Secondary Interactions Affecting the Dissociation Patterns of Arginine-Containing Peptide Ions

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Abstract: An explanation is proposed for the dominance of arginine in the dissociation patterns of peptides. Experiments measuring the kinetic energy lost by parent ions of a number of arginine-containing peptides in the formation of particular product ions provide a means of gauging the amount of energy required to observe the dissociation. It is proposed that the higher amounts of energy needed to observe dissociation adjacent to an arginine residue are due to secondary interactions between the arginine side chain and an adjacent amino acid. The appearance of the ($\mathbf{b}_{n-1}$ + OH) ion in the MS/MS spectra of many arginine-containing peptides and data acquired on a quadrupole ion trap help support these findings. We further suggest that the differences in the dissociation between peptides with arginine and those without may be due to the predominance of different reaction mechanisms, i.e. charge-remote versus charge-directed.

Introduction

Desorption techniques such as Fast-Atom Bombardment (FAB) and Matrix-Assisted Laser Desorption/Ionization (MALDI) are effective means for producing protonated peptide and protein ions $(M + H)^+$ in the gas phase.[1,2] When coupled with collision-induced dissociation (CID), these ionization methods have permitted the structural analysis of peptides and proteins by mass spectrometry.[3−6] CID involves activation of ions by collisions with neutral target gases, in which some of an ion's kinetic energy is converted into internal energy.[7] If a sufficient amount of internal energy has accumulated, ions begin to dissociate and the resulting product ions can be analyzed using tandem mass spectrometry (MS/MS). The structure of the parent ions can be postulated by examining the resulting MS/MS spectrum and rationalizing the specific structural features that lead to the observed dissociation patterns. Beam instruments involving both high-energy collisions (keV) and low-energy collisions (eV) have been used extensively to deduce the primary structure of peptides.[3−5] Much, though, is still not understood about the dissociation of peptide ions in the gas phase.

It is well-known that the presence of a strongly basic residue, such as arginine, significantly affects dissociation pathways of peptides and often limits the amount of structural information available.[8−10] It has been seen that the presence of arginine in either the N-terminal or C-terminal position results in spectra filled predominantly with N-terminal or C-terminal product ions.[8] Also the degree to which a charge is fixed on a peptide, as by a basic amino acid, controls the dissociation patterns for that peptide.[9] Experiments under low-energy-collision conditions show that peptides containing basic residues give little sequence information, presumably due to the localization of protons on highly basic sites rather than on the peptide backbone.[10]

The nature of sector instruments allows information about the energy content of ions to be obtained, and this ability has been especially useful in studying the thermochemical aspects of ion dissociation.[11] In particular, since the internal energy obtained during CID is converted from an ion's kinetic energy upon collision, the magnitude of the energy transfer can be gauged by measuring the kinetic energy lost by the incident ion.[12−14] According to Rice−Rampsberger−Kassel−Marcus (RRKM) theory, the rate of ion dissociation should be dependent on the internal energy of the parent ion, the critical energy of the particular dissociation, and the size of the ion. Since sector instruments provide a fixed time frame for dissociations to occur for a given ion, different dissociation pathways of different critical energies will result after distinct energy losses by the parent ion. In this paper we describe measuring energy losses of several arginine-containing peptides to better understand the dissociation processes of arginine-containing peptides.

Experimental Section

Sector instrument experiments were performed using a Finnigan MAT 900 double-focusing mass spectrometer which has the forward (EB) geometry. Ions were generated by FAB using an 8-keV argon beam. Glycerol was used as the liquid matrix with approximately 2 μL of a 1 mM peptide solution loaded on a probe tip.

Data were obtained by simultaneously scanning the magnetic sector (B) and the electric sector (E) with a constant ratio of B^2/E. This means of linked scanning provided the desired kinetic energy information of a parent ion when a particular product ion was selected to be transmitted to the detector. Helium (National Welders Supply, Co., Charlotte, NC)

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Table 1. Kinetic Energy Losses in the Formation of Various Product Ions of Leucine Enkephalin and Analogues

peptide	MW	energy loss (eV)		
		$\mathbf{a}_4$	$\mathbf{y}_4$	$\mathbf{y}_3$
leucine enkephalin	555	5.70	3.74	3.58
Y-G-L-F-L	611	5.73	3.66	3.32
Y-G-W-F-L	684	5.84	3.55	3.17
Y-G-R-F-L	654	13.01	8.82	4.89

was used as the collision gas, and the parent ion beam was attenuated 65 ± 5%. Determination of the energy losses by individual peptide ions using this type of scan has been described elsewhere.[15]

The peptides, substance P (RPKPQQFFGLM-NH_2), bradykinin (RPPGFSPFR), des-Arg[1]-bradykinin (PPGFSPFR), and leucine enkephalin (YGGFL), were purchased from Sigma Chemical Co. (St. Louis, MO). The leucine enkephalin analogues (YGRFL, YGLFL, YGWFL) were synthesized in the department.

Other MS/MS experiments were performed on a modified Finnigan quadrupole ion trap mass spectrometer of similar design as previously reported.[16] Ions were produced using a custom-built electrospray ionization source. The peptides were dissolved in a mixture of 20% water, 75% methanol, and 5% acetic acid at a concentration of 250 μM and infused into a capillary using a Harvard Apparatus syringe pump (South Natick, MA) at a flow rate of 0.5 μL/min. This capillary was connected to a needle that was held at about 4.5 kV, and the sample was sprayed into a differentially pumped region from which ion optics directed the ions into the mass spectrometer. MS/MS experiments were carried out when the singly-protonated peptide ions were resonantly excited by applying a variable supplementary low voltage (100's mV) AC signal to the endcaps which caused the ions to undergo energetic collisions with the bath gas, helium, which was held at a constant pressure of 8.6×10^{-4} Torr.

Molecular mechanics calculations were performed using the SYBYL 5.32 package from Tripos Associates, Inc. (St. Louis, MO). Energy minimizations were done interactively using the Tripos force field with conjugate gradient minimization and an energy gradient termination set at 0.005 kcal/mol. Charges applied were from the Kollman-all-atom[17] charge set and the dielectric constant was set at 1.0. The initial structures generated were in an all-trans conformation before energy minimization. The structures generated to examine hydrogen bonding interactions were produced by adding an unnatural bond between an atom on the adjacent residue in question and an arginine nitrogen hydrogen, then interactively minimized. This combination of minimization and unnatural bond placement served to pull the arginine side chain into a conformation which could possibly form a hydrogen bond. Once a possible conformation was achieved the unnatural bond was removed and energy minimization was performed on the altered ion.

Semiempirical molecular orbital calculations using the MOPAC package, version 6.0, were done in conjunction with the molecular modeling software program, PCMODEL (Serena Software). PCMODEL was first used to generate a structure based on molecular mechanics. This structure was then used as the starting structure for MOPAC, in which the AM1 (Austin Model 1) Hamiltonian was used.[18]

Results

Table 1 shows selected energy loss values for leucine enkephalin and analogues in which the third residue has been changed. It should be noted that energy loss values for three of the four peptides are very similar. However, the peptide YGRFL shows surprisingly high values for the $\mathbf{y}_4$ and $\mathbf{a}_4$ product ions. Other peptides such as bradykinin and des-Arg[1]-bradykinin also show anomalies in their energy loss values. We have shown elsewhere that the energy losses in the formation of **b**, **y**, and **a**-type product ions increase linearly with an increase in mass, with correlation coefficients of 0.990, 0.971, and 0.999, respectively.[15] For a given product ion, from the linear relationship between energy loss (ΔE) and mass, the deviation in energy loss ($\delta\Delta E$) from that expected can be determined. For example, ΔE for the $\mathbf{b}_6$ product ion of bradykinin is 12.77 eV, and the expected value for a **b**-type product ion from a peptide with the mass of bradykinin is 12.31 eV. Thus, $\delta\Delta E$ for the $\mathbf{b}_6$ product ion is 0.46 eV. Conversely, the $\mathbf{b}_8$ product ion from bradykinin has a ΔE of 17.85 eV, which gives a $\delta\Delta E$ of 5.54 eV. Likewise, the $\mathbf{a}_7$ product ion of des-Arg[1]-bradykinin has an energy loss that is over 5 eV higher than expected. In each of these cases an arginine is adjacent to the residue at which the dissociation occurs. Surprisingly, though, substance P, which has an arginine in the first position, shows the expected energy loss (i.e. $\delta\Delta E = -0.83$ eV) for the formation of the $\mathbf{y}_{10}$ product ion. These values are summarized in Table 2.

Table 2. Deviations from Expected Energy Losses for Various Product Ions of Different Peptides

peptide	MW	product ion	$\delta\Delta E$
des-Arg[1]-bradykinin	904	$\mathbf{a}_7$	5.87
		$\mathbf{y}_7$	−0.58
bradykinin	1060	$\mathbf{b}_8$	5.54
		$\mathbf{b}_6$	0.46
substance P	1347	$\mathbf{a}_7$	−0.39
		$\mathbf{y}_{10}$	−0.83

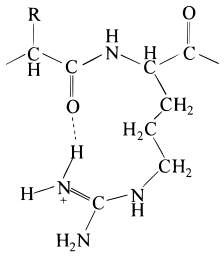

Figure 1. Proposed proton sharing between arginine side chain and adjacent carbonyl oxygen.

Three of the four arginine-containing peptides show anomalously high energy losses in the formation of product ions involving a dissociation adjacent to an arginine residue. The resulting high energy losses suggests that these product ions require additional internal activation to overcome some energy barrier. We propose that YGRFL, bradykinin, and des-Arg[1]-bradykinin can adopt a conformation where the side chain of the arginine is close to a carbonyl oxygen on the adjacent amino acid. If the proton is assumed to be localized on the side chain of arginine, it can then be shared between the carbonyl oxygen and a nitrogen on the arginine side chain (Figure 1). More energy then is needed to disrupt this interaction and produce the resulting product ion. In the case of substance P this intramolecular interaction is not possible due to the cyclic nature of proline which is the adjacent residue. Since the proton is not shared between the arginine nitrogen and the carbonyl oxygen in substance P, extra energy is not needed to generate the product ion, and the energy loss is what is expected on the basis of data for peptides which do not contain arginine residues.

Support for the possibility of this interaction can be found in computer-modeling studies. Molecular mechanics show that the protonated peptide $(M + H)^+$ can adopt a conformation where the proton is shared between the side chain of arginine and the carbonyl oxygen on the adjacent amino acid. For bradykinin, des-Arg[1]-bradykinin, and both cases of YGRFL this interaction is energetically possible. Molecular mechanics studies of substance P, though, show that it is unable to form

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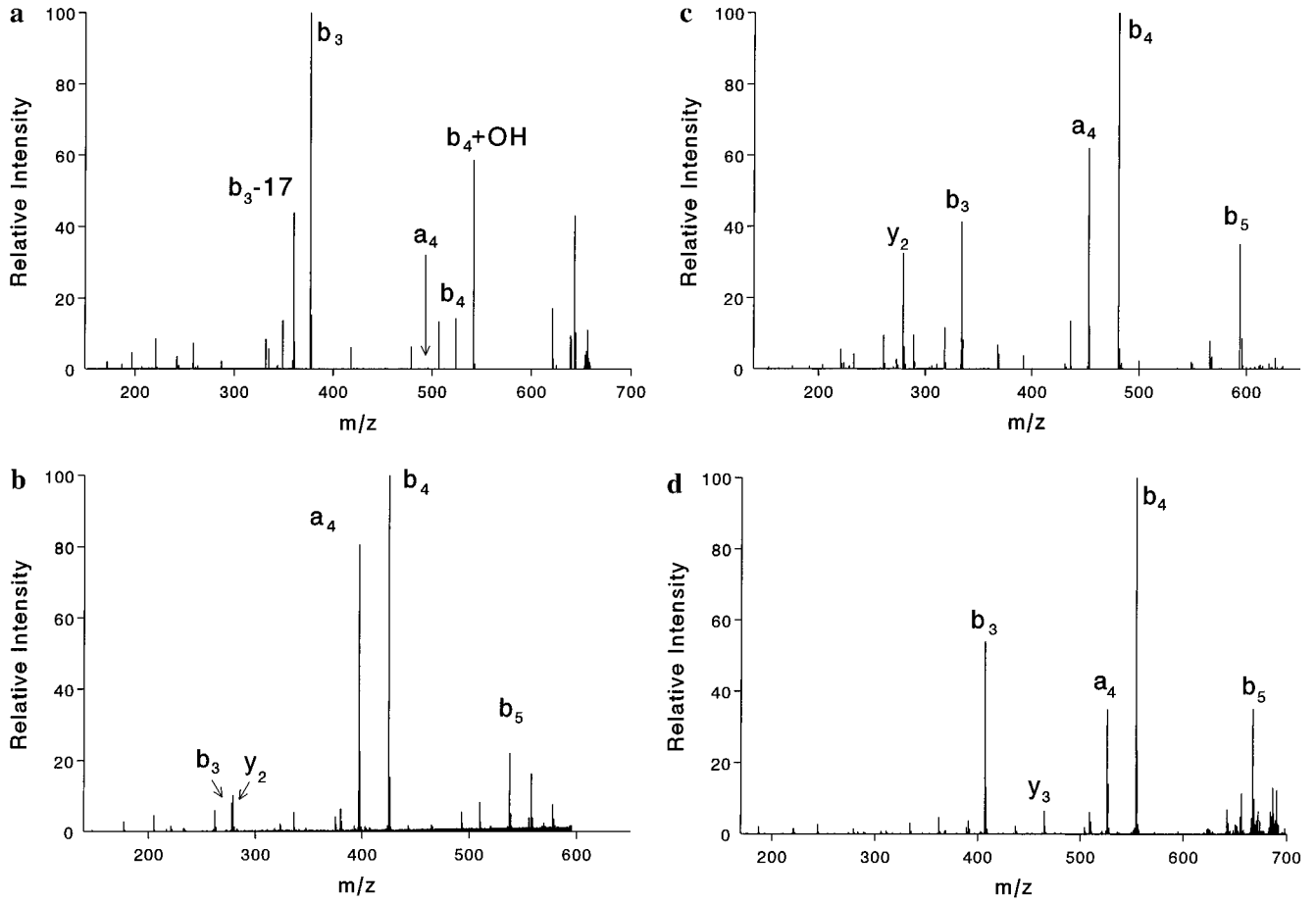

Figure 2. (a) Quadrupole ion trap MS/MS spectrum of YGRFL. (b) Quadrupole ion trap MS/MS spectrum of leucine enkephalin (YGGFL). (c) Quadrupole ion trap MS/MS spectrum of YGWFL. (d) Quadrupole ion trap MS/MS spectrum of YGLFL.

this interaction due to the twisting of the backbone caused by the proline residue. Another possibility is that the proton on the side chain of arginine could be shared with a backbone nitrogen on an adjacent residue, however; computer modeling shows that this interaction is not favorable for any of the above-mentioned peptides. Semiempirical molecular orbital calculations verified these results.

Previous experimental results suggest that 24−30 kcal/mol of stabilization should be obtained for the proposed proton bridge. It has been estimated from experiments with α,ω diamines that, without steric or strain factors, a stabilization of 24 kcal/mol should be obtained.[19] For proton-bound dimers similar values have been obtained. For example, the symmetrical pyridine[20] and symmetrical acetone[21] dimers have been measured to be stabilized by 24.6 and 32.1 kcal/mol, respectively. The strain energy expected from the structure in Figure 1 is 1−2 kcal/mol.[19] This leads to the estimate of a stabilization of 24−30 kcal/mol for the intramolecular bridge. Therefore, this interaction would in fact provide further stabilization of the ionizing proton.

Experiments conducted using a quadrupole ion trap also reveal information about the energy needed for these dissociations. The MS/MS spectrum of YGRFL under moderate excitation conditions shows that the $\mathbf{a_4}$ product ion is missing (Figure 2a), but the MS/MS spectra of leucine enkephalin, YGWFL, and YGLFL clearly show that the $\mathbf{a_4}$ product ion is one of the dominant peaks (Figures 2b, 2c, and 2d). As performed in the quadrupole ion trap, CID is generally considered to be a slow activation process where lower energy dissociation pathways are favored.[22−24] The absence of the $\mathbf{a_4}$ product ion in the YGRFL spectrum suggests that it is a higher energy process relative to the other dissociation pathways, and extra energy is needed to perhaps overcome secondary interactions. Also, observing the MS/MS spectra of leucine enkephalin and its analogues at the threshold of dissociation (data not shown) provides some information on which pathways are the lowest energy. It has been shown that the threshold resonance excitation amplitude can be related to the critical energy of a dissociation.[25] Thus, the product ions observed at the threshold are those that have the lowest critical energies for their formation. For leucine enkephalin (YGGFL), YGWFL, and YGLFL the resonance excitation voltages required to bring about the onset of dissociation are some 10−30 mV lower than those for the onset of dissociation of YGRFL. No attempt, however, was made to measure more accurately the voltages required for the onset of dissociation for any of the peptides.

To further determine that the $\mathbf{a_4}$ product ion of YGRFL is a higher energy dissociation than the $\mathbf{a_4}$ product ions of the other pentapeptides, the energy involved in the ion trap CID process was increased. This was accomplished by the addition of xenon to the buffer gas at a level of about 2%. It has been noted that the addition of a small percentage of heavy target gases, such

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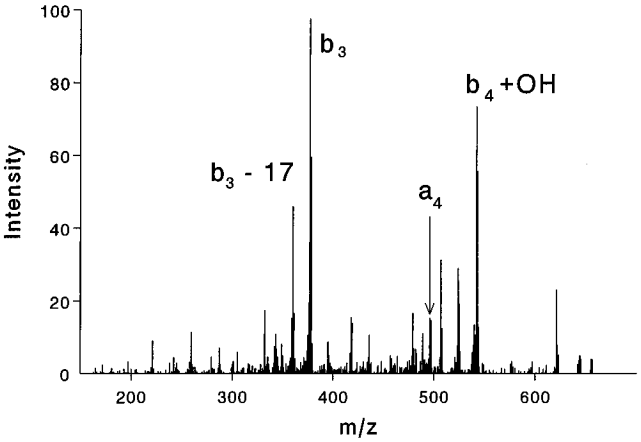

Figure 3. Quadrupole ion trap MS/MS spectrum of YGRFL with 2% xenon.

as xenon, increases the internal energy deposition in CID experiments performed on the ion trap.[26−28] Figure 3 shows the MS/MS spectrum of YGRFL after the addition of 2% xenon. The $\mathbf{a_4}$ product ion is now present in the spectrum, presumably due to the higher energy now involved in the CID experiment.

Another compelling piece of evidence for the proposed interaction is found in observing the presence of certain product ions in the MS/MS spectra of YGRFL, bradykinin, and des-Arg[1]-bradykinin. In each case an ion that corresponds to ($\mathbf{b_{n-1}}$ + OH), where *n* is the number of residues in the peptide, is present in the MS/MS spectrum. Previous studies using isotopic labeling have shown that, in the case of bradykinin, the product ion at *m/z* 904 results from the loss of the C-terminal arginine and the retention of one of the carboxyl oxygens, and not by the loss of the N-terminal arginine that would give the isomeric $\mathbf{y_8}$ product ion (Figure 4).[29] This rearrangement was seen to occur only appreciably in peptides containing arginine. Of the peptides studied here, only the MS/MS spectra of the three noted above (bradykinin, des-Arg[1]-bradykinin, and YGRFL) show the formation of this ion. As Figure 4 depicts, the formation of this ion was proposed to involve the transfer of a hydrogen to the carbonyl oxygen on the amino acid adjacent to the arginine and subsequent loss of CO and NH═CH-Arg. It is possible that this rearrangement occurs because the carbonyl carbon on the amino acid adjacent to arginine has a higher than normal partial positive charge and is therefore more susceptible to nucleophilic attack by the C-terminal hydroxyl group (Figure 5). The carbonyl carbon would have a higher than normal partial positive charge if the proton from the arginine side chain is being shared with the carbonyl oxygen. Electron density would be pulled from the carbon in the formation of the hydrogen bond between the carbonyl oxygen and the proton. Thus the same interaction that raises the energy of dissociations adjacent to arginine facilitates the formation of the ($\mathbf{b_{n-1}}$ + OH) product ions. This is illustrated experimentally in the quadrupole ion trap MS/MS spectrum of YGRFL (Figure 2a). The $\mathbf{a_4}$ product ion is absent from the spectrum, but the ($\mathbf{b_4}$ + OH) product ion is noticeably present.

Discussion

It has been suggested that the reason for the differences in dissociation behavior between peptides that contain arginine and those that do not is the inability of the proton to migrate along the backbone and charge-direct dissociations.[10] The results presented here indicate that while there is even greater stabilization of the proton than due to the proton affinity of arginine alone, this has little effect on the critical energy requirements for dissociations other than those adjacent to arginine, as indicated by energy loss measurements. The results in this and other studies[9,30,31] suggest that both charge-direct and charge-remote mechanisms are operative in peptide dissociation. It seems that in peptides that do not contain arginine, charge-directed dissociations are favored, and in peptides containing arginine, charge-remote dissociations predominate. There are sufficient data showing that the location of an arginine on the N- or C-terminus results in N- or C-terminal product ions.[8] These dissociations presumably occur by charge-remote mechanisms. If the proton is mobilized and directing dissociation, one would not expect to see such ordered series of product ions favoring the charge on the N- or C-terminus. The threshold measurements performed on the ion trap show that the lowest energy dissociation pathways in YGRFL require more energy than the lowest energy pathways in the other leucine enkephalin analogues. Along with the fact that the proton in YGRFL is strongly localized, these data suggest that the dissociations in YGRFL have different mechanisms than the dissociations in the other leucine enkephalin analogues. In YGRFL the

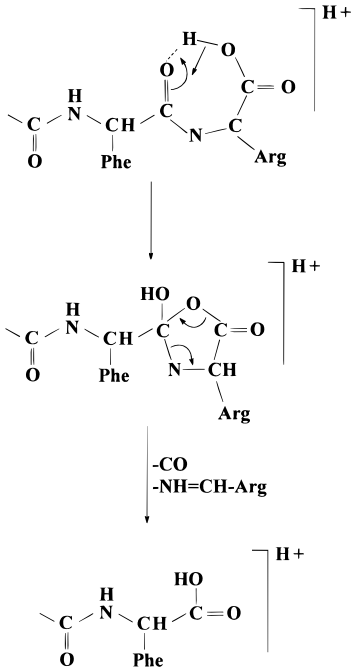

Figure 4. Previously proposed mechanism for the formation of the ($\mathbf{b_{n-1}}$ + OH) product ions.

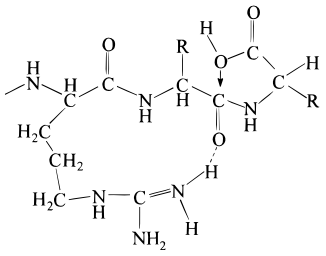

Figure 5. Currently proposed mechanism for the formation of the ($\mathbf{b_{n-1}}$ + OH) product ions.

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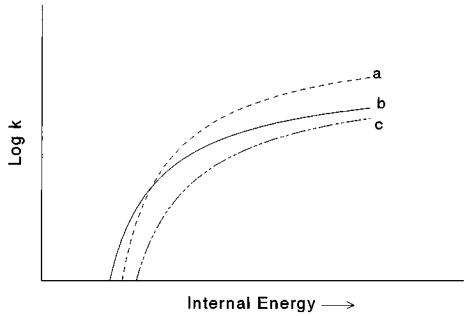

Figure 6. Theoretical plot of the log of the rate of dissociation versus internal energy for charge-remote and charge-directed dissociations: (a) charge-remote dissociations; (b) charge-directed dissociations for peptides without arginine; and (c) charge-directed dissociations for peptides with arginine.

predominant mechanisms are perhaps charge-remote ones, involving homolytic or heterolytic bond cleavages, which have higher critical energies than charge-directed dissociations. Recently, charge-remote dissociations in a quadrupole ion trap have been observed to be the dominant dissociation pathway for peptides containing both arginine and acidic residues.[32]

The anomalous trend in the energy losses for dissociations of bonds near arginine can be explained by the proton sharing, while the energy loss values for dissociations distant from arginine can be explained by considering the high-energy-collision conditions. Due to the time frame of the experiment, all dissociations in a sector-type instrument result with significant kinetic shifts. It is possible that dissociations distant from arginine, while still charge-remote, show similar energy losses to the charge-directed dissociations of other peptides because during the time frame and at the energies involved for dissociation, the rates of these two reactions are very similar. This agrees with a large amount of data that show charge-remote fragmentations compete more favorably at high collision energies.[33] This situation can be better understood by looking at Figure 6. At internal energies nearer the threshold (i.e. in the ion trap experiments) the charge-directed dissociations are the only ones seen for peptides without arginine. Since the proton is bound so tightly in arginine-containing peptides, charge-directed dissociations are not favored in these peptides. Therefore the arginine-containing peptides require more internal energy to observe their charge-remote dissociations. In the time frame for the sector experiments, the charge-remote fragmentations can now compete favorably with charge-directed dissociations at similar activation energies, thus explaining the similar energy loss values.

Data that show less sequence information is obtained from peptides that contain arginine[10] can be explained by recognizing the conditions of those experiments. The results in that paper were obtained using a triple quadrupole instrument where the time frame for the experiment is orders of magnitude shorter than the time frame for experiments on a quadrupole ion trap. It is possible that the energies involved in those experiments are not sufficient to observe extensive charge-remote dissociations in the time frame involved. However, the dissociations are observed in the quadrupole ion trap due to the significantly longer reaction times involved.

Conclusions

An attempt to give an explanation for the well-noted difficulty in obtaining structural information from peptides containing arginine has been presented here. It seems very likely that the greater amount of internal energy needed to observe fragmentations along the backbone of arginine-containing peptides may be due in some part to secondary interactions. The greater stabilization of the proton in peptides with arginine seems to precipitate the formation of product ions by charge-remote processes. Also, the secondary interaction facilitates the formation of certain rearrangement ions that are often observed in spectra of arginine containing peptides. These experiments provide a means of better understanding spectra from peptides containing basic residues. It may also have implications on the ability of peptides or proteins to retain some higher order structure in the gas phase.

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