $[M + H]^+$ ions, obtained on a BEqQ hybrid by scanning E with Q set to transmit the m/z 556 precursor ions; this spectrum was obtained in the absence of collision gas. (c) As in (b), with sufficient helium present in the collision cell between B and E to reduce the main beam intensity by 90%. The product ion spectra of Figure 2a–c were obtained from precursor ions corresponding to points A, B, and C, respectively.

The precursor ion tailing evident in Figure 1c could represent ions that have lost kinetic energy through collision, and therefore have been activated but have not fragmented, or ions that have undergone elastic collision and have simply been scattered without activation (or a combination of both of these). This issue has been addressed by Thibault et al. [4]. Using electron impact ionized n-butylbenzene as an indicator of internal energy content on a $B_1E_1E_2B_2$ instrument, these investigators selected (with E_1) precursor ions that had lost varying amounts of kinetic energy through collision with helium in the field-free region between B_1 and E_1; ions so selected were allowed to decompose unimolecularly between E_1 and E_2, and the abundance ratio of product ions of m/z 91 to those of m/z 92 was measured through linked scanning of E_2 and B_2. The relationship between this ratio and internal energy content was previously known from photodissociation studies [23, 24]. The results of those studies indicated that 8-keV ions, which had lost 12 eV in kinetic energy (laboratory frame of reference, KE_{lab}) through collision with helium, appeared to have gained approximately 1 eV in internal energy (compared to a maximum of 232 eV from the center-of-mass collision energy). Qualitatively similar results were obtained by Alexander and Thibault [3] on a BEqQ hybrid using FAB-ionized perdeuteroacetylgalactose, although calibration for the internal energy content of that system was not available. These authors were careful to point out, however, that the ions enriched in internal energy may have represented a subpopulation of the species that appeared to have lost kinetic energy, with the remainder of the population consisting of elastically scattered ions.

It has been proposed [15] that a sensitive indicator of the internal energy content of leucine-enkephalin is the m/z 278/279 ratio, representing the ratio of the formation of the B_3 and the Y_2'' ions (in the Roepstorff and Fohlman nomenclature [17]), respectively. Certainly the ratio of abundances of these ions in the low-energy CAD product ion spectrum varies markedly with small changes in center-of-mass collision energy (E_{cm}) [15, 25]. The formation of these ions involves cleavage of the same peptide bond, with charge retention on either the N-terminal (B_3 ion) or C-terminal (Y_2'' ion) portion of the molecule; the formation of the B_3 ion is expected to be the higher energy process [26]. During conventional product ion scanning analyses on the BEqQ hybrid, with unimolecular decomposition conditions employed in the rf-only quadrupole, scanning of Q gives an m/z 278/279 ratio of approximately 0.22. This ratio increases under low-energy CAD conditions (argon collision gas, multiple collision conditions) to 0.67 under 24-eV CAD conditions (laboratory frame of reference) and to 1.2 with 36-eV CAD. To compare the internal energies of the leucine-enkephalin ions comprising the tail of Figure 1c, ions at the points indicated by A, B, and C were selected with E and allowed to decompose under uni-

molecular decomposition conditions in q; the m/z 278/279 ratios were measured by scanning Q. The results of these analyses are shown in Figure 2a–c, respectively. The leucine-enkephalin ions at the centroid of the main beam gave a ratio indistinguishable from that obtained with no collision gas present in FFR2 (approximately 0.22). This ratio increased to about 0.4 for precursor ions that had lost 15 eV in KE_{lab}, and to roughly 0.5 for precursors that had lost 25 eV. Although no attempt has been made to calibrate this system during the present studies, the results clearly indicate that leucine-enkephalin ions that appear to have undergone deceleration through collision with helium include a population of ions enriched in internal energy. These results are qualitatively consistent with the findings of other investigators [3, 4] for other analytes.

Mass-to-Charge Ratio Analysis of the MIKES Tail

The mass-to-charge ratio–deconvoluted MIKE spectra of Figure 1b and c, as well as similar experiments with cesium iodide and des-Arg[1]-substance P (not shown), clearly indicate that the contribution of the precursor
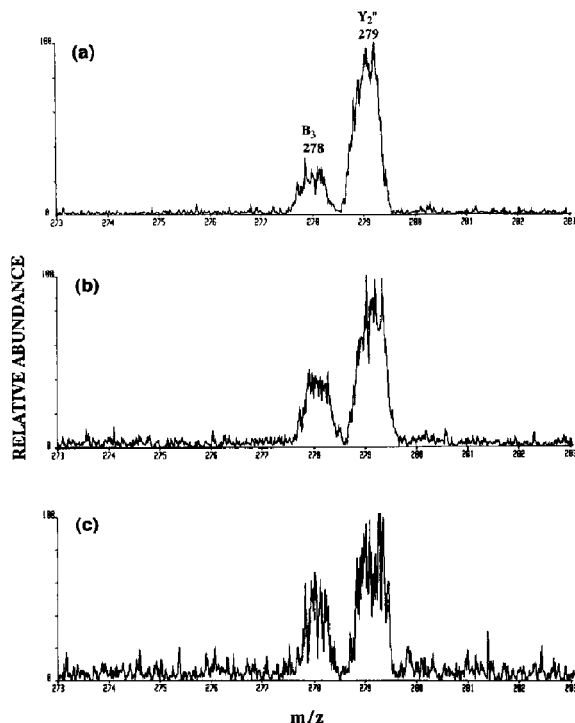

Figure 2. Portions of the product ion spectra [encompassing the B_3 (m/z 278) and Y_2''(m/z 279) ions] obtained on a BEqQ hybrid for leucine-enkephalin [M + H]$^+$ ions decelerated through collision with helium in the collision cell between B and E: (a) 8010-eV precursors (no deceleration; point A in Figure 1c); (b) 7995-eV precursors (ions decelerated by 15 eV; point B in Figure 1c); (c) 7985-eV precursors (ions decelerated by 25 eV; point C in Figure 1c).

ion to the protracted tail commonly observed in conventional MIKE spectra is insignificant, if present at all. Even with helium as a collision gas, the tailing precursor signal extends to only approximately 30 eV below the centroid of an 8-keV precursor, whereas the tailing signal in the conventional MIKE spectrum extends to at least 2500 eV below the main beam, even under unimolecular decomposition conditions. A simple means of gaining insight into the nature of the ions comprising the long MIKES tail is to measure the mass-to-charge ratio value for those ions at various points along the tail. This is a straightforward experiment with the BEqQ hybrid, again because Q is a true mass-to-charge ratio–measuring device. These experiments employed another simple pentapeptide, bradykinin (1-5), the MIKE spectrum (not shown) of which exhibits a protracted tail similar to that observed with leucine-enkephalin. The $[M + H]^+$ precursor ion (m/z 573) was selected with B, and E was set to transmit ions at various points along the MIKES tail. Q was then scanned to measure the mass-to-charge ratio values of these ions. Two such analyses are presented in Figure 3. For the scan in Figure 3a, E was set to transmit ions with a kinetic energy of 7900 eV, and Q was scanned to measure the mass-to-charge ratio value of the transmitted ions. The only signal detected in this region of the tail was due to ions of m/z 580, which is seven units *higher* in mass-to-charge ratio than the chosen precursor. At every point analyzed along the tail, ions of higher mass-to-charge ratio than the chosen precursor were detected, with the mass-to-charge ratio value increasing as the kinetic energy decreased. These results are summarized in Table 1, which shows the measured mass-to-charge values of ions at various points along the tail of the MIKES spectrum of m/z 573, along with the calculated velocity and momentum for ions of the measured mass-to-charge ratio and the selected kinetic energy (with unit charge assumed). In each instance, the higher mass-to-charge ratio ions that appeared in the MIKES tail had the same momentum as the chosen precursor, indicating that they were formed in either the ion source or
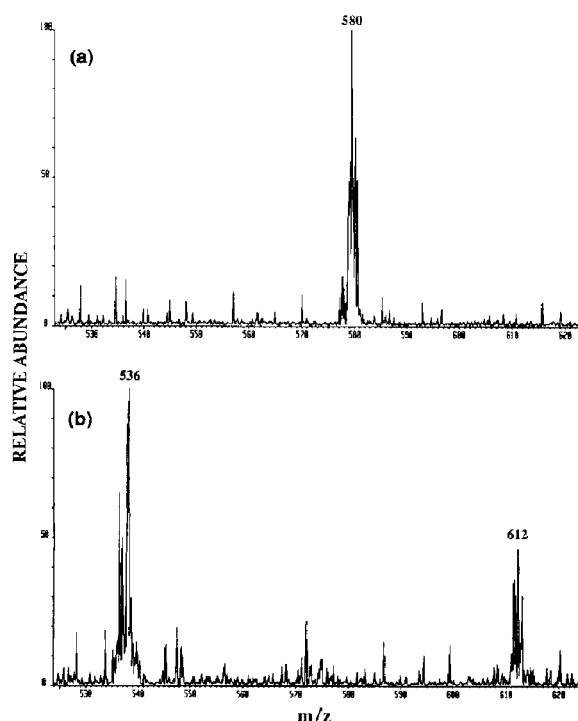

Figure 3. Mass-to-charge ratio analyses of tailing components in the MIKE spectrum of 8010-eV bradykinin (1-5) $[M + H]^+$ ions (m/z 573) obtained on a BEqQ hybrid. (a) The magnet was set to transmit the 8010-eV precursor, and E was set to transmit ions with a kinetic energy of 7900 eV; Q was then scanned to measure the mass-to-charge ratio values of the ions transmitted by E. (b) As in (a), but with E set to transmit 7500 eV ions.

FFR1, that is, at some point preceding the magnetic field. In two instances, one of which is shown in Figure 3b, ions lower in mass-to-charge ratio than the chosen precursor were detected, in addition to ions of higher mass-to-charge ratio. The lower mass-to-charge ratio ions were readily rationalized as product ions derived from the selected precursor. These ions had the same velocity as the chosen precursor but lower momentum,

Table 1. Mass-to-charge ratio, velocity, and momentum of ionic species identified in the tail of the MIKE spectrum of bradykinin (1-5) (m/z 573)

| Kinetic energy (eV) | Mass-to-charge ratio | | Velocity (10^6 cm /sec) | Momentum (10^{-15} g · cm /sec) |
	Ions with common momentum	Ions with common velocity		
8010	573	573	5.19	4.94
7950	577		5.15	4.94
7900	580		5.13	4.94
7850	584		5.09	4.94
7800	588		5.06	4.94
7768	591		5.04	4.94
7768		556	5.19	4.79
7500	612		4.86	4.94
7500		537	5.19	4.63

indicating that they were indeed product ions derived from the intended precursor, and were formed via metastable decomposition in FFR2.

The phenomenon of ions higher in mass-to-charge ratio than the selected precursor appearing in the tail of the MIKE spectrum was further examined using the mass-to-charge ratio–deconvoluted MIKES technique. In these analyses, 8-keV $[M + H]^+$ precursors were selected with B, and Q was set to transmit ions of higher mass-to-charge ratio than the chosen precursor at unit resolution. The electric sector was then scanned to determine the kinetic energy profiles of the higher mass-to-charge ratio ions within the MIKES tail. For precursors in the m/z 500–600 range, ions up to 130 u higher in mass-to-charge ratio than the chosen precursor were detected in this manner. Every arbitrarily selected mass-to-charge ratio value up to at least 10% larger than the chosen precursor was detected in the MIKES tail. This observation alone casts doubt upon the hypothesis that all of these ions originate through metastable decomposition of matrix ions in FFR1, since the precise precursor–product relationships involved in the detection of FFR1 decomposition products render it improbable that such a product would be formed for every mass-to-charge ratio value and for every momentum value examined. As an extreme example, m/z 635 ions were detected during the mass-to-charge ratio-deconvoluted MIKES analysis of m/z 503 from polyethylene glycol, with a peak maximum at 6345 eV (8-kV accelerating voltage). For this to represent a metastable decomposition product formed in FFR1, the m/z 635 ion would have to be formed from an ion of m/z 802, which is only a weak background ion in the conventional FAB spectrum of polyethylene glycol; the occurrence of such a decomposition would thus seem improbable. The m/z 635 ion is, however, an intense species in the conventional FAB spectrum of polyethylene glycol. If source-formed m/z 635 ions left the ion source with less than the full kinetic energy predicted from the ion source potential, some of these ions (6345-eV ions) could have the same momentum as the 8-keV m/z 503 ions, and thus would appear as a component of the tail in the MIKE spectrum of m/z 503.

Source-Formed Ions with Reduced Kinetic Energy

This possibility that the ions comprising the MIKES tail are intact source-formed species was examined through the further use of the mass-to-charge ratio–deconvoluted MIKES technique. In the mass-to-charge ratio–deconvoluted MIKE spectrum of Figure 4a, the 8-keV precursor selected with B was the $[M + H]^+$ ion of leucine-enkephalin (m/z 556), and Q was set to transmit ions of m/z 560. A signal maximizing at 7953 eV was detected. To the same sample was added a similar quantity of $[^{18}O_2]$leucine-enkephalin (m/z 560 for the $[M + H]^+$ ion) and the analysis was repeated. The resulting spectrum is shown in Figure 4b. The detected signal was approximately 50 times as intense
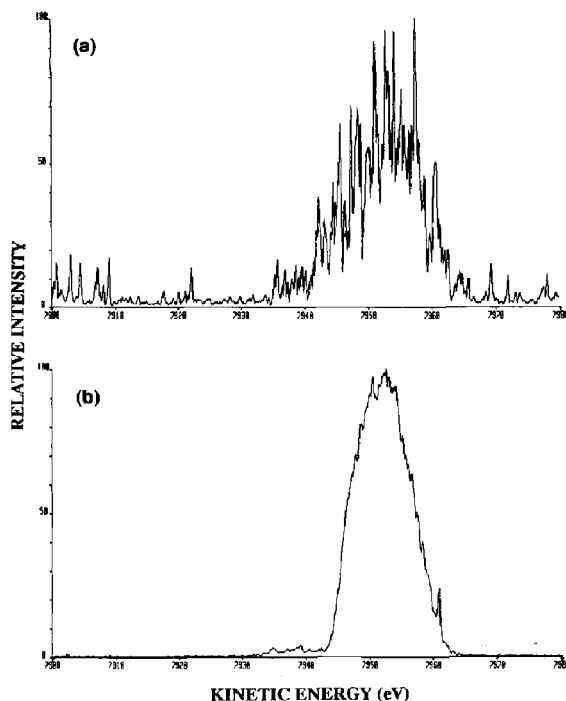

Figure 4. (a) Mass-to-charge ratio–deconvoluted MIKES analysis of m/z 560 ions in the MIKE spectrum of leucine-enkephalin $[M + H]^+$ ions (m/z 556), obtained on a BEqQ hybrid. The magnet was set to transmit 8010-eV m/z 556 ions, and Q was set to transmit m/z 560 ions; E was then scanned over a narrow range. (b) Similar spectrum obtained after a similar quantity of $[^{18}O_2]$leucine-enkephalin (m/z 560) was added to the sample; the absolute intensity of the detected signal increased approximately fiftyfold.

after the addition of the $[^{18}O_2]$leucine-enkephalin. Similar results were obtained for other higher mass-to-charge ratio components of the MIKES tail. In each case, when a higher mass-to-charge ratio species was enhanced in the conventional FAB spectrum, the corresponding signal from that mass-to-charge ratio component in the MIKES tail increased.

The conventional mass spectrum obtained by scanning the magnet on a double-focusing instrument can be viewed as an energy-selected momentum spectrum, with E set to transmit ions with kinetic energy equal to Vez. Such a spectrum of a mixture of leucine-enkephalin and bradykinin (1-5) is presented in Figure 5a. The spectrum shows only source-formed 8010 eV ions, with all metastable decomposition products formed in the acceleration region, FFR1, B, and FFR2 filtered out by the electric sector. Another energy-selected momentum spectrum of the same mixture, obtained with the ion source operated at 8010 V but with E set to transmit ions with a kinetic energy of 7960 eV, is shown in Figure 5b, with a similar spectrum for 7910 eV ions shown in Figure 5c. In principle, each of the major signals detected in Figure 5b and c could arise via decomposition of a higher mass-to-charge ratio species
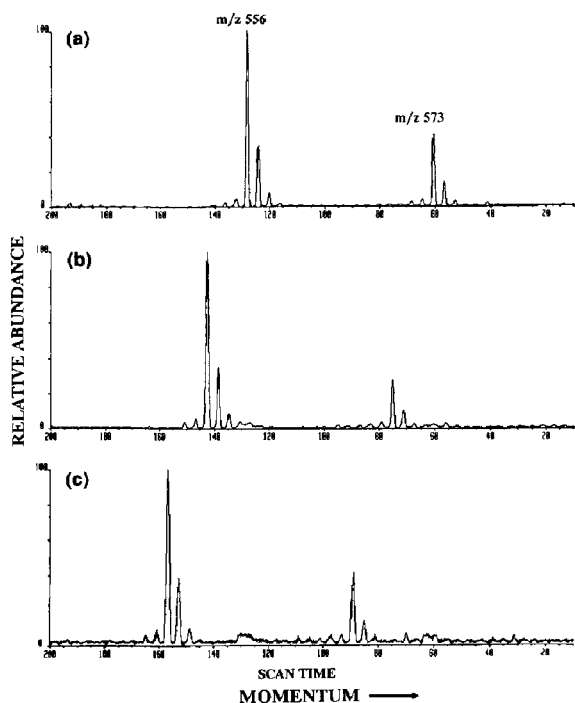
Figure 5. Energy-selected momentum spectra of a mixture of leucine-enkephalin and bradykinin (1-5), obtained using the BE portion of a BEqQ hybrid with the ion source operated at 8010 V. (a) Spectrum of FAB-desorbed 8010-eV ions, obtained by scanning B with E set to transmit ions with a kinetic energy of 8010 eV; these are the conditions for the conventional FAB spectrum. (b) Similar to (a) but with E set to transmit ions with a kinetic energy of 7960 eV. (c) As in (a) and (b), but with E transmitting 7910-eV ions. (The absolute intensities in (b) and (c) were reduced by 3–4 orders of magnitude as compared to (a).)

Figure 6. Mass-to-charge ratio–deconvoluted, energy-selected momentum spectra of leucine-enkephalin $[M + H]^+$ ions (m/z 556), obtained on a BEqQ hybrid with the ion source operated at 8010 V. E was set to a selected kinetic energy value, and Q was set to transmit m/z 556; B was then scanned to generate the momentum spectrum. Q was operated at reduced (3–4 u) resolution to permit the detection of the isotope cluster. E was set to transmit ions with kinetic energies of (a) 8010 eV; (b) 7900 eV; (c) 7800 eV; (d) 7700 eV; (e) 7600 eV. (The absolute intensities of the detected signals in (b–e) were reduced by 3–4 orders of magnitude as compared to (a).)

anywhere between the FAB target and the magnet. The close similarity of the relative abundances of the major peaks detected in Figure 5a–c, however, suggests that the same source-based processes that give rise to the 8010-eV ions are also involved in the generation of the 7960- and 7910-eV ions. Similar results were obtained with other precursor ion species.

With a BEqQ hybrid, spectra such as those in Figure 5 can be deconvoluted with respect to mass-to-charge ratio value (mass) by employing Q as a mass-to-charge ratio filter. A series of such spectra for leucine-enkephalin is shown in Figure 6. During these analyses, the ion source was operated at 8010 V, and Q was set to transmit the $[M + H]^+$ ion (m/z 556). The electric sector was set to a selected value, and B was scanned to generate the mass-to-charge ratio–deconvoluted, energy-selected momentum spectrum; Q was operated at decreased resolution (3–4 u) to permit the detection of the characteristic isotope cluster. During this series, representing 100 eV steps in kinetic energy from the full 8010 eV down to 7600 eV, the peak maxima occurred at successively lower values of momentum, and the characteristic shape of the isotope
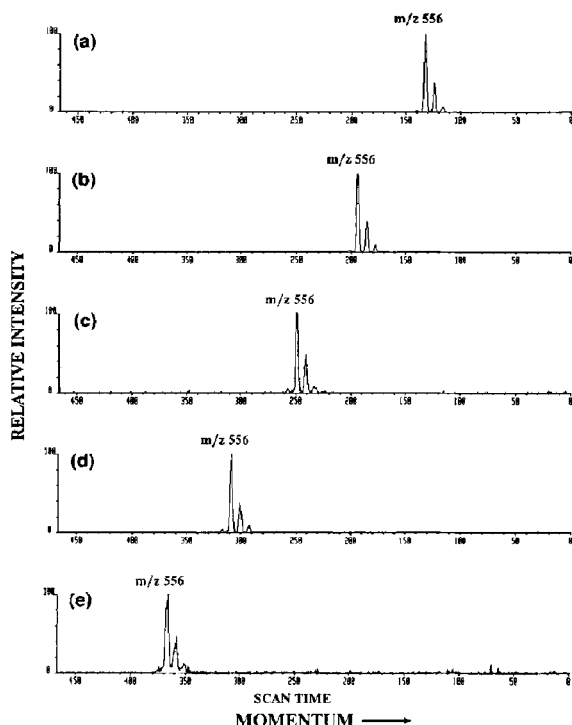
cluster was observed in each case. The consistent observation of the isotope cluster indicates that these were indeed leucine-enkephalin $[M + H]^+$ ions, and not product ions of m/z 556 arising via decomposition of higher mass-to-charge ratio background ions. These data, along with those of Figures 4 and 5, strongly suggest that a population of source-formed $[M + H]^+$ ions emerges from the source with less than the full kinetic energy predicted from the ion source potential.

Further evidence supporting this hypothesis was obtained through the product ion spectrum of the major peak detected in Figure 6b. The magnet was set to the value corresponding to the point of maximal intensity in Figure 6b (apparent mass-to-charge ratio approximately 549), and E was set to transmit ions with a kinetic energy of 7900 eV, with the ion source operated at 8010 V. Ions so selected were subjected to CAD in q (25 eV, argon collision gas), and Q was scanned. The resulting spectrum is presented in Figure 7. The $[M + H]^+$ ion and the major product ions characteristic of leucine-enkephalin were reproducibly ob-
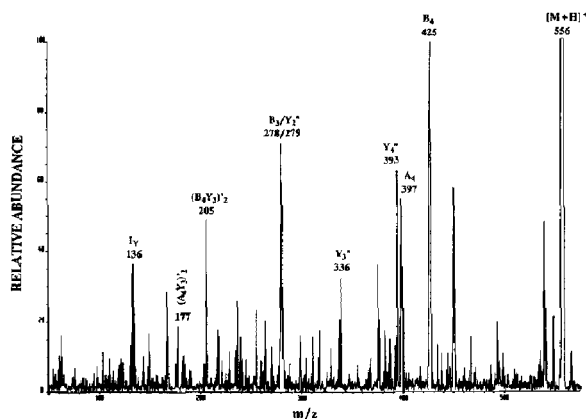
Figure 7. Low energy CAD (argon, 25 eV) product ion spectrum of leucine-enkephalin $[M + H]^+$ ions that emerged from an 8010-V ion source with a kinetic energy of 7900 eV. This spectrum was obtained on a BEqQ hybrid, with B set to the point of maximum intensity in Figure 6b, and with E set to transmit 7900 eV ions. CAD occurred in q, and Q was scanned to detect the product ions.

Figure 8. Portion of the unimolecular product ion spectrum of leucine-enkephalin $[M + H]^+$ ions that emerged from an 8010-V ion source with a kinetic energy of 7960 eV. The magnet on the BEqQ hybrid was set to the value appropriate for 7960-eV m/z 556 ions, and E was set to transmit 7960-eV ions. Unimolecular decomposition occurred in q, and Q was scanned over a narrow range to detect the product ions.

served. Figure 7 is thus a spectrum of the product ions derived from leucine-enkephalin $[M + H]^+$ ions that emerged from the ion source with 110 eV less than the maximum amount (8010 eV) of kinetic energy predicted by *Vez*.

One possible explanation for these observations concerns deceleration processes occurring within the ion source. An $[M + H]^+$ ion, originating at the FAB target (8 kV), could collide with surfaces or FAB gas in the acceleration region, resulting in the loss of a portion of the kinetic energy originally imparted through acceleration from the FAB target. If this is the case, one would expect such ions, which have not fragmented, to possess additional internal energy as compared to the fully accelerated species, as is seen for ions decelerated through collisional processes in FFR2 (as discussed above). The m/z 278/279 ratio from leucine-enkephalin was again employed as an indicator of internal energy to assess this possibility. Thus, B and E were set to transmit leucine-enkephalin $[M + H]^+$ ions with 50 eV less than the full available (*Vez*) amount of kinetic energy. These ions were allowed to decompose unimolecularly in q, and the m/z 278/279 ratio was measured by scanning Q. The resulting spectrum is shown in Figure 8. Although the signals involved were weak, there was no apparent increase in this ratio for these ions as compared to the fully accelerated species (Figure 2a). This observation is particularly important in light of the fact that leucine-enkephalin $[M + H]^+$ ions that were deliberately decelerated through collisional processes, leading to a drop in KE_{lab} of 15–25 eV in FFR2, reproducibly exhibited an increased m/z 278/279 ratio (Figure 2b and c), and one would expect this to be even more enhanced for ions decelerated by 50 eV. These ions do not appear to possess increased
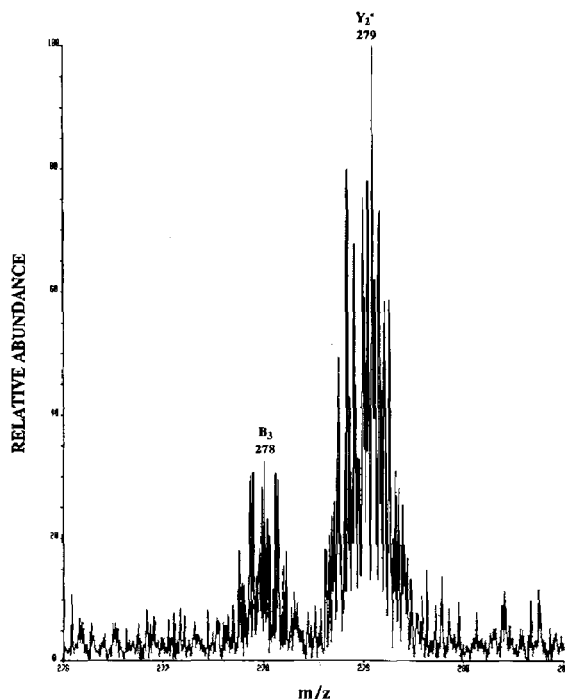
internal energy, and thus it is unlikely that they have undergone collisional deceleration.

Kinetic Energy Profiles of Unfragmented Fast Atom Bombardment-Desorbed Ions

An ideal experiment for obtaining information regarding the kinetic energy distribution of source-formed $[M + H]^+$ ions would be a mass-to-charge ratio–deconvoluted ion kinetic energy (IKE) scanning experiment. Such a scan would be simple on an EQ hybrid such as that built by Beynon's group [18] (who described the use of such a scan for kinetic energy release determinations in decomposition reactions). One would simply set Q to transmit the ion of interest, and scan E with the accelerating voltage held constant. This experiment is more complicated with a BeqQ hybrid, although in principle such a scan could be generated through linked scanning of B and E (with B^2/E constant), with Q set to the chosen mass-to-charge ratio value. During the present experiments, however, advantage was taken of the fact that the peak maxima of the mass-to-charge ratio–deconvoluted, energy-selected momentum spectra (Figure 6) represent individual data points in the mass-to-charge ratio–deconvoluted IKE spectrum. A series of such spectra, with Q

operated at unit resolution, was obtained for the [M + H]$^+$ ion of leucine-enkephalin and for the m/z 393 ion of cesium iodide. The intensities of the detected peaks are plotted against kinetic energy as mass-to-charge ratio–deconvoluted IKE "spectra" in Figure 9. The inset represents similar data for these two species, obtained on the same day under identical instrumental tuning conditions, with the signal intensities expressed as a fraction of the intensity of the main beam. Clearly, both leucine-enkephalin and cesium iodide exhibit a tailing kinetic energy distribution as they emerge from the ion source (although to differing degrees, as discussed below). Furthermore, the appearance of the tail in the mass-to-charge ratio–deconvoluted IKE spectra is remarkably similar to the MIKES tail, with the lower limit of its detectability apparently limited by instrumental sensitivity rather than by a lower limit on the occurrence of the phenomenon. (It should be noted that the apparent peaks in the leucine-enkephalin spectrum at 7017 and 7748 eV were reproducible, and are likely to represent contributions from metastable decompositions of higher mass-to-charge ratio precursors yielding m/z 556 product ions in FFR1; such peaks would be expected to appear in this type of analysis.)

The body of evidence described above establishes conclusively that the ionic species that are manifested as a broad, tailing signal in the conventional MIKE spectrum of FAB desorbed ions consist of intact,
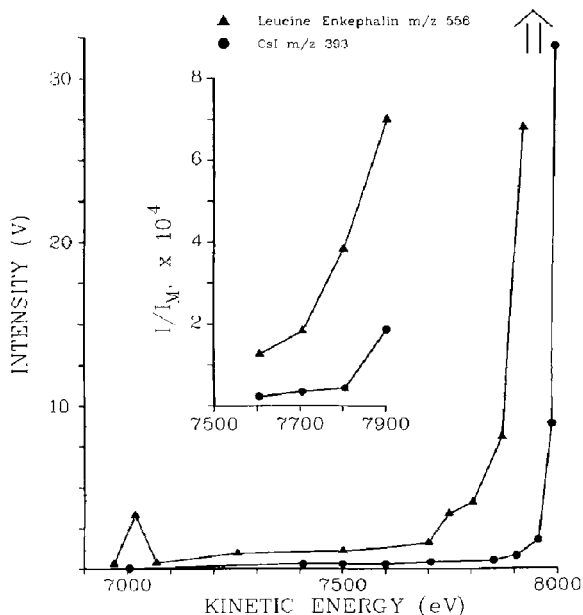
source-formed ions higher in mass-to-charge ratio than the selected precursor, which emerge from the ion source with kinetic energy less than that predicted by *Vez*. The tailing precursor ion signal observed under helium CAD conditions during mass-to-charge ratio–deconvoluted MIKES analyses is too short to account for the protracted MIKES tail, and is not observed at all under unimolecular decomposition conditions. Ions higher in mass-to-charge ratio than the chosen precursor are detected throughout the MIKES tail, with mass-to-charge ratio increasing as kinetic energy decreases. These ions possess the same momentum as the chosen precursor, and thus were formed prior to the magnetic field. The existence of intact [M + H]$^+$ ions with reduced kinetic energy is apparent from mass-to-charge ratio–deconvoluted MIKES analyses, from mass-to-charge ratio–deconvoluted, energy-selected momentum analyses, and from product ion scanning experiments. These [M + H]$^+$ ions with reduced kinetic energy do not appear to have undergone collisional deceleration because they do not appear to possess increased internal energy. The kinetic energy profiles of unfragmented FAB-desorbed ions, obtained through mass-to-charge ratio–deconvoluted IKE analyses, exhibit a tailing character very similar in appearance to that of the MIKES tail. The population of ions emerging from the source under FAB conditions thus incorporates the characteristics necessary to account for the MIKES tail, that is, the presence of ions of mass-to-charge ratio higher than the chosen precursor (due to matrix and other background ions), which possess reduced kinetic energy such that their momentum is identical to that of the selected precursor.

Origins of Source-Formed Ions of Reduced Kinetic Energy

There are at least three possible origins for the ions that leave the source with reduced kinetic energy without having undergone collisions. To facilitate the discussion of these possibilities, Figure 10 depicts the results of computer modeling of the conventional FAB source on the ZAB-SEQ using the SIMION ion optical modeling program [11]. The source was modeled using lens potentials experimentally determined with this instrument under typical operating conditions, as indicated. Of particular importance are the lines representing the equipotential contours within the acceleration region, several of which are labeled in the expanded plot of Figure 10b.

The first possible origin of [M + H]$^+$ ions with reduced kinetic energy arises from desolvation and declustering processes occurring during acceleration. [M + H]$^+$ ions solvated with the matrix (e.g., [M + H + glycerol]$^+$) are commonly observed in conventional FAB spectra, as are protonated multimers such as [2M + H]$^+$. If such species underwent desolvation (or declustering) in the acceleration region, the resulting [M + H]$^+$ ion would possess reduced kinetic energy,

Figure 9. Mass-to-charge ratio–deconvoluted ion kinetic energy "spectra" of leucine-enkephalin [M + H]$^+$ ions (m/z 556, filled triangles) and m/z 393 ions from cesium iodide (filled circles). The individual data points represent the maximum intensity values from analyses such as those in Figure 6 (obtained with Q operated at unit resolution). The inset shows similar data for the same analytes, obtained under identical instrumental conditions, with the detected signal intensity (I) expressed as a fraction of the main beam intensity (I_M).
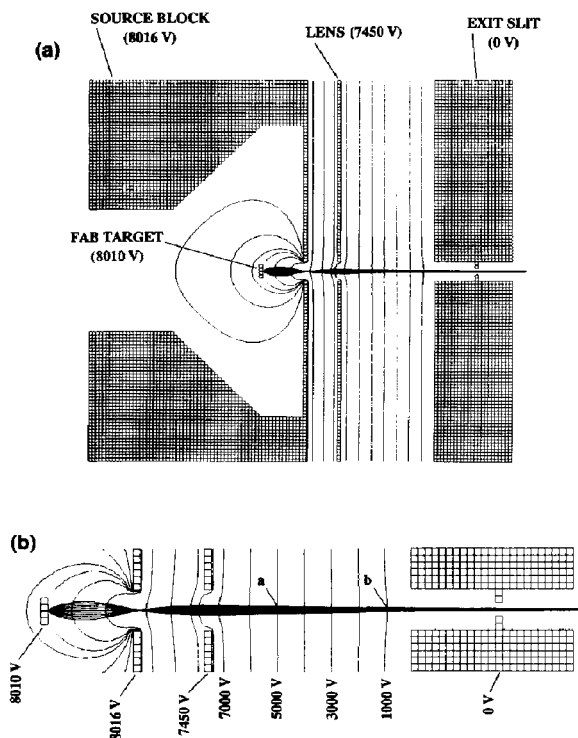
Figure 10. Plots resulting from computer modeling (SIMION) of the conventional FAB source for the ZAB-SEQ. The hatched areas represent horizontal cross sections (main axis) of the electrodes, which were modeled using the indicated potentials. The thin lines represent equipotential contours within the source. The ion trajectories were modeled for m/z 500 ions with a preacceleration kinetic energy of 0.2 eV and varying initial angles. (a) Overall plot. (b) Expanded plot.

and the value of the kinetic energy would depend upon both the nature of the desolvation and the position within the source at which the desolvation took place. As an example, consider an m/z 500 $[M + H]^+$ ion solvated with glycerol (molecular mass 92), accelerated from the 8010 V FAB target with a mass-to-charge ratio of 592 for the solvated species. If this ion underwent desolvation at point a in Figure 10b, that is, at any point on the 5000-V equipotential contour, then the resulting m/z 500 $[M + H]^+$ ion would emerge from the exit slit of the source with a kinetic energy of $[(8010 - 5000)500/592 + 5000] = 7542$ eV due to kinetic energy partitioning with the glycerol neutral. Similarly, if the protonated dimeric form of the same species ($[2M + H]^+$, m/z 999) underwent declustering at point a, the resulting m/z 500 species would leave the source with 6507 eV in kinetic energy. If the same protonated dimer declustered at a point on the 1000-V equipotential surface (point b in Figure 10b), the resulting m/z 500 ion would possess a kinetic energy of only 4509 eV. Clearly, the kinetic energy distribution of the ions resulting from these processes would depend upon the kinetics of the desolvation or declustering, and upon the time the ions spend in the various

portions of the acceleration region. Ions close to the target (the point of origin) are moving relatively slowly, while ions near the exit slit have high velocity; it is therefore likely that more desolvation or declustering events occur closer to the point of origin than near the exit slit. These considerations alone could account for the continuously decreasing nature of the intensity level in the mass-to-charge ratio–deconvoluted IKE profiles (Figure 9) as kinetic energy decreases.

The other two possible explanations for these observations both involve the ionization of desorbed neutral species at positions in the ion source removed from the FAB target (i.e., distinct from the equipotential contour corresponding to the nominal accelerating voltage). It is conceivable that small droplets, consisting of sample dissolved in matrix, are sputtered from the target during the FAB process. Such droplets could then be bombarded by fast atoms of the diffuse primary beam at points some distance away from the target, but within the accelerating region, giving rise to $[M + H]^+$ ions. (In the case of samples such as cesium iodide, these could be either hydrated droplets or microscopic crystals.) Any $[M + H]^+$ ions resulting from such a process would leave the source with the kinetic energy predicted from the equipotential contour present at the point of ionization. For instance, an $[M + H]^+$ ion, formed by the collision of a fast atom with a sputtered droplet at point a in Figure 10b, would exit the source with a kinetic energy of 5000 eV. If this phenomenon occurred, the shape of the kinetic energy profile of the source-formed ions would be determined by the physics of droplet desorption, and by any peculiarities in the focusing of the fast atom beam.

An alternative means by which ionization could occur remote from the FAB target is chemical ionization processes, which have been postulated to contribute (qualitatively and quantitatively) to the ion yield from FAB ionization [27–30]. It is well established [28, 30] that FAB results in efficient sputtering of neutrals as well as ions. A desorbed neutral molecular species could be ionized through interaction with ionized matrix in the gas phase (proton exchange), and this could occur remote from the target but within the acceleration region. Again, the kinetic energy of any resulting $[M + H]^+$ species would be determined by the position within the source where the ionization event occurred. The mass-to-charge ratio–deconvoluted IKE profile of leucine-enkephalin is significantly more intense at any given point than is that of the m/z 393 ion of cesium iodide, both in absolute and in relative terms (Figure 9), even though the main beam intensity with cesium iodide was approximately five times as intense as with leucine-enkephalin. This may reflect a contribution from chemical ionization processes to the kinetic energy profile of leucine-enkephalin, since chemical ionization could not be involved in the FAB ionization of cesium iodide. Equally, however, the difference in the intensities of the mass-to-charge ratio–deconvoluted IKE profiles may reflect differences in the kinetics of the desolvation and

declustering processes pertinent to the two sample types (i.e., a peptide dissolved in a liquid matrix as opposed to a dry crystalline salt adherent to the probe tip).

Conclusions

The broad, tailing signal in the conventional MIKE spectrum of FAB-desorbed ions obtained on BE instruments is a manifestation of intact, source-formed ions higher in mass-to-charge ratio than the intended precursor, which emerge from the ion source with kinetic energy less than that predicted by Vez. The tailing signal corresponding to decelerated precursor ions, observed under helium CAD conditions during mass-to-charge ratio–deconvoluted MIKES analyses, is too short to account for the protracted MIKES tail, and is not observed at all in the absence of collision gas. Measurements of the mass-to-charge ratio values of the ionic species comprising the MIKES tail demonstrated that ions higher in mass-to-charge ratio than the chosen precursor are present throughout the tail, with mass-to-charge ratio increasing as kinetic energy decreases. These ions possess the same momentum as the intended precursor, and thus were formed in some region of the instrument preceding the magnetic field. The existence of intact $[M + H]^+$ ions with reduced kinetic energy is apparent from mass-to-charge ratio–deconvoluted MIKES analyses, from mass-to-charge ratio–deconvoluted, energy-selected momentum analyses, and from product ion scanning experiments. The fragmentation patterns of the $[M + H]^+$ ions with reduced kinetic energy indicate that they do not possess increased internal energy, and thus it is unlikely that they have undergone collisional deceleration within the ion source. The kinetic energy profiles of unfragmented FAB desorbed ions, obtained through mass-to-charge ratio–deconvoluted IKE analyses, exhibit a tailing character similar in appearance to that of the MIKES tail. Ions higher in mass-to-charge ratio than the chosen precursor are always present under FAB conditions, and subpopulations of those ions possess reduced kinetic energy such that their momentum is identical to that of the intended precursor. The population of ions emerging from the source under FAB conditions thus incorporates the characteristics necessary to account for the MIKES tail.

If the broad tailing signal commonly observed in MIKE spectra corresponded to collided, unfragmented, and presumably excited precursor ions, one might devise mass spectrometric experiments to take advantage of their excited nature. This is not the case, however, and from an analytical point of view this tailing signal is a nuisance. Its presence can obscure low-intensity product ions so that they are not recognized, resulting in a loss of analytical information. The tailing signal can also cause distortion of the MIKES peak shape, resulting in erroneous conclusions during kinetic energy release determinations based upon peak width. These problems are particularly relevant with FAB, where ions higher in mass-to-charge ratio than the chosen precursor are always present, and where processes exist that result in a population of ions reduced in kinetic energy. These conditions are not, however, confined to the FAB ionization technique, and a tailing signal will be observed whenever higher mass-to-charge ratio ions with reduced kinetic energy are formed in the ion source. This potentially includes every known ionization technique.

The problems associated with the MIKES tail may be overcome in several ways. The simplest approach is to employ an E_1BE_2 instrument for MIKES analyses [31], because the first electric sector will filter out the reduced kinetic energy ions formed in the ion source and those formed between the source and E_1. Any ions unrelated to the chosen precursor appearing in the MIKE spectra with this instrument design would have to be formed between E_1 and B, and such species will generally appear as discrete peaks rather than as a tailing signal, as has previously been shown [7, 8]. Residual, minor tailing of the precursor ion signal is plausibly attributed to precursor ions decelerated by collision between B and E_2 [5, 6].

When the relative abundance and mass-to-charge ratio assignment of product ions formed under high energy CAD conditions are the primary information being sought, the best solution is to employ a four-sector instrument, where the ions with reduced kinetic energy formed prior to E_1 are again filtered out by E_1, and where such ions formed elsewhere in the instrument are largely eliminated through linked scanning of B_2 and E_2. The problems associated with the MIKES tail can be overcome with a BEqQ hybrid through either linked [18, 20] or unlinked [32–34] simultaneous scanning of E and Q. Both of these methods take advantage of the mass-to-charge ratio–measuring nature of the mass analyzer quadrupole, and can eliminate the interference from higher mass-to-charge ratio species with product ion detection and identification. When kinetic energy release information is being sought, the mass-to-charge ratio–deconvoluted MIKES technique yields kinetic energy profiles for ions of specified mass-to-charge ratio, and eliminates interference both from the tailing components and from neighboring, overlapping product ion signals in the MIKE spectrum [18, 19, 21].

Acknowledgment

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