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Electron Transfer Ion/Ion Reactions in a Three-Dimensional Quadrupole Ion Trap: Reactions of Doubly and Triply Protonated Peptides with $SO_2^{\cdot-}$

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Abstract

Ion–ion reactions between a variety of peptide cations (doubly and triply charged) and SO_2 anions have been studied in a 3-D quadrupole ion trap, resulting in proton and electron transfer. Electron transfer dissociation (ETD) gives many c- and z-type fragments, resulting in extensive sequence coverage in the case of triply protonated peptides with $SO_2^{\cdot-}$. For triply charged neurotensin, in which a direct comparison can be made between 3-D and linear ion trap results, abundances of ETD fragments relative to one another appear to be similar. Reactions of doubly protonated peptides with $SO_2^{\cdot-}$ give much less structural information from ETD than triply protonated peptides. Collision-induced dissociation (CID) of singly charged ions formed in reactions with $SO_2^{\cdot-}$ shows a combination of proton and electron transfer products. CID of the singly charged species gives more structural information than ETD of the doubly protonated peptide, but not as much information as ETD of the triply protonated peptide.

Most protein identification work today relies on information generated from peptides by mass spectrometry experiments, whether proteins are separated prior to enzymatic digestion (as with 2-D gels)[1–4] or peptides are separated after digestion (as with shotgun proteomics).[5–7] Particularly in the case of peptides formed from protein mixtures, sequence information derived from individual peptides via tandem mass spectrometry (or MS/MS) facilitates the identification of the proteins from which they were derived. Therefore, maximizing peptide sequence information via a tandem mass spectrometry experiment is of considerable interest. Collision-induced dissociation (CID) is the most common means for deriving peptide sequence information in MS/MS, and programs have been developed that automate analysis of the uninterpreted data.[8,9] CID of singly and multiply protonated peptides results primarily in the cleavage of the amide bonds of the peptide backbone, generating b- and y-type sequence ions, which are indicated in Scheme 1.[2] In favorable cases, all of the amide bonds in a polypeptide ion cleave to yield measurable fragments, yielding the full sequence of the peptide. But there are also a substantial number of cases in which the CID spectra of peptides are dominated by uninformative neutral losses or by cleavage of a limited set of specific peptide bonds. Furthermore, bonds associated with some of the common posttranslational modifications, such as glycosylation or phosphorylation, are relatively labile and are often the first bonds broken in CID.[10,11] Such fragmentation behavior can complicate derivation of peptide sequence and the identification of the site of modification. It has been noted that as many as one-quarter of the peptide ions subjected to CID can result in spectra which are insufficient to identify the peptide.[12]

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SUPPORTING INFORMATION AVAILABLE A product spectrum for the reaction of neurotensin $[M + 3H]^{3+}$ ions with $SO_2^{\cdot-}$ is provided for comparison with results for the same reaction in a linear ion trap.[25]

While CID has been highly useful in deriving structural information from polypeptide ions, means for deriving structural information that complements that derived from CID are of interest. In this context, McLafferty and co-workers introduced a new technique, called electron capture dissociation (ECD)[15] which has been recently reviewed by Zubarev.[16] In ECD, slow electrons are introduced along magnetic field lines of a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer and captured by trapped multiply charged peptide/protein cations. This induces cleavage of the peptide backbone at N–C_α bonds to yield complementary c and $z^{\cdot}$ sequence ions, as indicated in Scheme

1. The ECD process is clearly distinct from CID, and it generally results in almost complete sequence coverage for small peptide ions, with the exception of dissociation N-terminal of proline residues, which, unlike the case for all other amino acids, requires dissociation of two bonds. As the size of the peptide/protein increases, the sequence coverage tends to decrease, but even in multiply charged ions as large as ubiquitin (average mass = 8565 Da), 67 out of 75 peptide bonds are cleaved.[17] Another advantage of this method is that labile posttranslational modifications are generally preserved.[16] ECD, however, is generally limited to FT-ICR instruments. Other forms of tandem mass spectrometry, including those most commonly used for peptide ion tandem mass spectrometry, are not well-suited to ECD. To date, no examples of ECD effected in common beam-type MS/MS instruments have been reported. Electrodynamic ion traps, either 3-D or linear, are not well suited to ECD, because it is very difficult to trap both electrons and cations simultaneously in a RF field. Efforts are being made to overcome this limitation, however, by modifying ion traps to introduce a magnetic field to assist in trapping the electrons.[18,19]

Although 3-D and linear quadrupole ion traps are not suitable for trapping electrons and peptide/protein cations simultaneously, they are well-suited to the simultaneous storage of cations and anions in overlapping regions of space.[20–23] Under these conditions, reactions between the oppositely charged ions can take place. Ion/ion reactions have been observed to proceed via a variety of mechanisms, including inter alia proton transfer, electron transfer, and ion attachment. Syka and co-workers recently reported on anionic reagents that transfer electrons to polypeptide cations, giving rise to fragmentation that resembles that resulting from electron capture. The overall process has been referred to as electron-transfer dissociation (ETD).[22–26] In their experiments, +3 and +4 peptide ions are generated by electrospray ionization (ESI) and enter into one end of a linear ion trap. The anionic reagents are generated in a chemical ionization source (with a methane buffer gas) and injected into the other end of the linear ion trap, where they are allowed to react with the cations. Several anionic reagents, including negative ions derived from anthracene, SO_2, and fluoranthene, have been found to give rise to varying degrees of ETD. In addition to the production of the useful ETD c- and z-type sequence ions, there can also be electron transfer with no dissociation and proton transfer. So far, the highest reported efficiencies have been around 30–35% (precursor-to-ETD-product) with anthracene[25] and 40% with fluoranthene.[24] It has been demonstrated that this process can easily be automated and employed on a chromatographic time scale.[26]

Ion/ion reactions with $SO_2^{\cdot-}$ ions and several multiply charged cations had been studied previously with a 3-D quadrupole ion trap.[27] Electron transfer was noted as a major process with porphyrin dications, but ETD was not noted for polypeptide cations. Proton transfer was reported to be the dominant, if not exclusive, process. Coon et al. reported observing ETD with $SO_2^{\cdot-}$, although at relatively low efficiency, as compared with proton transfer. The ETD results identified using a linear ion trap but not a 3-D ion trap give rise to the possibility that the reaction dynamics may differ in some way in the two ion storage devices. On the other hand, considering that relatively low levels of ETD were observed in the LIT using $SO_2^{\cdot-}$, it is possible that low levels of ETD in the 3-D ion trap were overlooked.[25] For this reason, we have reexamined the reaction of SO_2 anions with peptide cations in a 3-D quadrupole ion trap.

We have also chosen a set of peptides to examine that allow us to compare results for the +3 and +2 charge states of the peptides. All the ETD results reported thus far have been for the +3 charge state or higher. Electron transfer to doubly protonated peptides is obviously of interest in that most tryptic peptides are observed as doubly charged ions when subjected to electrospray.

EXPERIMENTAL SECTION

Pyridine, methanol, and glacial acetic acid were purchased from Mallinckrodt (Phillipsburg, NJ). Angiotensin I, RKRARKE, bradykinin, des-Arg_1-bradykinin, and des-Arg_9-bradykinin were obtained from Sigma (St. Louis, MO); neurotensin and reduced somatostatin were obtained from Bachem (King of Prussia, PA); KGAILxGAILR (x = A, P, D, K) and GAILyGAILR (y = A, P, K) were synthesized by SynPep (Dublin, CA); and Lys_0–bradykinin was obtained from Anaspec (San Jose, CA). Where indicated, GAILKGAILR and KGAILxGAILR were guanidinated (lysine residues converted to homoarginine residues) using a method described previously.[28] All peptides other than the guanidinated peptides were used as obtained without further purification. The guanidinated peptides were purified on a Hewlett-Packard (Palo Alto, CA) 1090 liquid chromatograph as described previously.[28] For electrospray, 0.1 mg/mL solutions in 49.5/49.5/1 methanol/ water/acetic acid were prepared for each peptide.

Experiments were carried out on a modified Hitachi (San Jose, CA) M-8000 3-DQ ion trap that has been described previously,[29] which is capable of injecting ions generated in an atmospheric sampling glow discharge ionization (ASGDI) source into the trap through a hole in the ring electrode. Cations were generated using nanoelectrospray. Nanoelectrospray emitters were pulled from borosilicate glass capillaries with a 1.5-mm o.d. and a 0.86-mm i.d. using a P-87 Flaming/Brown micropipet puller (Sutter Instruments, Novato CA). The nanoelectrospray assembly consists of an electrode holder (Warner Instruments, P/N ESW-MISP, Hamden, CT) with a stainless steel wire inserted into the capillary.[30,31] Nanoelectrospray was accomplished by applying 1.2–2 kV to the wire. To form the +2 charge state of RKRARKE, a small dish of pyridine was placed under the nanospray tip to allow for ion/molecule proton-transfer reactions to occur in the ion sampling region. Anions were generated using the ASGDI source on the instrument. SO_2 gas (Scott Specialty Gases, Troy, MI) was leaked into the source to a pressure of ~530 mTorr. The discharge was pulsed using a software TTL trigger connected to a fast high-voltage pulser (GRX-1.5K-E, Directed Energy Inc., Fort Collins, CO).

In a typical experiment, cations are injected for ~1 s, and the desired charge state is then isolated using programmable filtered noise field (FNF)[32,33] waveforms (50 ms). Anions are then injected for ~200 to 300 ms to effect the reaction. During this time, an AC signal is applied to the endcaps of the trap resonant with *m*/*z* 80 to eject any SO_3^- ions formed in the discharge. We have observed that these ions are present in the initial discharge and that they are formed from ion/molecule reactions as SO_2^- ions are stored in the trap. This is one of the reasons that anions are continually injected throughout the reaction time. The other reason is that they help to trap the higher *m*/*z* positive ions formed in the reaction, a process termed “trapping by proxy”.[34] The low trap RF levels required to trap the low-mass (*m*/*z* 64) SO_2^- ions make this necessary for efficient trapping of the high-mass ions. After the injection/reaction time, the RF level of the trap is raised to eject the remaining anions, and the cations are mass-analyzed via resonance ejection. In some experiments, the +1 peptide ions generated by the reaction are then isolated and subjected to CID, using an auxiliary Agilent (Palo Alto, CA) 33120A arbitrary waveform generator controlled by a software TTL trigger to resonantly excite the ions of interest (~300 ms), before mass analysis is performed. Spectra reported herein are typically the result of 5 min of averaging (~250 scans). The number of scans required here is a result of the low ion

transmission efficiency for this particular instrument and the overall lower injection efficiency of 3D ion traps as compared to linear ion traps. These factors are not expected to affect the ion/ion reaction chemistry observed.

RESULTS AND DISCUSSION

Reactions of Triply vs Doubly Protonated Peptides with $SO_2^{\cdot-}$

Results from the reaction of triply protonated RKRARKE with $SO_2^{\cdot-}$ are shown in Figure 1a. It is expected that this reaction can occur through two major pathways, electron transfer from the anion to the cation or proton transfer from the cation to the anion. (The formation of a complex consisting of the two reactants can also occur. However, such a complex can be considered to be a stable intermediate associated with electron transfer or proton transfer.) On the basis of extensive experience with ion/ion proton-transfer reactions, fragmentation of the polypeptide ions is not expected to arise from proton transfer to the anionic reagent.[27] Electron transfer, on the other hand, is more likely to lead to fragmentation of the peptide, on the basis of the work reported by Coon et al., which, in turn, was anticipated on the basis of the work done with ECD.[25] The major products of the reaction are +2 and +1 peptide species, which can be composed of both proton transfer and electron transfer products. Previous studies with the 3-D ion trap and the more recent work with a linear ion trap showed that proton transfer products make up most of the +2 and +1 product ions. In addition to these ions, a variety of fragment ions are observed at relatively low levels. No such products are noted for ion/ion reactions with reagent anions known to react exclusively via proton transfer (data not shown). On the basis of the fact that the masses of the product ions correspond to expected c- and z-type ions and the fact that the same types of ions were noted in the linear ion trap study with $SO_2^{\cdot-}$ anions, it is strongly suggestive that electron-transfer dissociation gives rise to the fragments observed in Figure 1a. These c- and z-type fragments are analogous to those observed with ECD.[16] The dissociation shown here demonstrates cleavage at every N–C_α bond in the peptide, resulting in full sequence coverage. The dominant backbone cleavage gives rise to the complementary z_3^+/c_4^+ pair, which corresponds to cleavage between the Ala-4 and Arg-5 residues. The ion/ion reaction also leads to neutral losses from the peptide, corresponding to ammonia and fragmentation of the arginine side chain. Such fragmentation of arginine residues has also been reported in ECD studies and has implications for determining information about peptide amino acid composition.[35,36] A small amount of $SO_2^{\cdot-}$attachment is also observed, as evidenced by the $[\text{peptide} + SO_2]^+$ ion. No $[\text{peptide} + SO_2]^{2+}$ ion is observed, however, indicating that the attachment arises entirely from the reaction of the +2 peptide ion with $SO_2^{\cdot-}$ and not from the reaction of the +3 peptide ion. Figure 1b shows results from the reaction between doubly protonated RKRARKE and $SO_2^{\cdot-}$. This reaction does not lead to a significant extent of cleavage of any N–C_α bonds. The comparison of parts a and b of Figure 1 clearly suggests that most of the c- and z-type product ion signal in Figure 1a arises from the reaction of the triply charged ion with $SO_2^{\cdot-}$. Reaction of doubly protonated RKRARKE with $SO_2^{\cdot-}$ also results in neutral losses and $SO_2^{\cdot-}$ attachment similar to the reaction of triply protonated RKRARKE with $SO_2^{\cdot-}$, as shown in Figure 1a.

Another example of the different relative extents of sequence information obtained from reacting triply vs doubly charged species with $SO_2^{\cdot-}$ is provided by guanidinated KGAILKGAILR. The reaction between triply protonated guanidinated KGAILK-GAILR and $SO_2^{\cdot-}$ (Figure 2a) shows a variety of c- and z-type ions, in analogy with the case of triply protonanted RKRARKE in reaction with $SO_2^{\cdot-}$. The product ions once again show the dissociation of every N–C_α bond in the peptide, thereby yielding complete sequence coverage. Neutral losses of ammonia and arginine side-chain fragments are also seen as a result of this reaction. It is also interesting to note that a relatively large amount of $SO_2^{\cdot-}$ attachment is observed, as demonstrated by the $[\text{peptide} + SO_2]^+$ ion. As in the case of RKRARKE, no clear

evidence for attachment to the triply charged ion is observed. Apparently, attachment takes place with the doubly charged species. This particular peptide ion shows the largest relative $SO_2^{\bullet -}$ attachment signal of any peptide studied to date, although $SO_2^{\bullet -}$ attachment to doubly charged peptide cations is commonly observed, but only as a minor product. The adduct ion is of interest from a mechanistic point of view and will be discussed at greater length elsewhere.

As expected from the data for the +3 ion, the reaction of +2 guanidinated KGAILKGAILR with $SO_2^{\bullet -}$ shows $SO_2^{\bullet -}$ attachment as a major reaction channel (Figure 2b). The amount of $SO_2^{\bullet -}$attachment relative to the +1 peptide species is the same as in the case of +3 peptide reacted with $SO_2^{\bullet -}$, suggesting that most of the attachment is a result of the +2 to +1 step. In this experiment, the residual intact +2 peptide ions have been ejected from the trap prior to mass analysis. It was noted during the course of these studies that some doubly charged peptides gave rise to b- and y-type ions during resonance ejection that often were comparable in abundance to the c- and z-type product ions. Ejection of the residual +2 peptide ions prior to mass analysis avoided the formation of the b- and y-type products, thereby simplifying identification of product ions formed from electron transfer. The reaction of the +2 species with $SO_2^{\bullet -}$ shows only two fragment peaks that can be attributed to ETD along the peptide backbone (z_9^+ and z_{10}^+), resulting in only 20% sequence coverage, as opposed to 100% sequence coverage from the +3 reaction. The relative z_9^+ and z_{10}^+ abundances appear to be different from those derived from the reaction of the +3 peptide with $SO_2^{\bullet -}$, although it is not clear that enough ions were sampled to conclude that they are significantly different. Nevertheless, both the example of RKRARKE and guanidinated KGAILKGAILR suggest that the sequence coverage produced by ETD is much higher when $SO_2^{\bullet -}$ is reacted with a triply charged ion than when it is reacted with a doubly charged ion of the same peptide. In total, the ETD behavior of doubly vs triply charged peptides reacted with $SO_2^{\bullet -}$ was studied for seven different peptides. A summary of the fragmentation behavior is given in the first two columns of Table 1. In all cases, the triply charged peptide reaction yields more sequence coverage than the reaction with the doubly charged peptide. For six of the seven peptides shown, reaction of +3 peptides with $SO_2^{\bullet -}$ yields cleavage at all of the possible nonproline N–C_α bonds. As with ECD, N-terminal proline cleavage appears to be inhibited with ETD due to the cyclic nature of proline residues.[16] For reduced somatostatin, 11 of 14 N–C_α bonds are cleaved. For the reactions of +2 peptides with $SO_2^{\bullet -}$, fragmentation tends to be limited to primarily one or both ends of the peptide, thus limiting the sequence information that could be obtained. It is also worth noting that Coon and co-workers have studied the reaction between triply charged neurotensin and $SO_2^{\bullet -}$in a linear ion trap, which resulted in cleavage of every amide bond.[25] The reaction of $SO_2^{\bullet -}$ with neurotensin in a 3-D trap, as summarized in Table 1, shows results that are very similar to those reported for the linear ion trap. The similarity of the relative abundances of ETD products in a 3-D vs linear ion trap suggests that there is little difference in the reaction dynamics between the two instruments. (Figure S-1 in the Supporting Information shows results obtained here for +3 neurotensin with $SO_2^{\bullet -}$, which can be compared with the results reported by Coon et al.[25])

Several factors may play a role in the lower degree of fragmentation arising from electron transfer to form doubly versus singly charged ions of the same peptide. First, the overall reaction exothermicity is expected to increase with cation charge due to increasing recombination energy of the cation. Reaction exothermicity, ΔH_{rxn}, for the generic reaction,

$$MH_n^{n+} + N^{-\bullet} \rightarrow MH_n^{(n-1)\bullet +} + N \quad (1) \tag{1}$$

is given by

$$\Delta H_{rxn} = EA(N) - RE(MH_n^{n+}) \quad (2) \tag{2}$$

where EA(N) is the electron affinity of N and RE(MH_n^{n+}) is the recombination energy of the cation. Although the recombination energies of the peptide ions relevant to this study have not been measured, recent measurements of the ionization energies of protonated peptides relate to this point. Electron removal (ionization) is the inverse of recombination (electron capture). Therefore, trends in ionization energies are expected to be mirrored by the recombination energies. Budnik et al. recently reported that the ionization energies of polypeptide ions show an average increase in ionization energy of 1.1 eV/charge.[37] A similar difference in the recombination energies of the triply and doubly charged peptide ions discussed here can be expected, thereby making the electron transfer reaction significantly more exothermic for the triply charged ions. A second major factor follows from the fact that the doubly charged cation product, formed from the triply charged ion via electron transfer, can be expected to be less stable than the singly charged cation, formed from the doubly charged ion via electron transfer. This follows from the Coulomb repulsion that exists in the doubly charged product but is absent in the singly charged product. In general, multiply protonated species tend to be less kinetically stable than singly charged species. The electrostatic repulsion in the doubly charged product might be expected both to minimize intramolecular interactions that might otherwise form to solvate a single charge and to weaken existing covalent bonds situated between the charges. Some ECD studies have suggested that ion structure, and in particular the sites of charge solvation, may play a role in the fragmentation observed upon electron capture. Because doubly and triply charged ions are expected to have different structures and, thus, different charge solvation environments, this may also help explain the differences observed here.[36,38–40] Hence, the single electron transfer product ions formed from triply charged parent ions can be expected to be formed with greater internal energies than those formed from single electron transfer to doubly charged ions, and furthermore, the stabilities and three-dimensional structures of the products are also expected to differ.

Another factor that may play a role in the comparison of the products arising from the reaction of triply vs doubly protonated peptides with $SO_2^{\cdot-}$ is the amino acid composition of the peptides. To form triply charged ions, peptides must be relatively basic. All the peptides in Table 1 contain two or more basic amino acids distributed across the peptide, in addition to the N-terminus. These peptides readily form triply charged ions with the instrumentation used in this study. Table 2 shows the results for a variety of other peptides that did not readily form triply charged ions. Although some of these peptides contain two basic amino acids, in most cases, one is the N-terminal residue. These peptides show results that are similar to those of the doubly charged ions shown in Table 1. Fragmentation is limited in most cases to one or both ends of the peptide. This suggests that, at least for the peptides studied, amino acid composition is not responsible for the differences in fragmentation of the doubly and triply charged ions during reactions with $SO_2^{\cdot-}$.

It is worth noting that in ECD, peptide fragmentation does not show as distinct a charge state dependence as shown here with ETD. While the relative abundances of different c and z ions and overall fragmentation efficiency vary with precursor ion charge state in ECD, peptide sequence coverage does not seem to show as strong a dependence on precursor ion charge state.[41,42] The apparent difference in the influence of precursor charge state on sequence coverage between ECD and ETD may simply arise from energetic and kinetic considerations. The exothermicity associated with electron capture is simply the negative of RE(MH_n^{n+}). The electron capture process is, therefore, more exothermic than the electron transfer process by a value equal to EA(N). Furthermore, it remains unclear how the exothermicity of the electron transfer process is partitioned among the internal and translational energies of the products. In the case of electron capture, all of the reaction exothermicity must be partitioned into the internal energy of the electron capture product ion. The net effect is that electron capture is expected to be a significantly more energetic process than electron transfer. A second factor that might be expected to play a role in the extent to which dissociation follows electron transfer

or electron capture is product ion lifetime relative to the rates of removal of excess internal energy. Most electrodynamic ion traps are operated with helium as a bath gas at roughly 1 mTorr. This gives rise to a significantly greater cooling rate for internally excited peptide ions than prevails in the more highly rarefied environment of ion cyclotron resonance instruments ($\leq 10^{-7}$ Torr), where ECD is generally performed. The lower reaction exothermicities, the possibly lower energy partitioning into product ions, and higher product ion cooling rates associated with the electron-transfer experiment tend to disfavor the observation of fragmentation relative to the electron capture experiment at low pressures. For these reasons, it is not surprising that ETD might show more of a parent ion charge state dependence than ECD. However, it remains to be seen how general the ETD observations are for other reagents.

CID of the Singly Charged Reaction Product

Proton transfer and electron transfer compete when multiply protonated peptides react with $SO_2^{\bullet -}$, with electron transfer constituting a minor process. The intact peptide product ions can therefore be composed of mixtures of ions. For example, in the case of the reaction of a doubly protonated peptide, the following reaction scheme applies:

$$MH_2^{2+} + SO_2^{\bullet -} \rightarrow MH^+ + SO_2H^{\bullet} \quad (3)$$

$$MH_2^{2+} + SO_2^{\bullet -} \rightarrow MH_2^{+\bullet} + SO_2 \quad (4)$$

The resulting product cations differ in mass by 1 Da. While it is possible, in principle, to resolve these products with the present instrumentation, under the conditions used in this study, the pressure of heavy gases in the vacuum system was high enough to prevent a reliable determination of the relative contributions of these two product ions. Nevertheless, it is of interest to determine (a) if an intact electron transfer product is formed and (b) if so, how it fragments when subjected to collisional activation. The existence of a stable +1 electron transfer product would indicate that at least some of the initially formed +1 ions survive the electron transfer process and that the extent of electron transfer versus proton transfer cannot be ascertained by the abundances of the c- and z-type ions alone. The product ion spectrum derived from the +1 electron transfer product is of interest both from practical and fundamental perspectives. From a practical standpoint, it is of interest to determine the extent of structural information that can be derived from a doubly charged peptide, whether it is produced directly via ETD or indirectly via ET/CID. From a more fundamental perspective, comparison of the products formed via ET/CID versus direct ETD might reveal differences in behavior that arise from internal energy differences; time-scale differences; differences in structures of products formed initially, as probed relatively early by ETD and much later by ET/ CID; or mechanistic differences (e.g., nonergodic versus statistical behavior[43]).

Figure 3 compares the spectrum obtained for the reaction of doubly charged neurotensin with $SO_2^{\bullet -}$ (Figure 3a) with the CID spectrum derived from the isolation of the +1 product ion population (Figure 3b). This reaction produces almost no ETD fragments with the exception of a tentatively identified z_{12}^+ ion. The CID experiment shows cleavage at several N–C_α bonds, generating c- and z-type sequence ions, as well as cleavage of several amide backbone bonds to yield b- and y-type sequence ions. The $[M + H]^+$ ions produced via ion/ion reactions with perfluoro-1,3-dimethylcyclohexane anions, reactions known to proceed exclusively through proton transfer,[27] when subjected to CID, produce only the b- and y-type sequence ions indicated in the figure (data not shown). This suggests that the c- and z-type sequence ions arise from intact $[M + 2H]^{\bullet +}$ ions produced in the ion/ion reaction with $SO_2^{\bullet -}$. This result

indicates that at least some or most (as in the case of doubly charged neurotensin) of the $[M + 2H]^{\cdot+}$ product ions formed via electron transfer from $SO_2^{\cdot-}$ are stable on the ion trap time scale. It has been argued that the electron capture process in ECD gives rise to very rapid fragmentation of N–C_α bonds.[16] In cases in which stable electron-transfer ions have been formed, it has been argued that N–C_α bonds have been broken but that the fragments remain associated due to noncovalent interactions between one another.[44] If electron transfer also leads to nonergodic fragmentation of N–C_α bonds, a similar argument regarding the observation of an intact +1 ion must be made. Alternately, it could be interpreted that the initial electron-transfer product survives without cleavage of covalent bonds and that subsequent activation is required to dissociate the weakened N–C_α bonds.[43]

The comparison of Figure 3 obviously shows that more structural information from the c- and z-type sequence ions is apparent from the ET/CID (Figure 3b) experiment than from the ETD experiment (Figure 3a). Neurotensin is somewhat unique, however, in the relative lack of ETD products formed from the doubly charged ion (reduced doubly charged somatostatin and RKRARKE show even less). However, more structural information was obtained from the ET/CID experiment than from the ETD experiment for almost all of the doubly charged peptides studied. The results of the ETD and ET/CID experiments for all seven peptides studied as both doubly and triply charged ions are summarized in Table 1, where only cleavages represented by c-and/or z-type ions are indicated. The ETD results for the triply and doubly charged ions are shown in columns 1 and 2, respectively, and the CID results derived from the +1 ions resulting from ion/ion reactions with the doubly and triply charged peptides are shown in columns 3 and 4, respectively. A number of noteworthy observations can be drawn from this table. First, in all cases, the experiment that yielded the most extensive sequence information was the ETD reaction involving the triply protonated peptide. This observation probably reflects the likelihood that most of the products arise from the doubly charged peptide formed via electron transfer to the triply charged ion. Second, in almost all cases, both ET/CID experiments yielded more structural information than the electron transfer experiment with the doubly charged peptide. Third, the two ET/CID experiments yielded very similar results. And fourth, many of the same fragments observed by ETD with the doubly charged ions are also observed in the ET/CID experiment. The first observation probably reflects the likelihood that most of the products observed in the electron transfer experiment with the triply charged ions arise from the doubly charged peptide formed via electron transfer to the triply charged ion. The second observation demonstrates that, at least for the doubly protonated peptides studied here, an ET/CID experiment can yield greater structural information than an electron transfer experiment alone. The third observation suggests that similar +1 ion populations formed via electron transfer result from both the doubly and triply charged ions. Given that proton transfer is the predominant ion/ion reaction channel, it is unlikely that singly charged ions formed via two consecutive electron transfer reactions can contribute significantly to the +1 population. Furthermore, many, if not most, of the first generation electron transfer products formed from the triply charged ions may not survive the process (i.e., they undergo ETD). Hence, it is not surprising that the results of columns 3 and 4 are similar. Too few data have been collected to draw conclusions about the fourth observation, which relates to differences observed in direct ETD versus ET/CID. The energies and time frames of the experiments are clearly different. However, further study is required to determine if these differences alone can account for the observations.

The second observation, that the ET/CID experiments associated with the doubly charged peptide ions in reaction with $SO_2^{\cdot-}$ can give significantly more structural information than ETD, which is also generally reflected for the peptide ions of Table 2, may have important practical significance. Trypsin is by far the most commonly used enzyme in proteomics, and it cleaves proteins C-terminal to arginine and lysine residues. When the resulting peptides are ionized with electrospray, they produce predominantly +2 ions. Although the peptides studied here are

not tryptic peptides, these results suggest that, at least for some doubly charged peptides, ETD effected in the manner described herein can yield relatively little structural information. It has also been reported that ECD of some tryptic peptides has yielded limited structural information.[45] A possible means for increasing structural information would be to subject the +1 peptide ions produced by electron transfer to subsequent activation. At least for ion trap CID, more structural information is obtained, although the complete peptide sequence might not be forthcoming. Of course, the degree of structural information that can be derived from doubly charged peptides might also depend on the anion identity. Coon et al. have already shown that anthracene and fluoranthene are more efficient ETD reagents.[24,25] Further studies with other anionic reagents are in progress in our laboratory as well.

CONCLUSIONS

Ion/ion reactions involving a variety of doubly and triply charged peptide cations and $SO_2^{\bullet-}$ have been studied in a 3-D quadrupole ion trap. Evidence for both proton transfer and electron transfer was observed. Where direct comparisons could be made, the extent of electron transfer as well as the identities and relative abundances of the products formed via electron transfer were very similar to those reported in a linear ion trap.[25] There appears to be no significant difference between ion/ion electron-transfer dynamics in a linear ion trap versus a conventional Paul (3-D) ion trap. The previous report regarding ion/ion reactions between $SO_2^{\bullet-}$ and multiply protonated peptides in a Paul trap apparently missed the relatively low abundance electron-transfer dissociation products or regarded them as chemical noise.

Triply charged peptides have been shown to produce more ETD fragments, resulting in a greater degree of sequence coverage, than doubly charged peptides. Most of the fragment ions formed from the triply charged ions, therefore, arise from electron transfer to the triply charged ion, rather than from electron transfer to a doubly charged ion formed via a proton transfer ion/ion reaction. The extent to which doubly charged peptide ions fragment upon electron transfer from $SO_2^{\bullet-}$ appears to vary from peptide to peptide, but in no case was complete sequence coverage noted. CID of singly charged ions formed from ion/ion reactions with $SO_2^{\bullet-}$ implicated the presence of both proton transfer and electron transfer products. The former gave rise to the usual b- and y-type fragments typically observed from protonated peptides, whereas the latter gave rise to c- and z-type fragments as well as neutral losses primarily from the side chain of arginine. In most cases studied, CID of the intact singly charged ions that survived the electron transfer process gave more structural information than was present from direct ETD of the doubly protonated peptide. However, the ET/CID results did not yield as much structural information as ETD of the triply charged ions. Clearly, much has yet to be learned about electron-transfer ion/ion reactions involving polypeptide cations. Variables such as peptide charge state, composition, sequence, modification state, and the nature of the electron-transfer reagent remain to be studied in detail. However, this work demonstrates that a 3-D ion trap can be used to study these reactions and that peptide charge state is an important factor in determining peptide sequence via electron-transfer reactions, at least with $SO_2^{\bullet-}$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Henzel WJ, Billeci TM, Stults JT, Wong SC, Grimley C, Watanabe C. Proc Natl Acad Sci USA 1993;90:5011–5015. [PubMed: 8506346]
2. Hunt DF, Yates JR III, Shabanowitz J, Winston S, Hauer CH. Proc Natl Acad Sci USA 1986;83:6233–6237. [PubMed: 3462691]
3. Mann M, Wilm M. Anal Chem 1994;66:4390–4399. [PubMed: 7847635]
4. Busch, K. L.; Glish, G. L.; McLuckey, S. A. Mass Spectrometry/Mass Spectrometry. Techniques and Applications of Tandem Mass Spectrometry; VCH Publishers: New York, 1988.
5. Washburn MP, Wolters D, Yates JR III. Nat Biotechnol 2001;19:242–247. [PubMed: 11231557]
6. Davis MT, Beierle J, Bures ET, McGinley MD, Mort J, Robinson JH, Spahr CS, Yu W, Luethy R, Patterson SD. J Chromatogr, B 2001;752:281–291.
7. Wolters DA, Washburn MP, Yates JR III. Anal Chem 2001;73:5683–5690. [PubMed: 11774908]
8. Eng JK, McCormack AL, Yates JR III. J Am Soc Mass Spectrom 1994;5:976–989.
9. Perkins DN, Pappin DJ, Creasy DM, Cottrell JS. Electrophoresis 1999;20:3551–3567. [PubMed: 10612281]
10. Carr SA, Huddleston MJ, Bean MF. Protein Sci 1993;2:183–196. [PubMed: 7680267]
11. Huddleston MJ, Annan RS, Bean MF, Carr SA. J Am Soc Mass Spectrom 1993;4:710–717.
12. Simpson RJ, Connolly LM, Eddes JS, Pereira JJ, Moritz RL, Reid GE. Electrophoresis 2000;21:1707–1732. [PubMed: 10870958]
13. Roepstorff P, Fohlman J. Biomed Mass Spectrom 1984;11:601. [PubMed: 6525415]
14. Biemann K. Biomed Environ Mass Spectrom 1988;16:99–111. [PubMed: 3072035]
15. Zubarev RA, Kelleher NL, McLafferty FW. J Am Chem Soc 1998;120:3265–3266.
16. Zubarev RA. Mass Spectrom Rev 2003;22:55–77.
17. Zubarev RA, Horn DM, Fridriksson EK, Kelleher NL, Kruger NA, Lewis MA, Carpenter BK, McLafferty FW. Anal Chem 2000;72:563–573. [PubMed: 10695143]
18. Silivra OA, Kjeldsen F, Ivonin IA, Zubarev RA. J Am Soc Mass Spectrom 2005;16:22–27. [PubMed: 15653360]
19. Baba T, Hashimoto Y, Hasegawa H, Hirabayashi A, Waki I. Anal Chem 2004;76:4263–4266. [PubMed: 15283558]
20. Pitteri, S. J.; McLuckey, S. A. Mass Spectrom. Rev., in press.
21. Xia Y, Wu J, Londry FA, Hager JW, McLuckey SA. J Am Soc Mass Spectrom 2004;16:71–81. [PubMed: 15653365]
22. Syka JEP, Coon JJ, Schroeder MJ, Shabanowitz J, Hunt DF. Proc Natl Acad Sci USA 2004;101:9528–9533. [PubMed: 15210983]
23. Syka, J. E. P.; Coon, J. J.; Schwartz, J. C.; Shabanowitz, J. C.; Hunt, D. F Proc. 52nd ASMS Conf. Mass Spectrom. Allied Top., Nashville, TN, 2004.
24. Coon, J. J.; Syka, J. E. P.; Schroeder, M. J.; Shabanowitz, J.; Hunt, D. F. Proc. 52nd ASMS Conf. Mass Spectrom. Allied Top., Nashville, TN, 2004.
25. Coon JJ, Syka JEP, Schwartz JC, Shabanowitz J, Hunt DF. Int J Mass Spectrom 2004;236:33–42.
26. Schroeder, M. J.; Coon, J. J.; Syka, J. E. P.; Shabanowitz, J.; Hunt, D. F. Proc. 52nd ASMS Conf. Mass Spectrom. Allied Top., Nashville, TN, 2004.
27. Stephenson JL Jr, McLuckey SA. Int J Mass Spectrom Ion Processes 1997;162:89–106.
28. Pitteri SJ, Reid GE, McLuckey SA. J Proteome Res 2004;3:46–54. [PubMed: 14998162]
29. Reid GE, Wells JM, Badman ER, McLuckey SA. Int J Mass Spectrom 2003;222:243–258.
30. Van Berkel GJ, Asano KG, Schnier PD. J Am Soc Mass Spectrom 2001;12:853–862. [PubMed: 11444609]
31. Kelleher NL, Senko MW, Siegel MM, McLafferty FW. J Am Soc Mass Spectrom 1997;8:380–383.
32. Goeringer DE, Asano KG, McLuckey SA, Hoekman D, Stiller SE. Anal Chem 1994;66:313–318.
33. Kelley, P. E. Mass Spectrometry Method Using Notch Filter, U.S. Patent 5,-134,286, July 28, 1992.

34. McLuckey SA, Wu J, Bundy JL, Stephenson JL Jr, Hurst GB. Anal Chem 2002;74:976–984. [PubMed: 11925000]
35. Cooper HJ, Hudgins RR, Håkansson K, Marshall AG. J Am Soc Mass Spectrom 2002;13:241–249. [PubMed: 11908804]
36. Haselmann KF, Budnik BA, Kjeldsen F, Polfer NC, Zubarev RA. Eur J Mass Spectrom 2002;8:461–469.
37. Budnik BA, Tsybin YO, Håkansson P, Zubarev RA. J Mass Spectrom 2002;37:1141–1144. [PubMed: 12447890]
38. Adams CM, Kjeldsen F, Zubarev RA, Budnik BA, Haselmann KF. J Am Soc Mass Spectrom 2004;15:1087–1098. [PubMed: 15234367]
39. Mihalca R, Kleinnijenhuis AJ, McDonnell LA, Heck AJR, Heeren RMA. J Am Soc Mass Spectrom 2004;15:1869–1873. [PubMed: 15589763]
40. Breuker K, Oh H, Cerda BA, Horn DM, McLafferty FW. Eur J Mass Spectrom 2002;8:177–180.
41. Iavarone AT, Paech K, Williams ER. Anal Chem 2004;76:2231–2238. [PubMed: 15080732]
42. Budnik BA, Nielsen ML, Olsen JV, Haselmann KF, Hörth P, Haehnel W, Zubarev RA. Int J Mass Spectrom 2002;219:283–294.
43. Tureèek F. J Am Chem Soc 2003;125:5954–5963. [PubMed: 12733936]
44. Breuker K, Oh H, Horn DM, Cerda BA, McLafferty FW. J Am Chem Soc 2002;124:6407–6420. [PubMed: 12033872]
45. Håkansson K, Emmett MR, Hendrickson CL, Marshall AG. Anal Chem 2001;73:3605–3610. [PubMed: 11510824]

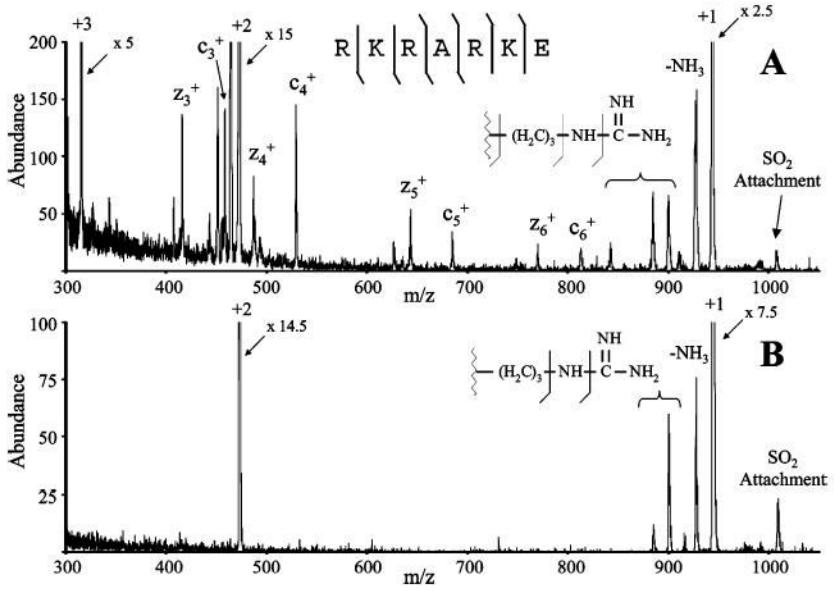


Figure 1.

(a) Product spectrum from the reaction of RKRARKE $[M + 3H]^{3+}$ with $SO_2^{\cdot-}$. b) Product spectrum from reaction of RKRARKE $[M + 2H]^{2+}$ with $SO_2^{\cdot-}$.

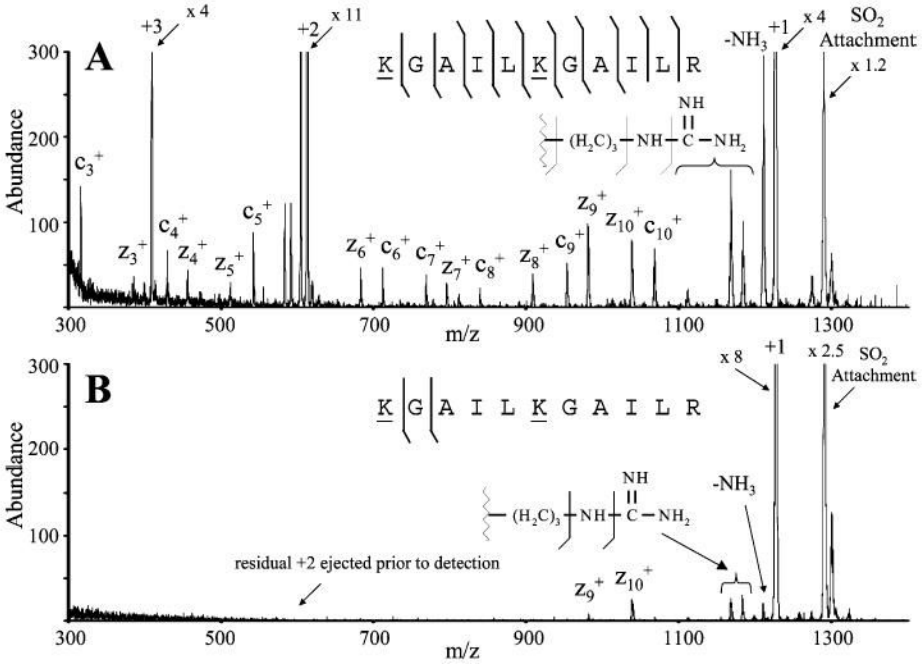


Figure 2.

(a) Product spectrum from the reaction of guanidinated KGAILKGAILR $[M + 3H]^{3+}$ with $SO_2^{\cdot-}$. (b) Product spectrum from the reaction of guanidinated KGAILKGAILR $[M + 2H]^{2+}$ with $SO_2^{\cdot-}$. K represents homoarginine.

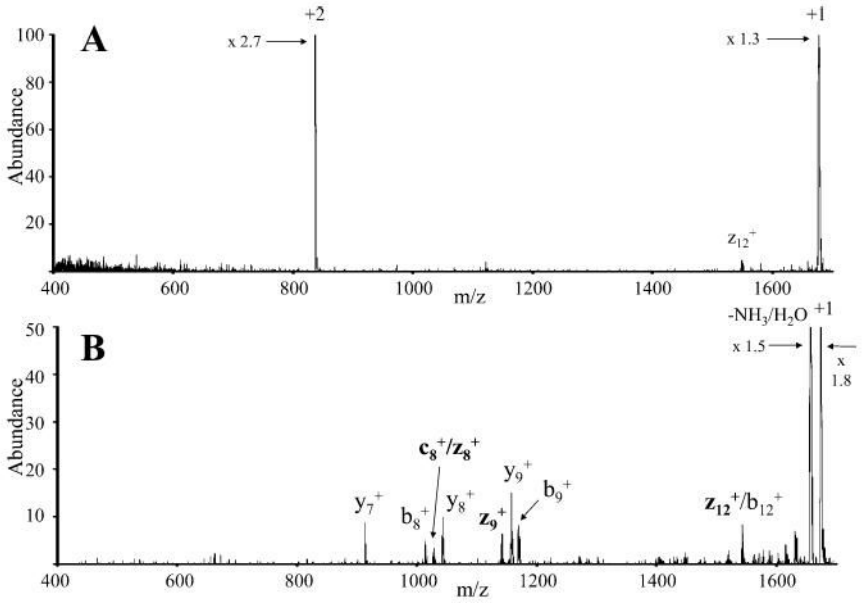
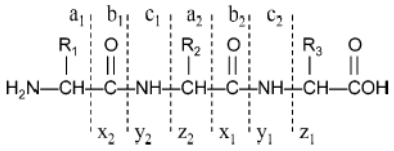


Figure 3.

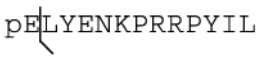
(a) Product spectrum from the reaction of neurotensin $[M + 2H]^{2+}$ with $SO_2^{\cdot-}$. (b) CID of the singly charged peptide species from (a).



Scheme 1.
Standard Fragmentation Nomenclature for Peptides[13,14]

Table 1

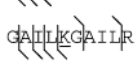
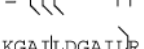
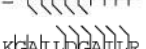
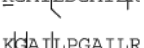
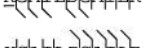
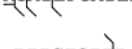
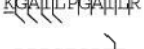
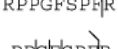
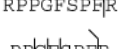
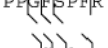
ETD and ET/CID Fragmentation Summary for Various Triply and Doubly Charged Peptides[a]

Peptide Name	$[M+3H]^{3+}$ with $SO_2^{-\bullet}$	$[M+2H]^{2+}$ with $SO_2^{-\bullet}$	CID of +1 ion after rxn. of $[M+2H]^{2+}$ with $SO_2^{-\bullet}$	CID of +1 ion after rxn. of $[M+3H]^{3+}$ with $SO_2^{-\bullet}$
RKRARKE		No fragmentation observed		
Guanidinated-KGAILKGAILR				
KGAILKGAILR				
Angiotensin				
Reduced Somatostatin		No fragmentation observed		
Neurotensin				
Lys_0-Bradykinin				

[a] Only c- and z-type fragments are indicated. K represents homoarginine.

Table 2

ETD and ET/CID Fragmentation Summary for Various Doubly Charged Peptides[a]

Peptide Name	$[M+2H]^{2+}$ with $SO_2^{-\bullet}$	CID of + 1 ion after rxn. of $[M+2H]^{2+}$ with $SO_2^{-\bullet}$
GAILAGAILR		
GAILPGAILR		
KGAILAGAILR		
KGAILPGAILR		
KGAILDGAILR		
Guanidinated GAILKGAILR		
Guanidinated KGAILAGAILR		
Guanidinated KGAILDGAILR		
Guanidinated KGAILPGAILR		
Bradykinin		
Des-Arg_1 Bradykinin		
Des-Arg_9 Bradykinin		

[a] Only c- and z-type fragments are indicated. K represents homoarginine.