Endothermic Ion Molecule Reactions

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Endothermic ion-molecule reactions in a tandem mass spectrometer have been used for a number of years for determining thermodynamic quantities, such as heats of formation and proton affinities, for gaseous ions. Recently, the reactive, endothermic collision has been exploited as an analytical technique for the structural analysis of peptides and other biomolecules. The technique is based upon the endothermic transfer of protons associated with amide bonds to ammonia. This reaction proceeds via a long-lived collision complex. When additional beam energy is supplied, other dissociation channels are opened up, leading to the production of sequence ions for the mass-selected, protonated analyte that are normally observed in high energy collision-induced dissociation spectra. The advantage, however, is that such spectra can be produced at very low beam energies. In this article, the rationale for developing this scheme, and its roots in previous ion-molecule studies, are explored. *(J Am Soc Mass Spectrom 1991, 2, 189–197)*

Tandem mass spectrometry (MS/MS) has become one of the most powerful techniques for the structural analysis of biological molecules. Tandem mass spectrometers consist of two separate mass analyzers, connected generally by a *collision chamber*. In the most common type of experiment, the first mass analyzer (MS-I) is not scanned but is tuned to transmit ions of a selected mass. These ions are then activated by collision with an inert target gas (commonly helium), and their decomposition products (fragment ions) are analyzed by the second mass spectrometer (MS-II). Such instruments can be used to obtain the sequences of peptides, carbohydrates, and other biomolecules even when these are presented to the instrument as mixtures.

Four-sector tandem instruments (EBEB) are constructed from two double-focusing mass spectrometers using combinations of electrostatic (E) and magnetic (B) fields. Because of their high mass ranges, four-sector instruments are well suited for the amino acid sequence analysis of peptides obtained from high performance liquid chromatography fractionation of a tryptic digest of a protein. One commonly encounters situations in which two or more peptides coelute, so the ability to obtain sequence spectra of individual components in a mixture becomes a tremendous advantage. In the four-sector instrument, ions selected by MS-I are most commonly fragmented by collision-induced decomposition (CID) at high energy, utilizing the full accelerating energy (8–10 keV) obtained from the ion source in MS-I, as shown in Figure 1a. Alternatively, intermediate collision energies are often utilized by electrically floating the collision cell. Because the energy spread of the product ions is thereby minimized, this improves mass resolution in MS-II, in which the product ion mass spectrum is recorded by a linked scan of E and B (Figure 1b). Such collisions are also considered to be high energy collisions.

In triple-quadrupole (QqQ) instruments, the first and third quadrupoles are the mass analyzers MS-I and MS-II, while the middle (rf-only) quadrupole passes ions of all masses and acts as a collision region. Such instruments can be used in much the same way as four-sector mass spectrometers, but are more limited in mass range. In addition, they employ low energy (10–100 eV) collisions for ion activation and dissociation. Fragmentation resulting from low energy collisions proceeds by somewhat different mechanisms than from high energy collisions. Nevertheless, low energy collisions are effective because the collision cross sections are high and also because the longer residence time in the collision chamber of these slower moving ions increases the probability for multiple collisions.

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1044–0305/91/$3.50

Received September 11, 1990
Accepted December 10, 1990

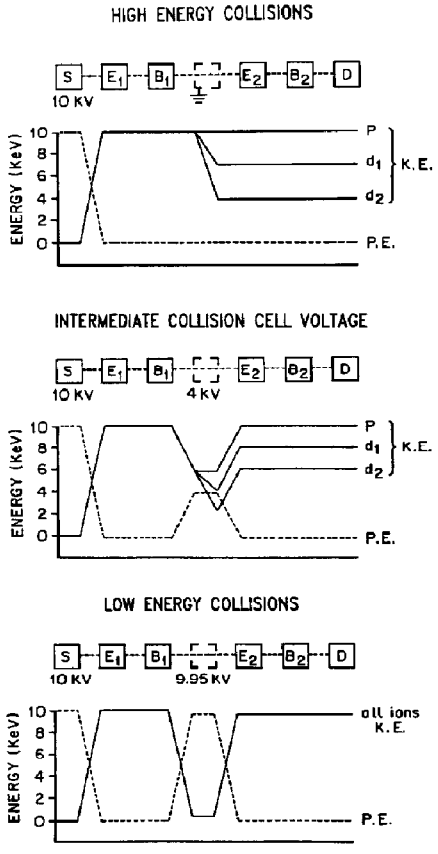

Figure 1. Potential and kinetic energies of ions in a four-sector tandem mass spectrometer. **(Top)** High energy collisions for a collision cell at ground potential. **(Middle)** High energy collisions when the collision cell is floated at voltage intermediate between the ion source and ground. **(Bottom)** Low energy collisions, such as those used for endothermic ion-molecule reactions.

Early Tandem Mass Spectrometers

In 1954, Lindholm [1] constructed the first tandem mass spectrometer. In this instrument ions selected by the primary mass spectrometer (PMS) have energies of 1 keV and are decelerated prior to entering the collision chamber. Deceleration lenses that provide excellent focusing at very low beam energies are critical in such instruments and were described early on by Futrell and Miller [2]. In Lindholm's instrument, product ions were extracted at 90°, with respect to the incoming primary ion beam, in order to discriminate against ions with appreciable forward momentum. Thus, the instrument was most suitably employed for the study of charge-exchange reactions.

In the following decade, tandem mass spectrometers were constructed by Fite et al. [3], Stebbings et al. [4], Lehrle et al. [5], Abbe and Adloff [6], and Weiner et al. [7], the latter used earlier by one of us (RJC) for the study of a number of endothermic reactions. These instruments were used (in part) for the determination of thermodynamic quantities (heats of formation, proton affinities, etc.) of gaseous ions. Prior to the introduction of tandem mass spectrometers, such quantities were determined by bracketing techniques, which were based upon the success or failure of observing an exothermic reaction occurring in the ion source of a single mass spectrometer. For example, in 1956 Tal'rose and Frankevich [8] observed the reaction

$$H_2S^+ + H_2O \rightarrow HS + H_3O^+ \quad (1)$$

which is exothermic, but did not observe the reaction

$$C_2H_2^+ + H_2O \rightarrow C_2H + H_3O^+ \quad (2)$$

which must therefore be endothermic. From these results they were able to establish a range for the proton affinity of water between 7.08 and 7.4 eV. Note that H_2S and acetylene (in reactions 1 and 2, respectively) were present in the source at much higher pressure than that of H_2O. This ensures that H_3O^+ (in both cases) is formed only from secondary processes and that the reverse reaction does not take place. The use of a reagent gas in excess of an analyte is, of course, the basis for chemical ionization (CI) mass spectrometry. In their original paper describing CI, Munson and Field [9] used methane as a reagent gas. In this case methane is ionized preferentially, and methane ions undergo a spontaneous (exothermic) proton transfer reaction as they collide with other reagent molecules

$$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3 \quad (3)$$

These reagent ions (CH_5^+) will then transfer a proton to a sample molecule (M) that has a higher proton affinity (i.e., for which the reaction

$$CH_5^+ + M \rightarrow MH^+ + CH_4 \quad (4)$$

is exothermic). When the proton affinity of the sample molecule is only slightly higher than that of the reagent molecule, ionization will be soft (i.e., there will be little fragmentation of the protonated molecule). Methane, isobutane, ammonia, and many other gases have been used and provide a range of proton affinities that can be used to control the degree of fragmentation. In addition, reagent gases can be chosen that will selectively ionize particular classes of compounds that have higher proton affinities than the reagent gas, but will not ionize those for which the proton transfer reaction is endothermic. This occurs because the kinetic energies of the ions and molecules in the source are essentially thermal. For example, ammonia is commonly used as a reagent gas for ionizing carbohydrates and glycosides. In this case, it is also interesting to note that this generally leads to

the formation of stable adduct ions (MNH_4^+), rather than the protonated species.

Tandem mass spectrometers offer the opportunity to separate the primary ionization processes from secondary reactions between these ions and neutral molecules. More important, these instruments make it possible to study endothermic reactions because the heats of reaction can be derived from the kinetic energies of the primary ions, or more precisely, from the relative energy (E_{rel}) between the projectile ion and the neutral target in the center-of-mass frame

$$E_{rel} = (E_{LAB} \times M_n)/(M_{ion} + M_n) \quad (5)$$

where M_{ion} is the mass of the projectile ion, M_n is the mass of the neutral target, and E_{LAB} is the primary ion beam energy. Such studies can be carried out using reactive or nonreactive (CID) collisions. Thus (continuing our example), Friedman and co-workers [10] studied the endothermic reaction

$$D_3O^+ + He \rightarrow D_2O + D^+ + He \quad (6)$$

using CID of D_3O^+ with helium and determined a proton affinity of 8.0 eV for D_2O, or 7.9 eV for H_2O (after correction for zero-point energies). Alternatively, in 1973 Cotter and Koski [11] studied the reactive, endothermic collision

$$D_3O^+ + D_2 \rightarrow D_3^+ + D_2O \quad (7)$$

and, by using the deuteron affinity of D_2 reported previously [12], determined the proton affinity of H_2O (corrected for zero-point energies) as 7.20 eV.

Collision Complexes and Spectator-stripping Mechanisms

Proton affinities, heats of formation, and other thermodynamic quantities for gaseous ions and neutrals [13] have been derived from studies of endothermic ion-molecule reactions on tandem mass spectrometers. In such studies, ions selected from the primary mass spectrometer are decelerated to varying beam energies as they enter the collision chamber. The beam energy (kinetic energy of the ion in the laboratory frame) at which the product ion is first observed (threshold) is converted to the relative energy according to eq 5. Because thresholds often exhibit considerable tailing due to the kinetic energy distributions of both projectile ions and target neutrals, some correction for this doppler broadening [14] can be made by deconvoluting these effects [11, 12]. In addition, the threshold is generally corrected by calibration against a reaction of known endothermicity, in order to account for instrumental uncertainties in determining the actual beam energy.

Using conditions in which single collisions predominate, the assumption is made that the threshold represents a point at which the beam energy (in the center-of-mass frame) corresponds to the (endothermic) heat of reaction. This is most easily understood in cases in which the beam energy is converted into the internal energy of collision complex formed between the reactant ion and neutral. In some cases, collision complexes are persistent or long-lived and can be observed as detectable ions; these are referred to as sticky collisions [15]. In other cases, their existence as intermediates in the ion-molecule reaction can be demonstrated by angular distribution measurements [15, 16]. In such cases the distribution of angular velocities of the product ion is symmetric about the center-of-mass velocity. Collision complexes generally occur only at or near the endothermic threshold. At higher energies, direct processes occur that result from reactions in which only a portion of the projectile ion or target molecule participates. Such spectator-stripping mechanisms generally show considerable asymmetry and forward momentum in their angular distributions about the center-of-mass. However, they should be distinguished from CID processes, which are also direct and carry the forward momentum of the primary ion, because they do involve a chemical reaction.

In 1965, the phase-space theory for ion-molecule reactions was introduced by Light [17]. The essential assumption of this theory is that all information about the initial states of the colliding particles (i.e., direction and magnitude of velocity) is lost when the collision complex forms. The distribution of product ion from the collision complex will reflect the internal (electronic, vibrational, rotational) energy states of the complex and those of the product ions and neutrals. If one monitors a product ion for a reaction that proceeds via a long-lived complex, the ion intensity will increase at beam energies above the threshold as the internal energy of the complex rises. At still higher beam energies, the product ion intensity will begin to decrease. The classical explanation for this decrease was provided by Gioumousis and Stevenson [18], who noted that the cross section for capture of the ion by the neutral molecule falls off as $(E_{rel})^{1/2}$. In the phase-space theory, however, the product ion decreases much more rapidly with beam energy as the higher internal energy of the complex becomes distributed over an increasing number of alternate dissociation channels.

Endothermic Reactions on Four-sector Instruments

The instrument constructed by Weiner and Koski (and used earlier by one of the authors) differed from the instruments devised by Lindholm and co-worker [1, 19] in that the primary mass spectrometer was floated at a voltage corresponding to the beam energy, obviating the need for decelerating while focusing the ion beam [7]. Like many other instruments of the time, product ions were extracted from the collision chamber at 90°, with respect to the primary beam, by

applying a low voltage on a repeller located in the collision chamber. Thus, the instrument was used initially for charge-exchange reactions [20] in which the product ions did not carry momentum in the primary ion beam direction. When used for the study of endothermic ion-molecule reactions, this arrangement discriminates in favor of reactions involving a collision complex, although somewhat higher repeller voltages could be used to extract ions from direct, stripping reactions at relatively low beam energies. This arrangement would be least favorable for CID at high beam energies for which the product ions carry appreciable forward momentum.

In contrast, tandem four-sector instruments are generally designed for CID at high energies. The entrance and exit slits on the collision chamber for the primary and product ion beams, respectively, are collinear. In its intended high energy mode, product ions enter the second mass analyzer with considerable forward momentum

$$p = (2m_2E)^{1/2} \quad (8)$$

corresponding to kinetic energies

$$KE = (m_2/m_1)eV \quad (9)$$

where m_1 = mass of the primary ion, m_2 = mass of the product ion, and V = accelerating voltage. One then accounts for the dependence of ion kinetic energy on the product mass by using linked scans of the electric (E) and magnetic sector (B) fields in MS-II.

Using Endothermic Reactions for the Structural Analysis of Biomolecules

We have recently developed and extended endothermic ion-molecule reactions proceeding through a collision complex for the structural analysis of peptides [21-23], carbohydrates containing amino sugars [24], and dimethyl amide derivatives of myristic acid [25]. Our experiments were conducted on a JEOL (Tokyo, Japan) HX110/HX110 four-sector (EBEB) tandem mass spectrometer. When endothermic ion-molecule reactions are carried out near threshold, both the collision complex and its fragment ions will have momentum distributions that are symmetric and thermal (in the center-of-mass frame), reflecting the thermal distributions of the primary particles. Because the center-of-mass velocity of the products ions is low, MS-II can be scanned in B only. This arrangement is shown in Figure 1c, in which the collision chamber is floated to within a few volts of the ion source. The beam energy was determined (approximately) by measuring the voltage difference between the source and the collision chamber.

Initially we used ammonia as a collision gas for the endothermic transfer of protons from small peptides to ammonia, based upon a 1973 study by Yamdagni and Kebarle [26] of the reaction between ammonium ions and acetamide

$$NH_4^+ + CH_3CONH_2 \rightarrow NH_3 + CH_3CONH_3^+ \quad (10)$$

which is slightly exothermic (−0.16 eV, although more recent values [13] for the proton affinities of acetamide place the heat of this reaction as low as −0.10 ev). Peptide samples were ionized in the ion source by fast atom bombardment, which generally yields protonated peptide ions. For peptides that do not carry very basic residues (arginine, lysine), we assumed that protonation occurs primarily at the amide bond, so that proton transfer to ammonia would resemble the reverse (acetamide) reaction, with an endothermicity of about 0.16 eV.

The reaction between protonated leucine enkephalin (MW 555) and ammonia as a function of laboratory beam energy is shown in Figure 2. Because it is observed, the proton-bound collision complex (MNH_4^+) is long-lived and has the same threshold as the product ion (NH_4^+) at 6 eV. On the basis of 5, this corresponds to an endothermicity of 0.18 eV, suggesting that our assumptions on the site of protonation are correct. As the beam energy is increased, the intensities of both ions rise to a maximum (at 7.5 eV), and then rapidly decrease. At this point the internal energy of the collision complex is sufficiently high as to open other fragmentation channels. These channels correspond to the production of those sequence ions normally observed for this peptide in CID spectra; the C-terminal sequence ions (m/z 425 and 397) and the N-terminal sequence ion (m/z 279) are shown in Figure 3. At 15 eV, the fragment ion intensities have decreased, presumably because they can no longer be formed via a long-lived complex. Their intensities rise slowly as they are produced by low energy CID processes, and in this higher energy regime the spectra are nearly identical with those produced when helium is used as a target gas [23].

It is interesting as well to examine the ratio of the intensity of the b_3 ion (m/z 278) to the y_2 fragment ion (m/z 279) from leucine enkephalin as a function of beam energy. Several investigators [27, 28] have noted that this ratio is dependent upon the collision energy, and at a high collision energy of 6 keV it reaches its maximum of 1.5 + /− 0.2. Figure 4 shows the ratio of the m/z 278/279 intensities for collisions between leucine enkephalin and ammonia. At 10 eV (when the highest intensity of sequence ions arising from dissociation of the collision complex is observed) this ratio reaches a maximum slightly above 1.5. It then decreases as the collision complex is no longer formed, and rises again with increasing beam energy. This similarity between the results obtained by endothermic reactions at low energy and high energy CID led to our subsequent investigations (see below) into the possibility that charge-remote fragmentation could also be observed from collision complexes.

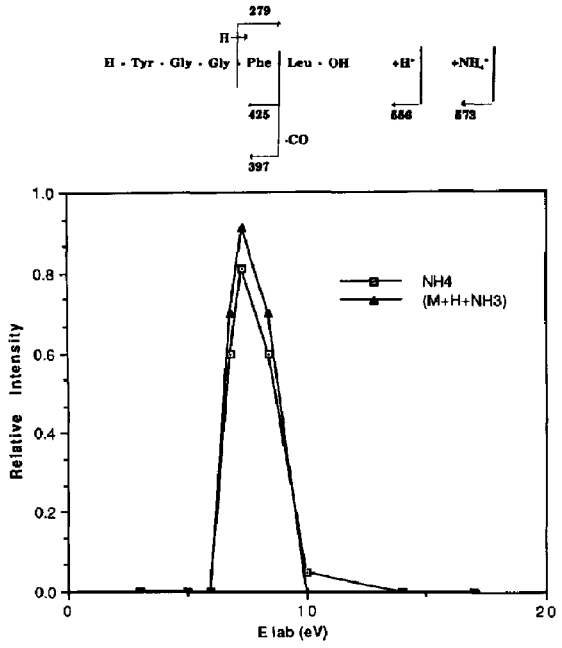

Figure 2. Relative intensities of the proton-bound complex and ammonium ions versus ion kinetic energy for the reaction of protonated leucine enkephalin with ammonia. (*Organic Mass Spectrometry*, R. Orlando, C. Fenselau, R. J. Cotter. ©1989, reprinted by permission of John Wiley & Sons, Ltd.)

Why the Endothermicity Must Be Low

For the hexapeptide Leu-Trp-Met-Arg-Phe-Ala (MW 823), the charge on the protonated molecular ion will be carried by the basic arginine residue. While possible, proton transfer from arginine to ammonia is a more endothermic process than transfer from the amide bond. The results for the collision between the protonated molecular ion of this hexapeptide and ammonia as a function of beam energy are shown in Figure 5. There is some indication of the formation of a collision complex at 22 eV in the laboratory frame (corresponding to an endothermicity of 0.45 eV); however, fragmentation by direct, low energy CID occurs much earlier and competes with the formation of a collision complex. Similar results were observed for luteinizing hormone-releasing hormone (MW 1182), which also contains arginine residues. In both cases, the onset of direct CID processes occurred at relative energies of 0.32 eV, suggesting that endothermic reactions must be selected that are below this limit.

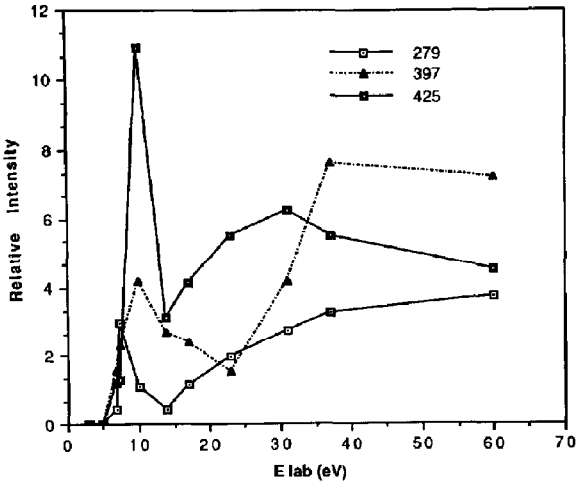

Figure 3. Relative intensities of the fragment ions versus ion kinetic energy for the reaction of protonated leucine enkephalin with ammonia. (*Organic Mass Spectrometry*, R. Orlando, C. Fenselau, R. J. Cotter. ©1989, reprinted by permission of John Wiley & Sons, Ltd.)

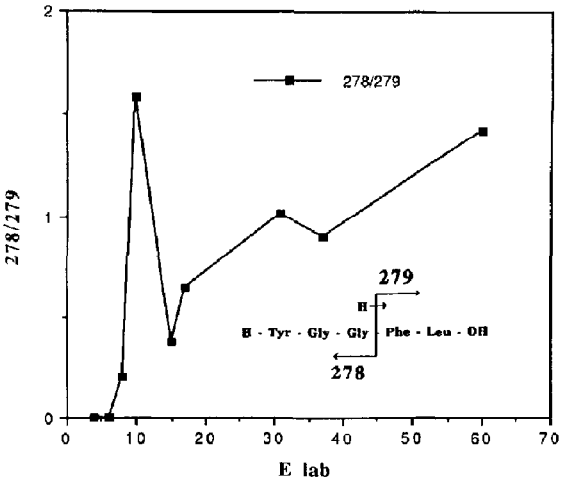

Figure 4. Ratio of the b_3 fragment ion (m/z 278) to the y_2 fragment ion (m/z 279) versus ion kinetic energy for the reaction of protonated leucine enkephalin with ammonia. (*Rapid Communications in Mass Spectrometry*, R. Orlando, C. Fenselau, R. J. Cotter. ©1990, reprinted by permission of John Wiley & Sons, Ltd.)

Charge-remote Fragmentation at Very Low Beam Energies

Ammonia is a suitable endothermic target for any compound containing an amide bond. Thus, it can be used to examine the structures of carbohydrates containing N-acetylated amino sugars. Figure 6 shows the structure of N,N′,N″-triacetyl chitotriose (MW 628) and the results for its reaction with ammonia. The onset of formation of the product ion (NH_4^+) and the collision complex (MNH_4^+) occurs at about 5 eV (or 0.13 eV in the center-of-mass frame). At 10 eV, these ions decrease in intensity as the first fragmentation channels are opened up. These channels produce fragment ions (m/z 586 and 60) that result from cleavages on either side of the amide nitrogen. At 15 eV, these ions give way to the formation of fragment

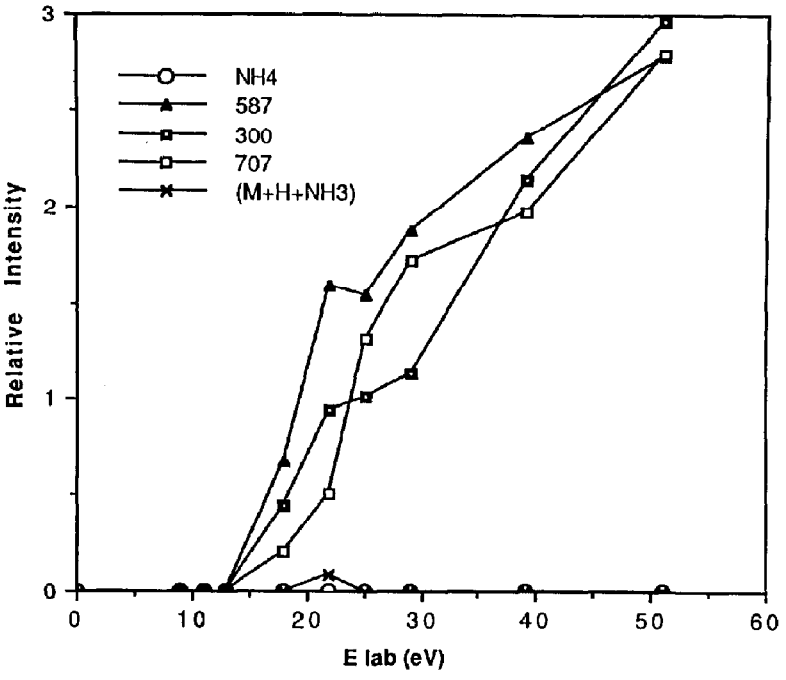

Figure 5. Relative intensities of the product ions from the reaction of the protonated molecular ion of the hexapeptide Leu-Trp-Met-Arg-Phe-Ala with ammonia as a function of the molecular ion kinetic energy. (*Organic Mass Spectrometry*, R. Orlando, C. Fenselau, R. J. Cotter. ©1989, reprinted by permission of John Wiley & Sons, Ltd.)

ions (m/z 407 and 204) resulting from cleavage of the glycosidic bond. That these ions may be attributed to dissociation of the collision complex is evidenced by their near disappearance at 30 eV. The glycosidic bond cleavages then rise slowly with beam energy as low energy CID processes predominate.

Similar results were also obtained for GalGlcNAc [24], and underscored the possibility that control of the beam energy could be used for site-directed fragmentation. Cleavage of the glycosidic bond (in this case) is remote from the protonation site, although the fragmentation might as well be explained as the result of the rearrangement. Charge-remote fragmentation was introduced by Gross and co-worker [29] for the structural analysis of long-chain fatty acids in high energy CID collisions. It has also been argued [30] that such fragmentation can also be observed in low energy CID, when multiple collisions can occur to pump up the internal energy of the protonated molecular ion. Our subsequent efforts, therefore, were directed to finding a suitable endothermic reaction scheme for long-chain fatty acids.

The reaction between protonated molecular ions of the dimethyl amide derivative of myristic acid (N,N-dimethyl myristamide, whose structure is shown in Figure 7) and monomethyl amine appeared to have the required low endothermicity. Based upon available thermodynamic data [13], the reaction

$$CH_3CONH(CH_3)_2^+ + CH_3NH_2 \rightarrow CH_3NH_3^+ + CH_3CON(CH_3)_2 \quad (11)$$

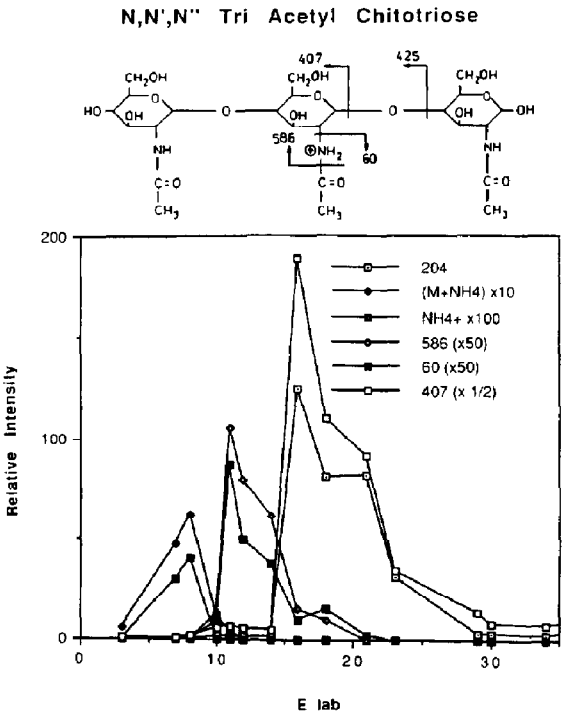

Figure 6. (**Top**) Structure and fragmentation scheme for the protonated molecular ion of N,N',N'' triacetyl chiotriose, and (**bottom**) relative intensities of the product ions from its reaction with ammonia as a function of ion kinetic energy. (Reprinted with permission from Cotter, R. J. *Analytical Chemistry* **1990**, *62*, 2389. Copyright 1990, American Chemical Society.)

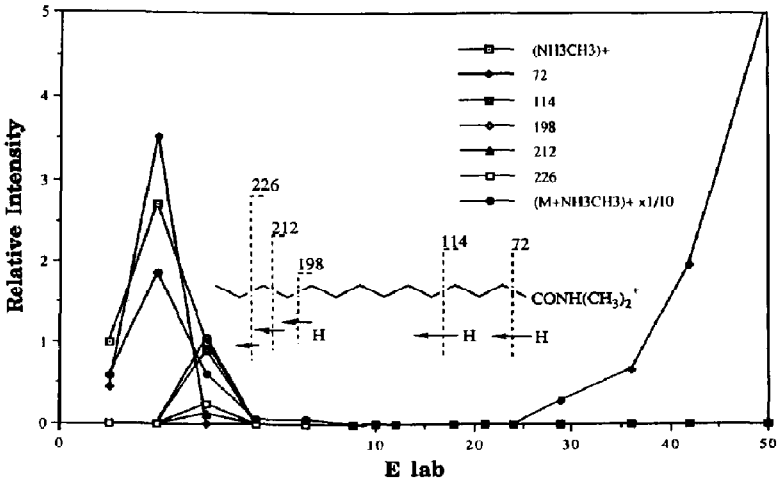

Figure 7. Structure and fragmentation scheme for the protonated molecular ion of N,N dimethyl myristamide, and relative intensities of the product ions from its reaction with monomethyl amine as a function of ion kinetic energy. The region (0-6 eV) has been expanded for clarity. (*Organic Mass Spectrometry*, R. Orlando, C. Fenselau, R. J. Cotter. ©1990, reprinted by permission of John Wiley & Sons, Ltd.)

has an endothermicity of 0.10 eV, and served as the model reaction. Results for the reaction of protonated N,N-dimethyl myristamide with monomethyl amine are also shown in Figure 7. The product of the proton transfer reaction ($NH_3CH_3^+$ at m/z 32) and the collision complex ($M + NH_3CH_3^+$ at m/z 273) are first observed at around 1.0 eV (in the laboratory frame), and reach their maximum intensity at 2 eV. This threshold corresponds to 0.11 eV in the center-of-mass frame, in agreement with the model reaction. An ion at mass 72, corresponding to the cleavage of the first C—C bond adjacent to the amide functional group, follows the same behavior. Assuming that the amide bond is the original site of protonation, formation of this fragment ion would be directed by the charge.

As the beam energy is increased, the abundances of these three ions decrease as additional dissociation channels are opened up. At 3 eV, cleavages corresponding to losses of neutral C_nH_{2n+2} remote from the charge site predominate and are associated with an intermediate collision complex with higher internal energy. Beyond 4 eV, fragment ions are no longer observed until the onset of low energy CID at 24 eV. Above 24 eV, only charge-directed ions are observed (up to 80 eV in this experiment).

Gross and co-workers [29, 31] have estimated the activation energy for charge-remote fragmentation to be between 1.4 to 2.9 eV. They have argued that such energies can more easily be transferred to the molecular ion by using high energy CID. Wysoki et al. [32] have estimated that 7–10 eV of excitation are required, and that this can be achieved on triple-quadrupole, hybrid, and ion cyclotron resonance mass spectrometers through multiple, low energy collisions. In contrast, for the endothermic reaction between dimethyl myristamide and monomethyl amine charge-remote fragmentation occurs at a relative energy of only 0.3 eV. Similarly, for the oligosaccharides N,N′,N″-triacetyl chitotriose and GalGlcNAc, cleavage localized at the amide bond occurs at 0.3 eV, while cleavages of glycosidic bonds occur at 0.43 and 0.47 eV, respectively. The fact that the onset of the collision complex and the products of the proton transfer reactions occurs at predictable beam energies corresponding to model reaction involving amides suggests that single collisions are involved, and that there is no substantial contribution from precollision energy, i.e., the protonated molecular ions are largely in their ground states.

In addition, fragmentation induced by endothermic reactions can be highly efficient, as is shown in Figure 8. For leucine enkephalin, the most abundant fragment ion (m/z 278) has an intensity of 23% relative to the surviving protonated molecular ion when reacted with ammonia at 10 eV. For high energy collisions with helium the intensity is only 2%. Alternatively, for the endothermic reaction 37.9% of the ion current was due to fragment ions, while for high energy CID it was only 1.5%. In general the reaction between protonated leucine enkephalin and ammonia (Figure 8c) produces a product ion spectrum that more closely resembles that produced by high energy CID (Figure 8a) than by low energy CID (Figure 8b) with helium. All three of these spectra were produced at target gas pressures that attenuate the ion beam by 80%.

Conclusions and Future Prospects

Prior to the introduction of desorption ionization methods, mass spectroscopists involved in the development of new techniques concerned themselves with the task of producing molecular ions from intractable (involatile) and high molecular weight compounds. These developments have been so extraordinary that it is now possible to observe molecular ions in excess of 100 kDa using ultraviolet laser desorption [33] and electrospray [34]. Thus, there is now considerable interest in improving methods for fragmenting large

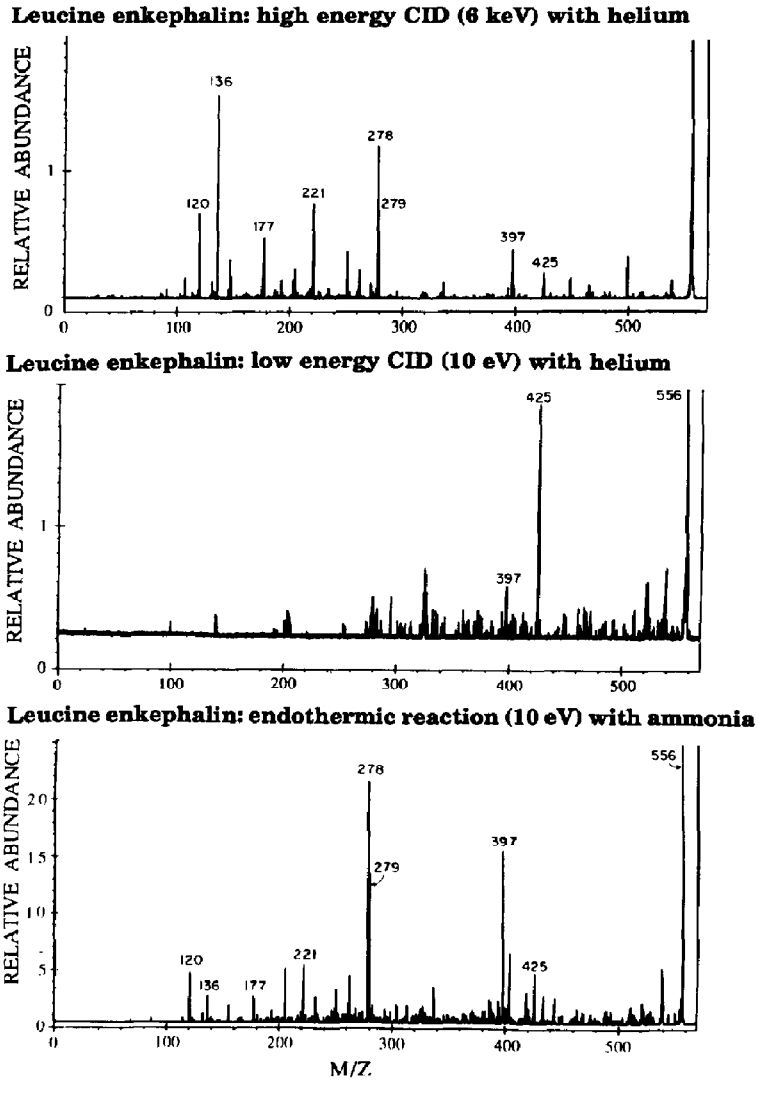

Figure 8. Product ion mass spectra for the protonated molecular ion of leucine enkephalin obtained by (Top) high energy (8 keV) collisions with helium, (**Middle**) low energy (10 eV) collisions with helium, and (**Bottom**) reactive collisions with ammonia at 10 eV. Product ion spectra were all obtained at 80% attenuation of the protonated molecular ion at m/z 556, and abundances are relative to the surviving molecular ion. (*Rapid Communications in Mass Spectrometry*, R. Orlando, C. Fenselau, R. J. Cotter. ©1990, reprinted by permission of John Wiley & Sons, Ltd.)

ions to improve structural information. While CID has been the most commonly used method, the limitations set by eq 5 motivated our efforts to apply reactive collisions to small peptides and oligosaccharides, and ultimately to much larger structures. In particular, eq 5 predicts that the relative energy (E_{rel}) available for activation decreases with the size of the protonated molecule (M_{ion}) at a particular beam energy (E_{LAB}), so that processes that occur at very low relative energies could, in effect, reduce the laboratory energy scale. Surface induced collisions [35] and laser photodissociation [36] have also been proposed as methods to overcome the limitations on relative energy imposed by eq 6. The alternative that we present allows chemistry to do the work, rather than increases in the impacting energy. That is, the formation of product ions depends upon the heat of the reaction which, through the choice of appropriate target gases, can enable reactions to occur at energies below those needed for the onset of activation by CID. Indeed, mildly exothermic reactions may also be employed, resulting in the appearance of product ions near zero beam energy, although in this case extraction and focusing of secondary ions would be somewhat more difficult to accomplish.

We would expect that such reactions may be extended to much larger molecules with the same efficiency and, indeed, our efforts are currently aimed in that direction. A major limitation has been in devising suitable reaction schemes for peptides containing very basic residues such as arginine and lysine, because tryptic fragments of proteins will generally carry one of these residues on the C-terminus. In this case, monomethyl amine shows promise as a suitable tar-

get gas. Additionally, because four-sector tandem instruments are generally optimized for high energy collisions in which product ions carry considerable forward momentum, we are developing improved deceleration and extraction optics for low velocity incoming and product ions, respectively.

At the same time, the improved efficiency of reactive collisions at low energy could prove to be beneficial to triple-quadrupole (QqQ), hybrid (EBqQ), ion trap, and Fourier-transform ion cyclotron resonance mass spectrometers, resulting in product ion spectra that resemble those obtained from the high energy collisions used in the more expensive four-sector instruments.

References

1. Lindholm, E. *Z. Naturforsch.* **1954**, *9a*, 535.
2. Futrell, J. H.; Miller, C. D. *Rev. Sci. Instr.* **1966**, *37*, 1521.
3. Fite, W. L.; Brackman, R. T.; Snow, W. R. *Phys. Rev.* **1958**, *112*, 1161.
4. Stebbings, R. F.; Smith, A. C. H.; Erhardt, H. *J. Chem. Phys.* **1963**, *39*, 968.
5. Lehrle, R. S.; Robb, J. C.; Thomas, D. W. *J. Sci. Instr.* **1962**, *33*, 458.
6. Abbe, J. C.; Adloff, J. P. *Compt. Rend.* **1964**, *258*, 3003.
7. Weiner, E. R.; Hertel, G. R.; Koski, W. S. *J. Am. Chem. Soc.* **1964**, *86*, 788.
8. Tal'rose, V. L.; Frankevich, E. L. *Dokl. Akad. Nauk SSSR* **1956**, *111*, 376.
9. Munson, M. S. B.; Field, F. H. *J. Am. Chem. Soc.* **1966**, *88*, 2621.
10. DePas, N.; Leventhal, J. J.; Friedman, L. *J. Chem. Phys.* **1968**, *48*, 1783; *J. Chem. Phys.* **1969**, *51*, 3748.
11. Cotter, R. J.; Koski, W. S. *J. Chem. Phys.* **1973**, *59*, 748–787.
12. Cotter, R. J.; Rozett, R. W.; Koski, W. S. *J. Chem. Phys.* **1972**, *57*, 4100–4103.
13. Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17 (Supp. 1)*.
14. Chantry, P. J. *J. Chem. Phys.* **1971**, *55*, 2746.
15. Futrell, J. H.; Tiernan, T. O. In *Ion-Molecule Reactions*; Franklin, J. L., Ed.; Plenum: New York, 1972, Vol. 2, pp. 485–552.
16. Shukla, A.; Qian, K.; Anderson, A.; Futrell, J. Presented at the *38th ASMS conference on Mass Spectrometry and Allied Topics*, Tucson, AZ, 1990.
17. Light, J. C. *J. Chem. Phys.* **1965**, *43*, 3209.
18. Gioumousis, G.; Stevenson, O. P. *J. Chem. Phys.* **1958**, *29*, 294.
19. Jonsson, B. O.; Lindholm, E. *Arkiv Fysik* **1969**, *39*, 65.
20. Hertel, G. R.; Koski, W. S. *J. Am. Chem. Soc.* **1964**, *86*, 1683.
21. Orlando, R.; Murphy, C.; Fenselau, C.; Hansen, G.; Cotter, R. J. *Anal. Chem.* **1990**, *62*, 125–129.
22. Orlando, R.; Fenselau, C.; Cotter, R. J. *Org. Mass Spectrom.* **1989**, *24*, 1033–1042.
23. Orlando, R.; Fenselau, C.; Cotter, R. J. *Rapid Commun. Mass Spectrom.* **1990**, *4*, 259–262.
24. Orlando, R; Fenselau, C.; Cotter, R. J. *Anal. Chem.* **1990**, *62*, 2388–2390.
25. Orlando, R.; Fenselau, C.; Cotter, R. J. *Org. Mass Spectrom.* **1990**, *25*, 485.
26. Yamdagni, R.; Kebarle, P. *J. Am. Chem. Soc.* **1973**, *95*, 355–358.
27. Baeten, W.; Claerboudt, J.; Van den Heuvel, H.; Claeys, M. *Biomed. Environ. Mass Spectrom.* **1989**, *18*, 727–732.
28. Alexander, A. J.; Boyd, R. K. *Int. J. Mass Spectrom. Ion Processes* **1989**, *90*, 211–240.
29. Adams, J.; Gross, M. L. *J. Am. Chem. Soc.* **1986**, *108*, 6915.
30. Wysoki, V. H.; Ross, M. M; Horning, S. R.; Cooks, R. G. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 214.
31. Deterding, L. J.; Gross, M. L. *Org. Mass Spectrom.* **1988**, *23*, 169.
32. Wysoki, V. H.; Bier, M. E.; Cooks, R. G. *Org. Mass Spectrom.* **1988**, *23*, 627.
33. Karas, M.; Ingendoh, A.; Bahr, U.; Hillenkamp, F. *Biomed. Environ. Mass Spectrom.* **1989**, *18*, 841.
34. Smith, R. D.; Berinaga, C. J.; Udseth, H. R. *Anal. Chem.* **1988**, *60*, 1948.
35. Mabud, Md. A.; Dekrey, M. J.; Cooks, R. G. *Int. J. Mass Spectrom. Ion Processes* **1985**, *67*, 285–294
36. Baykut, G.; Watson, C. H.; Weller, R. R.; Eyler, J. R *J. Am. Chem. Soc.* **1985**, *107*, 8036–8042.