ELSEVIER

International Journal of Mass Spectrometry 200 (2000) 137–161

International Journal of Mass Spectrometry

Novel quadrupole ion trap methods for characterizing the chemistry of gaseous macro-ions

Scott A. McLuckey*[,a], J. Mitchell Wells[a], James L. Stephenson Jr.[b], Douglas E. Goeringer[b]

[a]*Department of Chemistry, Purdue University, West Lafayette, IN, 47907-1393, USA* [b]*Chemical and Analytical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 38731-6365, USA*

Received 8 June 2000; accepted 14 August 2000

Abstract

A variety of ion manipulation techniques that have been developed to study the chemistry of gas-phase macro-ions are described and illustrated. These techniques take advantage of several unique characteristics of the quadrupole ion trap, including operation at relatively high (1 mTorr) bath gas pressure and the ability to simultaneously store positive and negative ions in overlapping regions of space. Efforts to characterize unimolecular dissociation in the ion trap via resonance excitation and thermal dissociation at elevated bath gas temperatures are described; such dissociation is of interest as a means for obtaining primary structural information and Arrhenius activation parameters for macro-ions. The use of ion/molecule chemistry to obtain composition and higher level structural information is illustrated with a description of novel HI attachment chemistry which yields information on the number and accessibility of neutral basic sites in macro-ions. Ion/ion chemistry for the manipulation of parent and product ion charge states is shown to expand the range of charge states accessible for tandem mass spectrometry (MS/MS) analysis, and to greatly simplify the interpretation of MS/MS spectra obtained from multiply charged parent ions. (Int J Mass Spectrom 200 (2000) 137–161) © 2000 Elsevier Science B.V.

Keywords: Quadrupole ion trap; Macro-ion; Unimolecular dissociation; Ion/molecule reaction; Ion/ion reaction

1. Introduction

The development of ionization methods such as electrospray [1] and matrix assisted laser desorption ionization [2], capable of forming gaseous ions from macromolecules, including proteins, oligonucleotides, carbohydrates, and synthetic polymers, presents new challenges and opportunities to developers of mass spectrometers, to analytical chemists, and to gas-phase ion chemists. The analysis of macro-ions places the mass spectrometer figures of merit of, for example, mass resolving power, mass accuracy, mass-to-charge range, and speed, at a premium. Much of the recent instrument development activity in mass spectrometry has therefore been directed toward improving the levels of performance for the characteristics just mentioned [3]. The ability to form and analyze gaseous macro-ions, which are loosely defined herein as ions comprised of more than 50 or so heavy atoms (e.g. C,N,O,P,S), has opened new applications areas to mass spectrometry to the extent that a significant

* Corresponding author. E-mail: mcluckey@purdue.edu

PII S1387-3806(00)00306-7

and growing fraction of practicing analytical chemists currently using mass spectrometers are pursuing problems related to the analysis of macromolecules, with the primary emphasis being on peptides and proteins. The rapidly growing application of mass spectrometry to the analysis of macromolecules presents new opportunities to the gas-phase ion chemistry community for making vital contributions to fields heretofore largely inaccessible for lack of means of forming the ions. Some of the most important contributions that mass spectrometry makes, and will continue to make in the coming decade, rely on the chemical reactions of gaseous macro-ions. A particularly important example is the dissociation of macro-ions for the purpose of deriving primary structural information, such as sequence information for linear polymers and branching information for nonlinear polymers. The chemistry of gaseous macro-ions remains poorly understood in spite of several already relatively mature applications of mass spectrometry that rely heavily on this chemistry. Improved understanding of the behavior of macro-ions in the gas phase will likely point to worthwhile directions for novel instrument development, improved analytical approaches to solving macromolecule measurement problems using mass spectrometry, and, possibly, altogether new analytical measurements.

In many ways, gaseous macro-ions present new challenges to the ion chemistry community insofar as these species are of higher "dimensionality" than the smaller ions familiar to most ion chemists. The higher degree of dimensionality derives from the possibility for multiple charge states, particularly in the case of electrospray ionization, as well as secondary and tertiary ion structure that can influence ion behavior. These new dimensions of complexity require the development of new tools. The development of such tools has been a highly active area for the past decade and will likely continue to see emphasis in the coming years amongst ion chemistry groups worldwide. Notable examples of novel techniques and methodologies that are proving to be highly useful for studying gaseous macro-ions include techniques sensitive to ion shapes, such as ion mobility [4–11] and collision cross-section measurements [12–18]. The use of chemical probes in ion/molecule reactions [19] giving rise to, for example hydrogen/deuterium exchange [20–28] and proton transfer [29–31] constitutes another group of novel measurements for studying the chemistry of gaseous macro-ions. An array of activation methods has been applied to macro-ions for the purpose of inducing fragmentation, particularly for peptides with masses less than about 2 kDa. Relatively little work, however, has been reported on the dissociation of ions of mass greater than about 2 kDa, within the context of a true tandem mass spectrometry experiment. The earliest reports employed collisional activation in triple quadrupole instrumentation [32–35]. By far, the most extensive line of work involving the tandem mass spectrometry of macro-ions has been reported by McLafferty and co-workers using high magnetic field strength Fourier transform ion cyclotron resonance (FTICR) instrumentation [36–42]. The McLafferty group has investigated an array of activation methods applied to ions derived from proteins and oligonucleotides. A particularly important activation method for deriving fundamental information from the dissociations of macro-ions is referred to as blackbody infrared dissociation (BIRD) [43–46], as pioneered by Williams et al. for the study of high mass ions using FTICR [46].

In this article, we review and discuss our efforts over the past decade in developing the quadrupole ion trap or Paul trap as a tool for studying the chemistry of gaseous macro-ions. The quadrupole ion trap is widely recognized as a useful tool for analytical mass spectrometry, particularly as the mass analyzer for on-line gas chromatography, liquid chromatography, and capillary electrophoresis. Many of the capabilities of the quadrupole ion trap that make it useful as an analytical mass spectrometer were developed over the course of the past two decades. The evolution of the ion trap as a tool in analytical chemistry research has been chronicled in several books [47,48] and recent reviews [49–54]. Further, a recent special issue of this journal [55] honoring the careers of John Todd and Ray March provides a cross section of the research currently being undertaken with quadrupole ion traps. In addition to its merits as an analytical mass spectrometer, the Paul trap, operated with a light bath gas

such as helium at a pressure of 1 mTorr, has a number of characteristics that, we believe, make it a powerful tool for studying fundamental aspects of high mass polyatomic ions.

Within the context as a tool for macro-ion chemistry research, it is important to distinguish the roles of the quadrupole ion trap as a reaction vessel and as a mass spectrometer. The ion trap's greatest strengths for macro-ion chemistry research lie in the former role rather than the latter. For all of the work reported here, the ion trap has served both as a reaction vessel and as a mass analyzer. However, the benefits of the ion trap as a reaction vessel and an ion manipulation device can also be enjoyed with hybrid instruments in which mass analysis is performed with another form of mass spectrometry [56–63]. In this article we place virtually exclusive emphasis on macro-ion chemistry issues. In particular, we describe the use of the quadrupole ion trap for the study of the unimolecular reactions of macro-ions, ion/molecule reactions involving macro-ions, and ion/ion reactions involving oppositely charged ions in which multiply charged macro-ions constitute at least one of the reactants. To limit the scope of this overview, we highlight recent work with defining ion internal energies for dissociation studies, the attachment of hydroiodic acid to polypeptides as an example of ion/molecule reaction chemistry, and ion/ion reactions involving proton transfer. Essential aspects of the experimental conditions are mentioned in the relevant sections. However, the reader is encouraged to refer to the original literature for experimental details associated with the various experiments alluded to below.

2. Unimolecular dissociation of macro-ions

Next to the chemistry associated with ionization, unimolecular fragmentation is the most important chemical reaction in many mass spectrometry experiments. It was demonstrated roughly 15 years ago that ions stored in a quadrupole ion trap in the presence of 1 mTorr of helium could be induced to fragment by accelerating a parent ion population at its fundamental *z*-dimension secular frequency by applying a sine wave of the appropriate frequency to the endcap electrodes [64]. This so-called "resonance excitation" procedure is one of several techniques intended to accelerate parent ions in the presence of the bath gas [65–71] and remains a widely used technique for collisional activation of ions in an ion trap. An alternative approach to resonance excitation for collisional activation in an ion trap is to heat the bath gas [72,73]. The two approaches are both intended to increase the relative velocities of the collision partners. Resonance excitation does so by accelerating the ion whereas heating the bath gas does so by "accelerating" the bath gas atoms. As discussed further in this section, both approaches are likely to be important in the study of the unimolecular fragmentation of gaseous macro-ions and each is discussed in turn below.

2.1. Thermal dissociation of macro-ions in a quadrupole ion trap with heated bath gas

To study dissociation reactions of mass-selected macro-ions at well-defined temperatures is highly desirable, so that Arrhenius parameters, which provide important insights into the energetic and entropic requirements of these reactions, can be derived. The environment for stored ions in a quadrupole ion trap is unique in mass spectrometry in that a relatively high pressure of background gases is usually present in the ion-trapping region. The background gas therefore provides a mechanism for relatively rapid energy exchange between the ion and its environment. Energy exchange by means of absorption and emission of infrared photons [74–76], which forms the basis for BIRD in the rarefied environment of the FTICR instrument [43–45], also occurs, of course, in the quadrupole ion trap. However, the four to six orders of magnitude greater background pressure in the quadrupole ion trap relative to that of the typical FTICR experiment results in significantly greater rates of energy exchange in the ion trap by means of collisional exchange with the bath gas. Thermal dissociation experiments involving macro-ions have been performed in electrospray interfaces [77–80] at even higher pressures than those used in the ion trap.

Although the use of thermal dissociation in the electrospray interface region can yield quantitative information about the energetics and dynamics of macro-ion dissociation, the experiment suffers from the disadvantage that it does not define the parent ion/product ion relationship as does a true tandem mass spectrometry experiment. The unique ability of the ion trap to select parent ions and then store them at relatively high background pressure motivated us to explore the use of the quadrupole ion trap as a tool for measuring macro-ion dissociation rates and collecting product ion spectra as a function of bath gas temperature.

Two key issues must be addressed in interpreting dissociation rate behavior of a macro-ion as a function of bath gas temperature in a quadrupole ion trap. The first is the relationship between the ion internal temperature and the bath gas temperature under the ion trap operating conditions used to collect the data. The second is the extent to which the ions approach the high pressure or "rapid energy exchange" limit in the dissociation rate ranges that are convenient for study using the ion trap (i.e. 0.01–100 s^{-1}). As demonstrated in BIRD studies, the likelihood that rapid energy exchange conditions prevail for a given dissociation rate increases with the number of degrees of freedom of the ion. It is highly desirable to acquire dissociation rate data under rapid energy exchange conditions because Arrhenius activation parameters can be derived directly from such data. Our initial study of these two issues was conducted by using protonated leucine enkephalin, a five residue polypeptide ion of *m/z* 556, as the test substrate. Although this ion does not strictly fit the definition of a macro-ion used herein, it constitutes a relatively stringent test for the use of heated bath gas measurements in deriving accurate Arrhenius parameters from dissociation rate kinetics of macro-ions because it is a small ion relative to most macro-ions of interest to us.

The first issue mentioned previously relates to the extent that a stored macro-ion undergoes internal heating while being stored in an electrodynamic ion trap with a bath gas present. In such a scenario, an ion accelerated by the rf trapping voltage undergoes inelastic collisions with the bath gas resulting in a

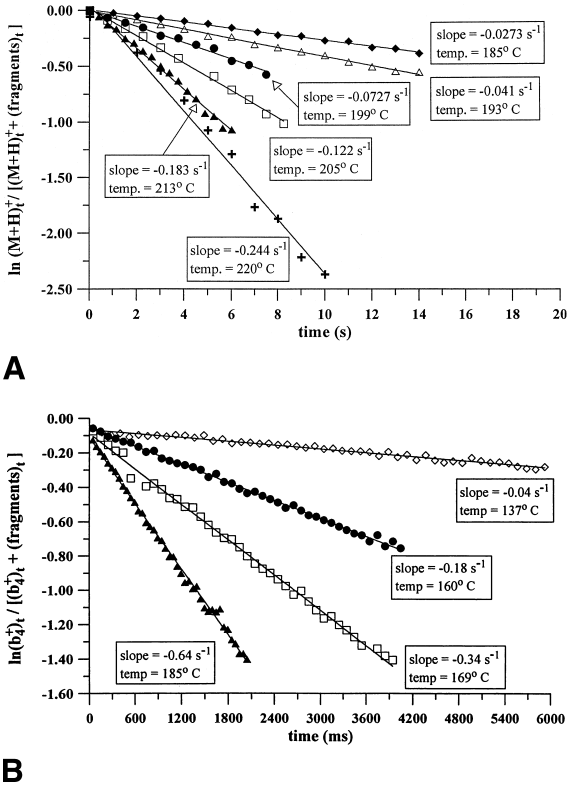

Fig. 1. Kinetic data collected for (a) protonated leucine enkephalin and for (b) b_4^+ product ion from protonated leucine enkephalin. Reprinted with permission from [73].

situation in which the parent ion internal temperature is elevated relative to the bath gas temperature. Ion acceleration due to the rf trapping voltage is sometimes referred to as "rf heating." The magnitude of rf heating is related to the coupling between ion motion and the trapping voltage [81–84] and is a function of how closely an ion approaches a stability boundary of the ion trap stability diagram. Advantage is taken of rf heating as a means for parent ion acceleration in ion trap collisional activation [70,85,86] by deliberately placing ions near a stability boundary in a technique referred to as "boundary-activated dissociation" (see the following). In the context of the present experiment, parent ion activation by means of rf heating is undesirable insofar as it introduces uncertainty in establishing the internal temperature of the parent ion. Fig. 1 shows the kinetic data collected for protonated leucine enkephalin [Fig. 1(a)] and its primary disso-

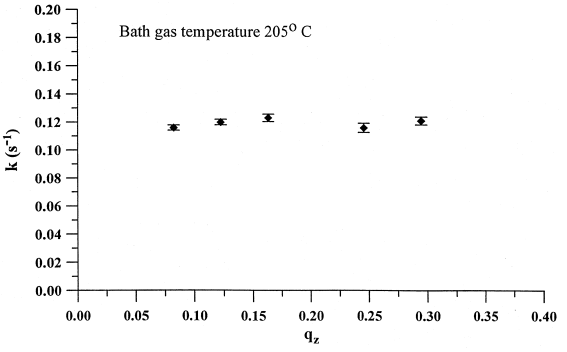

Fig. 2. Dissociation rate of protonated leucine enkephalin using a bath gas at 205 °C plotted as a function of the dimensionless storage parameter q_z. Error bars represent $\pm 1\sigma$ of the slopes from which the rate data were derived. Reprinted with permission from [73].

ciation product, the b_4^+ ion [Fig. 1(b)], from which dissociation rates are derived. It is clear that the rates at which these ions dissociate are highly sensitive to the bath gas temperature. For example, the dissociation rate of protonated leucine enkephalin increases nearly an order of magnitude over the temperature range of 185–220 °C. This ion can therefore serve as a sensitive thermometer for changes in the parent ion internal temperature that might arise due to ion storage in the electrodynamic field. Fig. 2 shows the result of a key experiment in which the dissociation rate of protonated leucine enkephalin using a bath gas at 205 °C is plotted as a function of the dimensionless storage parameter q_z, which is proportional to the magnitude of the rf trapping voltage. Within experimental error, no variation in the dissociation rate of leucine enkephalin is observed. This result indicates that no measurable change in parent ion internal temperature occurs over this range of ion storage conditions. If rf heating were important, an increase in dissociation rate would be expected as the q_z value (and the rf trapping voltage) is increased. Over this range of q_z values, the parent ions are relatively remote from any stability boundary such that ion acceleration due to the trapping field is expected to be small. Combined with the relatively inefficient conversion of translational to internal energy in collisions of heavy ions with light gases such as helium, rf heating is expected to be small for macro-ions provided they are stored remote from a stability boundary. At least for leucine enkephalin ions and, by extrapolation, any ion of higher mass this study suggests that the bath gas temperature is a very good approximation for the polyatomic ion internal temperature provided care is taken to avoid bringing the ions close to a stability boundary.

Regarding the second issue mentioned previously, the approach to rapid energy exchange, master equation modeling suggested that protonated leucine enkephalin approached rapid energy exchange conditions more closely than did the b_4^+ fragment ion under the conditions used to acquire dissociation rate data. Assumptions about collisional energy transfer required in the master equation modeling precluded a quantitative estimation of the approach to rapid energy exchange conditions for the leucine enkephalin ions; however, the modeling suggested that protonated leucine enkephalin begins to deviate significantly from rapid energy exchange conditions above a dissociation rate of about 1 s^{-1}. Interestingly, Arrhenius parameters for protonated leucine enkephalin measured with ion trap thermal dissociation [73], thermal dissociation in an electrospray ion source [80], and BIRD [87] do not agree within the errors of the reported measurements. Although the reasons underlying these differences are not clear, one possibility may be different extents of approach to the rapid energy exchange condition. A recent ion trap thermal dissociation study of ions derived from bradykinin, a nonapeptide, provided experimental evidence for "high pressure" limit (i.e. rapid energy exchange) behavior [72] over the rate ranges studied. Fig. 3 for example shows dissociation rate data for the $(M + H)^+$, $(M + 2H)^{2+}$, and $(M + 3H)^{3+}$ ions as a function of bath gas pressure over the range of 0.3–2.3 mTorr. The insensitivity of the observed dissociation rates to bath gas pressure over this range is consistent with rapid energy exchange behavior. In this case, Arrhenius parameters derived from the $(M + H)^+$ and $(M + 2H)^{2+}$ ions agreed with those reported using BIRD [88]. No independent measurements have been reported for the $(M + 3H)^{3+}$ ion. Note also that like the ions derived from leucine enkephalin, the dissociation rates measured for the bradykinin ions were

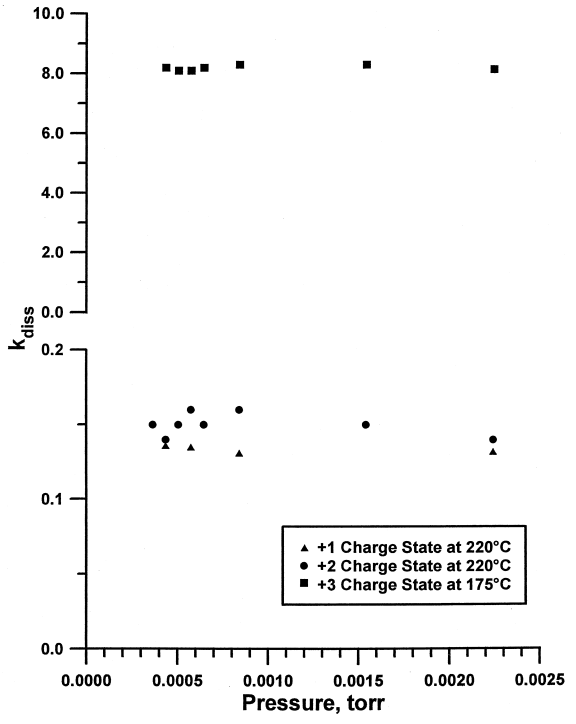

Fig. 3. Dissociation rate data for the $(M + H)^+$ (triangles), $(M + 2H)^{2+}$ (circles), and $(M + 3H)^{3+}$ (squares) ions of bradykinin as a function of bath gas pressure over the range 0.3–2.3 mTorr. Reprinted with permission from [72]

independent of the q_z value at which they were stored over the range of $q_z = 0.075–0.38$, providing further evidence that rf heating does not significantly affect the internal temperature of these large ions.

Although further studies are needed to establish, and to experimentally verify, the experimental conditions over which rapid energy exchange conditions are well approximated in ion trap thermal dissociation experiments, it is clear from the leucine enkephalin and bradykinin studies that rf heating is insignificant and hence the bath gas temperature is a close approximation to the parent ion internal temperature for macro-ions. Further, the bradykinin studies suggest that the rapid energy exchange condition is approximated by bradykinin ions at rates at least as high as 10 s^{-1}. It is therefore clear that ion storage conditions can be readily established whereby thermal dissociation experiments in the quadrupole ion trap can be used to measure Arrhenius parameters of macro-ions. This capability, combined with the well-known tandem mass spectrometry (MS^n) capabilities of the quadrupole ion trap [89,90], makes the quadrupole ion trap a flexible tool for the study of the unimolecular dissociation reactions of macro-ions.

2.2. Collisional activation via macro-ion acceleration in a quadrupole ion trap

Resonance excitation was the first approach developed for the dissociation of ions within the context of an ion trap tandem mass spectrometry (MS/MS) experiment and it is still widely employed. We have had a longstanding interest in developing theoretical tools for relating experimental conditions used to effect resonance excitation in the ion trap to the dynamics of energy transfer, parent ion internal energy distributions, and dissociation kinetics [91–94]. This work stems from observations made with relatively small organic ions that collision-induced dissociation kinetics in the ion trap are related to the critical energy for dissociation of the parent ion [95].

In our consideration of the factors that underlie collision-induced dissociation in the ion trap under resonance excitation conditions it was clear that Dunbar and co-workers had discussed a highly analogous situation involving the dissociation kinetics of ions undergoing continuous irradiation by infrared photons in the rarefied environment of the ion cyclotron resonance instrument [96–99]. Dunbar and coworkers have pointed out that under conditions of relatively low laser intensities, irradiated ions achieve a Boltzmann distribution of internal energies. Dissociation kinetics can therefore be modeled using Boltzmann statistics to describe the ion internal energies with some adjustments to account for the effect of dissociation on the shape of the internal energy distribution. Under rapid energy exchange conditions, this effect is minimal so that ions can be regarded as having a temperature. We have developed a collisional energy transfer model to simulate the resonance excitation process in the ion trap [91,92]. In analogy with continuous irradiation by low intensity infrared photons, ion trap resonance excitation leads to a steady-state Boltzmann parent ion internal energy

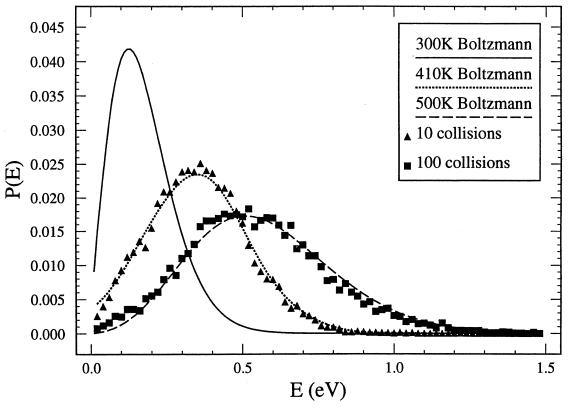

Fig. 4. Simulation of the internal energy distribution of ionized *n*-butylbenzene (no fragmentation) resulting from resonance excitation using an amplitude of 215 mV in 1 mtorr of 300 K helium after 10 ion/helium collisions (diamonds) and after 100 collisions (squares). Reprinted with permission from [93].

distribution in the absence of fragmentation or when fragmentation does not significantly deplete the high energy tail of the distribution (i.e. when the ions are in the rapid energy exchange condition). When fragmentation rates are significant compared with the rates for collisional excitation, a "truncated Boltzmann" distribution results in which the high-energy tail of the distribution is depleted [96]. Fig. 4 shows the results of a simulation of the resonance excitation of ionized *n*-butylbenzene (no fragmentation) using an amplitude of 215 mV in 1 mTorr of room temperature helium. The solid line represents the calculated internal energy distribution of the ions at 300 K. The data represented by the triangles reflects the distribution after 10 ion/helium collisions. At this point, the parent ion internal energy distribution is in transition from its initial condition to its final steady state condition. The data represented by the squares reflect the distribution after 100 collisions. By 100 collisions the distribution reaches a steady-state condition at an ion internal temperature of 500 K.

The relatively high collision rates in the ion trap that involve both activating and deactivating collisions allow a thermal analogy to be drawn for resonance excitation. Under resonance excitation conditions, the internal temperature that characterizes the parent ions is determined by bath gas temperature plus an increase in temperature arising from the ion acceleration process. For an ion trap with a pure quadrupolar field, on-resonance excitation leads to a parent ion internal temperature, T_{int}, of

$$T_{int} = T_{bath} + cm_T V^2/[3m_i\xi(T_i)^2] \quad (1)$$

where c is a collection of constants, T_{bath} is the bath gas temperature, m_T is the bath gas mass, m_i is the mass of the ion, V is the amplitude of the dipolar electric field applied to the end-cap electrodes of the ion trap, and $\xi(T_i)$ is the internal energy dependent reduced collision frequency [92]. Eq. (1) highlights the fact that ion internal temperatures can be manipulated either via resonance excitation or variation of the bath gas temperature. An attractive aspect of ion internal energy manipulation via resonance excitation is that temperature changes can be effected under software control during the course of a multistep ion trap experiment. Further, resonance excitation allows for ion temperatures that exceed those achievable by heating the bath gas. A disadvantage is that the relationship between resonance excitation conditions and ion internal temperature is ion dependent and is not yet sufficiently well understood to allow an accurate a priori prediction of the temperature change associated with a particular resonance excitation experiment.

We are currently engaged in studies designed to improve our understanding of the quantitative relationship between ion acceleration conditions and parent ion internal temperatures. Our published work to date has used protonated leucine enkephalin as the thermometer ion [100,101]. The experiments have involved measuring the dissociation rate of protonated leucine enkephalin under a variety of bath gas and ion acceleration conditions and relating the dissociation rate data to a parent ion internal temperature. If the protonated leucine enkephalin ion dissociates under rapid energy exchange conditions, the Arrhenius equation can be used directly to determine parent ion internal temperature using the Arrhenius parameters measured under thermal dissociation conditions. However, master equation modeling suggested that protonated leucine enkephalin begins to deviate sig-

nificantly from rapid energy exchange conditions above a dissociation rate of about 1 s^{-1} [93]. Most ion trap resonance excitation experiments lead to dissociation rates in the range of 10–100 s^{-1}. At dissociation rates where significant deviations from rapid energy exchange conditions prevail, the high energy tail of the internal energy distribution is depleted by fragmentation. It is therefore appropriate to use the term "effective internal temperature" to refer to the parent ion internal energy distribution since it is not strictly Boltzmann. We derived an approximate calibration for relating dissociation rate data and parent ion internal energy using an approach illustrated by the data in Fig 5. The top plot shows effective parent ion internal temperature as a function of bath gas temperature using 300 mV of resonance excitation (monopolar). The Arrhenius equation was assumed to give a reasonable estimate of internal temperature at the lowest dissociation rate. The solid line indicates the expected increase in effective parent ion internal temperature with increasing bath gas temperature, assuming a degree for degree increase in parent ion internal temperature with increasing bath gas temperature, whereas the dashed line with open circles shows the internal temperatures predicted from the experimental rate data by the Arrhenius equation. The curvature of the dashed line indicates deviation from rapid energy exchange conditions over the dissociation rate range reflected in the plot. The bottom panel of Fig. 5 shows a plot of effective parent ion internal temperature versus dissociation rate derived from use of the corrected internal temperature versus bath gas temperature relationship (solid line in the top panel) which accounts for deviation from rapid energy exchange conditions. The accuracy of the effective internal temperature correction is based on two assumptions: (1) the Arrhenius equation gives a good estimate of the internal temperature at the lowest dissociation rate and (2) the internal energy dependent reduced collision frequency of Eq. (1) changes insignificantly over the temperature range relevant to this study. Master equation modeling suggests that the first assumption will contribute little error [93]. Studies currently under way with systems in the rapid energy exchange condition over a relatively wide

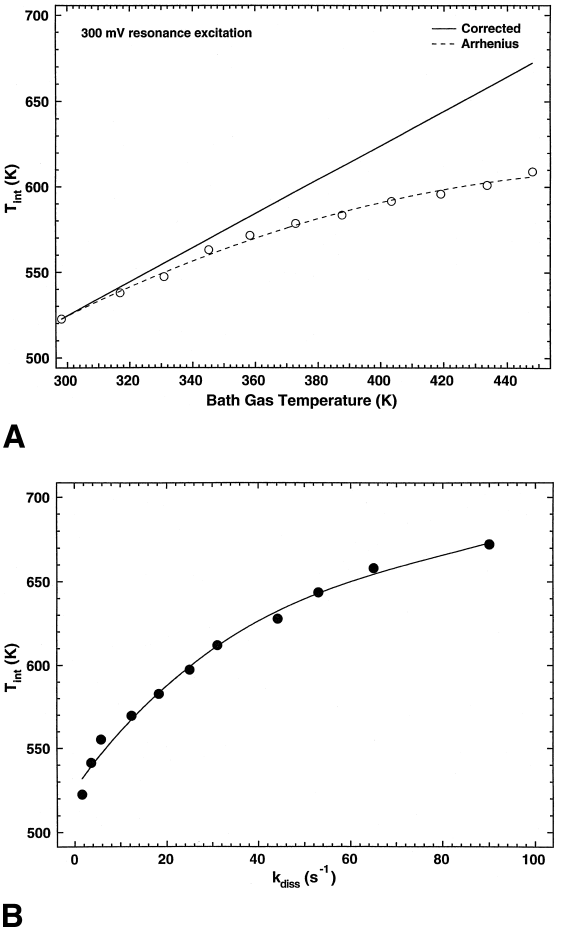

Fig. 5. (a) Parent ion internal temperature as a function of bath gas temperature using 300 mV resonance excitation (monopolar) for protonated leucine enkephalin. (b)Parent ion internal temperature vs. dissociation rate derived from use of the corrected internal temperature vs. bath gas temperature relationship (solid line in a), which accounts for deviation from rapid energy exchange conditions. Reprinted with permission from [100].

range of collisional activation conditions will shed light on the second assumption.

Two common collisional activation conditions have been studied using the effective internal temperature versus dissociation rate calibration described above, viz. resonance excitation [100] and boundary-activated dissociation [101]. Fig. 6 shows a plot of effective internal temperature versus resonance excitation voltage (monopolar excitation) for protonated leucine enkephalin. The dashed line with open circles is the curve resulting from use of the Arrhenius

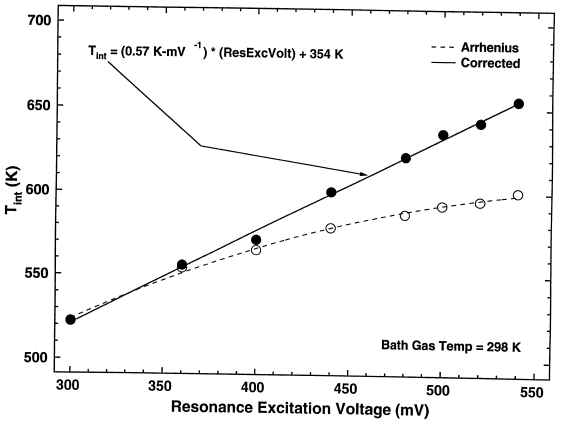

Fig. 6. Effective internal temperature vs. resonance excitation voltage (monopolar excitation) for protonated leucine enkephalin calculated using the Arrhenius equation (open circles) and corrected using the correction from Fig. 5(a) (filled circles) to account for deviation from rapid energy exchange conditions. Reprinted with permission from [100].

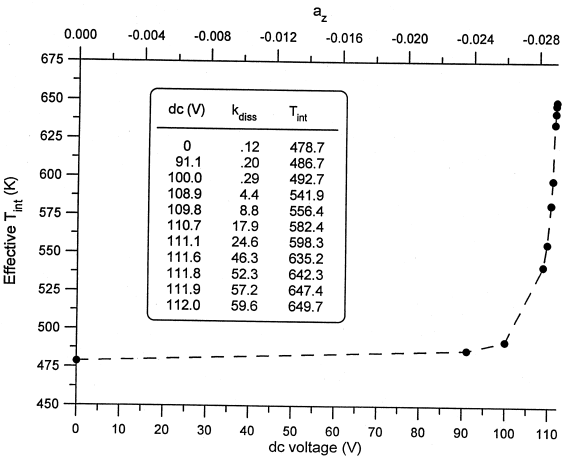

Fig. 7. Effective internal temperature of protonated leucine enkephalin ions as a function of a dc voltage applied to the ring electrode used to bring the ions into proximity of a stability boundary. Reprinted with permission from [101].

equation to yield the internal temperature. This curve is not expected to provide an accurate reflection of the relationship between resonance excitation and parent ion internal temperature due to deviation from rapid energy exchange conditions over the relevant dissociation rate range. The solid line reflects the results obtained with the corrected relationship between effective internal temperature and dissociation rate derived from the heated bath gas data discussed above (Fig. 5). The corrected data suggests that there is a linear relationship between effective internal temperature and resonance excitation voltage over the range of resonance excitation voltage amplitudes used in this study. The data also suggest that the protonated leucine enkephalin ions were elevated by about 357 K over the bath gas temperature to a total effective internal temperature of 655 K at a resonance excitation voltage of 540 mV.

We studied the use of boundary activation [70] for elevating parent ion internal temperatures due to its relevance to the issue of rf heating discussed in connection with thermal dissociation experiments. Boundary activation takes advantage of rf heating, the process by which ions are accelerated by the rf trapping voltage used to store the ions, to induce fragmentation by manipulating the applied voltages to place the ions near a stability boundary. Fig. 7 shows a plot of the effective internal temperature of protonated leucine enkephalin ions as a function of a dc voltage applied to the ring electrode used to bring the ions into proximity of a stability boundary. The q_z value used throughout was 0.245 whereas the dimensionless storage parameter a_z, which is proportional to the dc voltage applied to the ion trap ring electrode, is plotted as the top axis in the figure. Under these conditions, the parent ions approach a stability boundary as they near an a_z value of -0.03. Of particular interest is the fact that very little change in dissociation rate is observed until the ions are placed at a_z values in the range of -0.02–-0.03. These data provide support for the results taken as a function of q_z discussed above that rf heating of macro-ions only becomes significant as the ions approach a stability boundary. In this case, the effective temperatures that can be achieved with boundary activation were similar to those obtained using resonance excitation but a greater degree of parent ion loss from the ion trap was noted using boundary activation.

The work just described indicates that it is possible to use conditions for collisional activation in the ion trap where the internal temperature of the ions during activation can be determined. Further studies of this

nature whereby conclusions can be drawn regarding parent ion effective temperatures as functions of various ion acceleration variables, such as electric field strength, bath gas composition, and bath gas pressure, will enhance our understanding of the relationship between collisional activation conditions and parent ion temperatures. As this understanding improves, the value of the quadrupole ion trap as a tool for fundamental macro-ion chemistry studies will also improve. Further, this line of work is expected to provide valuable new insights into issues of collisional energy transfer involving small atomic targets and large multioscillator systems.

2.3. Bath gas temperature effects on the appearance of macro-ion MS/MS spectra derived from ion acceleration

It is appropriate here to point out an important characteristic of the quadrupole ion trap operated at relatively high background gas pressures that make it unusual, or even unique, in the tandem mass spectrometry of macro-ions. The conventional resonance excitation experiment leads to selective acceleration of the parent ion resulting in the elevation of the parent ion internal temperature over that of the background gas. The product ions are usually not themselves subject to acceleration. By virtue of the large number of internal states in a macro-ion, product ions are initially formed at nearly the same "temperature" as the parent ion. That is, they have a very similar amount of internal energy on a per-degree-of-freedom basis. Since this temperature is higher than that of the bath gas, first generation product ions can undergo active cooling via collisions with the bath gas such that sequential fragmentations that might otherwise occur are inhibited. This is a particularly important consideration for macro-ions because the lifetimes of excited first generation product ions are increasingly likely to overlap with the cooling rate afforded by ion/bath gas collisions due to the fact that the product ions can have a large number of internal states.

The importance of product ion cooling, assuming the product ions themselves are not subject to activation, via collisions with the bath gas is determined by

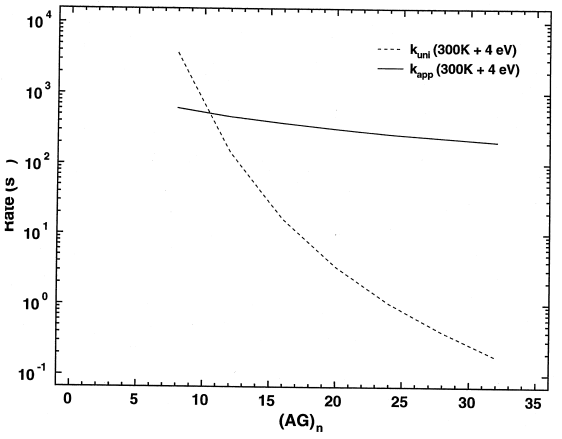

Fig. 8. Comparison of the unimolecular dissociation rate (k_{uni}) and the cooling rate (k_{app}) as a function of n for $(AG)_n$-mers having internal energy equal to 300 K + 4 eV. Reprinted with permission from [163].

competition between product ion fragmentation and cooling. We have recently modeled the competition between fragmentation and ion cooling for polypeptides containing multiple alanine-glycine (AG) dipeptide units in 1 mTorr of helium at 300 K. The cooling rates were obtained from two ion-cooling models that are expected to bracket the actual cooling rate in the ion trap. One was based on an inefficient colliders model and the other was based on a complete statistical redistribution of energy upon each ion/bath gas atom collision. Cooling rates for the species modeled [i.e. $(AG)_8$–$(AG)_{32}$] ranged from 200 to 2000 s^{-1} with the statistical model giving rise to cooling rates 3–4 times greater than those resulting from the inefficient colliders model. Fig. 8 shows plots of unimolecular dissociation rates derived from Rice-Ramsperger-Kassel-Marcus theory and using a critical energy of 1 eV for each $(AG)_n$-mer and the average internal energy of a 300 K ion plus 4 eV. This scenario was intended to simulate the dissociation of an initially room temperature ion that was activated by absorption of a 308 nm photon. Fig. 8 also shows a plot of the calculated cooling rates using the inefficient colliders picture, indicated as k_{app}(300 K + 4 eV) in the plot, for the various $(AG)_n$-mers. Of particular importance is the fact that the plots show that while the cooling

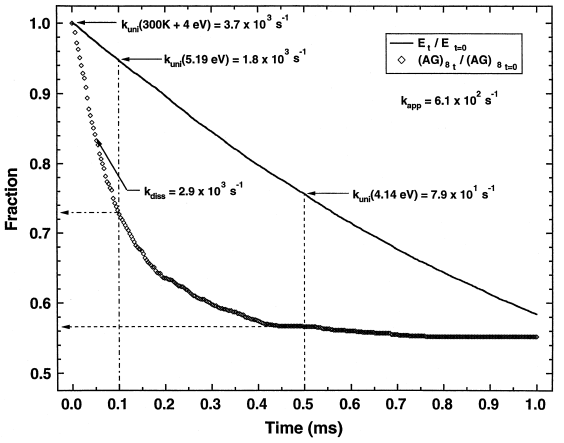

Fig. 9. Fraction of surviving $(AG)_8$ ions as a function of time following the input of 4 eV of internal energy into a 300 K ion population assuming 1 mTorr of room temperature helium bath gas (diamonds), and fraction of average internal energy remaining in the surviving $(AG)_8$ ion population as a function of time (solid line), illustrating that collisional cooling can inhibit the formation of product ions. Reprinted with permission from [163].

rate decreases with ion size, the unimolecular dissociation rates decrease even faster.

In MS/MS experiments, the net effect of the presence of the bath gas is that it can actively inhibit sequential fragmentation if the first generation product ions are not subjected to activation, and if the collisional cooling rate exceeds the dissociation rate. Further, fast activation methods that rely on a rapid input of energy, as opposed to the continuous input of energy associated with slower activation methods like resonance excitation, must drive dissociation at rates sufficiently high to avoid cooling of the activated parent ion. Fig. 9 illustrates how collisional cooling can inhibit the formation of product ions that might otherwise be formed in the absence of a background gas. The diamond data points show the fraction of surviving $(AG)_8$ ions as a function of time following the input of 4 eV of internal energy into a 300 K ion population assuming 1 mTorr of room temperature helium bath gas. The line represents the fraction of average internal energy remaining in the surviving $(AG)_8$ ion population as a function of time. A collisional cooling rate of 610 s^{-1} was used throughout the simulation. At $t = 0$, all parent ions have sufficient

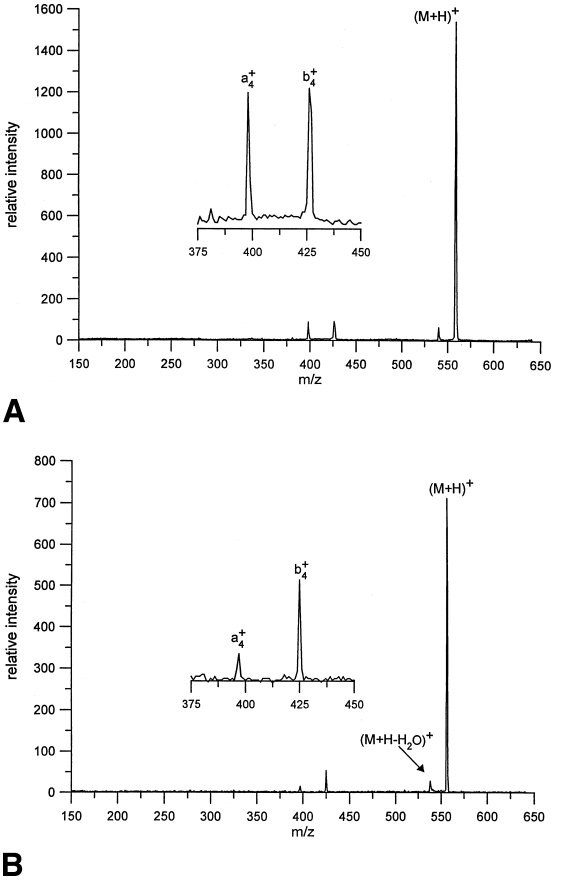

Fig. 10. MS/MS spectra of protonated leucine enkephalin under boundary activation conditions at a bath gas temperature of (a) 480 K and (b) 300 K. The parent ion excitation conditions were controlled to ensure that the parent ions were dissociating at nearly the same rate for the two spectra. Reprinted with permission from [101].

internal energy to fragment and the initial fragmentation rate is 3700 s^{-1}. Within 1 ms, the average internal energy drops to roughly 60% of its initial value and the dissociation rate drops to nearly zero after roughly 44% of the initial parent ion population fragmented. By contrast, in the absence of the bath gas over 97% of the parent ion population would be expected to dissociate by 1 ms.

The identity and number density of the bath gas are major factors in establishing the cooling rate for excited ions. The temperature of the bath gas can also play a noticeable role. This is illustrated in the comparison of Fig. 10 which shows MS/MS spectra

of protonated leucine enkephalin under boundary activation conditions. The spectra were recorded for parent ions that dissociate at rates of 4.4 s^{-1} (top) and 4.8 s^{-1} (bottom) but the top spectrum was recorded with a bath gas temperature of 480 K whereas the bottom spectrum was recorded with a bath gas temperature of 300 K. Recording spectra at similar dissociation rates ensures that the parent ion internal temperatures were similar (a dissociation rate of 4.4 s^{-1} corresponds to an effective internal temperature of 542 K and a dissociation rate of 4.8 s^{-1} corresponds to an effective internal temperature of 543 K) but it is clear that the a_4^+ ion is significantly more abundant at the higher bath gas temperature. The a_4^+ ion is formed from the b_4^+ ion. The Arrhenius parameters for the b_4^+ ion have been measured [72,88] and they indicate it is less stable than the protonated molecule and therefore fragments at a higher rate than the parent ion at the same temperature. In fact, in the thermal dissociation experiments, the a_4^+ ion is much more abundant than the b_4^+ ion even at far lower parent ion dissociation rates than 4.4 s^{-1}, presumably due to the fact that the b_4^+ ions are formed and are maintained at the same temperature as the parent ion. This observation and the spectra of Fig. 10 clearly indicate that the temperature of the bath gas plays an important role in the extent of consecutive dissociation involving the b_4^+ ion. This system and the role of bath gas temperature in determining the appearance of ion trap tandem mass spectra of high mass ions has recently been discussed in some detail [102].

3. Macro-ion/molecule reactions: hydroiodic acid attachment to polypeptide ions

The use of quadrupole ion traps for the study of ion/molecule reactions has a long history [103,104]. A large and diverse set of groups around the world use quadrupole ion traps to study ion/molecule reaction chemistry. A somewhat smaller subset has focused on the ion/molecule reactions of macro-ions or smaller molecules intended to serve as model systems [105–107]. We have reported on ion/molecule proton transfer reactions involving multiply charged protein ions [108–111] as well as nucleophilic substitution reactions involving multiply charged oligonucleotide ions and trimethylsilyl chloride [112]. In this overview, we emphasize novel ion/molecule reaction chemistry that has thus far only been studied using a quadrupole ion trap and involves the attachment of hydroiodic acid molecules to polypeptide cations [113–118]. The fact that this chemistry involves the attachment of neutral molecules to polypeptide cations makes it especially suitable for the quadrupole ion trap due to the relatively high cooling rates associated with the presence of the bath gas, as discussed in Sec. 2. Within the context of gas-phase adduct formation, the high collisional cooling rates in the ion trap facilitate attachment by removing the energy associated with condensation of the attaching species to the polypeptide cation.

In the course of studying the reactions of oppositely charged ions in the ion trap, as discussed further in the next section, we noted inter alia that anions derived from acids such as nitric acid, hydrochloric acid, hydrobromic acid, and hydroiodic acid tend to attach to multiply protonated polypeptides. Of all of the anions studied to date, I^- shows the strongest tendency for attachment relative to proton transfer. The ion/ion reaction with I^- can be written as

$$MH_n^{n+} + I^- \rightarrow (MH_n^{n+} - I^-)^{(n-1)} \quad (2)$$

or

$$MH_n^{n+} + I^- \rightarrow (MH_{(n-1)}^{(n-1)+} - HI) \quad (3)$$

where reaction (2) implies the formation of an ion pair in which all of the protons are located on the polypeptide and reaction (3) implies a proton transfer to I^- with the resulting molecule of HI remaining bound to the polypeptide cation. Of course, these are the two limiting cases. The degree of charge separation in the product is likely to be intermediate between these two extremes. However, a significant degree of electrostatic bonding, as represented by reaction (2), can rationalize the strength of the observed interaction [116]. The adduct ion is represented in the following discussion as an ion pair.

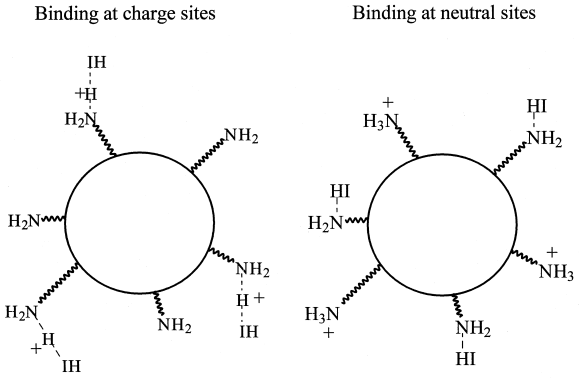

Fig. 11. Schematic illustration of the two most likely types of interactions between a protonated peptide and a molecule of HI.

The selective attachment of particular anions, I^- providing the most striking example, to protonated polypeptides was a curious observation and led us to investigate the phenomenon in some detail. The first experiment was to subject the adduct ion, $(MH_n^{n+} - I^-)^{(n-1)}$, to collisional activation. This experiment resulted in the exclusive loss of HI, i.e.

$$(MH_n^{n+} - I^-)^{(n-1)} \rightarrow MH_{(n-1)}^{(n-1)+} + HI \quad (4)$$

The fact that energy was required to dissociate a molecule of HI from the adduct ion suggested that the reverse reaction should be exothermic, implying that the ion/molecule reaction,

$$MH_n^{n+} + HI \rightarrow (MH_{(n+1)}^{(n+1)} - I^-) \quad (5)$$

should take place in the ion trap. Indeed, this was found to be the case, at least for most polypeptide ions (see the following). The fact that neutral hydroiodic acid would attach to polypeptide ions when water was not observed to attach to these ions under identical reaction conditions suggested that the nature of the interaction in these ions was not simply a charge solvation interaction. Water, having a significantly greater proton affinity than HI, would be expected to show a greater tendency for solvating a charge site on the polypeptide than HI. We then conducted a series of experiments to distinguish between the two most likely types of interactions that might be involved, shown schematically in Fig. 11. One is a charge-centered interaction whereby the HI molecules solvate the charge site and the other is an interaction between HI and a neutral basic site to form either an ion pair or a strong dipole–dipole interaction. Every experiment designed to distinguish between these two types of interactions strongly supported the interaction of HI with a neutral basic site [113]. This reaction, therefore, is not an ion/molecule reaction in the conventional sense in that it does not involve a charge site. The role of charge in this case is simply to allow us to study the reaction by means of mass spectrometry.

An important piece of evidence to support the interaction with neutral basic sites, as opposed to a charge solvation interaction, is the observation that the rates of reaction and the total number of HI molecules that readily attach to a polypeptide ion are inversely related to charge. Fig. 12 which shows spectra derived from neurotensin [114] provides an illustration. The triply protonated molecule is observed to be essentially unreactive toward HI attachment whereas the doubly protonated molecule readily attaches one and only one molecule of HI. Upon examining a range of polypeptides and charge states it became apparent that, in general, the total number of HI molecules that will eventually attach to a particular ion is related to the ion charge state and the total number of *N*-termini and arginine (R), lysine (K), and histidine (H) residues, i.e.

$$\#HI_{max} = \Sigma(R,K,H,\ N\text{-termini}) - z \quad (6)$$

An example where this relationship holds is shown in Fig. 13, which illustrates the behavior of the $(M + 12H)^{12+}$ ion of bovine cytochrome *c*. The total number of arginine, lysine, and histidine residues and *N*-termini in this protein is 23. After a relatively long storage period (300 ms) in the presence of neutral HI in the ion trap, a total of 11 molecules of HI are observed to attach to the protein thereby obeying relationship (6). Despite the use of much longer storage times, no measurable abundances of a product with 12 molecules of HI attached is observed. Having examined many charge states of several dozen peptides and proteins, we have found relationship (6) to

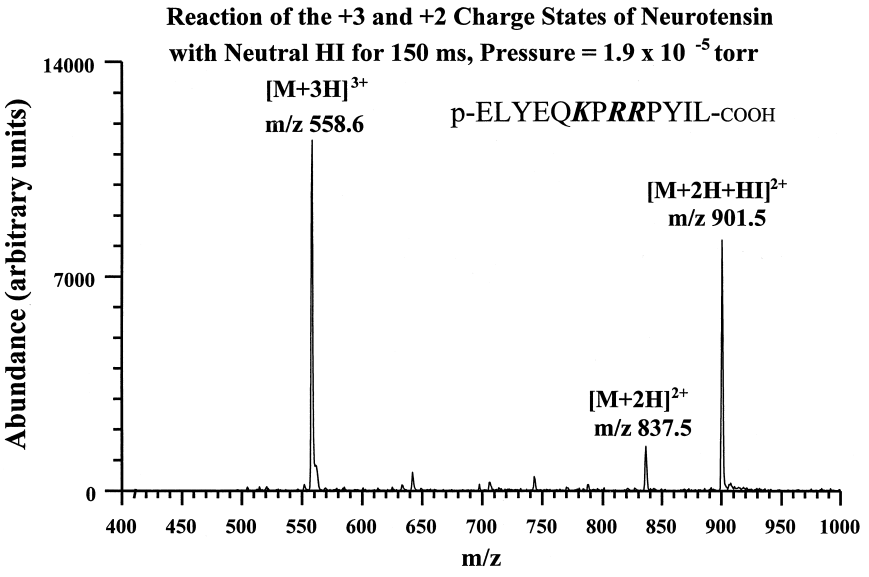

Fig. 12. Spectrum of neurotensin after a reaction time of 50 ms in the presence of 1.9×10^{-5} Torr of HI. Adapted with permission from [114].

hold in the vast majority of cases. Hydroiodic attachment to polypeptide cations in the gas phase provides a means for counting the total number of arginines, lysines, histidines, and *N*-termini in a peptide or protein [114]. This is an exceptional gas-phase "ion/molecule" reaction in that it can provide information about the composition of a protein.

To test the validity of Eq. (6), reactions are allowed

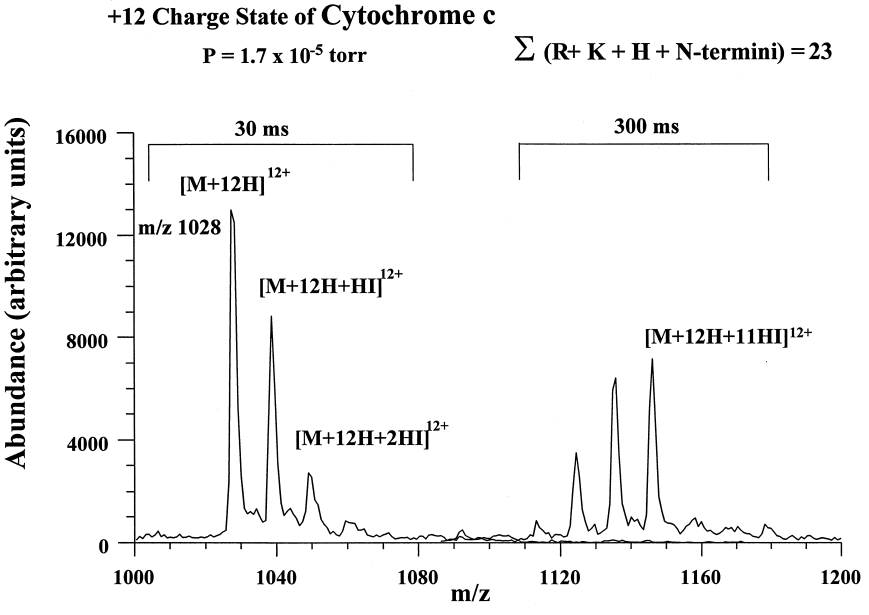

Fig. 13. Spectra of the +12 charge state of cytochrome *c* after reaction times of (a) 30 ms and (b) 300 ms in the presence of 1.7×10^{-5} Torr of HI. Adapted with permission from [114].

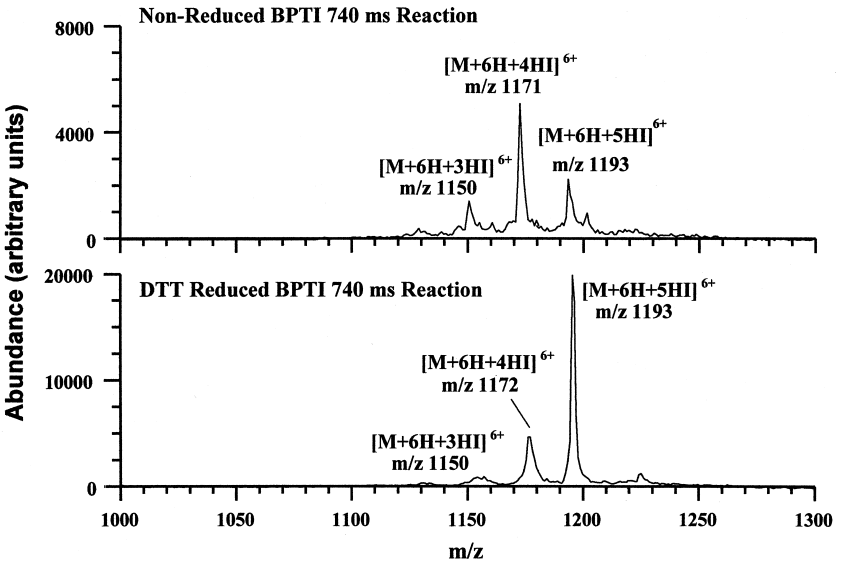

Fig. 14. Spectra of the +6 charge state of (a) native and (b) reduced bovine pancreatic trypsin inhibitor (BPTI) after a reaction time of 740 ms in the presence of 1.2×10^{-5} Torr of HI. Reproduced with permission from [115].

to proceed until the product ion spectrum is essentially invariant. That is, sufficient reaction time is allowed for reactions with an appreciable rate constant to proceed essentially to completion. However, the kinetics of HI attachment are highly variable among ions with available basic sites for reaction. The kinetics of attachment can be influenced by a number of factors. These include the inherent reactivity of the neutral basic site (i.e. arginine versus lysine versus histidine versus *N*-terminus), exposure of the basic site to attack by HI, and intramolecular interactions that might affect the binding strength of HI. The latter two factors are related to the three-dimensional structures of the ions. The kinetics of HI attachment might therefore serve as a chemical probe of three-dimensional ion structure. Bovine pancreatic trypsin inhibitor (BPTI), a roughly 6.5 kDa protein with two disulfide linkages, was chosen as an initial test substrate because ion mobility [119,120] and collision cross-section studies [17] have been reported for ions derived from this protein. Fig. 14 shows the results obtained after storage of the $(M + 6H)^{6+}$ ions derived from native and reduced forms of BPTI under identical conditions [115]. At the reaction time reflected here (740 ms) the product ion spectra for each reaction are essentially invariant as the storage time is increased. Close inspection of the time evolution of the product ions indicates that the major differences between the $(M + 6H)^{6+}$ ions of the native and reduced forms of BPTI are noted in the kinetics associated with the addition of the fourth and fifth molecules of HI [115]. This observation has been interpreted as resulting from greater exposure of the neutral basic sites to attack by HI in the ions of the reduced protein relative to ions of the native protein.

Recent studies have focused on the reactivity of hydroiodic acid and deuteriodic acid with ions derived from bradykinin and its analogues [116,117] and the reactivity of hydroiodic acid with angiotensin ions [118]. These studies have shown several cases in which non-linear kinetics are observed indicating the presence of two or more ion populations with distinct reactivities. Attention has also been focused on H/D exchange reactions involving DI leading to the conclusion that the mechanism for H/D exchange with DI is similar to that associated with H/D exchange with D_2O [23,26] except that the rate constants associated with H/D exchange with DI are higher due to the higher gas-phase acidity of DI relative to D_2O [117]. The BPTI study and the studies just mentioned have

shown that the kinetics of HI attachment to polypeptide ions can be used as a chemical probe of intramolecular interactions and steric effects in macro-ions. Given the unusual nature of the interaction, HI attachment kinetics might be able to provide information complementary to that provided by other chemical probes, such as proton transfer and H/D exchange, which are charge-site mediated.

4. Macro-ion/ion chemistry: proton transfer

In addition to the capability for operation with a relatively high background gas pressure, the ion trap has the capability for storage of oppositely charged ions simultaneously and in overlapping regions of space. This capability, combined with the ability to form multiply charged macro-ions, opens up the possibility for the study of the reactions of oppositely charged ions in the gas phase using all of the ion trap tools for manipulating ions. In recent years, we have been studying the reactions of multiply charged ions derived from electrospray, most of which fit the criterion of a macro-ion used herein, with singly charged ions of opposite polarity in the ion trap. Most of our work in this area published through 1998 has been summarized in a recent review [121]. It has involved studies focused on the kinetics and thermodynamics of ion/ion chemistry [122,123], mechanisms of reactions [113,122,124–128], instrumentation considerations [129,130], and analytical applications [131–136].

To date, two general approaches to effecting macro-ion/ion chemistry have been employed. In one approach, oppositely charged ions are mixed prior to sampling into the mass spectrometer [137–140]. The other approach, which is the focus here, uses the quadrupole ion trap as the reaction vessel for ion/ion chemistry. The latter approach enjoys the flexibility of MS^n and can therefore employ ion/ion reactions within the context of tandem mass spectrometry. The instrumentation used for the bulk of our ion/ion chemistry work has been described [129]. The apparatus is a quadrupole ion trap mass spectrometer equipped with both an electrospray ion source and a glow discharge ion source. The instrument is configured for the injection of electrospray ions through an aperture in an endcap electrode. Glow discharge ions are injected through a hole drilled in the side of the ring-electrode. A typical ion/ion reaction experiment involves first the accumulation of electrospray ions. This step may or may not be followed by an ion isolation step and, perhaps, a collisional activation step, depending upon the nature of the experiment. Glow discharge ions are then injected into the ion trap through the ring-electrode for a period of roughly 20 ms and the ions of both polarities are stored together for a period of time ranging from a few tens of milliseconds to about 100 ms. The negative ions are then ejected prior to mass analysis of the positive ions. In some cases, there may be more ion accumulation and ion/ion reaction steps. For example, a short ion/ion reaction period may be used to form an ion of low charge that is not formed directly by electrospray. Following an ion isolation and collisional activation period, the products ions may be subjected to ion/ion reactions to yield a product ion spectrum comprised largely of singly charged ions. The ability to perform experiments such as these, stems in part from the highly flexible nature of ion trapping instruments in conducting multistep experiments.

Within the context of the present subject, we emphasize the use of ion/ion reactions for the study of macro-ion chemistry. The range of possible experiments involving macro-ion/ion chemistry is prohibitively broad to cover in an overview of this type. In keeping with our objective to illustrate rather than to catalog novel quadrupole ion trap approaches for studying the chemistry of gaseous macro-ions, we limit the scope of the discussion to ion/ion reactions involving proton transfer. In particular, we emphasize the use of proton transfer reactions to manipulate the charge states of parent ions for the purpose of providing a wide range of parent ion charge states for subsequent tandem mass spectrometry experiments and to manipulate the charge states of product ions for the purpose of simplifying interpretation of product ion spectra derived from multiply charged parent ions.

Electrospray usually forms ions from macromolecules with a distribution of charge states. For rela-

tively small macromolecules (i.e. <1.5 kDa) the distribution of charges may include singly charged ions as well as some higher charge states, depending largely upon the number of basic sites in the molecule. As the size, and the number of potential charge carrying sites, of a macromolecule increases, the charge state distribution shifts to higher charge with ions of low charge states decreasing in abundance. For moderate to high mass species (i.e. >10 kDa) with many potential charge sites, such as a large majority of proteins, only multiply charged ions are observed. The charge state of a macro-ion plays an integral role in its chemistry; therefore, it is desirable to be able to study as wide a range of charge states as possible in characterizing the ion chemistry of a macromolecule. This fact led us to develop the ability to manipulate macro-ion charges states through ion/ion chemistry in the ion trap.

Several approaches have been used to manipulate the charge states of ions formed by electrospray. These include the manipulation of solution and electrospray interface conditions [141], ion/molecule proton transfer reactions [28,29,108,109], and ion/ion proton transfer reactions [121,137–140]. The manipulation of solution and/or interface conditions is the simplest approach but can lead to compromises in ion yields and ion transmission on the one hand and observed charge state distribution on the other. Ion/molecule proton transfer reactions are effective for high charge states but are often not effective in converting moderate to high mass multiply charged ions to singly charged ions. Further, adduct formation involving the neutral base used to deprotonate the macro-ion can lead to complications [109]. Ion/ion proton transfer reactions, while perhaps the least straightforward approach to implement of those mentioned here, enjoy clear advantages for charge state manipulation. Use of ion/ion reactions (or ion/molecule reactions) decouples the ionization process from the charge state manipulation process such that each can be optimized independently. With use of the appropriate ionic reagents, ion/ion proton transfer can be observed without adduct formation and has been observed to be capable of partially neutralizing any multiply charged ion to an arbitrary degree, including complete neutralization.

The underlying advantages of ion/ion proton transfer over ion/molecule proton transfer in reducing charge states of multiply charged ions lie in differences in the thermodynamics of the reactions and the energy surfaces over which they proceed. Ion/ion reactions leading to a net reduction in charge are typically exoergic by 50–200 kcal/mol, even for singly charged ions reacting with singly charged ions, whereas ion/molecule proton transfer reactions involving macro-ions are usually endoergic at low macro-ion charge states [29]. Further, multiply charged ion/molecule reactions involving proton transfer give rise to two products of the same charge leading to a so-called “Coulomb barrier” in the exit channel which can inhibit the reaction [29]. No such barrier is present with multiply charged ion/ion reactions because at least one of the products is neutral. The lack of a Coulomb barrier and the high exothermicities of ion/ion reactions suggests that they should be “unit efficient.” That is, every ion/ion capture collision should lead to a charge reduction reaction. The ion/ion capture rate increases as the square of the charges of the reactant ions [122,123]. For every system studied thus far in our laboratory, ion/ion proton transfer reactions appear to show this charge-squared dependence, which is consistent with the reactions having unit efficiency.

It is significant that despite the high exothermicities associated with ion/ion proton transfer reactions, we rarely observe any fragmentation resulting from macro-ion reactants. While we have observed extensive fragmentation of small oligonucleotide anions (4-mers and smaller) following highly exothermic electron transfer reactions with ionized rare gases [125], we have not observed fragmentation of polypeptide or other macro-ions following proton transfer reactions. This includes noncovalently bound species such as duplex DNA [142] and holomyoglobin [127]. The lack of fragmentation arising from ion/ion reactions in the quadrupole ion trap has been ascribed to the high collisional cooling rate in the ion trap, as discussed previously in the unimolecular fragmentation section. The lifetimes of macro-ions

that undergo an ion/ion reaction are sufficiently long to allow for collisional cooling to remove any excess internal energy arising from the reaction. The likelihood for fragmentation resulting from an ion/ion reaction is a function of the reaction exothermicity and ion lifetime. The importance of reaction exothermicity was demonstrated by showing that the fragmentation of oligonucleotide anions by means of ion/ion electron transfer could be minimized by use of a cation with a relatively low recombination energy [143]. The importance of the number of degrees of freedom of the ion and its influence on ion lifetime was illustrated by showing that 16-mer oligonucleotide anions could undergo multiple electron transfer reactions with ionized xenon without showing any measurable fragmentation [144]. The lack of fragmentation arising from macro-ion/ion proton transfer reactions is significant in that it means that such reactions can be used for charge manipulation without creating further complications by inducing fragmentation.

To illustrate the role of parent ion charge state in the fragmentation chemistry of macro-ions, and the utility of ion/ion proton transfer in studying this role, we present results obtained for a poly(propylene imine) dendrimer synthesized from a 1,4-diaminobutane core shown schematically in Fig. 15 [145]. The parent molecule is designated as DAB-*dendr*-$(NH_2)_{32}$ according to convention [146]. This scheme indicates nomenclature used to designate the product ions from the major possible competitive fragmentation reactions of the parent ion. The tertiary nitrogens are identified as G_n where n represents the synthesis step, with the two nitrogens in the 1,4-diaminobutane core denoted as G_0. The major fragmentation mechanism observed for protonated dendrimers of this type involves the attack of a carbon alpha to a protonated nitrogen by an adjacent nitrogen to yield a quaternary nitrogen and a neutral amine [147,148]. This mechanism has been referred to as an intramolecular nucleophilic substitution (S_Ni) reaction. This general mechanism accounts for a large fraction of the total fragmentation observed for all charge states. Each tertiary nitrogen contains three nearest neighbor nitrogens. Two are closer to the exterior of the molecule and one is closer to the core. For a given protonation

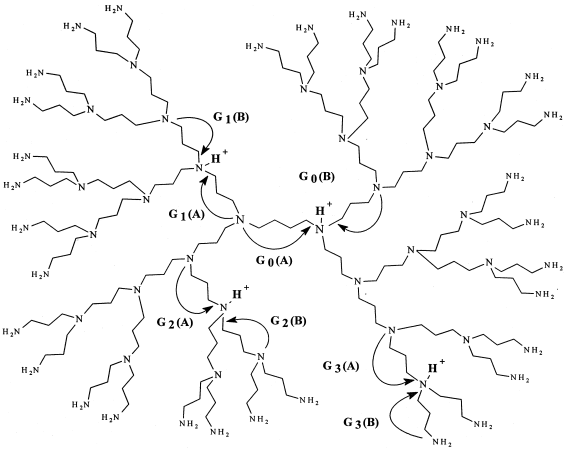

Fig. 15. Schematic representation of generation 4 of the poly(propylene imine) dendrimer DAB-*dendr*-$(NH_2)_{32}$ indicating the nomenclature used to designate the product ions from the major possible competitive fragmentation reactions of the parent ion (see text). Reproduced with permission from [145].

site, attack from a nitrogen closer to the core is labeled (A) and attack from a nitrogen closer to the exterior is labeled (B). For singly charged parent ions, the charge always resides on the quaternary nitrogen produced via the S_Ni reaction but multiply protonated parents can also give rise to charged amine fragments. The nomenclature scheme accounts for this with a subscript *a* or *q* denoting either an amine or quaternary amine fragment, respectively.

Spectra for the DAB-*dendr*-$(NH_2)_{32}$ dendrimer are shown in Fig. 16 to illustrate the utility of ion/ion proton transfer reactions for manipulating parent ion charge states. The main plot shows the post-ion/ion reaction mass spectrum using a reaction time sufficient to make the singly protonated ions dominate the spectrum whereas the insert shows the normal (pre-ion/ion reaction) electrospray mass spectrum. Essentially no singly protonated ions are formed directly from electrospray. Ion/ion proton transfer reactions allow product ion spectra to be collected from every parent ion charge state ranging in charge from +1 to the highest charge state observed via electrospray, which is +4 in this case. The product ion spectra from the $(M + H)^+$ (Fig. 17) and $(M + 3H)^{3+}$ (Fig. 18) ions are shown here for comparison. The favored dissociation channels are seen to be highly dependent

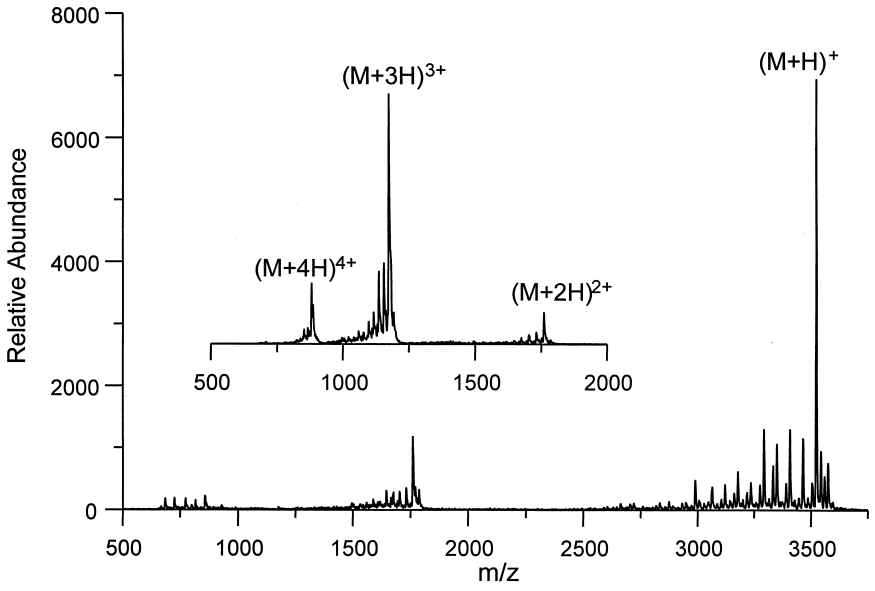

Fig. 16. Mass spectra of the DAB-*dendr*-$(NH_2)_{32}$ (a) before ion/ion reactions and (b) after an ion/ion reaction period of 50 ms. The negative ion reagents were derived from glow discharge of perfluoro-1,3-dimethylcyclohexane. Reproduced with permission from [145].

upon parent ion charge. In the case of the $(M + H)^+$ parent, by far most of the fragmentation is initiated at a core nitrogen to give the $G_0(B)_q^+$ and $G_0(A)_q^+$. Much weaker signals are observed to arise from the higher generation nitrogens. This result suggests that the core nitrogens are the favored protonation sites in

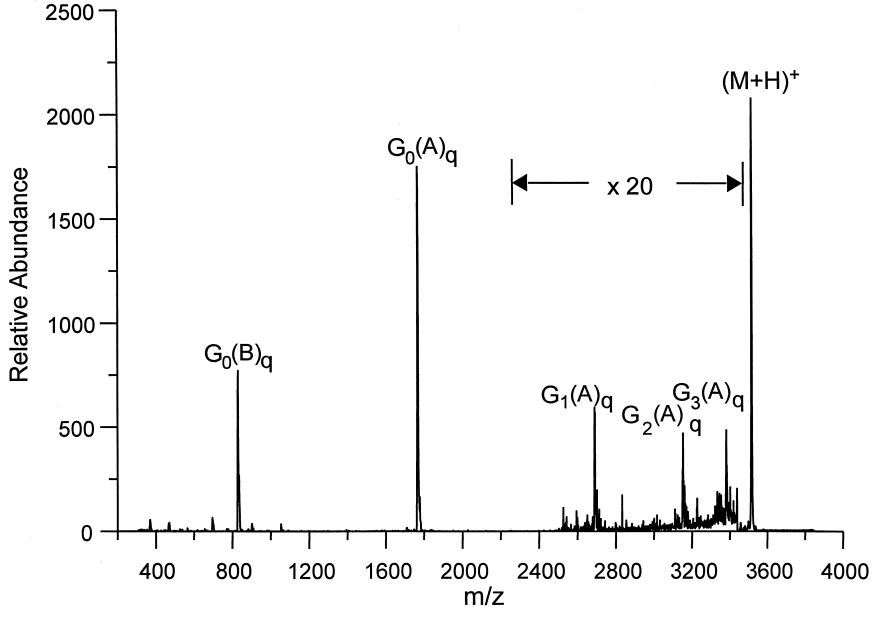

Fig. 17. Product ion spectrum from the $(M + H)^+$ ion of DAB-*dendr*-$(NH_2)_{32}$. Reproduced with permission from [145].

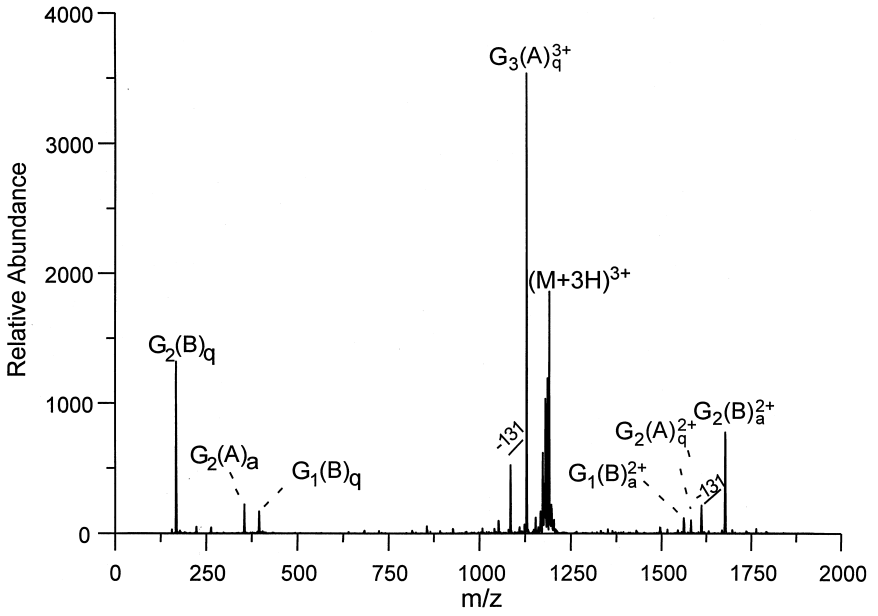

Fig. 18. Product ion spectrum from the $(M + 3H)^{3+}$ ion of DAB-*dendr*-$(NH_2)_{32}$. Reproduced with permission from [145].

the singly charged ions. The $(M + 3H)^{3+}$ ion, on the other hand, shows fragmentation that arises primarily from nitrogens which are closer to the exterior of the ion. For example, the most abundant fragment, $G_3(A)_q{}^{3+}$, arises from tertiary nitrogens closest to the exterior of the ion. These results, which are illustrative of data from ions derived from the other generations studied, reflect the role that parent ion charge can play in determining sites of protonation which, in turn, largely determine which of the possible competing fragmentation channels dominate. In the case of the poly(propylene imine) dendrimers, it was shown that complementary information about the structures of incomplete synthesis products (the peaks observed on the low mass side of the singly charged ion of DAB-*dendr*-$(NH_2)_{32}$ in Fig. 18), could be obtained from the various parent ion charge states. The key point is that ion/ion proton transfer reactions in the ion trap allowed for MS/MS data to be acquired for every charge state ranging from +1 to the highest charge state formed by means of electrospray.

The product ion spectra derived from the poly(polypropylene imine) dendrimer ions are relatively straightforward to interpret as a result of the relatively limited number of competing dissociation reactions. However, the product ion spectra for many macroions are far more difficult to interpret, particularly with the resolving power of our quadrupole ion trap at high mass-to-charge ($M/\Delta M \approx 500$–1000). Multiply charged parent ions can give rise to a complex mixture of ions in the product ion spectrum ranging in mass and charge up to the mass and charge of the parent ion. For highly charged proteins, most of the product ion signal falls within a relatively narrow range of mass-to-charge centered around that of the parent ion. As the protein increases in mass and charge, the product ion spectrum can become too congested to interpret. Ion/ion proton transfer reactions can simplify the situation by converting most of the product ions to singly charged ions thereby eliminating charge state ambiguity and dispersing the product ion signal over a much wider mass-to-charge range. These points are illustrated in Fig. 19 which compares the product ion spectra of the $(M + 16H)^{16+}$ ion of human hemoglobin β-chain before (top) and after (bottom) an ion/ion proton transfer reaction period used to reduce the majority of product ions to +1 [149]. Although ion trap reso-

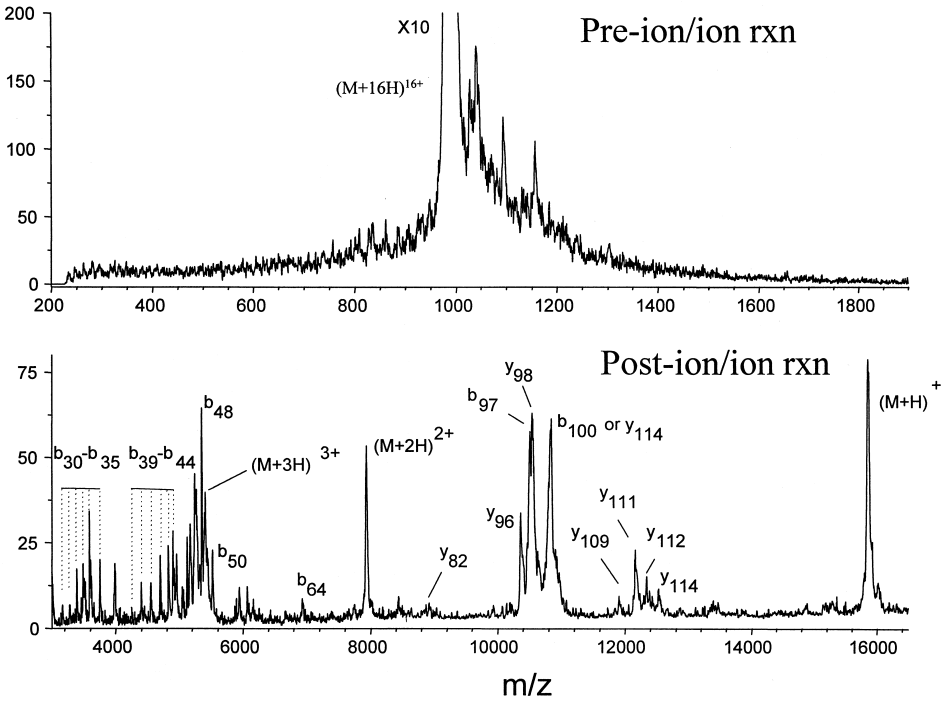

Fig. 19. Product ion spectra for the +16 charge state of human hemoglobin β-chain recorded (a) before ion/ion reactions and (b) after an ion/ion reaction period of 100 ms. The negative ion reagents were derived from glow discharge of perfluoro-1,3-dimethylcyclohexane. Reproduced with permission from [149].

nance excitation is clearly capable of dissociating this macro-ion, it is not possible to interpret the product ion spectrum without recourse to ion/ion reactions. After ion/ion reactions, the majority of fragment ion peaks in the spectrum can be assigned to sequence-informative *b*- or *y*-type fragmentation. These data illustrate that ion/ion proton transfer chemistry significantly expands the range of macro-ions amenable to study with the ion trap to ions of much higher mass and charge than would otherwise be tractable. This range now extends to ions of several tens of kilodaltons in mass.

5. Summary

Gaseous macro-ions present interesting and important avenues for research. To understand the chemistry of these ions will require the development of new tools and methodologies to augment information obtainable with existing tools. The quadrupole ion trap has several unusual characteristics as a device for manipulating and storing gaseous ions that allow for the development of novel approaches to study gaseous macro-ions. Of particular importance is operation of the device with a light bath gas present at about 1 mTorr. The bath gas serves as a medium through which relatively rapid energy exchange can occur between the macro-ion and its environment. As a result, measurements acquired as a function of bath gas temperature can be used to derive Arrhenius parameters for dissociation reactions of macro-ions at rates as high as 10 s^{-1} for relatively small macro-ions ($\approx$1 kDa), and perhaps higher for larger macro-ions. Determining the Arrhenius parameters for the decompositions of macro-ions will provide important insights into the energetic and entropic requirements of these reactions, as already illustrated with BIRD measurements. This kind of information will also be

useful in the study of collisional energy transfer dynamics. Once the Arrhenius parameters for an ion are established, it can then be used as a thermometer in studies designed to investigate energy transfer in ion trap collisional activation experiments. The relatively high bath gas pressures make a thermal analogy applicable to the collisional activation of macro-ions via ion acceleration in the ion trap. As this experiment becomes better characterized, ion trap resonance excitation can be used to study macro-ions at higher temperatures than can be readily accessed by heating the bath gas.

The relatively high energy transfer rates in the ion trap that result from use of a bath gas at relatively high pressures has important implications in several common experiments. For example, in cases in which the parent ion is selectively accelerated to induce fragmentation, the first generation product ions are actively cooled by means of the bath gas, thus they tend to inhibit sequential fragmentation. Interpretation of product ion spectra can be greatly simplified when most of the fragments arise directly from the parent ion. The extent to which sequential fragmentation occurs can be affected to a degree by control of the bath gas temperature. The relatively high cooling rate in the ion trap also can affect the study of ion/molecule reactions. In particular, reactions that involve two bodies becoming one body, as in adduct formation or clustering, will be favored in the ion trap provided the product is stable at the temperature of the bath gas. This is because the bath gas tends to remove the energy of condensation relatively quickly thereby stabilizing the incipient adduct. This scenario was illustrated here using the attachment of hydroiodic acid to polypeptide ions as an example.

The ability to store ions of opposite polarity in overlapping regions of space in the ion trap enables the study of ion/ion reactions. The ability to form multiply charged macro-ions allows for a wide range of possible studies in which at least one of the products of the ion/ion encounter retains a charge. The ability to manipulate charge states via ion/ion proton transfer reactions was emphasized here for creating a wide range of parent ion charge states for study and for manipulating product ion charge states to aid in spectral interpretation. This facile capability for manipulating parent and product ion charge states within the context of an MS/MS experiment greatly expands the range of macro-ions amenable to study with the ion trap.

Acknowledgements

The unimolecular ion chemistry studies described herein were supported by the U.S. Department of Energy, Office of Basic Energy Sciences, at Oak Ridge National Laboratory. The ion/molecule reaction studies were supported by the National Institutes of Health under grant no. GM 45372. Two of the authors (S.A.M. and J.M.W.) acknowledge support of the Purdue Research Foundation in the preparation of this report. Oak Ridge National Laboratory is managed and operated by UT-Battelle, LLC under contract no. DE-AC05-00OR22725 with the U.S. Department of Energy.

References

[1] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Mass Spectrom. Rev. 9 (1990) 37.
[2] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1989) 229.
[3] S.A. McLuckey, J.M. Wells, Chem Rev., In press.
[4] G. von Helden, T. Wyttenbach, M.T. Bowers, Science 267 (1995) 1483.
[5] S. Lee, T. Wyttenbach, M.T. Bowers, Int. J. Mass Spectrom. 167 (1997) 605.
[6] D.E. Clemmer, R.R. Hudgins, M.F. Jarrold, J. Am. Chem. Soc. 117 (1995) 10141.
[7] D.E. Clemmer, M.F. Jarrold, J. Mass Spectrom. 32 (1997) 577.
[8] J. Woenckhaus, Y. Mao, M.F. Jarrold, J. Phys. Chem. B 101 (1997) 847.
[9] S.J. Valentine, J.G. Anderson, A.D. Ellington, D.E. Clemmer, J. Phys. Chem. B 101 (1997) 3891.
[10] C.S. Hoaglund-Hyzer, A.E. Counterman, D.E. Clemmer, Chem. Rev. 99 (1999) 3037.
[11] S.C. Henderson, S.J. Valentine, A.E. Counterman, D.E. Clemmer, Anal. Chem. 71 (1999) 291.
[12] C.A. Srebalus, J.W Li., W.S. Marshall, D.E. Clemmer, Anal. Chem. 71 (1999) 3918.
[13] T. Covey, D.J. Douglas, J. Am. Soc. Mass Spectrom. 4 (1993) 616.
[14] K.A. Cox, R.K. Julian, R.G. Cooks, R.E. Kaiser, J. Am. Soc. Mass Spectrom. 5 (1994) 127.

[15] B.A. Collings, D.J. Douglas, J. Am. Chem. Soc. 118 (1996) 4488.
[16] Y.L. Chen, B.A. Collings, D.J. Douglas, J. Am. Soc. Mass Spectrom. 8 (1997) 681.
[17] V. Nesatiy, Y.L. Chen, B.A. Collings, D.J. Douglas, Rapid Commun. Mass Spectrom. 12 (1998) 40.
[18] A.C. Gill, K.R. Jennings, T. Wyttenbach, M.T. Bowers, Int. J. Mass Spectrom. 195/196 (2000) 685.
[19] M.K. Green, C.B. Lebrilla, Mass Spectrom. Rev. 16 (1997) 53.
[20] B.E. Winger, K.J. Light-Wahl, A.L. Rockwood, R.D. Smith, J. Am. Chem. Soc. 114 (1992) 5897.
[21] D. Suckau, Y. Shi, S.C. Beu, M.W. Senko, J.P. Quinn, F.M. Wampler III, F.W. McLafferty, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 790.
[22] T.D. Wood, R.A. Chorush, F.M. Wampler III, D.P. Little, P.B. O'Connor, F.W. McLafferty, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 2451.
[23] S. Campbell, M.T. Rodgers, E.M. Marzluff, J.L. Beauchamp, J. Am. Chem. Soc. 117 (1995) 12840.
[24] M.A. Freitas, C.L. Hendrickson, M.R. Emmett, A.G. Marshall, J. Am. Soc. Mass Spectrom. 9 (1998) 1012.
[25] M.A. Freitas, A.G. Marshall, Int. J. Mass Spectrom. 182/183 (1999) 221.
[26] T. Wyttenbach, M.T. Bowers, J. Am. Soc. Mass Spectrom. 10 (1999) 9.
[27] F.W. McLafferty, S. Guan, U. Haupts, T.D. Wood, N.L. Kelleher, J. Am. Chem. Soc. 120 (1998) 4732.
[28] C.J. Cassady, S.R. Carr, J. Mass Spectrom. 31 (1997) 247.
[29] E.R. Williams, J. Mass Spectrom., 31 (1996) 831.
[30] C.J. Cassady, S.R. Carr, J. Mass Spectrom. 31 (1996) 247.
[31] R.R. Ogorzalek Loo, R.D. Smith, J. Am. Soc. Mass Spectrom. 5 (1994) 207.
[32] R.D. Smith, C.J. Barinaga, H.R. Udseth, J. Phys. Chem. 93 (1989) 5019.
[33] R.D. Smith, C.J. Barinaga, Rapid Commun. Mass Spectrom. 4 (1990) 54.
[34] J.A. Loo, C.G. Edmonds, R.D. Smith, Anal. Chem. 65 (1993) 425.
[35] J.A. Loo, C.G. Edmonds, R.D. Smith, Anal. Chem. 63 (1991) 2488.
[36] J.P. Speir, M.W. Senko, D.P. Little, J.A. Loo, F.W. McLafferty, J. Mass Spectrom. 30 (1995) 39.
[37] N.L. Kelleher, S.V. Taylor, D. Grannis, C. Kinsland, H.J. Chiu, T.P. Begley, F.W. McLafferty, Protein Sci. 7 (1998) 1796.
[38] N.L. Kelleher, H.Y. Lin, G.A. Valaskovic, D.J. Aaserud, E.K. Fridriksson, F.W. McLafferty, J. Am. Chem. Soc. 121 (1999) 806.
[39] Z.Q. Guan, N.L. Kelleher, P.B. O'Connor, D.J. Aaserud, D.P. Little, F.W. McLafferty, Int. J. Mass Spectrom. Ion Processes 158 (1996) 357.
[40] J.P. Speir, M.W. Senko, D.P. Little, J.A. Loo, F.W. McLafferty, J. Mass Spectrom. 30 (1995) 39.
[41] R.A. Chorush, D.P. Little, S.C. Beu, T.D. Wood, F.W. McLafferty, Anal. Chem. 67 (1995) 1042.
[42] F.W. McLafferty, Acc. Chem. Res. 27 (1994) 379.
[43] R.A. Jockusch, P.D. Schnier, W.D. Price, E.F. Strittmatter, P.A. Demirev, E.R. Williams, Anal. Chem. 69 (1997) 1119.
[44] W.D. Price, P.D. Schnier, R.A. Jockusch, E.F. Strittmatter, E.R. Williams, J. Am. Chem. Soc. 118 (1996) 10640.
[45] W.D. Price, E.R. Williams, J. Phys. Chem. A 101 (1997) 8844.
[46] E.R. Williams, Anal. Chem. 70 (1998) 179A.
[47] R.E. March, R.J. Hughes, Quadrupole Storage Mass Spectrometry, Wiley, New York, 1989.
[48] Practical Aspects of Ion Trap Mass Spectrometry, R.E. March, J.F.J. Todd (Eds.), CRC Press, Boca Raton, FL, 1995, Vols. I–III.
[49] C.S. Creaser, J.W. Stygall, Trac-Trends Anal. Chem. 17 (1998) 583.
[50] R.E. March, Rapid Commun. Mass Spectrom. 12 (1998) 1543.
[51] R.E. March, J. Mass Spectrom. 32 (1997) 351.
[52] K.R. Jonscher, J.R. Yates, Anal. Biochem. 244 (1997) 1.
[53] M.E. Bier, J.C. Schwartz, Electrospray Ionization Mass Spectrometry, R.B. Cole, (Ed.), Wiley New York, 1997, Chap. 7.
[54] J.C. Schwartz, I. Jardine, Meth. Enzymol. Part A 270 (1996) 552.
[55] S.A. McLuckey, Int. J. Mass Spectrom. 190/191 (1999).
[56] M.G. Qian, D.M. Lubman, Anal. Chem. 67 (1995) 234A.
[57] K.P. Aicher, M. Müller, U. Wilhelm, J. Grotemeyer, Eur. Mass Spectrom. 1 (1995) 331.
[58] Q. Ji,, M.R. Davenport, C.G. Enke, J.F. Holland, J. Am. Soc. Mass Spectrom. 7 (1996) 1009.
[59] P. Kofel, M. Stöckli, J. Krause, U.P. Schlunegger, Rapid Commun. Mass Spectrom. 10 (1996) 658.
[60] R.W. Purves, L. Li, J. Microcol. Sep. 7 (1995) 603.
[61] W. Gabryleski, L. Li, Rev. Sci. Instrum. 70 (1999) 4192.
[62] M. Baril, Nucl. Instrum. Methods, Phys. Res. A 427 (1999) 141.
[63] V.M. Doroshenko, R.J. Cotter, J. Mass Spectrom. 33 (1998) 305.
[64] J.N. Louris, R.G. Cooks, J.E.P. Syka, P.E. Kelley, G.C. Stafford, J.F.J. Todd, Anal. Chem. 59 (1987) 1677.
[65] S.A. McLuckey, D.E. Goeringer, G.L. Glish, Anal. Chem. 64 (1992) 1455.
[66] R.K. Julian, R.G. Cooks, Anal. Chem. 65 (1993) 1827.
[67] L.L. Lopez, P.R. Tiller, M.W. Senko, J.C. Schwartz, Rapid Commun. Mass Spectrom. 13 (1999) 663.
[68] S.A. Lammert, R.G. Cooks, Rapid Commun. Mass Spectrom. 6 (1992) 528.
[69] M.D. Wang, S. Schachterle, G. Wells, J. Am. Soc. Mass Spectrom. 7 (1996) 668.
[70] C. Paradisi, J.F.J. Todd, P. Traldi, U. Vettori, Org. Mass Spectrom. 27 (1992) 251.
[71] J. Qin, B.T. Chait, Anal. Chem. 68 (1996) 2108.
[72] D.J. Butcher, K.G. Asano, D.E. Goeringer, S.A. McLuckey, J. Phys. Chem. A 103 (1999) 8664.
[73] K.G. Asano, D.E. Goeringer, S.A. McLuckey, Int. J. Mass Spectrom. 185/186/187 (1999) 207.
[74] R.C. Dunbar, T.B. McMahon, Science 279 (1998) 194.
[75] D. Thölmann, D.S. Tonner, T.B. McMahon, J. Phys. Chem. 98 (1994) 2002.

[76] D.S. Tonner, D. Thölmann, T.B. McMahon, Chem. Phys. Lett., 233 (1995) 324.
[77] M. Busman, A.L. Rockwood, R.D. Smith, J. Phys. Chem. 96 (1992) 2397.
[78] A.L. Rockwood, M. Busman, H.R. Udseth, R.D. Smith, Rapid Commun. Mass Spectrom. 5 (1991) 582.
[79] S.G. Penn, F. He, M.K. Green, C.B. Lebrilla, J. Am. Soc. Mass Spectrom. 8 (1997) 244.
[80] M. Meot-Ner, A.R. Dongré, Á. Somogyi, V.H. Wysocki, Rapid Commun. Mass Spectrom. 9 (1995) 829.
[81] F.G. Major, H.G. Dehmelt, Phys Rev. 170 (1968) 91.
[82] Y. Moriwaki, M. Tachikawa, T. Shimizu, Jpn. J. Appl. Phys. 35 (1996) 757.
[83] Y. Moriwaki, M. Tachikawa, Y. Maeno, T. Shimizu, Jpn. J. Appl. Phys. 31 (1992) L1640.
[84] R. Blatt, P. Zoller, G. Holzmuller, I. Siemers, Z. Phys. D 4 (1986) 121.
[85] P. Traldi, S. Catinella, R.E. March, C.S. Creaser, in Practical Aspects of Ion Trap Mass Spectrometry, R.E. March, J.F.J. Todd (Eds.), CRC Press, New York, 1995, Vol. I, Chap. 7.
[86] R.W. Vachet, G.L. Glish, Anal. Chem. 70 (1998) 340.
[87] P.D. Schnier, W.D. Price, E.F. Strittmatter, E.R. Williams, J. Am. Soc. Mass Spectrom. 8 (1997) 771.
[88] P.D. Schnier, W.D. Price, R.A. Jockusch, E.R. Williams, J. Am. Chem. Soc. 118 (1996) 7178.
[89] J.N. Louris, J.S. Brodbelt-Lustig, R.G. Cooks, G.L. Glish, G.J. Van Berkel, S.A. McLuckey, Int. J. Mass Spectrom. Ion Processes 96 (1990) 117.
[90] S.A. McLuckey, G.L. Glish, G.J. Van Berkel, Int. J. Mass Spectrom. Ion Processes 106 (1991) 213.
[91] D.E. Goeringer, S.A. McLuckey, J. Chem. Phys. 104 (1996) 2214.
[92] D.E. Goeringer, S.A. McLuckey, Rapid Commun. Mass Spectrom. 10 (1996) 328.
[93] S.A. McLuckey, D.E. Goeringer, J. Mass Spectrom. 32 (1997) 461.
[94] D.E. Goeringer, S.A. McLuckey, Int. J. Mass Spectrom. Ion Processes 177 (1998) 163.
[95] K.J. Hart, S.A. McLuckey J. Am. Soc. Mass Spectrom. 5 (1994) 250.
[96] R.C. Dunbar, J. Chem. Phys. 95 (1991) 2537.
[97] G.T. Uechi, R.C. Dunbar, J. Chem. Phys. 96 (1992) 8897.
[98] R.C. Dunbar, R.C. Zaniewski, J. Chem. Phys. 96 (1992) 5069.
[99] G.T. Uechi, R.C. Dunbar, J. Chem. Phys. 98 (1993) 7888.
[100] D.E. Goeringer, K.G. Asano, S.A. McLuckey, Int. J. Mass Spectrom. 182/183 (1999) 275.
[101] K.G. Asano, D.J. Butcher, D.E. Goeringer, S.A. McLuckey, J. Mass Spectrom. 34 (1999) 691.
[102] K.G. Asano, D.E. Goeringer, D.J. Butcher, S.A. McLuckey, Int. J. Mass Spectrom. 190/191 (1999) 281.
[103] R.F. Bonner, G. Lawson, J.F.J. Todd, Int. J. Mass Spectrom. Ion Phys. 10 (1972/73) 197.
[104] J.F.J. Todd, R.E. March, Int. J. Mass Spectrom. 190/191 (1999) 9.
[105] T. Felix, M. Reyzer, J. Brodbelt, Int. J. Mass Spectrom. 190/191 (1999) 161.
[106] G.E. Reid, R.J. Simpson, R.A.J. O'Hair, Int. J. Mass Spectrom. 190/191 (1999) 209.
[107] I.A. Kaltashov, V.M. Doroshenko, R.J. Cotter, Proteins-Struct. Func. Genet. 28 (1997) 53.
[108] S.A. McLuckey, G.J. Van Berkel, G.L. Glish, J. Am. Chem. Soc. 112 (1990) 5668.
[109] S.A. McLuckey, G.L. Glish, G.J. Van Berkel, Anal. Chem. 63 (1991) 1971.
[110] S.A. McLuckey, D.E. Goeringer, Anal. Chem. 67 (1995) 2493.
[111] R.S. Ramsey, S.A. McLuckey, J. Microcol. Sep. 9 (1997) 523.
[112] R.A.J. O'Hair, S.A. McLuckey, Int. J. Mass Spectrom. Ion Processes 162 (1997) 183.
[113] J.L. Stephenson Jr., S.A. McLuckey, J. Am. Chem. Soc. 119 (1997) 1688.
[114] J.L. Stephenson Jr., S.A. McLuckey, Anal. Chem. 69 (1997) 281.
[115] J.L. Stephenson Jr., T.G. Schaaff, S.A. McLuckey, J. Am. Soc. Mass Spectrom. 10 (1999) 552.
[116] T. G. Schaaff, J.L. Stephenson Jr., S.A. McLuckey, J. Am. Chem. Soc. 121 (1999) 8907.
[117] T.G. Schaaff, J.L. Stephenson Jr., S.A. McLuckey, J. Am. Soc. Mass Spectrom. 11, (2000) 167.
[118] T.G. Schaaff, J.L. Stephenson Jr., S.A. McLuckey, Int. J. Mass Spectrom., In press.
[119] K.B. Shelimov, D.E. Clemmer, R.R. Hudgins, M.F. Jarrold, J. Am. Chem. Soc. 119 (1997) 2240.
[120] R.R. Hudgins, J. Woenckhaus, M.F. Jarrold, Int. J. Mass Spectrom. Ion Processes 165/166 (1997) 497.
[121] S.A. McLuckey, J.L. Stephenson Jr., Mass Spectrom. Rev. 17 (1998) 369.
[122] J.L. Stephenson Jr., S.A. McLuckey, J. Am. Chem. Soc. 118 (1996) 7390.
[123] S.A. McLuckey, J.L. Stephenson Jr., K.G. Asano, Anal. Chem. 70 (1998) 1198.
[124] W.J. Herron, D.E. Goeringer, S.A. McLuckey, J. Am. Soc. Mass Spectrom. 6 (1995) 529.
[125] W.J. Herron, D.E. Goeringer, S.A. McLuckey, J. Am. Chem. Soc. 117 (1995) 11555.
[126] S.A. McLuckey, W.J. Herron, J.L. Stephenson Jr., D.E. Goeringer, J. Mass Spectrom. 31 (1996) 1093.
[127] J.L. Stephenson Jr., G.J. Van Berkel, S.A. McLuckey, J. Am. Soc. Mass Spectrom. 8 (1997) 637.
[128] J.L. Stephenson Jr., S.A. McLuckey, J. Am. Soc. Mass Spectrom. 9 (1998) 957.
[129] J.L. Stephenson Jr., S.A. McLuckey, Int. J. Mass Spectrom. Ion Processes 162 (1997) 637.
[130] J.L. Stephenson Jr., S.A. McLuckey, Anal. Chem. 69 (1997) 3760.
[131] W.J. Herron, D.E. Goeringer, S.A. McLuckey, Anal. Chem. 68 (1996) 257.
[132] J.L. Stephenson Jr., S.A. McLuckey, Anal. Chem. 68 (1996) 4026.
[133] J.L. Stephenson Jr., S.A. McLuckey, J. Am. Soc. Mass Spectrom. 9 (1998) 585.
[134] J.L. Stephenson Jr., S.A. McLuckey, J. Mass Spectrom. 33 (1996) 664.
[135] J.L. Stephenson Jr., S.A. McLuckey, Anal. Chem. 70 (1998) 3533.

[136] B.J. Cargile, S.A. McLuckey, J.L. Stephenson Jr., Anal. Chem., submitted.
[137] R.R.O. Loo, H.R. Udseth, R.D. Smith, J. Am. Soc. Mass Spectrom. 3 (1992) 695.
[138] R.R.O. Loo, H.R. Udseth, R.D. Smith, J. Phys. Chem. 95 (1991) 6412.
[139] M. Scalf, M.S. Westphall, J. Krause, S.L. Kaufman, L.M. Smith, Science, 283 (1999) 194.
[140] M. Scalf, M.S. Westphall, L.M. Smith, Anal. Chem. 72 (2000) 52.
[141] D.C. Muddiman, X.H. Cheng, H.R. Udseth, R.D. Smith, J. Am. Soc. Mass Spectrom. 7 (1996) 697.
[142] J.L. Stephenson Jr., S.A. McLuckey, Int. J. Mass Spectrom. Ion Processes 165/166 (1997) 419.
[143] S.A. McLuckey, J.L. Stephenson Jr., R.A.J, O'Hair, J. Am. Soc. Mass Spectrom. 8 (1997) 89.
[144] J.L. Stephenson Jr., S.A. McLuckey, Rapid Commun. Mass Spectrom. 11 (1997) 3760.
[145] S.A. McLuckey, K.G. Asano, T.G. Schaaff, J.L. Stephenson Jr., Int. J. Mass Spectrom. 196 (2000) 419.
[146] J.C. Hummelen, J.L.J. van Dongen, E.W. Meijer, Chem Eur. J. 3 (1997) 1489.
[147] J.W. Weener, J.L.J. van Dongen, J.C. Hummelen, E.W. Meijer, Polym. Mater. Sci. Eng. 77 (1997) 147.
[148] J. de Maaijer-Gilbert, C. Gu, A. Somogyi, V.H. Wysocki, P.G. Kistemaker, T.L. Weeding, J. Am. Soc. Mass Spectrom. 10 (1999) 414.
[149] T.G. Schaaff, B.J. Cargile, J.L. Stephenson Jr., S.A. McLuckey, Anal. Chem. 72 (2000) 899.