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Bath gas temperature and the appearance of ion trap tandem mass spectra of high-mass ions

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Abstract

Bath gas temperature effects upon the appearance of ion trap tandem mass spectra of protonated leucine enkephalin have been studied under a variety of ion activation conditions and over a bath gas temperature range of 298–486 K. Bath gas temperature was found to have two possible effects upon the identities and abundances of product ions observed in tandem mass spectra. At high parent ion dissociation rates ($>10\ s^{-1}$) and short activation times (<30 ms), bath gas temperature principally affects the appearance of tandem mass spectra via its role in determining the kinetics for first generation product ion cooling. In the case of protonated leucine enkephalin, this is primarily reflected in the extent to which the first generation b_4^+ ion fragments further to yield the a_4^+ ion, the energy supplied for this process arising from the parent ion internal energy. Bath gas temperature is most likely to be an important factor in the appearance of tandem mass spectra collected under the conditions just mentioned, when a first generation product ion tends to fragment at rates comparable to or greater than the parent ion dissociation rate. At low parent ion dissociation rates ($>10\ s^{-1}$), relatively long ion activation times (<100 ms), and elevated bath gas temperatures, dissociation resulting from thermal activation of the first and higher generation fragments can make significant contributions to the tandem mass spectra. (Int J Mass Spectrom 190/191 (1999) 281–293) © 1999 Elsevier Science B.V.

Keywords: Quadrupole ion trap; Bath gas temperature; Collisional activation; Collisional relaxation; Thermal dissociation

1. Introduction

The identities and relative abundances of fragmentation products reflected in a mass spectrum are generally determined by instrumental discrimination effects, the distribution of parent ion internal energies, the time over which dissociation products are allowed to accumulate, and the energetic and entropic requirements of the relevant competitive and consecutive fragmentation channels. The latter three factors comprise the picture associated with statistical theories of mass spectra [1–3], whereby in the usual case, the internal energy initially present in the parent ion population drives all subsequent dissociation. This situation also applies for fast activation methods, defined as those where the input of internal energy into a parent ion population occurs much faster than fragmentation [4], and where the excited parent ions are maintained in a collision-free environment. This scenario is exemplified by single photon UV photo-

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Dedicated to J.F.J. Todd and R.E. March in recognition of their original contributions to quadrupole ion trap mass spectrometry.

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dissociation [5,6] and single collision condition collisional activation [7–11] in beam-type tandem mass spectrometers. The appearance of a tandem mass spectrum or MS/MS spectrum, defined as the signal intensities and mass-to-charge ratios of the product ions, is generally determined by the same factors that determine the appearance of a mass spectrum [12,13]. MS/MS spectra, however, are collected under a wide variety of experimental conditions using a wide variety of instrumental types such that, at least in some scenarios, factors beyond those just mentioned might affect the appearance of MS/MS spectra. A particularly striking case is that associated with the quadrupole ion trap MS/MS experiment employing single frequency resonance excitation [14] as the means for collisional activation.

Ion trap collisional activation is one of several "very slow" or "slow heating" activation methods now employed in ion trapping instruments [6], as are continuous wave infrared multiphoton dissociation [15–18], and blackbody infrared dissociation [19–23]. These methods share the characteristic that ion activation, ion deactivation, and ion dissociation processes all occur in parallel. This characteristic distinguishes these methods from faster ion activation methods wherein the activation process is fast relative to either ion deactivation or ion dissociation. Despite their commonalities, however, the very slow activation methods can differ from one another in ways that can lead to significant differences in observed MS/MS behavior. The quadrupole ion trap experiment is unique in that a relatively high pressure bath gas, usually helium at roughly 1 mTorr, is present throughout the ion excitation period. It is widely recognized that product ions formed in a single-frequency resonance excitation ion trap MS/MS experiment are not subsequently subjected to further activation by the applied excitation frequency because the product ions tend to be out of resonance with the activation signal [14,24,25]. Subsequent fragmentation of the first generation product ions is therefore not actively promoted in this type of experiment. It is not widely appreciated, however, that first generation product ions can be rapidly cooled internally once they are formed, thereby *actively inhibiting* further fragmentation. The inhibition of sequential fragmentation is most likely for relatively long-lived product ions and is therefore most likely to be important for product ions with many degrees of freedom (see below).

We illustrate here the important role that bath gas temperature can play in affecting the appearance of an MS/MS spectrum acquired with a quadrupole ion trap using protonated leucine enkephalin as the parent ion. This ion has been studied extensively both in the ion trap [26–32], and by other slow activation methods [33,34] such that the genealogy of the most abundant product ions (i.e. first generation versus higher generation) are reasonably well established. It will be shown that the temperature of the bath gas is a variable in the extent to which first generation product ions are internally cooled after formation. This example highlights the unusual situation the ion trap collisional activation experiment creates in that the internal temperature to which the parent ion is elevated can be significantly higher than the temperature of its environment, and that the ion relaxation rate due to ion/helium collisions, is sufficiently high to inhibit sequential fragmentation. Single frequency ion trap collisional activation is unusual relative to other slow activation methods, because with other methods (e.g. blackbody infrared dissociation) the environment may be at the same temperature as the parent ions. The product ions are therefore themselves subjected to activation conditions similar to those experienced by the parent ion.

2. Experimental

2.1. Materials and electrospray conditions

Leucine enkephalin (tyr-gly-gly-phe-leu or YGGFL) obtained commercially (Sigma, St. Louis, MO) was dissolved in a solution of 50:50 methanol:water to a concentration of roughly 2160 μM with 1% acetic acid. Working solutions ($\sim$30 μM) were prepared by diluting the stock solution with 99% methanol/1% acetic acid. The solution was infused at a rate of 1.0 μL/min through a 100 μm inner diameter (i.d.) stainless steel capillary held at $+3.5$–4.0 kV.

2.2. Mass spectrometry

All experiments were performed with a Finnigan ion trap mass spectrometer (ITMS, San Jose, CA), modified for electrospray ionization. An electrospray interface/ion injection lens/ion trap assembly, which has been described previously [35], was attached to the vacuum system of the ITMS. The ITMS vacuum system is equipped with infrared heaters and a closed-loop temperature control system. A constant bath gas temperature was achieved by suspending the platinum resistance thermometer of the temperature control system in the vacuum system (i.e. it was disconnected from its normal attachment to the surface of the vacuum system). The location of the platinum resistance thermometer is somewhat remote from the ion trap itself. The temperature indicated by the platinum resistance thermometer was therefore calibrated against a temperature measurement made by suspending a temperature probe (Omega) adjacent to the ion trap (in the absence of any applied voltage) and systematically altering the temperature set point of the temperature feedback system. Essentially identical temperatures were also measured when the probe was physically touching a mounting bracket of the ion trap electrodes. In all cases, helium was introduced into the vacuum chamber to a total pressure (uncorrected) of 1.1×10^{-4} Torr, as measured on the ion gauge.

Following an ion accumulation period of 100–300 ms, protonated leucine enkephalin ions were isolated using a single resonance ejection ramp. That is, isolation of the parent ion of interest was effected using a single scan of the rf-voltage amplitude applied to the ring electrode while simultaneously applying a single frequency, in monopolar fashion [36], chosen to sweep out ions of mass-to-charge ratio greater than that of the ion of interest. Lower mass-to-charge ratio ions were swept out by passing the ions through the $q_z = 0.908$ exclusion limit. A relatively broad ion isolation window (i.e. several mass-to-charge ratio units) was employed to avoid collisional activation of the parent ion by off-resonance power absorption during the isolation step.

Product ion spectra were recorded as a function of ion activation time. Ion activation was effected either by storing the ions in the heated bath gas that was held at a temperature sufficiently high to drive dissociation over the time frame of interest, or by monopolar resonance excitation [36]. In all cases, the parent ion was held at a q_z value of 0.163. The spectrum used for each reaction time was the average of 100 repetitions of the experimental sequence described above.

3. Results and discussion

This work is particularly focused on “high-mass” ions, where “high-mass” is intended to imply “many internal degrees of freedom.” Several factors underlie the sensitivity to bath gas temperature of ion trap MS/MS spectra derived from parent ions with many internal degrees of freedom. For example, the relative degree of internal cooling of fragments associated with dissociation of a many oscillator system is lower than with a system with fewer internal degrees of freedom. Some internal energy must be expended to break a bond and some internal energy can go into relative translation of the fragments. This implies that the fragments have less internal energy than the parent on a “per-degree-of-freedom” basis. As the number of internal degrees of freedom in the parent ion increases, however, the fraction of parent ion internal energy that goes into bond breaking and relative fragment translation decreases such that, in the extreme, the internal energy per degree of freedom in the fragments approximates that of the parent. Therefore, internally hot high-mass parent ions tend to produce internally hot product ions. The product ions themselves may be formed with sufficient energy to fragment further but the rate at which this occurs is partly a function of product ion size. Product ion size is also a factor in determining the ion/helium collision rate. The relative rates for sequential fragmentation and collisional relaxation, along with the time frame of the experiment, determine the extent to which sequential fragmentation is observed. As discussed further below, bath gas temperature plays a role in both the rate and extent of collisional relaxation.

The first factor discussed above implies that product ions from high-mass parent ions are likely to undergo further fragmentation if the energetic and entropic requirements for dissociation are comparable to or are more relaxed than those of the parent ion. In other words, if there is sufficient energy in the parent ion for fragmentation at a given rate on a per-degree-of-freedom basis, a product ion of comparable or lower kinetic stability would be expected to fragment further at a similar or faster rate. However, if the bath gas temperature is significantly lower than the effective internal temperature of the parent ion undergoing resonance excitation, first generation product ions with large numbers of degrees of freedom can have energy removed via ion/helium collisions, thereby diminishing the probability for further fragmentation. Protonated leucine enkephalin is a parent ion that illustrates well the dramatic influence that ion trap bath gas temperature can have on the appearance of an ion trap MS/MS spectrum through its influence on sequential decomposition. Protonated leucine enkephalin contains a moderate number of degrees of freedom (i.e. 228) such that loss of internal energy on a per degree of freedom basis associated with fragmentation is not trivial (see below). That is, it does not represent the extreme case wherein the energy required for fragmentation is only a very small fraction of the total ion internal energy. Nevertheless, it is sufficiently large for the bath gas temperature to affect the appearance of its MS/MS spectra.

Protonated leucine enkephalin primarily fragments to yield the b_4^+ ion with a relatively minor competitive channel involving loss of water ($MH^+ - H_2O$). The b_4^+ ion fragments further by loss of CO to yield the a_4^+ ion. The a_4^+ ion, in turn, fragments to several other products, as discussed below. These observations are consistent with a recent blackbody infrared dissociation study of protonated leucine enkephalin [33]. It has recently been argued, based on ion trap double resonance experiments [29], that there is also a mechanism for direct formation of the a_4^+ ion from protonated leucine enkephalin. Experiments in our laboratory at significantly higher resonance excitation amplitudes than those used to acquire the data shown here resulted in a_4^+ ion abundances that rivaled those of the b_4^+ ion in the MS/MS spectrum. These conditions were presumably similar to those used in the double resonance experiments [29]. However, at the resonance excitation voltage amplitudes used in this study and at a bath gas temperature of 298 K, relatively little a_4^+ ion is observed in the MS/MS data. Therefore, essentially all of the a_4^+ ions observed in this work are expected to have been formed through the intermediate b_4^+ ion.

We recently described a series of studies in which the dissociation rates of protonated leucine enkephalin and the b_4^+ ion derived therefrom were reported as a function of bath gas temperature [31] (no resonance excitation was employed). Dissociation via use of a heated bath gas is, of course, a form of collisional activation whereby collision energies are raised by increasing the bath gas temperature rather than by increasing the translational energy of the ion via externally applied electric fields. From the dissociation rates obtained in the heated bath gas experiments, Arrhenius activation parameters were determined to be $E_a = 1.28 \pm 0.08$ eV and $\log A = 12.55 \pm 0.87$ for protonated leucine enkephalin and $E_a = 0.98 \pm 0.07$ eV and $\log A = 10.6 \pm 0.79$ for the b_4^+ ion. The activation parameters derived for protonated leucine enkephalin reflect both the water loss process and the channel leading to the b_4^+ ion. Master equation modeling suggested that rapid energy exchange conditions were approximated for the protonated leucine enkephalin ion over the dissociation rate range used to derive the activation parameters just listed. Modeling of the dissociation of the b_4^+ ion was not performed. Therefore, the activation parameters just listed may not reflect true high pressure limit conditions for the b_4^+ ion. Nevertheless, these activation parameters suggest and experimental data show that under identical conditions, the b_4^+ ion fragments at higher rates than does protonated leucine enkephalin, at least at temperatures less than ~800 K. For example, it was found experimentally that the b_4^+ ion fragments at a rate of 0.64 s^{-1} at 458 K, whereas the protonated molecule fragments at a rate of 0.027 s^{-1} at this temperature. These results, which are consistent with those reported for the blackbody infrared dissociation of protonated leucine enkephalin, show that protonated leucine enkephalin is a parent ion in which at least one of the major first generation

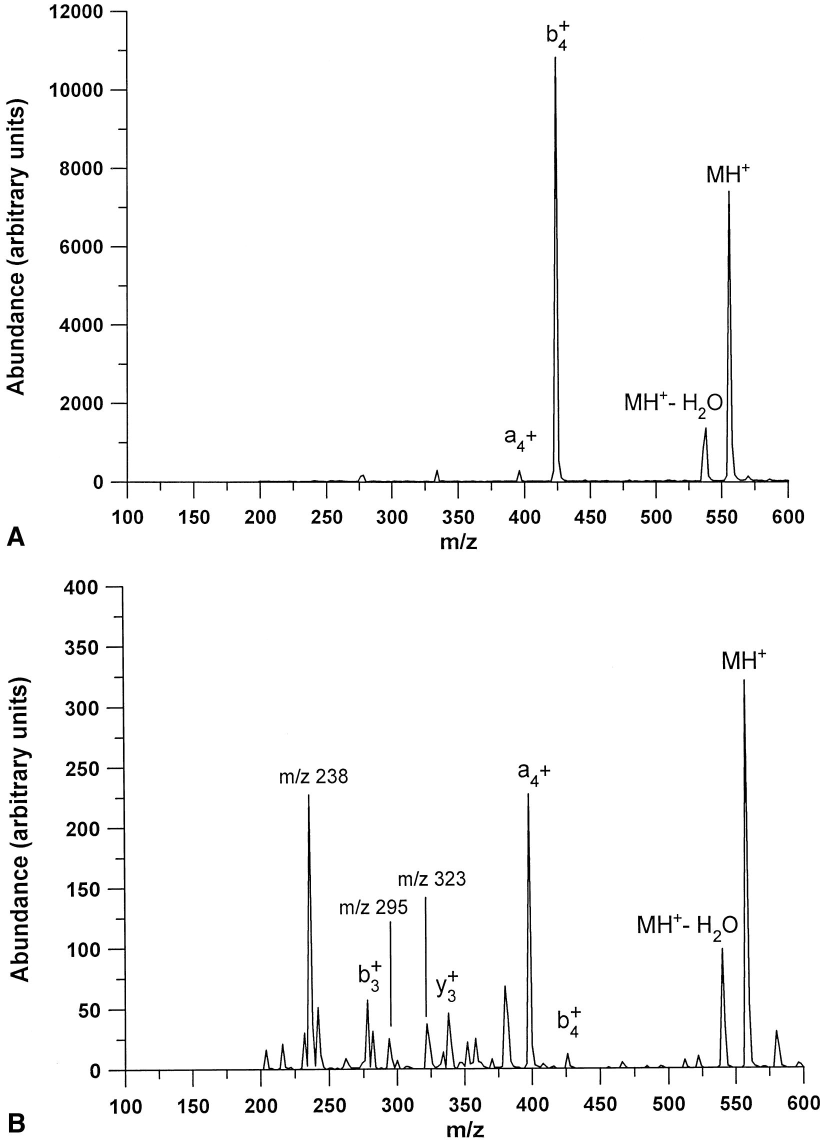


Fig. 1. (a) MS/MS spectrum of protonated leucine enkephalin using a resonance excitation voltage of 300 mV ($q_z = 0.163$), room temperature bath gas, and an ion activation time of 1 s. (b) MS/MS spectrum of protonated leucine enkephalin acquired at a bath gas temperature of 486 K ($q_z = 0.163$) without resonance excitation after an activation period of 6 s.

product ions (the b_4^+ ion, in this case) fragments more readily than does the parent ion under similar conditions.

When a first generation high-mass product ion can itself undergo facile fragmentation, the environment in which it is formed and stored can have a significant effect on the extent to which further fragmentation occurs. To illustrate, Fig. 1 compares protonated leucine enkephalin MS/MS spectra acquired using a resonance excitation voltage of 300 mV and a bath gas temperature of 298 K after 1 s of resonance excitation [Fig. 1(a)] and a bath gas temperature of 486 K without resonance excitation after storage for 6 s [Fig. 1(b)]. The first generation products (MH^+ −

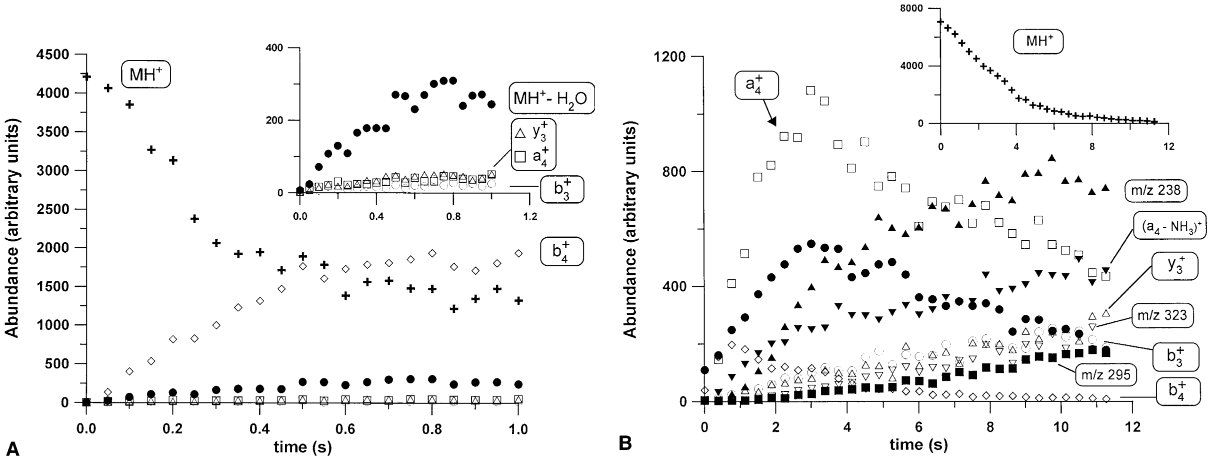
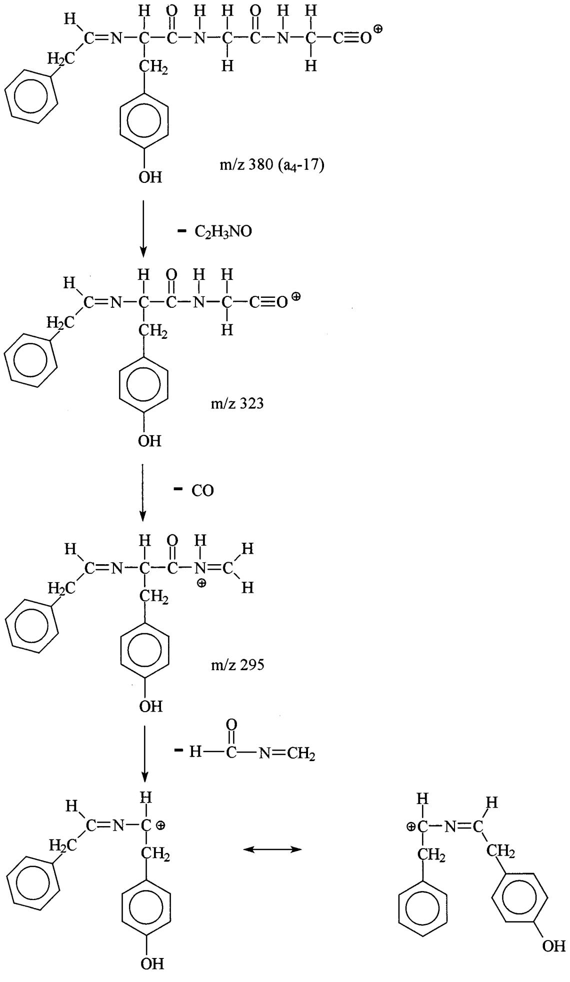


Fig. 2. (a) Ion abundances as a function of ion activation time derived from MS/MS spectra of protonated leucine enkephalin collected using a resonance excitation voltage of 300 mV and a bath gas temperature of 298 K (parent ion dissociation rate = 1.6 s^{-1}). (b) Ion abundances as a function of ion activation time derived from MS/MS spectra of protonated leucine enkephalin collected using a bath gas temperature of 486 K without resonance excitation (parent ion dissociation rate = 0.183 s^{-1}).

H_2O) and b_4^+ ion clearly dominate the MS/MS spectrum in Fig. 1(a) whereas products from sequential decompositions, e.g. a_4^+, $a_4^+ - NH_3$, y_3^+, and the ion labeled *m/z* 238, combine to dominate the MS/MS spectrum in Fig. 1(b). These observations are made despite the fact that the parent ions leading to Fig. 1(a) were higher in internal energy than those that led to Fig. 1(b), as indicated by the parent ion dissociation rates. The data in Fig. 1(a) were collected with a parent ion dissociation rate of 1.6 s^{-1}, which corresponds to a parent ion internal temperature of 522 K, whereas the dissociation rate for the data in Fig. 1(b) was 0.183 s^{-1}, corresponding to a parent ion internal temperature equal to that of the bath gas, 486 K. The greater extent of sequential decomposition arising from parent ions at lower internal temperature [Fig. 1(b)] therefore points to, as the underlying cause, the fact that the product ions were formed and stored in a bath gas at a temperature of 486 K whereas the product ions arising from a parent ion internal temperature of 522 K [Fig. 1(a)] were formed in a bath gas of 298 K.

The role of bath gas temperature in the data of Fig. 1 is further clarified in Fig. 2 which summarizes the time dependencies of MS/MS spectra derived under the conditions used to collect the spectra in Fig. 1(a) and (b). Fig. 2(a) summarizes MS/MS spectra derived using a resonance excitation voltage of 300 mV and a bath gas temperature of 298 K and Fig. 2(b) summarizes the MS/MS spectra derived from the use of bath gas heating to 486 K as the means for ion activation. The data in Fig. 2(a) show the usual exponential loss of parent ions as a function of ion activation time. A corresponding increase in total product ion abundance is also observed. However, the relative abundances of the product ions are essentially invariant with time, indicating that fragmentation of the first generation products ions is minimal. Product ion relative abundances continue to be essentially unchanged at activation times extending to as long as 12 s (data not shown). Fig. 2(b), however, shows that both the absolute and relative product ion abundances change with time when the bath gas temperature is held at 486 K. The relative abundance of the b_4^+ ion is largest at short times. This is consistent with its relatively rapid dissociation to yield the a_4^+ ion. Eventually, the *m/z* 238 ion, which arises from the a_4^+ ion (see below), becomes the most abundant product ion in the spectrum. This ion is rarely noted under normal ion trap collisional activation conditions because room temperature bath gases and relatively short activation periods, usually less than about 30 ms, are used. The results in Fig. 2 are consistent with the activation parameters mentioned above for protonated leucine enkephalin. At 486 K, the parent ion dissociates at a rate of 0.183 s^{-1} whereas the b_4^+ ion is expected to fragment at a rate of ~2.8 s^{-1}, based on the Arrhenius equation. The higher dissociation rate associated with the b_4^+ ion relative to that of the parent ion accounts for the fact that the a_4^+ product ion, a second generation fragment formed from the b_4^+ ion, tends to dominate the 486 K MS/MS spectra even at relatively short activation times. Very little sequential fragmentation of the b_4^+ ion is observed in Fig. 2(a) because the expected dissociation rate at 298 K is about 1×10^{-6} s^{-1}.

The data in Fig. 2(b) also illustrate the sometimes rather complex phenomenology that can occur with use of continuous ion activation over long periods of time. The relatively rapid emergence of the a_4^+ ion as the base peak in the thermal dissociation data illustrates how a higher generation product ion can come to dominate an MS/MS spectrum when each generation of product ions is held at the same temperature. The a_4^+ ion then goes on to lose 17 Da (ammonia loss), and to fragment further to several of the other ions observed in the MS/MS spectra at longer times. For example, the *m/z* 323 ion, the origin of which was identified by Vachet et al. [28] as a rearrangement reaction leading to the loss of ammonia and the third residue glycine, is noted at relatively long times. Furthermore, the *m/z* 295 ion that arises from the loss of CO from the *m/z* 323 ion is also observed. Eventually, the *m/z* 238 ion comes to dominate the MS/MS spectra at long times. This ion was identified as c_2^+ in the blackbody infrared dissociation study [33] but can be formed from several of the ions that derive from the *m/z* 323 rearrangement product, none of which is likely to lead to a c_2^+ ion. Rather, this ion appears to consist of elements of the tyrosine and phenylalanine residues. A proposed structure of the *m/z* 238 ion is shown in Scheme 1, along with those of the *m/z* 380 ($a_4^+ - NH_3$), *m/z* 323, and *m/z* 295 ions as proposed earlier [28]. When the *m/z* 238 ion was subjected to ion trap collisional activation (data not shown), it fragmented to two products of roughly equal abundance that corresponded either to loss of toluene (side chain of the phenylalanine residue) to yield a product ion at *m/z* 146 or to loss of hydroxytoluene (side chain of the tyrosine residue) to yield a product ion at *m/z* 130. These results are fully consistent with expectations based on the rearrangement reactions identified in the study of Vachet et al. [30]. MS^n data reported in that study and those observed in our laboratory suggest that the *m/z* 238 product might arise via several pathways. These include, for example, direct formation from the a_4^+ ion and the series of decompositions shown in part in Scheme 1 involving the following product ions: $a_4^+ \rightarrow a_4^+ - NH_3 \rightarrow$ *m/z* 323 → *m/z* 295 (CO loss from *m/z* 323) → *m/z* 238



Scheme 1.

(glycine loss from the ion at *m/z* 295). We have made no effort to fully reconstruct the genealogy of all of the various generation products in Fig. 2(b), but it is clear that a variety of rearrangement and fragmentation reactions occur leading to the point at which the ion at *m/z* 238 dominates. It is important to recognize that, whereas signals attributed to the *m/z* 323 and *m/z* 295 ions never become highly abundant, it is possible that much of the *m/z* 238 signal can arise from the sequence of reactions listed above. If the rates of dissociation at 486 K for the *m/z* 323 and *m/z* 295 ions are greater than those that lead to the *m/z* 323 ion, it is quite possible that these ions would not be observed in high abundance at any point in the overall reaction period. Much of the product ion signal eventually accumulates into the *m/z* 238 ion presumably because it does not fragment further at these time frames at 486 K [e.g. no signals at *m/z* 146 or *m/z* 130 were noted in any of the MS/MS spectra used to construct Fig. 2(b)].

The comparisons of Figs. 1 and 2 show how significantly bath gas temperature can affect the appearance of ion trap MS/MS spectra. However, the data for Figs. 1 and 2 were recorded at parent ion dissociation rates of 1.6 s^{-1} [Figs. 1(a) and 2(a)] and 0.183 s^{-1} [Figs. 1(b) and 2(b)], which are rates much lower than typically associated with most ion trap MS/MS experiments. Ion trap MS/MS spectra are usually recorded using resonance excitation voltages sufficiently high to drive reactions at rates such that much, if not all, of the parent ion population is made to dissociate within about 30 ms. Fig. 3 shows a comparison of data collected under conditions in which the parent ion dissociation rate was 65–66 s^{-1}—a rate that depletes most of the parent ion signal within 30 ms. Parent ion internal energy is held constant by comparing data at the same parent ion dissociation rate. By comparing data acquired at the same parent ion internal energy the role of bath gas temperature can be observed clearly. Fig. 3(a) shows data collected using a resonance excitation voltage of 540 mV and room temperature bath gas and Fig. 3(b) shows data collected using a resonance excitation voltage of 300 mV and a bath gas temperature of 434 K. Before comparing Fig. 3(a) to (b), it is instructive to compare data acquired using room temperature helium and different resonance excitation amplitudes [e.g. Figs. 3(a) and 2(a)]. It is clear that the room temperature/540 mV resonance excitation data [Fig. 3(a)] shows a greater abundance of the a_4^+ ion than the room temperature/300 mV resonance excitation data in Fig. 2(a). This is probably because of a greater degree of sequential fragmentation (i.e. $MH^+ \rightarrow b_4^+ \rightarrow a_4^+$) at the higher resonance excitation voltage. The initially formed b_4^+ ions are expected to be hotter with the use of the higher resonance excitation voltage, which would increase the likelihood for sequential fragmentation despite the ion cooling resulting from collisions with the room temperature helium. In the data of Fig. 3(b) the relative abundance of the a_4^+ ion is clearly greater than in either Figs. 2(a) or 3(a) despite the fact that the parent ion internal energy distribution leading to both plots of Fig. 3 was very similar. In contrast with Fig. 2(b), where at high bath gas temperatures the a_4^+ ion becomes the dominant product ion even at short activation times, the a_4^+ ion in Fig. 3(b) does not become the dominant product ion over the activation period shown. This is probably because of the differences in parent ion and product ion temperatures. At 434 K, the b_4^+ ion is expected to dissociate at a rate of about 0.17 s^{-1}. This rate is far less than that of the parent ion (65 s^{-1}), in contrast with the situation leading to the data of Fig. 2(b) (viz. bath gas temperature of 486 K, no resonance excitation) where the parent ion dissociation rate was less than that of the b_4^+ ion. The relatively small expected dissociation rate of the b_4^+ ion at 434 K leads to the conclusion that most of the a_4^+ ions observed in Fig. 3(b) arose from sequential fragmentation of the b_4^+ ion before it could be cooled to 434 K. At 434 K, less than 1% of the b_4^+ ions formed early in the activation period would be expected to fragment to the a_4^+ ion within 25 ms. In this case, the major influence that the bath gas temperature plays is in its effect upon the rate of ion cooling. Very few of the a_4^+ ions reflected in Fig. 3(b) were generated via activation by the bath gas at 434 K, whereas essentially all of the a_4^+ ions observed in the data of Fig. 2(b) were formed via activation by the bath gas at 486 K.

The data in Figs. 2 and 3 illustrate the two possible

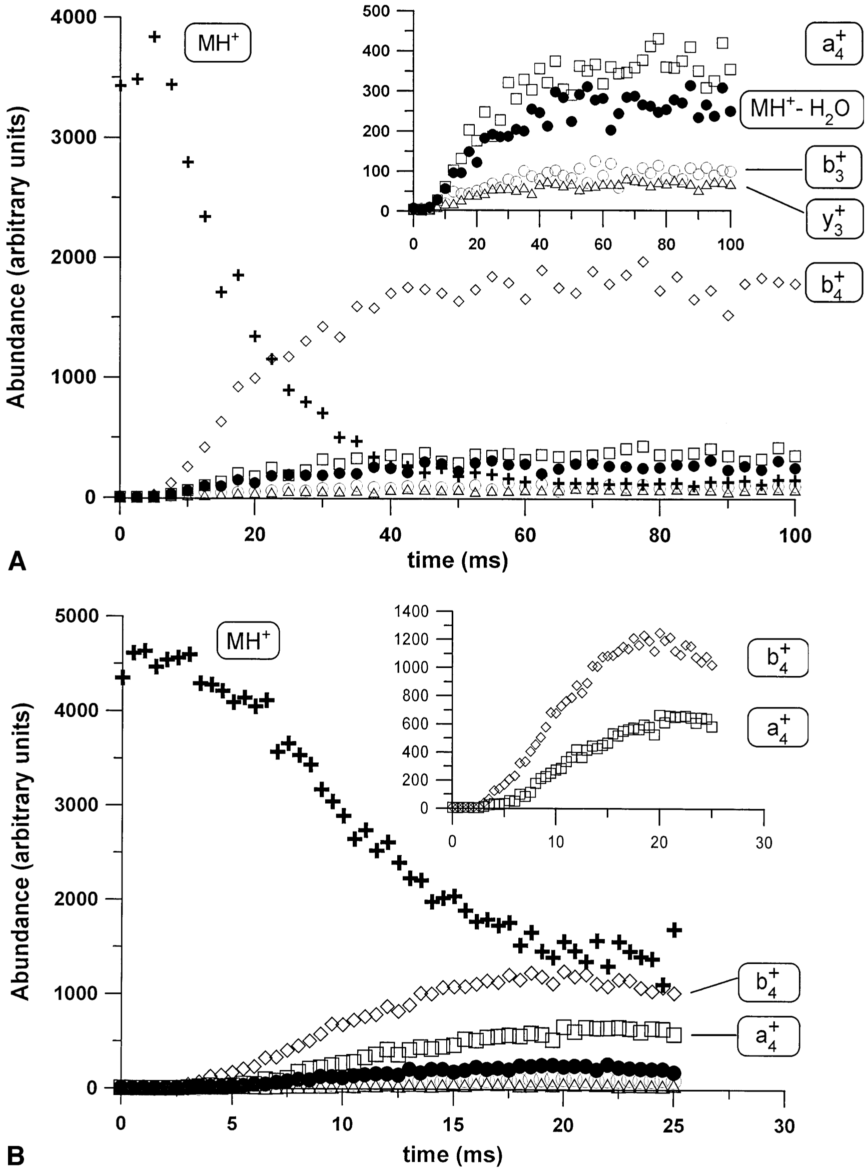


Fig. 3. (a) Ion abundances as a function of ion activation time derived from MS/MS spectra of protonated leucine enkephalin collected using a resonance excitation voltage of 540 mV and a bath gas temperature of 298 K. (b) Ion abundances as a function of ion activation time derived from MS/MS spectra of protonated leucine enkephalin collected using a resonance excitation voltage of 300 mV and a bath gas temperature of 434 K. The parent ion dissociation rate for each set of conditions falls in the range of 65–66 s^{-1}.

roles that the bath gas can play in affecting the appearance of MS/MS spectra derived from high-mass ions. The bath gas itself can drive dissociation and it can affect the rate of internal energy removal from first generation product ions. The latter effect can play a role in determining the extent of sequential fragmentation observed from the product ions initially formed with internal energies greater than those corresponding to the bath gas temperature. This effect can apply whenever collisional activation is effected by accelerating the parent ion (i.e. the normal ion trap collisional activation experiment) because the parent ion internal temperature and the bath gas temperature differ. In the most common scenario in which the bath gas temperature is close to room temperature and resonance excitation voltages are employed to drive most parent ion dissociation within a few tens of milliseconds, dissociation of first and higher generation product ions driven by the bath gas is minimal. That is, essentially all of the dissociation arises from the energy supplied by the ion acceleration process. In this scenario, the major effect of bath gas temperature arises from its role in determining the rate of removal of internal energy from highly excited first generation product ions.

We have recently simulated the relaxation of internally excited high-mass ions under typical ion trap storage conditions [37] (viz. 1 mTorr helium at 300 K). In that work, the highly excited ions were assumed to be stored at the center of the ion trap. That is, the collisional relaxation of internal modes was not complicated by a process in which excess translational energy was being removed. Such a scenario, however, does not typically apply to the ion trap collisional activation experiment because the product ions are initially formed with either excess kinetic or potential energy. That is, product ions formed remote from the center of the ion trap, as at the turning points of the ion oscillation, are in a region of relatively high potential energy. Product ions formed near the center of the ion trap, where parent ion velocities are highest, are formed with a velocity close to that of the parent ion. In both cases, the excess potential or kinetic energy is removed via momentum transfer collisions with the bath gas. The net effect is to extend the time frame for collisional relaxation of internal modes relative to the case in which the excited ions are already kinetically stabilized at the center of the ion trap. Nevertheless, the ion relaxation simulation study is highly relevant to this subject because it provides insight into the factors that affect the rate of internal relaxation of excited ions and the competition between ion relaxation and dissociation for systems where the rates for these processes are comparable. Of principle interest here is the role of bath gas temperature. Two models to describe collisional relaxation of internally excited ions were discussed in the simulation study. One was based on the exponential model for inefficient colliders [38] and the other was based on a so-called "diffuse scattering" model [39]. The two models tended to yield relaxation rates on the order of 200–2000 s^{-1} that differed by about a factor of three, with diffuse scattering yielding the higher cooling rates. Both models, however, shared the characteristic that the cooling rate is dependent upon the difference between the ion internal temperature and the temperature of the bath gas. Such a condition must hold for a highly excited ion to come to thermal equilibrium with its surroundings. The degree to which the bath gas is effective in inhibiting sequential fragmentation of first generation product ions is determined by the relative rates of unimolecular dissociation and ion cooling. The ion cooling rate is a function of the ion/bath gas collision rate, the composition of the bath gas, and the difference between the initial ion internal energy and its internal energy when it has equilibrated with the bath gas. Unimolecular dissociation rates are dependent upon the internal energy distribution and the internal energy-dependent microscopic rate constants. The extent to which product ion spectra are sensitive to bath gas temperature is therefore expected to be highly case specific. However, the case in which a first generation product ion tends to fragment at a higher rate than the parent ion is one that is most conducive to leading to a bath gas temperature effect on the appearance of the MS/MS spectrum.

Simulations have been performed on the protonated leucine enkephalin case to determine if the experimental observations described herein are consistent with a previously employed collisional cooling model, viz. the exponential model for inefficient colliders [37,38]. Specifically, the consecutive dissociation sequence:

$$\mathrm{MH}^+ \rightarrow \mathrm{b}_4^+ \rightarrow \mathrm{a}_4^+ \tag{1}$$

was modeled under conditions similar to those used to obtain the experimental data in Fig. 3. That is, the

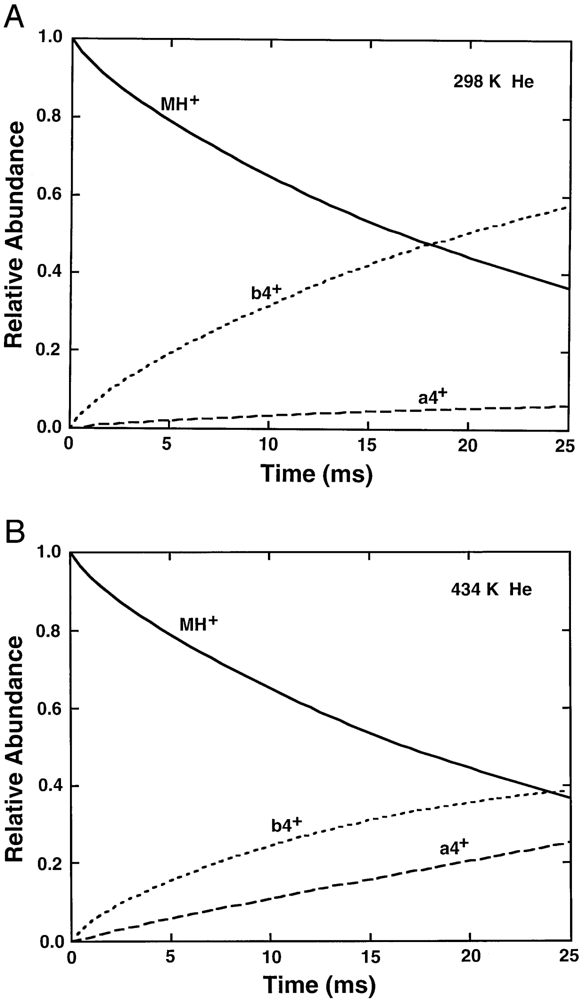


Fig. 4. Simulated ion abundances for the MH^+, b_4^+, and a_4^+ ions of leucine enkephalin as a function of time for a parent ion internal temperature of 655 K and bath gas temperatures of (a) 298 K and (b) 434 K.

MH^+ ion population had an internal energy distribution corresponding to an internal temperature of 655 K and the helium bath gas was at either 298 K [Fig. 4(a)] or 434 K [Fig. 4(b)]. The results were obtained using a numerical technique (4th order Runge–Kutta) to solve the complete set of simultaneous differential equations (i.e. master equation) describing the kinetics of MH^+ and b_4^+ internal energy evolution and dissociation. Internal energy-dependent unimolecular dissociation rates for MH^+ and b_4^+ were calculated via RRKM theory using vibrational frequencies obtained from molecular modeling [33] and critical dissociation energies of 1.38 eV and 0.80 eV, respectively. During the simulation, the internal energy of dissociating MH^+ ions was partitioned between the b_4^+ ion and neutral fragment according to the b_4^+/MH^+ ratio of degrees of freedom (162/228), after subtracting the MH^+ critical dissociation energy from the transition state. The exponential model for inefficient colliders was used to model the collisional energy transfer during ion-neutral collisions. In this case, the product ions are assumed to be formed in the center of the ion trap at thermal kinetic energies. That is, momentum transfer collisions necessary to decelerate translationally excited product ions were not incorporated into this simulation. The process of “translational” cooling is expected to extend somewhat the process of “internal” cooling relative to the situation in which the product ions are already translationally cooled to the center of the ion trap. The fairly close agreement between the simulation and the experimental data [e.g. compare Figs. 3(b) and 4(b)] was obtained by use of a b_4^+ critical energy (0.80 eV) somewhat lower than the experimentally measured value (0.98 ± 0.08 eV). The simulation therefore overestimates the kinetics for dissociation of the b_4^+ ion. This adjustment of the b_4^+ critical dissociation energy was necessary to make the simulation resemble the experimental data, presumably because the simulation overestimates the collisional internal cooling rate by not accounting for the translational cooling requirement in the ion trap collisional activation experiment.

Although the simulations used to derive Fig. 4 probably overestimate the collisional (internal) cooling rates of the initially formed b_4^+ ions, the comparison of Figs. 4(a) and (b) clearly suggests that the difference in cooling rates between 298 and 434 K helium is sufficiently different to lead to the notable increase in the abundance of the a_4^+ ion acquired using the higher bath gas temperature. The simulation therefore illustrates how bath gas temperature can affect the appearance of MS/MS spectra of high-mass ions even when the dissociation rate of product ions at the bath gas temperature is too low to allow for significant contributions from dissociation reactions driven by the bath gas itself. That is, the rate of removal of internal energy from excited first-generation product ions is a function of bath gas temperature. It is also noteworthy that this effect is observed for a system as small as that of protonated leucine enkephalin. Dissociation to form the b_4^+ ion (1.38 eV in the simulation), consumes a significant fraction of the total excess internal energy of the system. Despite the internal cooling associated with the dissociation reaction, a clearly observable temperature effect is noted in the MS/MS spectra (Fig. 3). Similar, or even larger bath gas temperature effects might be expected for higher mass ions wherein the fraction of total excess internal energy that is consumed by the dissociation reaction is expected to be inversely related to ion size. The most important factor in determining if a bath gas temperature effect is observed under typical ion trap MS/MS conditions is the relative dissociation rates of the parent ion and first-generation product ions.

4. Conclusions

Bath gas temperature can have a significant effect on the appearance of ion trap MS/MS spectra collected at the same parent ion internal energy. In the case of single frequency resonance excitation, whereby parent ion internal temperatures are elevated above the bath gas temperature, the rate at which first generation product ions are internally cooled is determined in part by the difference between the internal energy of the initially formed product ion and its internal energy when it comes into thermal equilibrium with the bath gas. In cases in which this collisional relaxation rate is comparable to or greater than the unimolecular dissociation rates of the first generation product ions, sequential fragmentation is actively inhibited. Under usual ion trap collisional activation conditions (e.g. parent ion dissociation rates of 1–250 s^{-1}, room temperature helium at 1 mTorr), further dissociation of first generation product ions with dissociation rates roughly equal to or less than that of the parent ion is almost completely inhibited. The inhibition of sequential fragmentation is reduced at elevated bath gas temperatures because of the role of ΔT on the collisional cooling rate. At relatively short ion activation times ($<$30 ms), the major effect of bath gas temperature at values less than ~500 K on MS/MS spectra stems from its role in removing excess internal energy from first generation fragments. At relatively long storage times ($>$100 ms), contributions arising from dissociation driven by the bath gas itself can become significant. Such a phenomenon is straightforward to identify from the time evolution of MS/MS spectra. In cases in which bath gas driven dissociation is inconsequential, product ion relative abundances are constant with time following the first few milliseconds of dissociation. In this instance, product ion spectra are largely determined by parent ion internal energy and product ion cooling rates. When dissociation is being driven by the bath gas, relative product ion abundances continue to evolve with time because of the continuous heating of the product ions by the bath gas.

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