RECENT DEVELOPMENTS IN THE ION/ION CHEMISTRY OF HIGH-MASS MULTIPLY CHARGED IONS

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The ability to form multiply charged high-mass ions in the gas-phase, most notably via electrospray ionization (ESI), has allowed the study of many different combinations of positively and negatively charged ions. The charged products are directly amenable to study with mass spectrometry. Ion/ion reactions have proved to be "universal" in the sense that the high exothermicities and large rate constants associated with essentially any combination of oppositely charged ions lead to reaction regardless of the chemical functionalities associated with the ions. These characteristics make ion/ion reactions potentially analytically useful provided reagent ion densities and spatial overlap of the oppositely charged ions are high. These conditions can be readily met by several instrumental configurations. The focus of this review is to highlight developments in this field since 1998. Novel instrumentation has been developed to study ion/ion reactions, such as atmospheric pressure ion/ion reactors followed by mass analysis, or electrodynamic ion trap mass spectrometers, which are used as reaction vessels at sub-atmospheric pressures. A wide variety of reaction phenomenologies have been observed in various ion/ion reactions, with proton transfer being the most common. New phenomenologies have been observed in the reactions of multiply charged positive ions with singly charged negative ions, including cation transfer and cation exchange. A new series of reactions between multiply charged positive ions and multiply charged negative ions have been made possible by recent instrumentation developments. These reactions have led to the observation of proton transfer and complex formation. These observations have provided new insights into ion/ion reaction dynamics and a bound orbit model appears to best account for experimental results. New applications are also discussed for a several ion/ion reaction.

Keywords: *ion/ion reactions; multiply charged Ions; charge permutation; mass spectrometry*

I. INTRODUCTION

More than century ago Thomson and Rutherford first studied the interactions between oppositely charged gas-phase ions (Thomson & Rutherford, 1896; Rutherford, 1897). These early experiments, and the majority of subsequent studies, involved reactions between singly charged ions. Studies of this type were complicated by the fact that all of the reaction products are neutral species. Nevertheless, the neutralization kinetics from such reactions have important applications in atmospheric chemistry, combustion studies, and plasmas (Mahan, 1973; Flannery, 1982; Bates, 1985). The development of electrospray ionization (ESI) in the 1980s and early 1990s (Fenn et al., 1989) enabled the formation of multiply charged high-mass gas-phase ions. Reactions between multiply charged ions of opposite polarities can produce charged products that are amenable to study by mass spectrometry. To date, there have been a relatively limited number of such studies of ion/ion reactions of this type. However, the utility of ion/ion chemistry is becoming increasingly apparent and it is an area that is likely to grow in coming years.

A comprehensive review of the ion/ion chemistry of high-mass multiply charged ions was published in 1998 (McLuckey & Stephenson, 1998). It discussed in detail the instrumentation, reaction phenomenologies, thermodynamics, and kinetics, and applications of ion/ion reactions up to late 1997. The aim of the current review is primarily to describe developments that have occurred since the previous review was published. In particular, there have been several new developments in the instrumentation used for ion/ion reactions, particularly those which enable the reaction of multiply charged ions of opposite polarities, and the applications of ion/ion reactions. To provide a comprehensive overview, the reaction phenomenologies mentioned in the previous review are briefly mentioned here with greater emphasis on new reaction phenomenologies not covered in the earlier review. Thermodynamics and kinetics discussed in the previous review are not repeated in detail here. However, new insights into reaction dynamics revealed by the study of the reactions of multiply charged cations with multiply charged anions are discussed. A selection of applications is highlighted in this review, including the use of ion/ion reactions in top-down proteomics, complex mixture analysis, charge inversion, metal ion chemistry, and noncovalent complex formation. Finally, future directions of the field are discussed.

II. INSTRUMENTATION

To date, ESI, or one of its variations, has been used to form multiply charged ions in all ion/ion reaction instruments that involve highly charged ions. The way in which reactions between oppositely charged ions are implemented in these instruments fall into two general categories. The first group of instruments

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utilizes ion/ion reactions at atmospheric pressure, or near atmospheric pressure, between oppositely charged ions prior to their introduction into the mass spectrometer. The second category of instruments uses quadrupole ion trap mass spectrometers as reaction vessels for ion/ion chemistry. Instrumentation associated with each of the two categories is discussed here.

A. Atmospheric Pressure Ion/Ion Reactions Followed by Mass Analysis

Early instruments that used a Y-tube reactor for atmospheric pressure ion/ion reactions have been reviewed previously (McLuckey & Stephenson, 1998) and the reader is directed to the original references for information (Ogorzalek Loo, Udseth, & Smith, 1991, 1992).

In recent years, Smith and co-workers have pioneered the use of atmospheric pressure ion/ion reactions coupled with a time-of-flight (TOF) mass analyzer (Scalf et al., 1999). One arrangement of the instrument uses a ^{210}Po source to produce α particles for charge reduction (Scalf, Westphall, & Smith, 2000). The setup for this instrument is shown in Figure 1. Multiply charged ions are formed via ESI and are then directed into a neutralization chamber via a flow of gas. The gas is ionized by the α particles to form both positive and negative singly charged ions. The primary ions react rapidly with background species, such as water, to form secondary ions that, in turn, can react with analyte ions of opposite polarity resulting in charge reduction. The degree of charge neutralization can be adjusted by controlling the flux of α particles in the neutralization chamber (by masking the α particle emitter) or by adjusting the amount of time that the analyte ions reside in the neutralization chamber (by adjusting the gas flow rate). After charge reduction, the analyte ions are focused into an orthogonal TOF mass spectrometer for mass analysis (Scalf, Westphall, & Smith, 2000).

In more recent work, Smith and co-workers have replaced the radioactive source with a corona discharge source to produce the singly charged reactant ions (Ebeling et al., 2000). The corona discharge current can be adjusted to control the flux of ions used for charge reduction. This arrangement eliminates the need for a radioactive source, which is an obvious advantage. Smith and co-workers have also reported the use of a cylindrical capacitor source, (Wang & Hackett, 1998) in place of a conventional ESI source, to generate multiply charged ions for charge reduction (Ebeling et al., 2001). The cylindrical capacitor source is a variation on conventional electrospray that does not require an externally applied high voltage, and generates ions by passing sample through a concentric cylindrical capacitor. Such a source

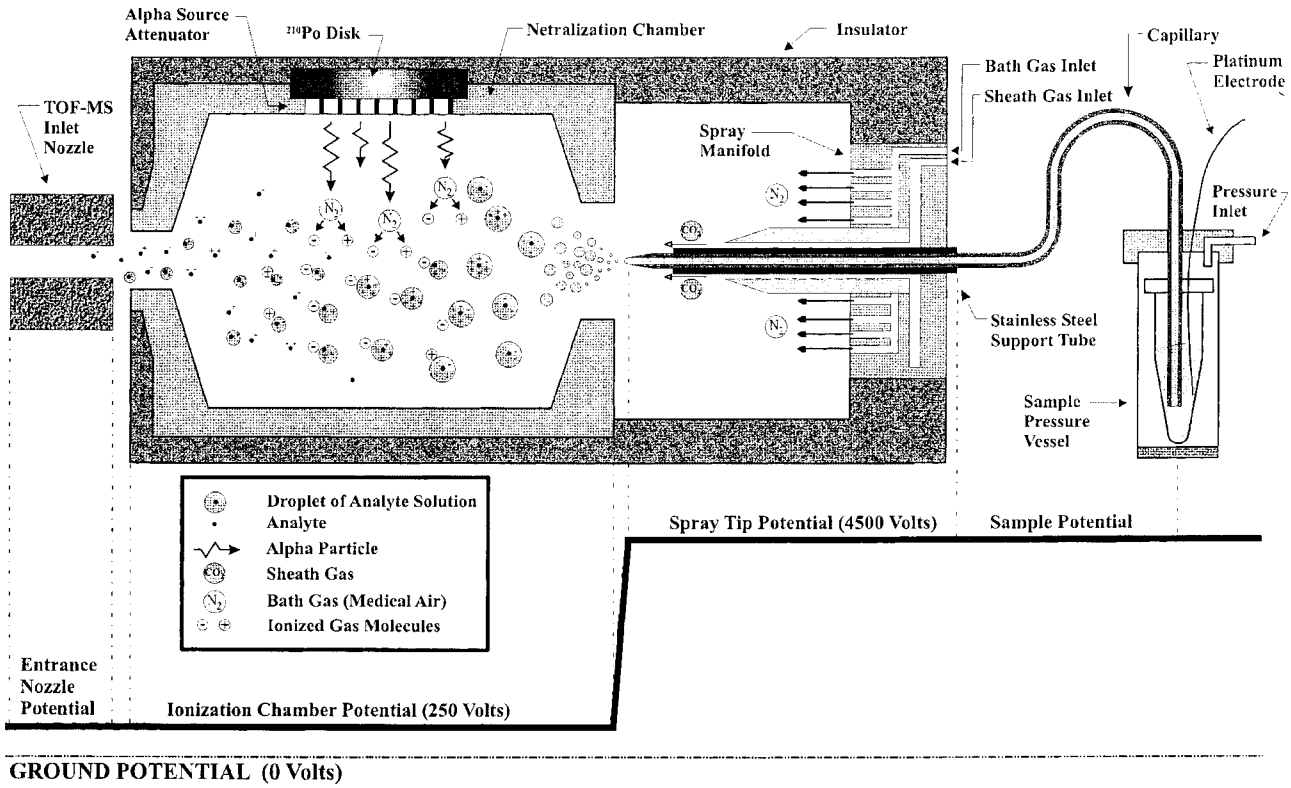


FIGURE 1. Schematic diagram of charge neutralization apparatus, which uses α-particles to reduce the charge of ions formed from electrospray ionization, coupled to a time-of-flight mass spectrometer. Reprinted from Scalf et al. with permission from Anal Chem 2000, 72, 52–60. Copyright 2000 American Chemical Society.

is reported to have advantages over conventional electrospray (i.e., more stable ion production and the ability to form ions not readily formed by conventional electrospray) (Wang & Hackett, 1998; Ebeling et al., 2001).

There are several advantages to performing charge reduction reactions using a setup similar to those used by Smith and coworkers, relative to an ion trap instrument (Scalf et al., 1999). First, the ions used for charge reduction need not be stored in the same space as the electrospray ions. That is, mutual storage of oppositely charged ions via an external field is not required when a sufficient flux of ions of at least one polarity is present. Therefore, the range of reactant species is not constrained by the need to establish storage conditions suitable for both reactants. Second, the charge reduction sources described here either produce bipolar ions or have the ability to switch the polarity of the ions quickly. This feature makes switching experiments fairly straightforward because the same neutral reagents can be used to form both positive and negative ions. Third, because ion/ion reactions are accomplished by modification of only the ESI source, adaptation of the charge reduction apparatus to different mass analyzers is relatively simple. These reactors produce singly charged ions to react with multiply charged ions, so ion/ion reactions are limited to these interactions. Proton transfer is the most dominant mechanism thus far reported using such a reactor (Scalf et al., 1999; Ebeling et al., 2000; Scalf, Westphall, & Smith, 2000).

B. Quadrupole Ion Traps as Reaction Vessels for Ion/Ion Chemistry

Quadrupole ion trap mass spectrometers have seen extensive use for selectivity of ion/ion chemistry in recent years. Ion trap instruments have several advantages for these types of reactions over the instrumentation described above. First, three-dimensional quadrupole ion traps are able to store ions of opposite polarities in overlapping regions of space (Mather & Todd, 1980). Second, quadrupole ion trap instruments allow the use of multiple stages of ion isolation/analysis (i.e., MS^n) (Louris et al., 1990) in conjunction with ion/ion reaction periods between mass analysis stages. Bipolar ions are usually introduced into these instruments via injection through the end-cap electrode and/or the ring electrode.

To date, most studies involving reactions between multiply charged positive ions and singly charged negative ions have been conducted using an instrumental setup shown schematically in Figure 2. In this arrangement, all ions are formed outside the ion trap. Multiply charged positive ions are formed via electrospray and are focused into the ion trap mass analyzer through a hole in the end-cap electrode. The ions can then be subjected to isolation and/or activation if desired. An atmospheric sampling glow discharge ionization (ASGDI) source is used to form negative ions, which are introduced into the ion trap via an aperture in the ring electrode. The oppositely charged ions are

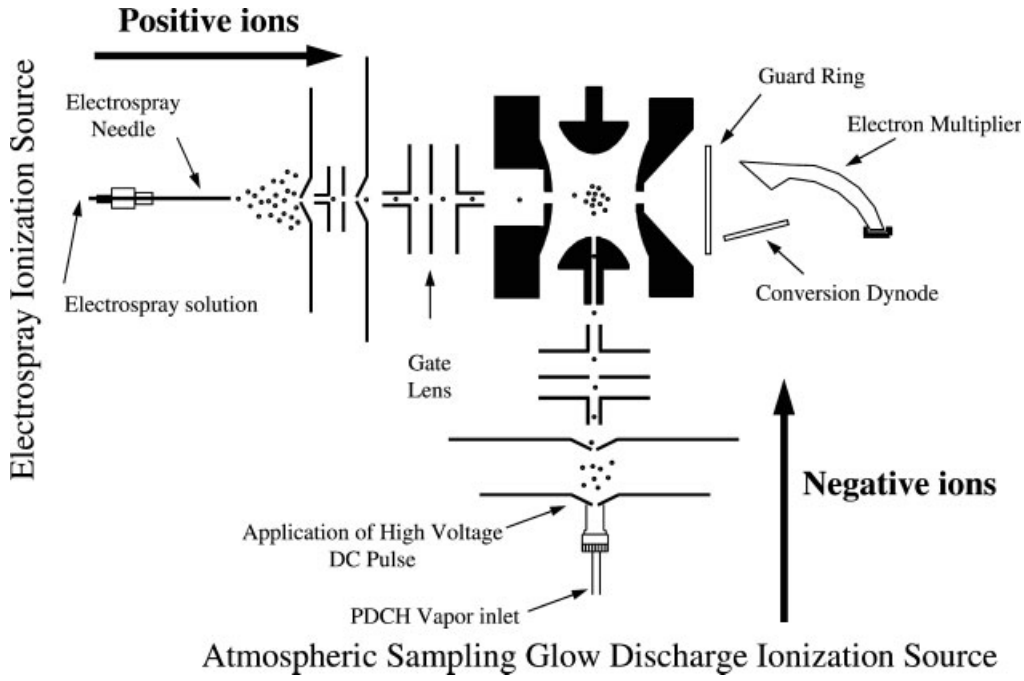


FIGURE 2. Schematic diagram of quadrupole ion trap mass analyzer that allows for reactions between ions generated by electrospray ionization (through the end cap electrode) and atmospheric sampling glow discharge ionization (through the ring electrode). Reprinted from The Encyclopedia of Mass Spectrometry, Vol. I, McLuckey SA, Ion-ion reactions, pp 866–877. Copyright 2003 with permission from Elsevier.

allowed to react in the ion trap for a controlled period of time, which determines the extent of charge reduction (Stephenson & McLuckey, 1997a). A Hitachi M-8000 ion trap mass spectrometer has recently been modified to provide a similar experimental setup. This instrument has shown significant improvement in the figures of merit for mass analysis over previous ion/ion reaction instrumentation. For example, the upper m/z limit is at least 150,000 Th, mass accuracy of better than 200 ppm at m/z <20,000 Da has been noted, and a mass resolving power of greater than 1500 at m/z 20,000 Da has been demonstrated. These values are all at least a factor of two better than the corresponding values obtained with the original ion trap instrumentation used for ion/ion reaction studies. Figure 3 shows an example of multiply charged protein ions from (a) bovine serum albumin, and (b) bovine immunoglobulin IgG, which have been charge reduced by ion/ion reactions with perfluoro-1,3-dimethylcyclohexane (PDCH) anions, on the modified Hitachi M-8000 (Reid et al., 2003).

Glish and co-workers have recently developed an apparatus similar to that of Figure 2 perform ion/ion reactions. Multiply charged positive ions are generated using ESI and introduced to the ion trap via a hole in an end-cap electrode. Singly charged ions are formed using laser desorption from a stainless steel surface, which enter the ion trap via a hole in the ring electrode. A study has been published using this instrument for the ion/ion reactions of iron and iron-containing ions with oppositely charged peptide and protein ions (Payne & Glish, 2001).

While the experimental setup discussed above that allows for ion/ion reactions between ions injected through a hole in the end cap and a hole in the ring electrode have proved to have great utility, there are several disadvantages associated with accumulation of ions admitted through a hole in the ring electrode. To date, the realm of possible ion/ion reactions has been limited by the formation of only singly charged ions to be injected through the ring electrode. Ion injection through the ring electrode via a continuous ionization source is very inefficient (less than 1%) and relatively energetic (yielding extensive ion dissociation during injection) (Stephenson & McLuckey, 1997a). These disadvantages make very difficult studies of multiply charged positive ions with multiply charged negative ions with such an experimental setup.

Instrumentation has been developed to overcome the disadvantages of ion injection through the ring electrode by admitting both positive and negative ions through the end cap electrode using a ''turning'' quadrupole to guide ions into the ion trap. Such an experimental setup is illustrated in Figure 4 (Wells, Chrisman, & McLuckey, 2002). Here a DC turning quadrupole is used to guide ions into the trap. The two ion sources (Source A and Source B) are mounted 180° from each other and the turning quadrupole directs ions from each source sequentially through a 90° turn into a hole in the center of an end-cap electrode. Various ionization sources have been used to generate ions using this setup including: ESI, glow discharge ionization, and corona discharge atmospheric pressure ionization. A wide array of ion/ion reactions can be studied with this instrument, including reactions of multiply charged negative ions with multiply charged positive ions. Such studies were not practical with previous ion trap instrumentation, which only had a single electrospray source. This type of instrumentation also allows reactions of multiply charged anions with singly charged cations. Only a limited set of such reactions could be effected with previous ion trap instrumentation.

A quadrupole ion trap mass spectrometer with up to four independent ion sources for the study of gas-phase ion/ion reactions has recently been described, and is shown in Figure 5

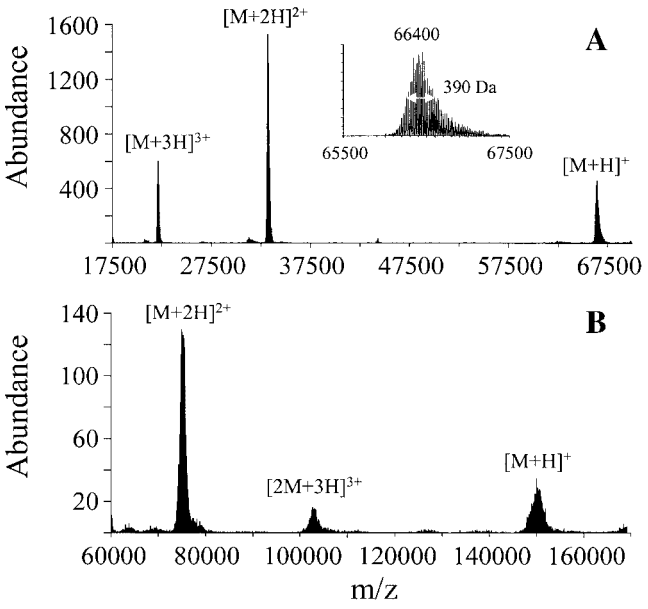


FIGURE 3. Post ion/ion reaction mass spectrum of (**A**) bovine serum albumin, and (**B**) bovine immunoglobulin IgG. Reprinted from Int J Mass Spectrom, Vol. 222, Reid GE, Wells JM, Badman ER, McLuckey SA, Performance of a quadrupole ion trap mass spectrometer adapted for ion/ion reaction studies, pp 243–258. Copyright 2003 with permission from Elsevier.

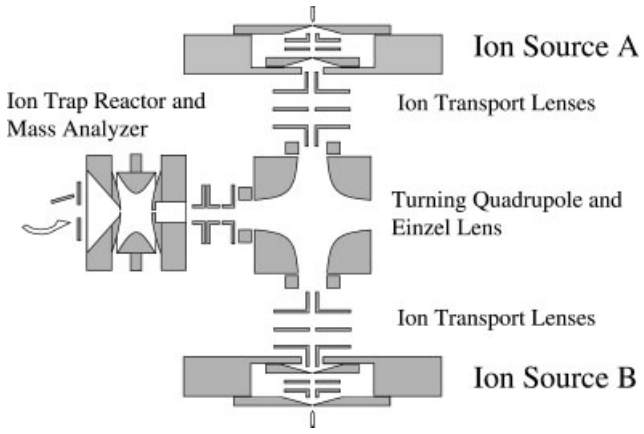


FIGURE 4. Schematic diagram of dueling electrospray quadrupole ion trap mass spectrometer Reprinted from The Encyclopedia of Mass Spectrometry, Vol. I, McLuckey SA, Ion-ion reactions, pp 866–877. Copyright 2003 with permission from Elsevier.

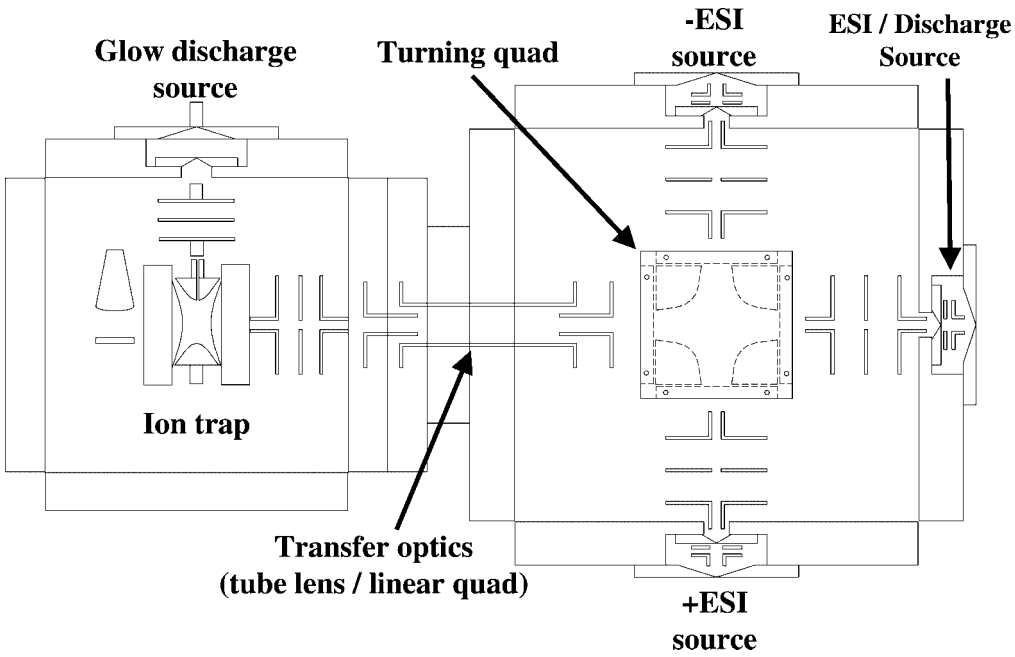


FIGURE 5. Schematic diagram of multiple-source quadrupole ion trap mass spectrometer. Adapted from Badman et al., Anal Chem 2002, 74, 6237–6243. Copyright 2002 American Chemical Society.

(Badman, Chrisman, & McLuckey, 2002). This instrument has three ESI and one ASGDI source, which allows for remarkable flexibility with respect to the reagents that can be used in a single experiment. For example, formation of gas phase complexes can be effected with two (or three) of the ESI sources and the charge states of the collision induced dissociation products of these complexes may be manipulated using anions formed with the glow discharge source. The flexibility of this instrument enables a wide array of possible cation-anion combinations in addition to the MS^n capabilities provided by an ion trap.

Several groups have recently reported the study of ion/ion reactions in linear ion traps (Coon et al., 2004; McLuckey et al., 2004; Syka et al., 2004; Wu et al., 2004). Such devices hold significant advantages over three-dimensional ion traps in terms of dynamic range and the efficiency with which they can be coupled with ion sources, detectors, and other mass analyzers. These developments are potentially highly significant for incorporating ion/ion reactions into various mass spectrometric experiments. It is still very early in the exploration of the use of linear ion traps as reaction vessels for ion/ion reactions. However, the initial results have revealed no important disadvantages relative to three-dimensional quadrupole ion traps. It is likely that extensive use of linear ion traps as reaction vessels for ion/ion reactions will be made in the coming years.

III. REACTION PHENOMENOLOGY

Although the novel instrumentation described above allows for a wide range of new reactions between anions and cations to be studied, only a small fraction of the possibilities have been investigated thus far. Nevertheless, from the limited number of combinations studied to date, a remarkable variety of new reaction phenomenologies have been observed, which are discussed here.

A. Multiply Charged Positive Ions/Singly Charged Negative Ions

Most ion/ion reaction studies to date have been conducted with multiply charged positive ions reacting with singly charged negative ions. For the most part, multiply charged positive ions are comprised of relatively high-mass biological molecules, usually proteins with an excess of cations. $(M + nX)^{n+}$ represents a generic multiply charged positive ion, where X represents H^+ (or a singly charged metal ion in a few select cases). Y^- represents an anion for even-electron species and $Y^{\bullet -}$ for odd-electron species. Observed reaction phenomenologies currently include charge transfer, attachment, and exchange reactions.

1. Proton Transfer

Proton transfer is the most commonly observed reaction to date in the study of the reaction of multiply charged positive ions with singly charged negative ions to date. In early Y-tube reactor studies, proton transfer was probably a major reaction that took place, although solvent transfer in conjunction with proton transfer may also have contributed to charge state reduction (Ogorzalek Loo, Udseth, & Smith, 1991, 1992). In atmospheric pressure ion/ion reactions coupled with TOF mass spectrometry, proton transfer reactions appear to dominate (Scalf et al., 1999; Ebeling et al., 2000; Scalf, Westphall, & Smith, 2000). In all ion

trap studies that have reacted even-electron perfluorocarbon anions with multiply charged cations, proton transfer has been observed (Stephenson & McLuckey, 1996a).

The transfer of a proton to a singly charged negative ion can be represented as:

$$(\mathrm{M} + n\mathrm{H})^{n+} + \mathrm{Y}^- \rightarrow (\mathrm{M} + (n-1)\mathrm{H})^{(n-1)+} + \mathrm{HY} \qquad (1)$$

The neutral product is neither analyzed nor detected by any tools currently used to study ion/ion reactions. Therefore, if fragmentation of the neutral product occurs, it cannot be determined. However the fragmentation of the ionic product would be detectable if it occurred at a measurable level. To date, there has been little evidence of fragmentation resulting from proton transfer ion/ion reactions involving macro-molecular ions. An example of singly charged anions produced from an atmospheric pressure corona discharge source, reacted with multiply charged protein cations formed via electrospray, is shown in Figure 6. Figure 6a shows an electrospray mass spectrum of an equimolar mixture of seven proteins. Figure 6b shows singly charged protein cations that have been charge reduced by multiple proton transfer reactions. These experiments were performed on a TOF mass spectrometer.

The reaction of singly charged anions with multiply charged cations to give proton transfer has also been shown in quadrupole ion trap mass spectrometers. An example of this is the reduction of multiply charged bovine ubiquitin cations, via reactions with singly charged anions derived from PDCH, to yield predominantly singly charged ubiquitin cations, which was summarized in a previous review (McLuckey & Stephenson, 1998) and in the original reference (Stephenson & McLuckey, 1996a; Reid et al., 2001). Ion/ion reactions with PDCH derived anions have proven to be a very effective means to reduce the charge of multiply charged ions (Reid et al., 2001; Engel et al., 2002; Hogan & McLuckey, 2003a) because they react exclusively by proton transfer. A selection of applications of these proton transfer reactions is discussed in a later section.

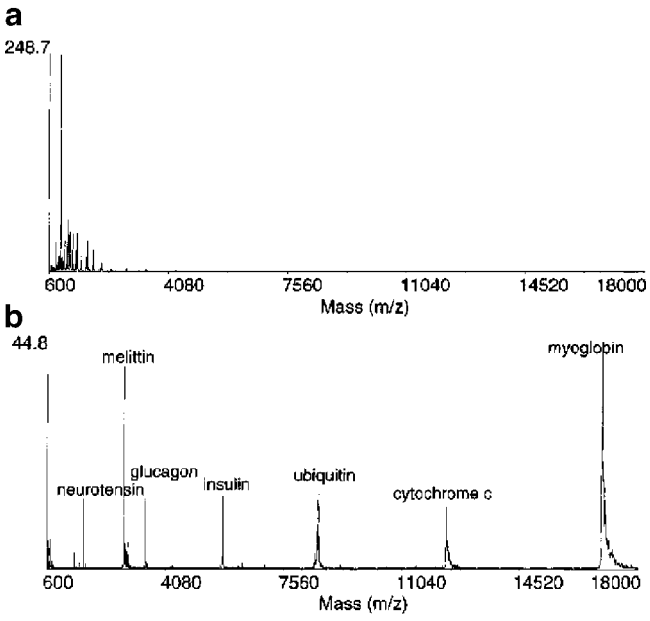


FIGURE 6. Mass spectra of (**a**) electrospray distribution of seven-component protein mixture, and (**b**) seven-component protein mixture after charge reduction. Reprinted from Ebeling et al. with permission from Anal Chem 2000, 72, 5158–5161. Copyright 2000 American Chemical Society.

2. *Electron Transfer*

Observations of electron transfer resulting from ion/ion reactions between multiply charged cations with singly charged anions have been limited, by comparison with observations of proton transfer reactions. The transfer of an electron from a singly charged negative ion to a multiply charged positive ion can be indicated generically as:

$$(\mathrm{M} + n\mathrm{X})^{n+} + \mathrm{Y}^- \rightarrow (\mathrm{M} + n\mathrm{X})^{(n-1)+} + \mathrm{Y} \qquad (2)$$

Electron transfer must compete with cation (usually proton) transfer as indicated by the following reaction:

$$(\mathrm{M} + n\mathrm{X})^{n+} + \mathrm{Y}^- \rightarrow (\mathrm{M} + (n-1)\mathrm{X})^{(n-1)+} + \mathrm{XY} \qquad (3)$$

Reaction (1) and (3) differ in that the negative ion in reaction (1) is an even-electron species, whereas the negative ion in reaction (3) is an odd-electron species, although the role of spin state on reactivity has not been established. To date, proton transfer reactions have been the dominant mechanism for most multiply charged positive ions, including a limited set of odd-electron anions studied. Electron transfer has been observed with highly conjugated cations. This observation was reviewed previously (McLuckey & Stephenson, 1998) and discussed in the original reference (Stephenson & McLuckey, 1996a).

Electron transfer to polypeptide cations via ion/ion chemistry resulting in the formation of c/z-type ions has very recently been described (Coon et al., 2004; Syka et al., 2004). These products correspond to those associated with electron capture dissociation (ECD) (Zubarev, Kelleher, & McLafferty, 1998; Kruger et al., 1999; Zubarev et al., 1999, 2000) and they often provide information complementary to conventional collision-induced dissociation. An example of electron transfer dissociation, resulting from anions derived from negative ion methane chemical ionization of anthracene, reacted with a phosphorylated peptide, is shown in Figure 7. These results demonstrate that electron transfer to polypeptide cations can be a favored ion/ion reaction mechanism with the appropriate selection of anionic reagents. This new development has important implications for the use of ion/ion reactions in complex peptide and protein mixture analysis because it provides an information rich alternative to collision-induced dissociation for the derivation of structural information from polypeptide ions.

3. *Anion Attachment*

Anion attachment involves two ions of opposite polarity associating to form one ion. This reaction is represented generically as:

$$(\mathrm{M} + n\mathrm{X})^{n+} + \mathrm{Y}^- \rightarrow (\mathrm{M} + n\mathrm{X} + \mathrm{Y})^{(n-1)+} \qquad (4)$$

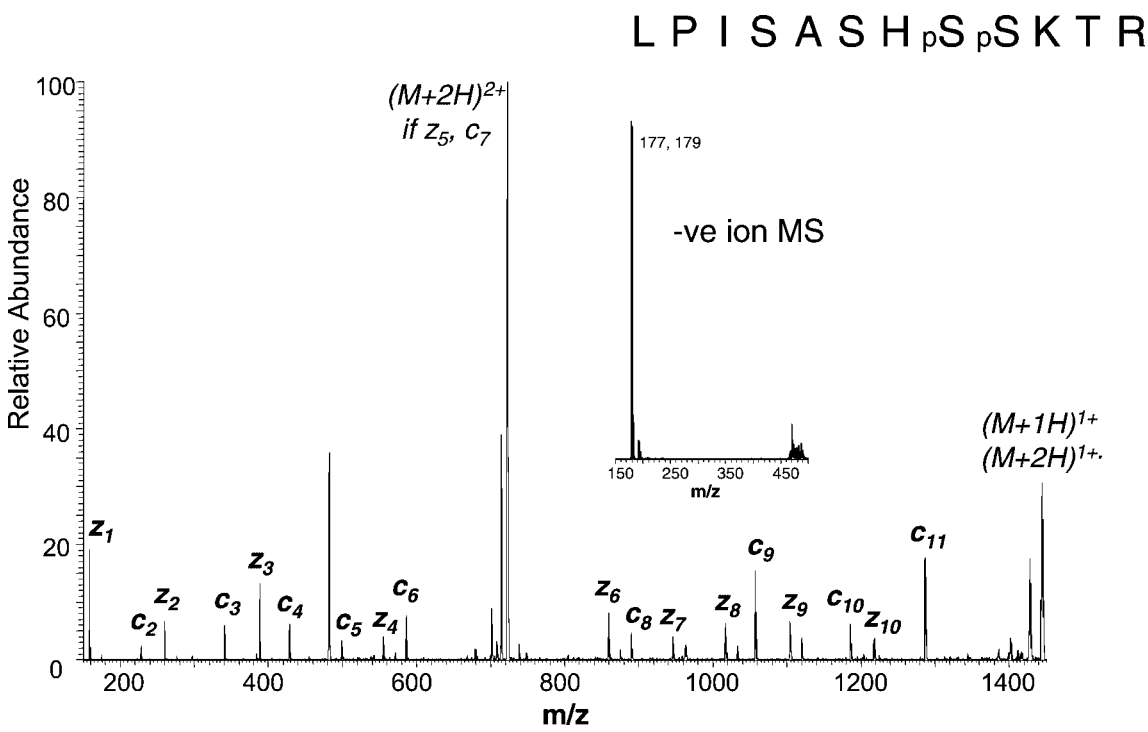


FIGURE 7. Electron transfer dissociation mass spectrum of a phosphorylated peptide resulting from ion/ion reactions with *m/z* 179 anions derived from chemical ionization of anthracene with methane gas (negative ion mass spectrum shown in inset). Figure courtesy of Syka J, Coon J, Shabanowitz J, and Hunt D of the University of Virginia.

The energy of condensation must be dissipated to avoid fragmentation. Iodide (I^-) is an example of an ion that readily attaches to peptides and proteins without causing fragmentation. An ion/molecule reaction analog to this ion/ion reaction has also been reported. Neutral HI attaches to polypeptide cations at basic sites (arginine, lysine, histidine, and the N-terminus) of bovine ubiquitin. This work has been reviewed previously (McLuckey & Stephenson, 1998) and is discussed in the original reference (Stephenson & McLuckey, 1997b).

Anion attachment in ion/ion reactions between PF_6^- and doubly charged bradykinin (RPPGFSPFR) $[M + H + Na]^{2+}$ ions has also been noted recently. The mass spectra of the isolated cation and anion prior to the ion/ion reaction are shown in Figure 8a and b, respectively. The ion/ion reaction yields the attachment product $[M + H + Na + PF_6]^-$ as the major reaction product, as shown in Figure 8c. Sodium transfer and proton transfer to produce the $[M + H]^+$ and $[M + Na]^+$ products, respectively, are also observed to make minor contributions. There is no observation of fragmentation of either peptide or PF_6 bonds from the ion/ion reaction. Calculations suggest that PF_6^- forms a relatively stable intermediate structure in a model ion/ion reaction, which is a prerequisite for the observation of attachment (Newton et al., 2004).

Evidence of anion attachment in conjunction with fragmentation has been recently reported in the case of $FeCO_2^-$ reacted with the doubly charged bradykinin (RPPGFSPFR) (Payne & Glish, 2001). The results of this reaction are shown in Figure 9. The intact attachment product $[RPPGFSPFR + 2H + FeCO_2]^+$ is not observed. However, several product ions contain $FeCO_2^-$ including the $[a_6 + FeCO_2]^+$ and $[y_5 + FeCO_2]^+$ products as well as the attachment products minus water(s) and/or ammonia: $[RPPGFSPFR + FeCO_2 - NH_3 - 2H_2O]^+$, $[RPPGFSPFR + FeCO_2 - NH_3 - H_2O]^+$, and $[RPPGFSPFR + FeCO_2 - NH_3]^+$.

4. Anion Transfer

Although not commonly observed in reported ion/ion reactions to date, anion transfer is another possible type of charge transfer that occurs as a result of reactions between multiply charged positive ions and singly charged anions. Anion transfer is written generically as:

$$(M + nX)^{n+} + YZ^- \rightarrow (M + nX + Z)^{(n-1)+} + Y \quad (5)$$

To date, the only reported example of anion transfer has been the transfer of fluoride (F^-) to cations that are not expected to readily undergo proton transfer. Multiply charged poly(ethylene gycol) 20 cations (in particular, the $[M + 2Na]^{2+}$ ion) reacted with anions derived from PDCH have been shown to undergo anion (F^-) transfer and proton transfer, but no sodium ion transfer. This work has been reviewed previously (McLuckey & Stephenson, 1998) and is discussed in the original reference (Stephenson & McLuckey, 1998).

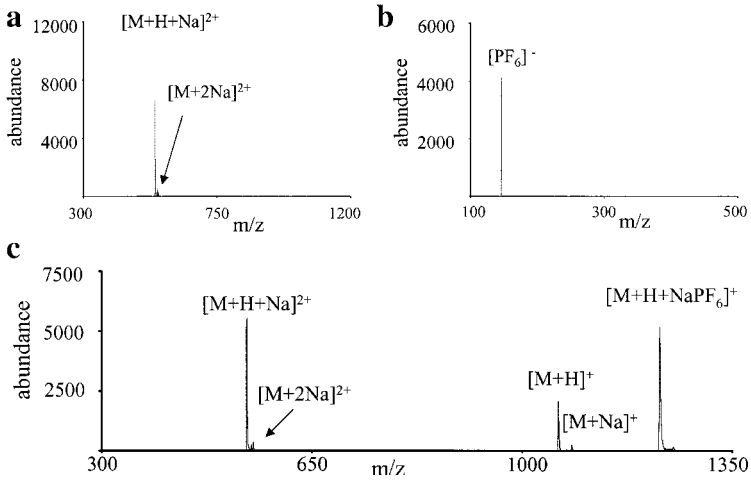


FIGURE 8. (**a**) Mass spectrum of isolated $[M + Na + H]^{2+}$ bradykinin. (**b**) Mass spectrum of PF_6^- anions. (**c**) Ion/ion reaction product ion spectrum of reaction between (a) and (b). From Newton et al., Phys Chem Chem Phys 2004, 6, 2710–2717. Reproduced by permission of the PCCP Owner Societies, copyright 2004.

5. Cation Transfer Other Than Proton Transfer

Similar to anion transfer, cation transfer (where the cation is not H^+) is another possible charge transfer reaction that has not been commonly reported in ion/ion reactions to date. A generic cation transfer reaction can be represented by:

$$(M + nX)^{n+} + Y^- \rightarrow (M + (n-1)X)^{(n-1)+} + XY \qquad (6)$$

Cation transfer has been recently observed in the ion/ion reaction between PF_6^- and the doubly charged bradykinin $[M + H + Na]^{2+}$ ion, as discussed previously. Evidence for Na^+ transfer is apparent in Figure 8c from the appearance of the $[M + H]^+$ peak in the ion/ion reaction product spectrum. The calculation of cation affinities and stabilities of species relevant to this experiment suggest that sodium ion transfer is competitive with proton transfer during reactions with PF_6^-, which is consistent with the experimental results (Newton et al., 2004). Furthermore, collisional activation of the adduct species $[M + H^+ + Na^+ + PF_6^-]$ shows loss of $NaPF_6$ to be competitive with loss of HPF_6.

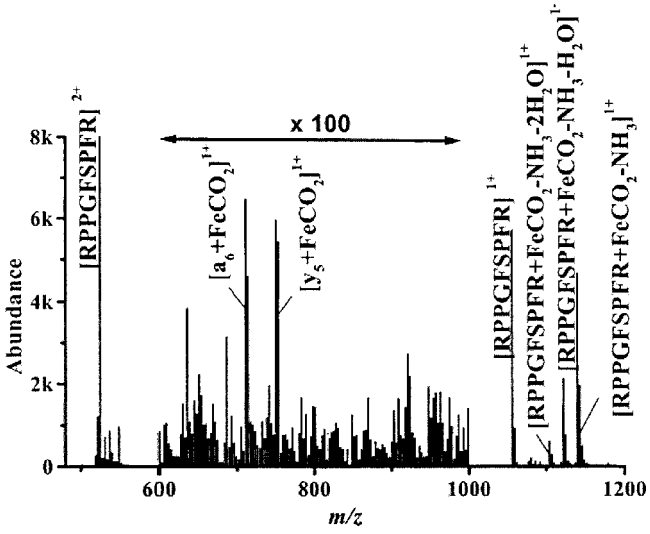


FIGURE 9. Ion/ion reaction product spectrum of reaction between $[RPPGFSPFR + 2H]^{2+}$ and $FeCO_2^-$. Reprinted from Int J Mass Spectrom, Vol. 204, Payne AH, Glish GL, Gas-phase ion/ion interactions between peptides or proteins and iron ions in a quadrupole ion trap, pp 47–54. Copyright 2001 with permission from Elsevier.

6. Multiple Proton Transfer

Reactions between multiply charged positive ions and singly charged negative ions have been shown to result in multiple proton transfer, which can be represented generically as:

$$(M + nH)^{n+} + Y^- \rightarrow (M + (n-m)H)^{(n-m)+} + YH_m^{(m-1)+} \qquad (7)$$

The first Y-tube study showed evidence of double proton transfer, giving rise to the charge inversion of the initially singly charged anion. In these Y-tube studies, deprotonated molecules (fluorescein and adenosine 5′-monophosphate (ATP)) were reacted with multiply protonated polypeptides (melittin, myoglobin, and cytochrome *c*). These ion/ion reactions produced protonated fluorescin or ATP in the positive ion mass spectrum. Evidence was presented to suggest that the most likely mechanism for this charge inversion involved double proton transfer in a single encounter. They suggested a relatively long-lived collision complex in this mechanism in which both an ion/ion proton transfer reaction and an ion/molecule proton transfer reaction take place. This work has been previously reviewed (McLuckey & Stephenson, 1998) and readers are referred to the original reference (Ogorzalek Loo, Udseth, & Smith, 1991).

Recently, evidence for double proton transfer has also been observed in ion trap studies. The reaction of the singly deprotonated peptide GLSDGEWQQVLNVWGK (Fig. 10a) with

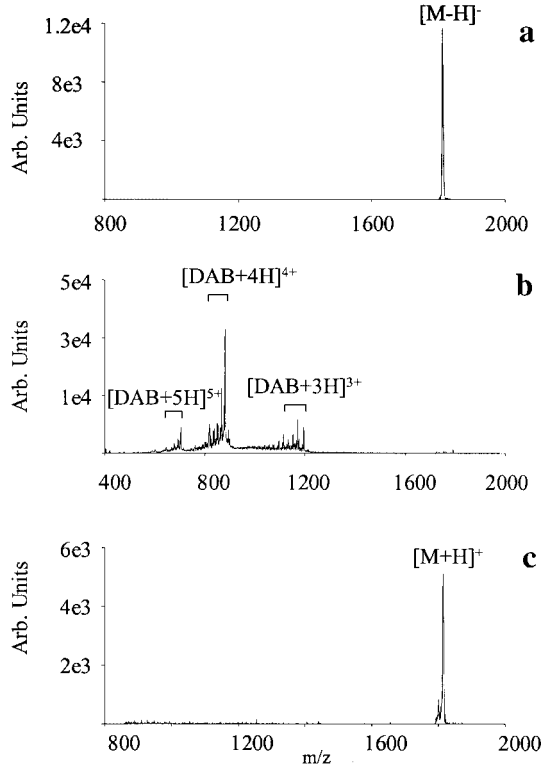


FIGURE 10. (**a**) Negative electrospray mass spectrum of isolated $[M-H]^-$ GLSDGEWQQVLNVWGK. (**b**) Positive electrospray mass spectrum of DAB cations. (**c**) Positive ion mass spectrum of isolated $[M+H]^+$ from the reaction between (a) and (b). Reproduced from He & McLuckey with permission from Anal Chem 2004, 76, 4189–4192. Copyright 2004 American Chemical Society.

multiply charged 1,4-diaminobutane (DAB) generation 4 dendrimer cations (Fig. 10b) results in double proton transfer and the formation of singly protonated peptide cations (Fig. 10c) (He & McLuckey, 2004a). Triple proton transfer has also been observed in the reaction of singly deprotonated bradykinin anions with multiply protonated DAB dendrimer cations to form doubly protonated bradykinin cations (He & McLuckey, 2003). The applications of this reaction are discussed below.

7. Solvent Transfer With Proton Transfer

The second Y-tube study described an experiment where the presence of negative ions in the Y-tube resulted in the partial reduction of protein positive ion charge states and an increased relative degree of solvation of the protein ions. Bradykinin positive ions introduced from one arm of the Y-tube were reacted with negative ions introduced from a negative corona discharge via the other arm of the Y-tube. With the negative discharge in operation, there is an apparent increase in the relative abundances of doubly charged ions with 1–17 water molecules attached. This phenomenon is attributed to proton transfer from, and solvent transfer to, triply protonated bradykinin ions. A single collision process that leads to this kind of observation might be represented as:

$$(M+nH)^{n+} + YS_m^- \rightarrow (M+(n-1)H)^{(n-1)+}(m-x)S + YH + xS \quad (8)$$

where S represents a solvent molecule. The assumptions behind this mechanism include that a solvated anion is one of the reactants (the major negative ion reactants present in the discharge were $O_2^-(H_2O)_n$ ions) and that solvent is transferred within the collision complex that leads to proton transfer. Since it is not clear from the Y-tube studies that a single collision mechanism is responsible for this observation, reaction (8) remains to be demonstrated unambiguously. This study is described in further detail in a previous review (McLuckey & Stephenson, 1998) and in the original study (Ogorzalek Loo, Udseth, & Smith, 1992). To date, solvated anions have not been used as negative ion reagents in ion trap experiments.

8. Cation Exchange

The observation of a new type of phenomenology, cation exchange, resulting from ion/ion reactions between multiply charged positive ions and singly charged negative ions has recently been reported (Newton & McLuckey, 2003). In particular, cation exchange has resulted from the reaction of multiply charged peptide and protein cations with metal containing anions to lead to the incorporation of a metal ion into a peptide or protein. The observed cation exchange phenomenon can be represented generically by:

$$[M+2X]^{2+} + [YZ_2]^- \rightarrow [M+Y]^+ + 2XY \quad (9)$$

where X represents a cation (either a proton or metal cation), Y represents a metal, and Z represents a counter ion. An example of cation switching is illustrated in Figure 11. The left side of Figure 11a shows a mass spectrum of isolated triply protonated *Trp*-11 neurotensin, which was subjected to ion/ion reactions with sodium diethyldithiocarbamate anions (shown on the right side of Fig. 11a). The resulting ion/ion reaction products are shown in Figure 11b. The major product from this reaction is $[M+H+Na]^{2+}$, which is a result of the addition of one sodium ion and the removal of two protons. This reaction product is likely formed via a relatively long-lived complex between the sodium-containing anions and protonated peptide ions. After the transfer of a sodium cation to the peptide and two protons to the sodium diethyldithiocarbamate anion, the complex breaks up resulting in the net effect of cation exchange. In addition to reaction with sodium diethyldithiocarbamate anions, triply charged *Trp*-11 neurotensin has undergone ion/ion reactions with calcium acetate, silver nitrate, and nickel acetate anions leading to cation exchange products of $[M-2H+2Ca]^{2+}$, $[M+H+Ag]^{2+}$ and $[M+2Ag]^{2+}$, and $[M+Ni]^{2+}$ and $[M-2H+2Ni]^{2+}$, respectively. All reactions of metal containing anions reported to date with triply charged *Trp*-11 neurotensin have also shown evidence of proton transfer by the formation of the $[M+2H]^{2+}$ product. Multiply charged ubiquitin cations have also been shown to undergo both proton transfer and cation exchange when reacted with copper nitrate anions (Newton & McLuckey, 2003).

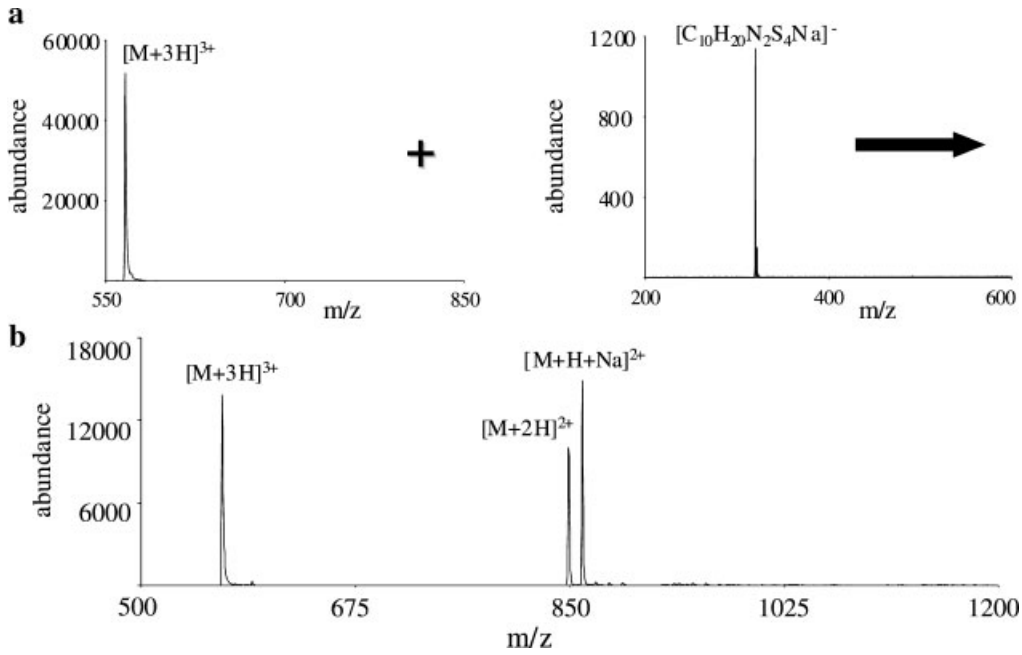


FIGURE 11. (**a**) Mass spectra of $[M + 3H]^{3+}$ *Trp*-11 neutrotensin (left) and sodium diethyldithiocarbamate anions (right). (**b**) Ion/ion reaction product ion spectrum for reaction of ions in (a). Reproduced from Newton & McLuckey with permission from J Am Chem Soc 2003, 125, 12404–12405. Copyright 2003 American Chemical Society.

B. Multiply Charged Negative Ions/Singly Charged Positive Ions

To date, all multiply charged negative ions involved as reactants in ion/ion reactions have been deprotonated species. The generic multiply charged negative ion is represented as $(M - nH)^{n-}$. Singly charged positive ion reactants have generally been either protonated molecules (YH^+) or radical cations ($Y^{\bullet+}$).

1. Proton Transfer

Multiply charged oligonucleotide anions have been subjected to ion/ion reactions with solvated protons (protonated water clusters, protonated methanol clusters, and mixed solvent clusters) from atmospheric pressure discharge (Ogorzalek Loo, Udseth, & Smith, 1991, 1992; Scalf et al., 1999; Ebeling et al., 2000; Scalf, Westphall, & Smith, 2000). The major reaction type observed was proton transfer, which can be represented generically by:

$$(M - nH)^{n-} + YH^+ \rightarrow (M - (n-1)H)^{(n-1)-} + Y \qquad (10)$$

In the Y-tube study, the ion/ion reactions of anions from 5′-d(pAAA)-3′ show evidence of fragmentation, possibly arising from the exothermicity of the proton transfer reaction. However, firm conclusions about the origin of the fragment ions cannot be drawn from these studies, as any product ion formed via ion/ion reactions must also survive transmission through the atmospheric interface.

Ion/ion reactions between multiply charged oligonucleotide and polypeptide anions and singly protonated pyridine ions have been performed in an ion trap mass spectrometer (Herron, Goeringer, & McLuckey, 1995a, 1996a). These cases, and all even-electron anions studied thus far, including those discussed by Herron, appear to react exclusively with protonated pyridine ions through proton transfer. Identifiable fragmentation as a result of proton transfer has only been reported in the cases of doubly deprotonated 2-hydroxynaphthalene-3,6-disulfonic acid (Herron, Goeringer, & McLuckey, 1996b), and the doubly deprotonated dye Direct Red 81 (Herron, Goeringer, & McLuckey, 1995b). The observation of ion/ion reaction-induced fragmentation from the latter two molecules is consistent with the claim that fragmentation arising from ion/ion reactions is most likely to occur in the ion trap with relatively small ions for reasons discussed in detail elsewhere (McLuckey & Stephenson, 1998).

More recently, proton transfer has been observed with reactions between multiply charged protein anions and protonated benzoquinoline (BQH^+) (Chrisman & McLuckey, 2002). Reactions of various protein anions with benzoquinoline were shown to give rise exclusively to proton transfer and were useful in reducing the protein anions to singly charged species. These reactions were the first example of the use of benzoquinoline as a charge reduction agent for negative ions. BQH^+ has also been used to manipulate the charge states of multiply charged anions derived from PAMAM dendrimers by proton transfer without observable fragmentation (He & McLuckey, 2004b).

Reactions between oligonucleotide anions and a variety of cations in an ion trap have also resulted in proton transfer.

Oligonucleotide anions have been reacted with protonated quinoline, isoquinoline, and benzoquinoline cations derived from nano-ESI (Wu & McLuckey, 2003). Each cationic reagent underwent proton transfer reactions with the multiply charged oligonucleotide anions with only a small amount of fragmentation. BQH^+ has been the most extensively studied reagent cation and it tends to show essentially exclusive proton transfer (i.e., little or no adduct formation and fragmentation). A few exceptions have been reported where benzoquinoline showed extensive attachment to 5′-d$(T)_{20}$-3′ and 5′-d$(CGGG)_5$-3′ (Wu & McLuckey, 2003). A comparison of the ion/ion reaction spectra of triply charged 5′-d(AAAA)-3′ anions with $O_2^{\bullet+}$, $C_4H_9^+$, and BQH^+ is shown in Figure 12. Reaction with $O_2^{\bullet+}$, as shown in Figure 12a, shows no proton transfer but leads to extensive fragmentation. The reaction with $C_4H_9^+$, as shown in Figure 12b, shows predominantly proton transfer (apparent from the doubly and singly charged oligonucleotide anions) with some signs of fragmentation (i.e., w_2, w_3, and $(a_2\text{-}A_2)$ anions). The reaction of BQH^+, shown in Figure 12c, yields essentially exclusive proton transfer products with little, if any, fragmentation or adduct formation. The degree of fragmentation noted here correlates with the relative ion/ion reaction exothermicities. The reaction exothermicities associated with the reagents used here follow the order $O_2^{\bullet+} > C_4H_9^+ > BQH^+$ (Wu & McLuckey, 2003). The reaction with $O_2^{\bullet+}$, which occurs by electron transfer, as discussed below, leads to a radical anion species the stability of which relative to the even-electron species of the same charge is not known. Hence, product anion stability may also play a role in the differences between the $O_2^{\bullet+}$ results, on the one hand, and those obtained with $C_4H_9^+$ and BQH^+, on the other hand.

2. *Electron Transfer*

By selecting positive ion reagents that cannot readily undergo cation transfer, electron transfer reactions that involve multiply charged closed-shell negative ions are relatively straightforward to effect (Herron, Goeringer, & McLuckey, 1995b). The majority of studies to date have used rare gas cations to achieve electron transfer reactions. This reaction can be represented generically as:

$$(M - nH)^{n-} + Y^+ \rightarrow (M - nH)^{(n-1)-} + Y \qquad (11)$$

Extensive dissociation has been commonly observed as a result of electron transfer with reactions between relatively small polyanions and rare gas cations. The high reaction exothermicities and the relatively small ion size are conducive to fragmentation, as discussed previously (McLuckey & Stephenson, 1998). The reaction of Fe^+ and anions including various peptides and insulin have been shown to result in electron transfer

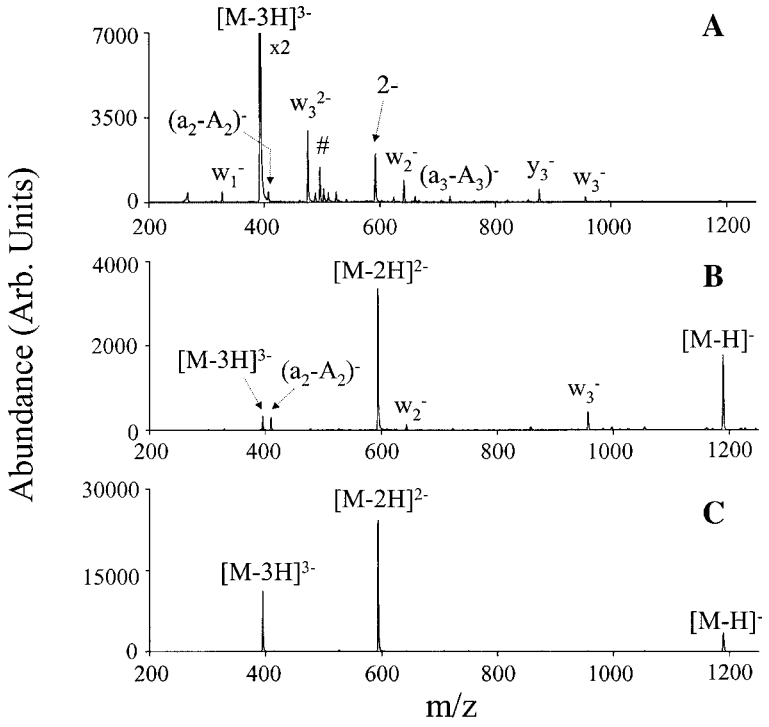


FIGURE 12. Ion/ion reaction production spectra of $[M - 3H]^{3-}$ of 5′-d(AAAA)-3′ with (**A**) $O_2^{\bullet+}$, (**B**) $C_4H_9^+$, and (**C**) BQH^+. # indicates the loss of the terminal base and the terminal sugar. Reprinted from Int J Mass Spectrom, Vol. 228, Wu J, McLuckey SA, Ion/ion reactions of multiply charged nucleic acid anions: Electron transfer, proton transfer, and ion attachment, pp 577–597. Copyright 2003 with permission from Elsevier.

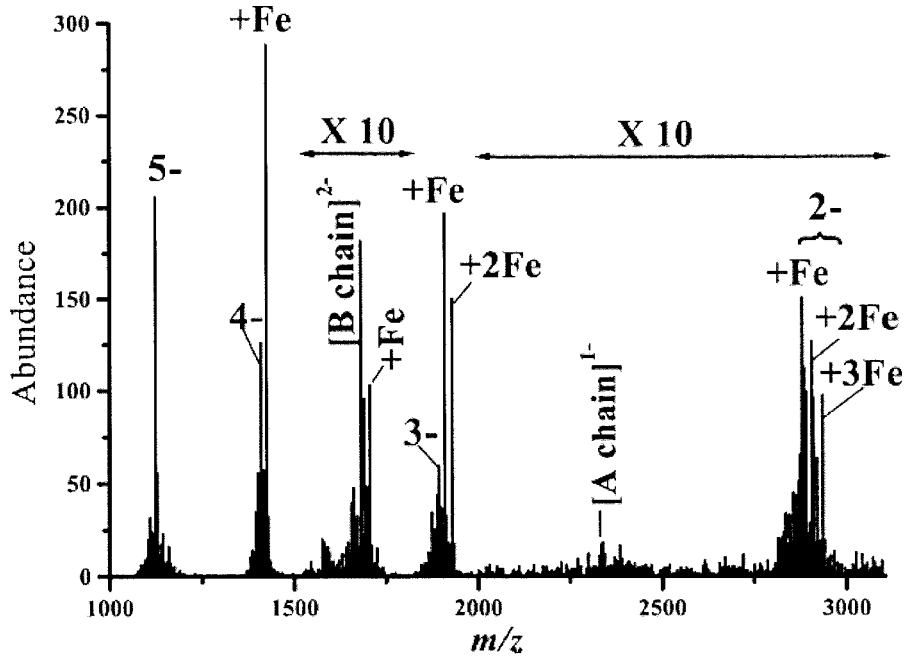


FIGURE 13. Ion/ion reaction product ion spectrum of $[\text{insulin} - 5\text{H}]^{5-}$ and Fe^{+}. Reprinted from Int J Mass Spectrom, Vol. 204, Payne AH, Glish GL, Gas-phase ion/ion interactions between peptides or proteins and iron ions in a quadrupole ion trap, pp 47–54. Copyright 2001 with permission from Elsevier.

reactions. In the case of $[\text{insulin} - 5\text{H}]^{5-}$ reacted with Fe^{+} (Fig. 13), cleavage of disulfide bonds (apparent by the appearance of $[\text{A chain}]^{2-}$ and $[\text{B chain}]^{2-}$ anions) has been reported to result from electron transfer reactions (Payne & Glish, 2001).

Oxygen cations ($O_2^{+\bullet}$) have recently been reacted with oligonucleotide anions resulting in electron transfer (see also Fig. 12) (McLuckey, Reid, & Wells, 2002a). Varying degrees of fragmentation have been observed as a result of electron transfer, depending on the composition of the oligonucleotides. Figure 14a–d shows post ion/ion reaction mass spectra resulting from the reaction of oxygen cations with $[M - 7H]^{7-}$ ions derived from several 20-residue oligomers: 5′-d$(A)_{20}$-3′, 5′-d$(C)_{20}$-3′, 5′-d$(T)_{20}$-3′, and 5′-d$(CGGG)_5$-3′, respectively. Each oligonucleotide shows as major fragmentation processes resulting from ion/ion reactions with oxygen cations, the loss of a base as well as formation of w_{19} ions via loss of the 5′ base and sugar. PolydA (Fig. 14a) and PolydC (Fig. 14b) homopolymers show w- (or d-), z-, and some low abundance (a_n-B_n) fragment ions in series. In contrast to the results from 5′-d$(A)_{20}$-3′ and 5′-d$(C)_{20}$-3′, 5′-d$(T)_{20}$-3′ (Fig. 14c) and 5′-d$(CGGG)_5$-3′ (Fig. 14d) show no evidence of z-, w- (except w_{19}), or a-B fragment ions. Although general conclusions regarding the degree of fragmentation from electron transfer as a function of oligonucleotide sequence require the observation of results from a wider variety of oligonucleotides, some preliminary observations can be made. The polydA and polydC homopolymer anions tend to fragment more than the polydT and mixed-base CG oligomer anions, which does not correlate directly with the order of the proton affinities of the nucleobases (G > A > C > T). These results suggest that nucleobase proton affinity is not likely to be a dominant factor in determining the degree to which fragmentation ensues as a result of electron transfer involving oligonucleotide anions. However, further studies are needed to determine the contribution of oligonucleotide anion sequence composition to fragmentation with electron transfer (Wu & McLuckey, 2003).

3. *Cation Attachment*

Analogous to anion attachment to multiply charged positive ions, cation attachment to multiply charged negative ions has also been observed. The reaction can be generically represented as:

$$(M - nH)^{n-} + Y^{+} \rightarrow (M - nH + Y)^{(n-1)-} \qquad (12)$$

The first reported case of cation attachment resulted from the reaction between deprotonated oxidized bovine insulin anions and various pyridine cations (McLuckey et al., 1996). More recently, cation attachment has been reported in reactions between Fe^{+} ions and insulin anions and in reactions between bradykinin cations and $FeCO_2^{-}$ (Payne & Glish, 2001).

Ion/ion attachment in reactions between singly protonated leucine enkephalin (YGGFL) ions and oligonucleotide anions has also recently been reported. An example involving the reaction between a $[M - 4H]^{4-}$ ion derived from an oligonucleotide 12-mer and $[M + H]^{+}$ of leucine enkephalin (LE) is illustrated in Figure 15a. $[M + LE - 3H]^{3-}$ is the dominant product in this reaction, showing the attachment of leucine enkephalin to the oligomer. $[M - 3H]^{3-}$ is also a major product as the result of single-proton transfer. The appearance of $[M + 2LE - 2H]^{2-}$ and $[M - 2H]^{2-}$ ions are indicative of

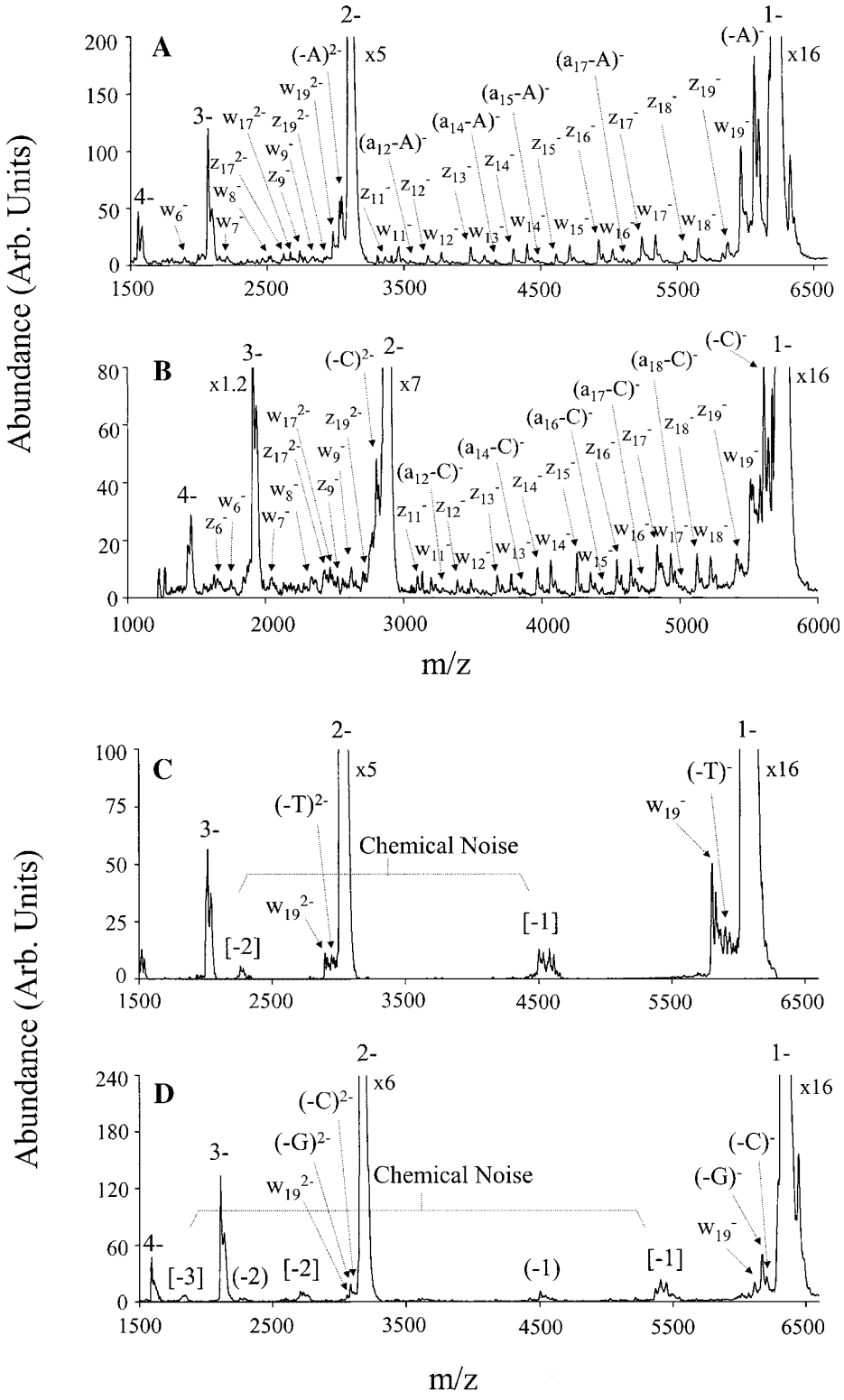


FIGURE 14. Ion/ion reaction product ion spectra of $O_2^{\bullet+}$ with $[M-7H]^{7-}$ of (**A**) 5′-d$(A)_{20}$-3′, (**B**) 5′-d$(C)_{20}$-3′, (**C**) 5′-d$(T)_{20}$-3′, and (**D**) 5′-d$(CGGG)_5$-3′. The vertical scale is expanded by a factor of 16 to highlight low-abundance ions. Reprinted from Int J Mass Spectrom, Vol. 228, Wu J, McLuckey SA, Ion/ion reactions of multiply charged nucleic acid anions: Electron transfer, proton transfer, and ion attachment, pp 577–597. Copyright 2003 with permission from Elsevier.

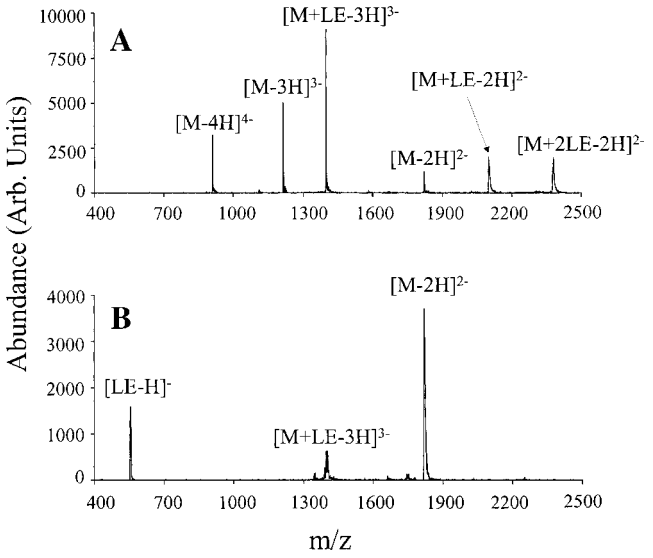


FIGURE 15. (**A**) Ion/ion reaction product ion spectrum of $[M-4H]^{4-}$ of the 12-mer with $[M+H]^{+}$ leucine enkephalin (LE). (**B**) MS/MS spectra of $[M+LE-3H]^{3-}$ isolated from (A). Reprinted from Int J Mass Spectrom, Vol. 228, Wu J, McLuckey SA, Ion/ion reactions of multiply charged nucleic acid anions: Electron transfer, proton transfer, and ion attachment, pp 577–597. Copyright 2003 with permission from Elsevier.

sequential attachment and proton transfer reactions, respectively. $[M+LE-2H]^{2-}$, which can be formed from $[M-3H]^{3-}$ and $[M+LE-3H]^{3-}$ via LE attachment and proton transfer, respectively, is also present. Since LE is not expected to bind specifically with the 12-mer in solution, it is unlikely that cation attachment in this case is "specific" in the biological sense. Fragmentation of the $[M+LE-3H]^{-}$ ions (Fig. 15b) under ion trap CID conditions shows two main complementary product ions, $[M-2H]^{2-}$ and $[LE-H]^{-}$. No $[M-3H]^{3-}$ product ions are observed from the loss of neutral LE. This result suggests that either the $[M-3H]^{3-}$ anions in Figure 15b were not formed via a long-lived excited $[M+LE-3H]^{3-*}$ complex (see discussion of ion/ion reaction dynamics) or that the time frames and energies of complex decomposition under ion trap collisional activation conditions are sufficiently different from those associated with the break-up of a chemically bound complex under ion/ion reaction conditions that significantly different product ion distributions are observed (Wu & McLuckey, 2003).

C. Multiply Charged Positive Ions/Multiply Charged Negative Ions

A significant increase in the number of studies involving multiply charged positive ions in reaction with multiply charged negative ions have been reported since the original review. Some early experiments using the Y-tube reactor combined multiply charged ions of opposite polarities, each formed by electrospray. Relatively little information about these reactions could be derived, however, due to the limited mass-to-charge range of the analyzer. Recently, multiply charged positive ions have been reacted with multiply charged negative ions in quadrupole ion trap instruments (Wells, Chrisman, & McLuckey, 2001). In these experiments, two general categories of reaction phenomenologies have been observed: proton transfer reaction and complex formation.

1. Proton Transfer

Both single and multiple proton transfers in a single encounter have been observed when reacting multiply charged positive ions with multiply charged negative ions. Proton transfer between multiply charged ions where x is the number of protons transferred, can be written generically as:

$$(M+nH)^{n+} + (Y-mH)^{m-} \rightarrow (M+(n-x)H)^{(n-x)+} + (Y-(m-x)H)^{(m-x)-} \quad (13)$$

An example of ion/ion reactions between positive and negative ubiquitin (U) ions is shown in Figure 16. When 7+ ions are reacted with 5− ions of ubiquitin (Fig. 16a), several proton transfer products are observed: viz., $(U)^{4+}$, $(U)^{3+}$, $(U)^{2+}$, and $(U)^{1+}$. There is also evidence of complex formation from the appearance of the abundant product at the nominal mass-to-charge ratio of $(U)^{1+}$. The relative magnitude of this signal suggests that it is likely due, at least in part, to $(2U)^{2+}$ ions (definitive evidence for complex formation has been obtained from experiments in which mixed dimers were formed as well as odd charge states of homodimers). The extent of proton transfer relative to complex formation is dependent upon the charge states of the reactants. For example, the results of the reaction of the

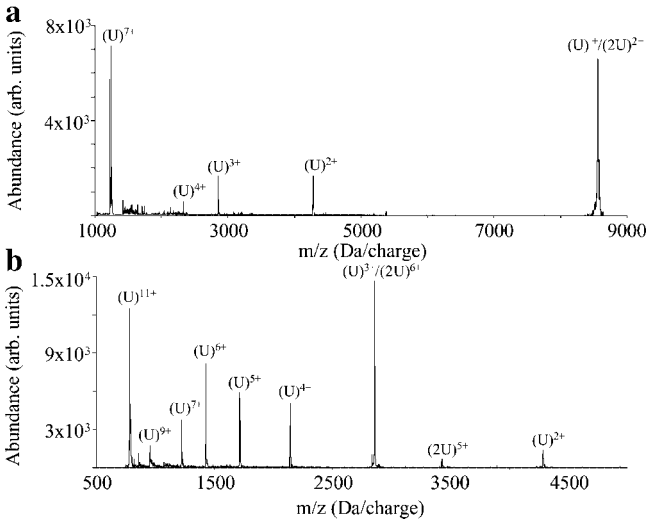


FIGURE 16. Ion/ion reaction spectra from the reactions between (**a**) 7+ and 5− ubiquitin (U) with 120 ms reaction time and (**b**) 11+ and 5− ubiquitin with 80 ms reaction time. Reproduced from Wells et al. with permission from J Am Chem Soc 2003, 125, 7238–7249. Copyright 2003 American Chemical Society.

11+ ubiquitin charge state with 5− ions of ubiquitin (U) are shown in Figure 16b. Multiple proton transfer products are apparent in this spectrum. Complex formation is also apparent from the appearance of the $(2U)^{6+}$ product. (The $(2U)^{6+}$ and U^{3+} ions cannot be distinguished on the basis of the mass-to-charge measurement. However, the abundance of the signal relative to those of the proton transfer products indicates that the signal is likely to be comprised of a mixture of the two species.) Most protein and charge state combinations studied to date in a quadrupole ion trap mass spectrometer have shown complex formation in conjunction with proton transfer. The degree of proton transfer tends to increase with the total absolute charge associated with the reactants. Proton transfer and complex formation products are formed by competitive processes. Both types of products arise from single ion/ion encounters as shown experimentally by Wells, Chrisman, and McLuckey (2003).

2. *Complex Formation*

The second phenomenology observed in reactions between multiply charged positive ions and multiply charged negative ions is complex formation. Complex formation can be represented generically as:

$$(\mathrm{M} + n\mathrm{H})^{n+} + (\mathrm{Y} - m\mathrm{H})^{m-} \rightarrow (\mathrm{M} + \mathrm{Y} + (n-m)\mathrm{H})^{(n-m)+} \quad (14)$$

The extent of products from proton transfer and complex formation is dependent upon the charge states of the reactant ions (see above) and the reactant identities (Wells, Chrisman, & McLuckey, 2003). An example of how reactant ion identities affect the extent of complex formation is shown in Figure 17.

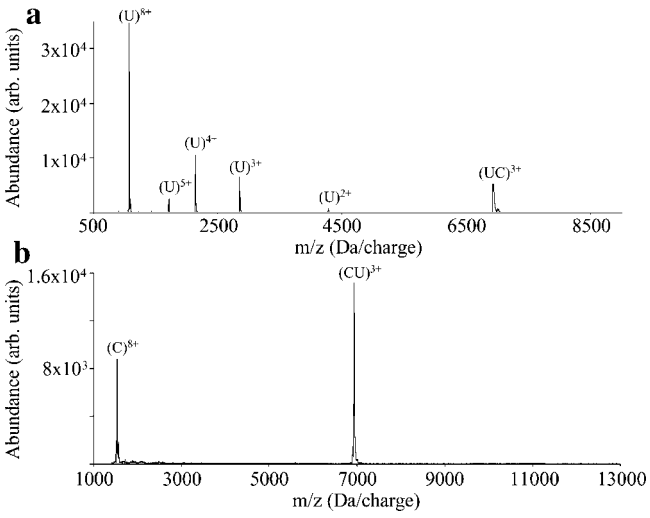


FIGURE 17. Ion/ion reaction between (**a**) 8+ ubiquitin (U) and 5− cytochrome *c* (C) and (**b**) 8+ of C and 5− of U. Reaction time was 150 ms. Reproduced from Wells et al. with permission from J Am Chem Soc 2003, 125, 7238–7249. Copyright 2003 American Chemical Society.

When 8+ ions of ubiquitin (U) are reacted with 5− ions of cytochrome *c* (C), proton transfer ($(U)^{5+}$, $(U)^{4+}$, $(U)^{3+}$, and $(U)^{2+}$) and complex formation $(UC)^{3+}$ are observed (Fig. 17a). In Figure 17b, the identities of one of the reactants are changed to C^{8+} and U^{5-} ions of ubiquitin under the same conditions. This reaction shows primarily complex formation $(CU)^{3+}$. This clearly demonstrates that structural or chemical aspects of ions, such as physical cross-section or gas-phase basicity, play a role in determining the relative contribution of proton transfer and complex formation (Wells, Chrisman, & McLuckey, 2003).

IV. REACTION DYNAMICS

The correlation between ion/ion reaction rate constant and ion charge state for multiply charged ions has been previously studied. Under conditions in which there is a large excess of singly charged ions such that pseudo first-order kinetics are obtained, the rate of proton transfer to singly charged negative ions from multiply charged positive ions has shown a charge-squared dependence (Stephenson & McLuckey, 1996a). This is consistent with the formation of a stable ion/ion orbit as the rate-determining step for eventual reaction. A rate constant for the formation of such an orbiting complex under the influence of the long-range attractive Coulomb potential, k_c, is given by:

$$k_c = v\pi\left[\frac{Z_1 Z_2 e^2}{4\pi\varepsilon_0 \mu v^2}\right]^2 \quad (15)$$

where v is the relative velocity of the oppositely charged ions, Z_1 and Z_2 are the cation and anion charges, respectively, e is the electron charge, ε_0 is permittivity of vacuum, and μ is the reduced mass of the collision pair. This model assumes that because these reactions are highly exothermic, the oppositely charged ions will eventually react upon formation of a complex. However, this model is not entirely suitable because the long range $1/r$ attraction can give rise to a stable orbiting complex between the oppositely charged ions at values of r that are significantly larger than distances normally associated with chemical reactions (e.g., proton transfer, electron transfer, and complex formation). Once the ions are in orbit, r must somehow be reduced to a distance where a reaction can occur.

Recent experimental data has provided new insights into ion/ion reaction dynamics from the study of reactions between multiply charged positive and multiply charged negative ions. Two different phenomenologies, proton transfer and complex formation, have been observed from the reaction of multiply charged ions of opposite polarities. The degree to which each of these phenomenologies occurs is variable and has prompted the examination of several different models to account for these observations. A useful model should account for overall reaction rates, protein–protein complex formation, the degree of incomplete proton transfer, the role of total charge, and the role of relative translational energy. Three different models have been considered: hard-sphere collision, proton transfer during a transient encounter, and the formation of bound orbits. The latter model was found to best account for the experimental observations (Wells, Chrisman, & McLuckey, 2003).

A. Bound Orbit Model to Account for Magnitudes and Z Rate Dependence

A "three-body" interaction, first suggested by Thomson (1924) to account for ion/ion reaction kinetics, appears to provide at least a semi-quantitative description of ion/ion reactions under the conditions used in the quadrupole ion trap. This model postulates the formation of a bound orbit, with a range of eccentricities, for the oppositely charged ions, if the initial relative translational energy from the ionic reactant pair is removed when the reactants are at a critical distance, d_{orbit}. The maximum cross-section for the bound orbit formation ($\sigma_{orbit,\max}$) has a charge-squared dependence, and is defined as (Mahan, 1973):

$$\sigma_{orbit,\max} \approx \pi d_{orbit}^2 \approx \frac{4\pi Z_1^2 Z_2^2 e^4}{4\pi\varepsilon_0(\mu v^2)^2} \qquad (16)$$

The actual value of $\sigma_{orbit,\max}$ is also dependent on the probability that adequate relative translational energy is removed when the ionic reactant pair is at a critical distance. The mechanism of removal of such translational energy in the case of structureless ions is via collisions with a third-body. However, a bound orbit involving ions with internal structure, such as protein ions, could be formed in the absence of a third-body, if tidal effects are significant. The relatively large number of internal degrees of freedom in the large ions involved in these reactions can provide a repository for the translational energy of the reactant pair, and this is most likely to occur as the orbital eccentricity increases. Tidal processes (Bates & Morgan, 1990; Morgan & Bates, 1992) can potentially contribute to the efficiency of bound orbit formation in the absence of a third-body. In the case of multiply charged macro-ions, a tidal effect could result from intramolecular proton transfer resulting from the electric field of the oppositely charged ion as the two species approach and recede from one another in an eccentric orbit. The net effect for such a process would be vibrational excitation of the ions and a decrease in relative translational energy. Collisions and tidal effects can both potentially contribute under the conditions used to date to study ion/ion reactions of multiply charged ions (Wells, Chrisman, & McLuckey, 2003).

B. Proton Transfer vs. Complex Formation

The formation of a bound orbiting complex accounts for the charge dependence and the magnitudes of the rates, at least within the error associated with estimation of ion densities. The shapes of the orbit are likely to play a major role in determining product ion partitioning between complex formation and proton transfer. Elliptical orbits allow for the reactants to come into close proximity at high relative velocities. Provided the orbit does not bring the reactants into physical contact, there is the possibility that one or more protons can transfer, giving rise to a significantly smaller attractive potential. In this scenario, the two ions may retain sufficient relative translational energy to escape their mutual attraction. Ions that do not escape their mutual attraction eventually collide with one another thereby increasing the likelihood of complex formation. The degree to which each of these processes is observed is variable, depending upon the shapes of orbits, the physical size of the ions, ion charge states, binding strengths of charge sites, etc. When elliptical orbits bring the reactants close together (but not close enough for a physical collision), the ions tend to interact at high kinetic energy (i.e., highly eccentric orbits), which results in more proton transfer and less formation of a chemically bound complex. When ions interact at low kinetic energy (i.e., low eccentricity orbits), the probability for escape from the attractive potential is minimized and the orbit will tend to collapse due to collisions with neutral species and tidal effects. This collapse may allow for additional proton transfers or the formation of a chemically bound complex. The likelihood of escape of proton transfer products is predicted to increase as the total charge of the products decreases because of the decrease in the attractive Coulombic forces. When the reactants approach distances that are conducive to physical collisions, complex formation is most likely via either bound or unbound orbits (Wells, Chrisman, & McLuckey, 2003). The two scenarios described are pictorially represented in Figure 18a (high eccentricity orbit) and Figure 18b (low eccentricity orbit).

Of the models thus far investigated, only the stable orbiting complex model predicts product ion relative abundances that reflect, at least qualitatively, experimental results using physically reasonable rate constants. A kinetic scheme consistent with the orbiting complex model for the reaction between cytochrome c $[M+8H]^+$ and $[M-5H]^{5-}$, depicted here as $C^{8+}+C^{5-}$, is shown in Scheme 1. In this picture, the formation of a Coulombically bound complex ($[C^{8+}$–$C^{5-}]$), $k_{capture}$, is the rate-limiting step and the abundances of the various products are determined by the relevant rate constants associated with proton transfer and the rate constants for subsequent escape from a stable orbit or collapse into a protein complex. The reader is referred to the original publication for details about how the individual rate constants were estimated (see Wells, Chrisman, & McLuckey, 2003). The key result is that this picture predicts a distribution of proton transfer products with a maximum abundance corresponding to an intermediate number of transferred protons, as well as protein complex formation.

V. APPLICATIONS

Ion chemistry plays a central role in many applications of mass spectrometry. Although ion/ion chemistry involving relatively high-mass ions is a relatively new field, several applications have already been explored. Ion/ion proton transfer reactions, for example, have been used to simplify the complexity associated with electrospray of mixtures of macromolecules by reducing spectral overlap due to ions of different mass and charge but similar mass-to-charge ratios. Ion/ion reactions have also been shown to be useful in simplifying product ion spectra from unimolecular dissociation of multiply charged ions, in concentration and purification of parent ions, and in other applications. We focus here predominantly on applications involving proton transfer reactions, because they are by far the most mature. Most applications reported thus far involve charge state manipulation. Proton transfer reactions have played a particularly important role in these applications. All of the applications described in this section have been developed on quadrupole ion trap mass spectrometers, unless otherwise noted.

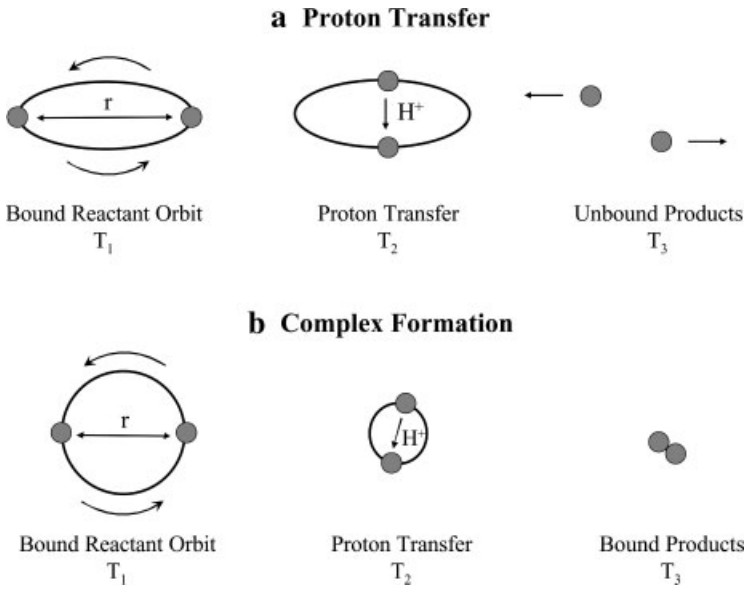
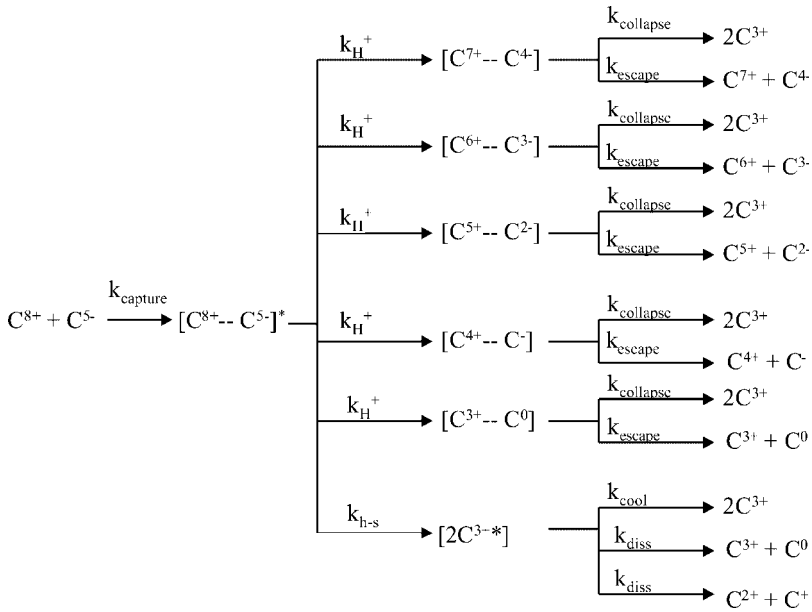


FIGURE 18. Schematic representation of the two possible processes resulting from a bound reactant orbit (**a**) proton transfer (high kinetic energy), and (**b**) complex formation (low kinetic energy). T = time.

A. Charge State Manipulation—Proton Transfer

The use of ion/ion reactions to manipulate multiply charged ions has found several applications. Proton transfer reactions are particularly useful in altering the charge states of parent ions to form charge states not directly accessible from ESI and to concentrate ions into a particular charge state for tandem mass spectrometry (MS/MS) analysis. Proton transfer reactions are also useful for simplifying product ion spectra by reducing product ion charge states to predominantly singly charged species thereby allowing ions with different masses but similar initial *m*/*z* to be distinguished form one another. Ion/ion reactions have also been shown recently to play a central role in a strategy designed to increase the net charge of an ion.



SCHEME 1. "Orbital Complex" Kinetic Scheme. Reproduced from Wells et al. with permission from J Am Chem Soc 2003, 125, 7238–7249. Copyright 2003 American Chemical Society.

1. Precursor Ion Manipulation

a. Macromolecule mixtures. The first analytical application of ion/ion proton transfer reactions was for the simplification of electrospray mass spectra of protein mixtures (Stephenson & McLuckey, 1996b). This application has been demonstrated both using an ion trap and with ion/ion reactions at atmospheric pressure followed by TOF mass analysis (Scalf et al., 1999; Ebeling et al., 2000, 2001; Scalf, Westphall, & Smith, 2000). Application of this approach to oligonucleotide mixtures has also been described (Scalf et al., 1999; Ebeling et al., 2000, 2001; Scalf, Westphall, & Smith, 2000; McLuckey, Reid, & Wells, 2002a). The charge-squared dependence of the reaction kinetics is particularly important in this application because it gives rise to the desirable situation that a single ion/ion reaction period can be used to reduce the charges of all mixture components largely to the +1 or −1 charge state with relatively little differential neutralization. The net effect is to convert an electrospray mass spectrum into one that resembles a MALDI mass spectrum. Such ion/ion chemistry has recently been used to analyze intact proteins for expected site-directed mutagenesis products. Figure 19a shows an example of an electrospray mass spectrum of a purified site-directed mutant overexpression product. Figure 19b shows a post ion/ion reaction mass spectrum of the protein mixture in Figure 19a reacted with PDCH anions. From the post ion–ion reaction mass spectrum, the various components of the protein mixture can be determined, including the correct site-directed mutagenesis product and degradation of the N-terminus of the correct site-directed mutagenesis product (VerBerkmoes et al., 2002).

As mentioned above, the range of masses that can be accommodated by this approach in the ion trap is limited by the range of mass-to-charge ratios that can be stored simultaneously. In particular, the singly charged proton transfer reagent must be stored while the analyte ions are converted to singly charged ions. The trapping well becomes increasingly shallow as the mass-to-charge ratio of the analyte ions increases and the point is eventually reached where trapping conditions are insufficient to prevent escape of the high mass ions. This limitation is absent in the case of ion/ion reactions conducted in a high pressure ion source. However, it can be a serious limitation in the ion trap when high mass reagents are unavailable for proton transfer reactions. In the case of protein mixtures, singly charged protein ions of mass-to-charge ratio as high as 150,000 have been observed using anions derived from PDCH (Reid et al., 2003). It is straightforward to form relatively high mass anions in a gas-type source, such as a glow discharge, due to the high volatilities and large electron capture cross-sections of perfluorocarbons. An analogous situation does not prevail for high mass negatively charged analytes, such as oligonucleotides ionized in negative ESI. That is, it is more challenging to form abundant high mass cations in a gas-type source than it is to form high mass anions. An approach to overcome this problem, referred to as ''trapping by proxy,'' was developed since the last review.

It was found that it is possible to store high mass ions using the electric field of low mass ions of opposite polarity when the low mass ions are stored by an electrodynamic quadrupole field (McLuckey et al., 2002b). That is, provided a sufficient number of low mass ions can be formed to create the necessary electric field, the ion trap can be used to store the low mass ions which, in turn, prevent high mass ions of opposite polarity from escaping the ion trap even when the trapping potential of the ion trap itself is too weak to store the high mass ions. A representation of this ''trapping by proxy'' approach is shown in Figure 20. The positive ions are stored in the usual way via the oscillatory quadrupole field in the ion trap, and the negative DNA ions are stored by the electric field of the positive ions. This approach has been demonstrated with negatively charged oligonucleotide mixtures using the cationic charge transfer reagents $O_2^{+\bullet}$ and $C_4H_9^+$, each formed via glow discharge. In the former case, electron transfer was the means for charge state reduction whereas in the latter case, proton transfer was the mechanism for charge state reduction. Figure 21a shows the post-ion/ion mass spectrum that resulted from the application of trapping by proxy with $O_2^{+\bullet}$ to a population of multiply charged anions derived from a 40- to 60-mer mixture of polyadenylate oligomers (i.e., 5′-pd$(A)_{40-60}$-3′). It was demonstrated that extensive fragmentation accompanied the charge state reduction using $O_2^{+\bullet}$, which is the origin of most of the signals that appear below that of the singly charged 40-mer. When trapping by proxy was effected with protonated isobutylene, much less fragmentation was noted, as illustrated in the spectrum of Figure 21b. This work demonstrated that the trapping by proxy approach is an option when suitable high mass charge transfer reagents are unavailable (McLuckey et al., 2002b).

b. Parent ion charge state manipulation. ESI produces a distribution of ion charge states dependent on the composition of the molecules of interest and the ionization/interface conditions (Wang & Cole, 1997). As macromolecules increase in size, the average charge state tends to increase and ions of lower charge states decrease in abundance. It is sometimes desirable to be able to study the widest range of charge states possible because the charge state of an ion plays a central role in its chemistry, particularly in its gas-phase fragmentation behavior. Several approaches have been taken to alter the charge states of ions formed from ESI, including the manipulation of solution and electrospray interface conditions (Muddiman et al., 1996) and ion/molecule proton transfer reactions (McLuckey, Van Berkel, & Glish, 1990; McLuckey, Glish, & Van Berkel, 1991; Williams, 1996; Cassady & Carr, 1997). While ion/ion proton transfer reactions are less straightforward to implement than changes of solution and electrospray conditions or ion/molecule proton transfer reactions, they have several distinct advantages for charge state manipulation. Unlike modification of solution and electrospray conditions, ion/ion proton transfer reactions can be optimized independently of the ionization step. Furthermore, proton transfer ion/ion reactions also have the advantage of being able to form ions of lower charge states that are free of adducts (given an appropriate choice of charge transfer reagent ions) than can ion/molecule proton transfer reactions.

Ion/ion reactions are capable of providing a controlled means for charge state reduction of multiply charged precursor ions to form lower charge state precursor ions that are not initially produced from ESI. Due to the exothermic nature of ion/ion reactions, charge state reduction by proton transfer is highly efficient and allows for an arbitrary degree of charge state

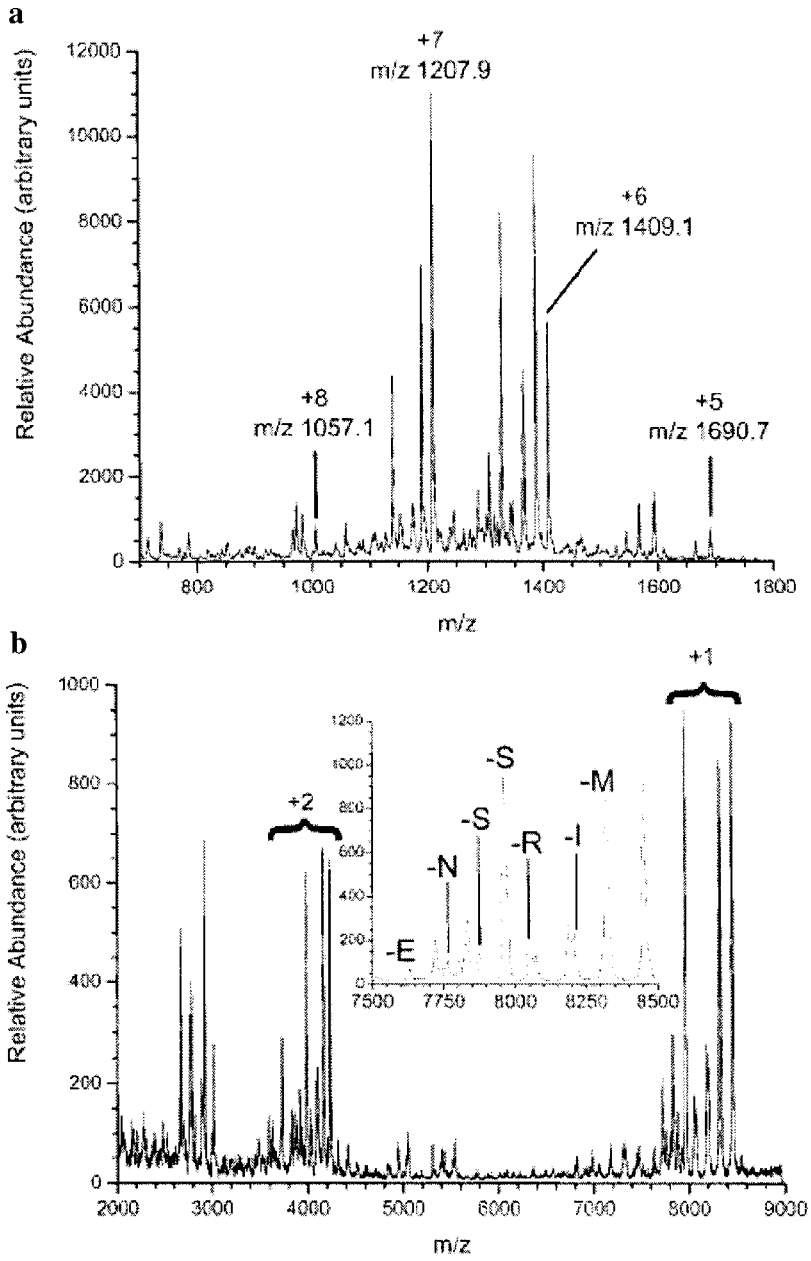


FIGURE 19. (**a**) Electrospray mass spectrum of purified site-directed mutagenesis overexpression product. (**b**) Post ion/ion reaction mass spectrum of (a). Reprinted from Anal Biochem, Vol. 305, VerBerkmoes NC, Strader MB, Smiley RD, Howell EE, Hurst GB, Hettich RL, Stephenson JL, Jr., Intact protein analysis for site-directed mutagenesis overexpression, pp 68–81. Copyright 2002 with permission from Elsevier.

reduction. The ability to access lower charge states than those produced directly from ESI can produce precursor ions not formed from the initial charge state distribution. When these lower charged precursor ions are subjected to collisional activation, different fragments may arise than those produced from CID of the more highly charged precursor ions. An example of this is shown in Figure 22. In Figure 22a, $[M+12H]^{12+}$ bovine ubiquitin ions have been isolated directly from the initial electrospray charge state distribution and subjected to CID. The fragments were then subjected to proton transfer reactions to simplify the product ion mass spectrum. Figure 22b shows the product ion spectrum derived from bovine ubiquitin $[M+4H]^{4+}$ ions that were formed from higher charge states using proton transfer ion/ion reactions, subjected to CID, and followed by ion/ion proton transfer reactions for product ion charge reduction. By comparing these two spectra, one can observe various fragments in the $[M+4H]^{4+}$ ions dissociation (i.e., b_{32}^{+}, b_{34}^{+}, b_{39}^{+}, etc.) that were not observed from the fragmentation of the

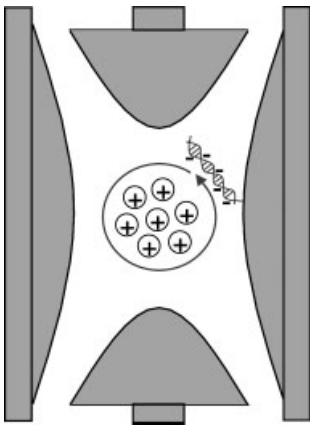


FIGURE 20. Representation of trapping by proxy. Positive ions are stored by the electrodynamic ion trap's radio-frequency field. Negative ions are stored by the electric field of the positive ions.

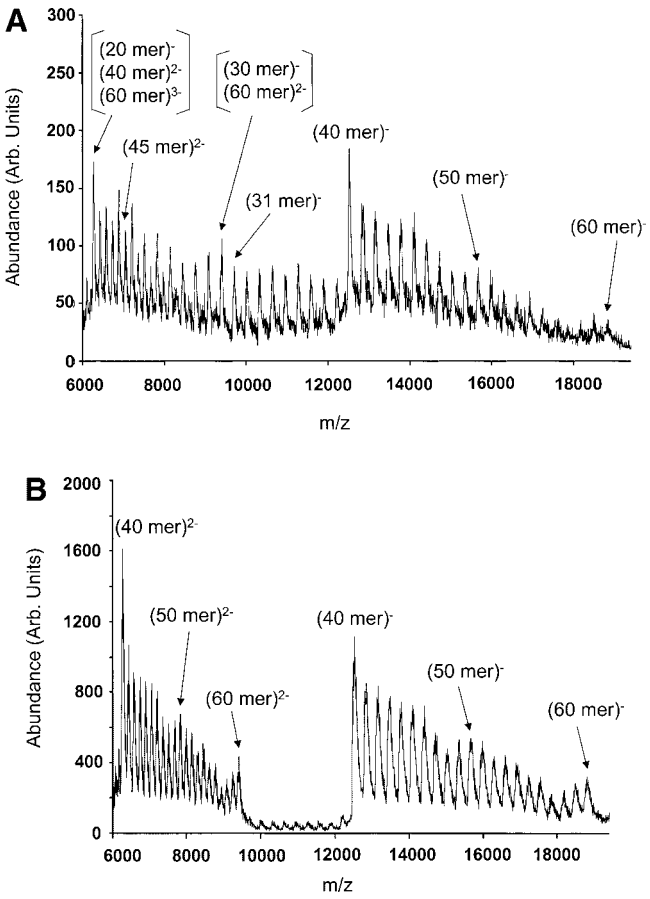


FIGURE 21. Ion/ion reaction spectra between a 5′-pd(A)$_{40-60}$-3′ mixture and (**A**) $O_2^{\bullet+}$, and (**B**) $C_4H_9^+$. Reproduced with permission from McLuckey et al., Anal Chem 2002b, 74, 976–984. Copyright 2002 American Chemical Society.

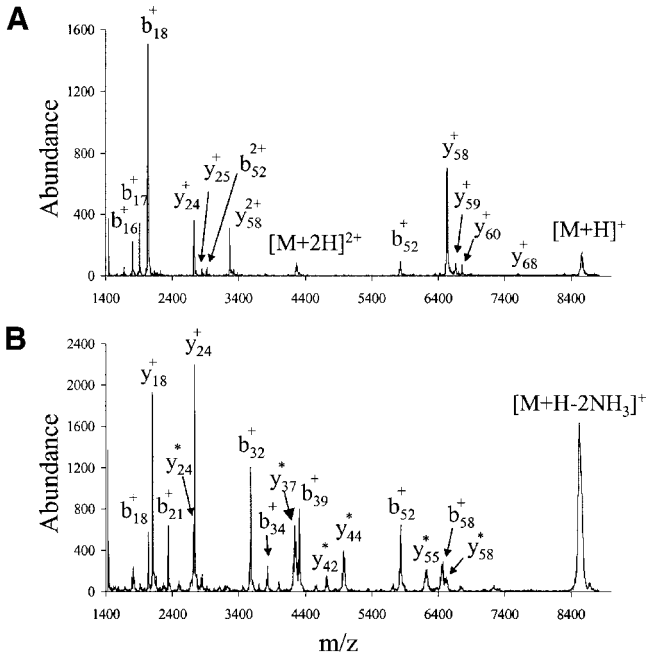


FIGURE 22. Post ion/ion reaction MS/MS spectra of (**A**) $[M + 12H]^{12+}$ ubiquitin and (**B**) $[M + 4H]^{4+}$ ubiquitin. Reproduced from Reid et al. with permission from Anal Chem 2001, 73, 3274–3281. Copyright 2001 American Chemical Society.

$[M + 12H]^{12+}$ ions. The activation of lower charge states (+4 in this example) can allow for more fragmentation channels to be observed in the CID of protein ions and therefore provide more information, which can be advantageous in protein identification applications. In particular, dissociations of lower charge state protein ions have been shown to lead to preferred cleavages at sites C-terminal to aspartic acid (Reid et al., 2001; Hogan & McLuckey, 2003a; Pitteri, Reid, & McLuckey, 2004). Knowledge of such preferred cleavages can be incorporated into database searches to increase confidence in protein ion identification (Reid et al., 2002a).

A useful technique that provides a degree of control over ion/ion reaction product ion charge states, referred to as ''ion parking,'' has recently been described (McLuckey, Reid, & Wells, 2002a). This technique involves the application of a dipolar resonance excitation voltage to the end-caps of an electrodynamic ion trap mass spectrometer. This results in the selective inhibition of the ion/ion reaction rate for ions in the mass-to-charge range that corresponds to the excitation frequency (McLuckey, Reid, & Wells, 2002a). One application of ion parking involves its ability to concentrate ions into a particular charge state, which is illustrated in Figure 23. The initial electrospray distribution of bovine serum albumin (BSA) cations is shown in trace (a). Anions (from PDCH) underwent a mutual storage time with the BSA cations during which proton transfer reactions took place to form a lower charge state distribution shown in trace (b). Trace (c) shows the results of an ion parking experiment in which a resonance ejection frequency was applied to the end-caps of the ion trap mass spectrometer

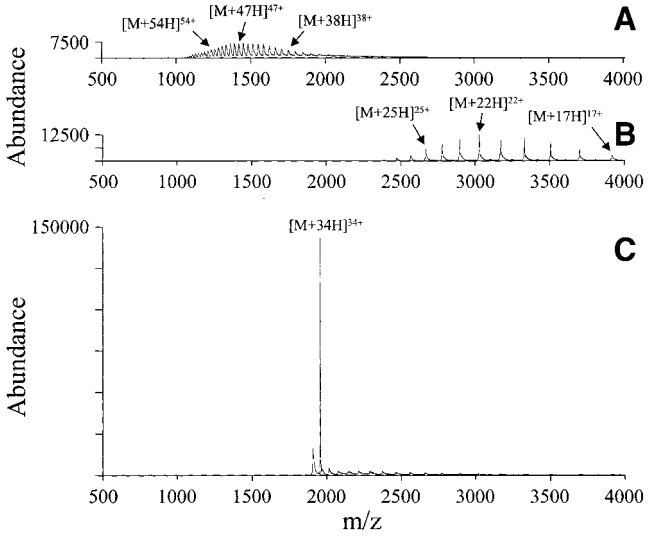


FIGURE 23. Mass spectra of bovine serum albumin: (**A**) native electrospray distribution, (**B**) after charge reduction by proton transfer ion/ion reactions, and (**C**) ion parking mode (18 kHz). Reprinted from Int J Mass Spectrom, Vol. 222, Reid GE, Wells JM, Badman ER, McLuckey SA, Performance of a quadrupole ion trap mass spectrometer adapted for ion/ion reaction studies, pp 243–258. Copyright 2003 with permission from Elsevier.

intended to inhibit the ion/ion reaction rate of species with the mass-to-charge ratio of the $[M + 34H]^{34+}$ ion. This experiment effectively concentrates ions from higher charge states ($\sim +54$ to $+38$) into the $[M + 34H]^{34+}$ peak. The concentration of ions into a particular charge state can be applied to the analysis of mixtures of ions from various compounds. The ions of interest are parked into one charge state while the other components in the mixture without a charge state that corresponds in frequency to the resonance excitation frequency continue to be neutralized. Typically, the ion/ion reactions are allowed to proceed to the point where facile ion isolation of the parked species can be effected.

The ion parking technique is also useful for the charge state purification of ions of interest using multiple parking steps. In complex protein mixture analysis, it is possible that a single ion parking step can concentrate several proteins with disparate masses into a single band of mass-to-charge ratios, if each of the proteins has a charge state that matches the frequency of the ion parking signal. Proteins with the same nominal mass-to-charge ratio will have different mass and charge and hence they will overlap in the initial mass spectrum. To deal with this possibility, a sequential ion parking procedure has been developed. This technique was illustrated in the gas-phase concentration, purification, and identification of five proteins in a complex mixture derived from a whole cell lysate fraction of *E. coli* (Reid et al., 2002a). In these experiments, after an ion accumulation period, some of the ions were concentrated into a band of mass-to-charge ratios through the use of proton transfer ion/ion reactions in conjunction with ion parking. Ion isolation was performed on the "parked" ions followed by an additional ion/ion reaction parking step. The second ion parking step dispersed the ions of different mass and charge that were initially present in the first parked ion population and isolation steps helped accomplish the removal of proteins that may overlap in the mass spectra by resolving their charge states (ion parking) and ejecting those not of interest (isolation). These steps resulted in charge state purified protein ions of interest by resolving the charge states of the overlapping ions. A schematic of the described scan function is shown in Figure 24. The concentrated and purified ions of interest were then subjected to CID. The fragments were then subjected to ion/ion reactions to reduce them to predominantly singly charged species and the product ion spectra were searched against a database and ranked using a novel scoring scheme. These experiments resulted in the identification of four of the five proteins. This experiment demonstrates that "top-down" protein identification (analysis of the whole protein without digestion) can be accomplished on proteins from a complex mixture using a relatively low-resolution instrument. Ion/ion proton transfer reactions played a role both in the concentration and charge-state purification of the parent ions and in the identification of the product ions (Reid et al., 2002a).

Gas-phase purification and concentration has also been used for the analysis of a bacteriophage MS2 viral coat protein (He et al., 2002). In these experiments, a single charge state of the protein, $[M + 8H]^{8+}$, was subjected to ion/ion proton transfer reactions and ion parking to accomplish the gas-phase purification and concentration as described previously. The isolated charge state was then subjected to more ion/ion proton transfer reactions to give $[M + 6H]^{6+}$ and $[M + 7H]^{7+}$ ions in addition to the $[M + 8H]^{8+}$ ions. These three charge states were sequentially activated off resonance and the products were reduced to predominantly singly charged species through ion/ion reactions. These experiments allowed for structural information to be obtained from both high and low charge states in a single experiment (He et al., 2002).

2. *Product Ion Manipulation*

As alluded to in the previous section, proton transfer ion/ion reactions can be used to simplify the charge states of product ions resulting from collision-induced dissociation experiments. Product ion spectra resulting from the dissociation of multiply charged parent macro-ions can be challenging to interpret, particularly when it is not possible to resolve product ion isotope spacings. The product ion spectra of highly charged proteins usually consist of fragment ions that surround the parent ion in a relatively small mass-to-charge window. The complexity and congestion of the product ion spectra tends to increase as protein ions increase in mass and charge. Ion/ion proton transfer reactions have proven to be useful for the simplification of such product ion spectra by reducing product ions to predominantly singly charged species using suitable reagents.

An example of the use of ion/ion proton transfer reactions in a quadrupole ion trap mass spectrometer is shown in Figure 25. The $[M + 19H]^{19+}$ charge state of porcine elastase was isolated and subjected to collisional activation as shown in Figure 25A, which cannot be interpreted unambiguously due to spectral

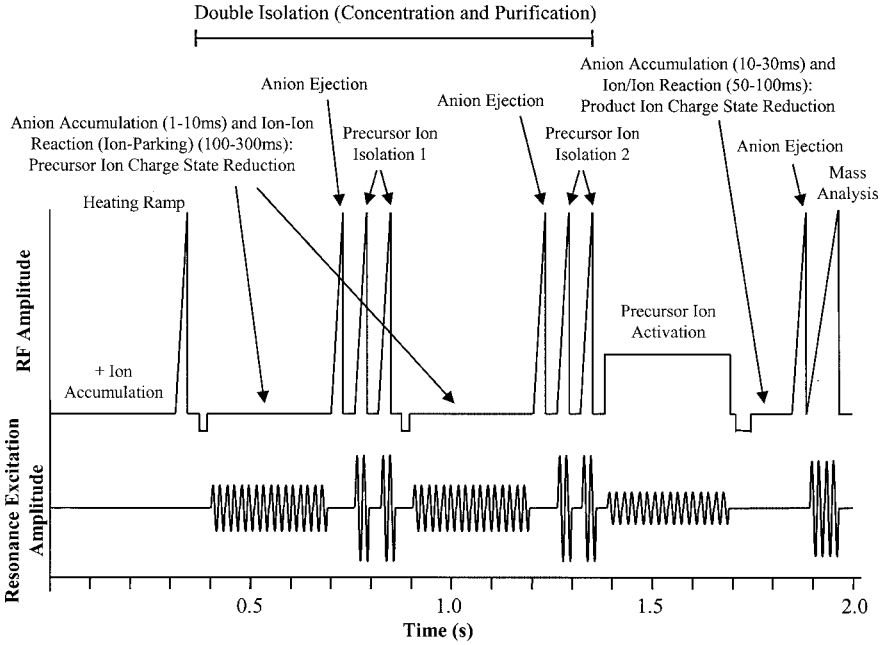


FIGURE 24. Schematic diagram of a quadrupole ion trap scan function used in gas-phase concentration, purification, and dissociation of whole protein ions from complex mixtures. Reproduced from Reid et al. with permission from J Am Chem Soc 2002, 124, 7353–7362. Copyright 2002 American Chemical Society.

congestion. These ions were then subjected to proton transfer reactions with anions from PDCH, which reduced the species to predominantly singly charged ions (as shown in Fig. 25B). Simplification of the product ion spectra in such a manner and distributing the fragment ions over a much larger mass-to-charge range, allows the various *b*- and *y*-type fragment ions to be assigned without charge state ambiguities (Hogan & McLuckey, 2003a). This approach has been applied to a variety of protein ions to date, both in the study of model proteins and in the identification of *a priori* unknown proteins in complex mixtures (Wells, Stephenson, & McLuckey, 2000; Cargile, McLuckey, & Stephenson, 2001; Newton et al., 2001; Wells et al., 2001; Engel et al., 2002; He et al., 2002; Reid et al., 2002a; Reid, Stephenson, & McLuckey, 2002b; Hogan & McLuckey, 2003a; Hogan, Pitteri, & McLuckey, 2003b; Amunagama et al., 2004).

B. Charge Inversion

While proton transfer ion/ion reactions are particularly well-suited to reducing the charge state of an ion, increasing the charge state of an ion is a significantly more challenging task. There are several instances where it would be desirable to increase the charge state of an ion. For example, the detector response in most forms of mass spectrometry is a function of an ion's charge. Because of this charge dependence, increasing the charge of an ion can enhance detector response and hence improve the signal-to-noise ratio. Potential benefits for increasing the charge states of ions could also be seen in the improved resolution in some instruments. For example, in Fourier transform ion cyclotron resonance mass spectrometers, the resolution (defined as M/ΔM) is inversely related to mass-to-charge ratio. Therefore reducing the mass-to-charge ratio by increasing charge can allow the instrument to make a measurement at higher resolving power. In addition to these qualities, the ability to access a variety of parent ion charge states can lead to the study of a wider range of chemistries, as mentioned above. For example, matrix assisted laser desorption ionization forms predominantly singly charged ions which upon collisional induced fragmentation often results in noninformative losses of neutral molecules such as water and ammonia. By increasing the charge states of such molecules, additional charge states of ions are available for study, potentially resulting in a greater variety of structurally informative fragmentation.

A single ion/ion reaction encounter is unlikely to increase the charge state of an ion due to the endothermic nature of such a reaction and the long range Coulombic repulsion in bringing an additional charge to an ion of similar polarity. However, a two-step process can be used to increase ion charge. Experiments have been recently described whereby a doubly protonated peptide can be formed from a singly protonated peptide in the gas-phase using two sequential ion/ion reactions:

$$(\mathrm{M}+\mathrm{H})^{+} + (\mathrm{N}-n\mathrm{H})^{n-} \rightarrow (\mathrm{M}-\mathrm{H})^{-} + (\mathrm{N}-(n-2)\mathrm{H})^{(n-2)-} \quad (17)$$

$$(\mathrm{M}-\mathrm{H})^{-} + (\mathrm{R}+m\mathrm{H})^{m+} \rightarrow (\mathrm{M}+2\mathrm{H})^{2+} + (\mathrm{R}+(m-3)\mathrm{H})^{(m-3)+} \quad (18)$$

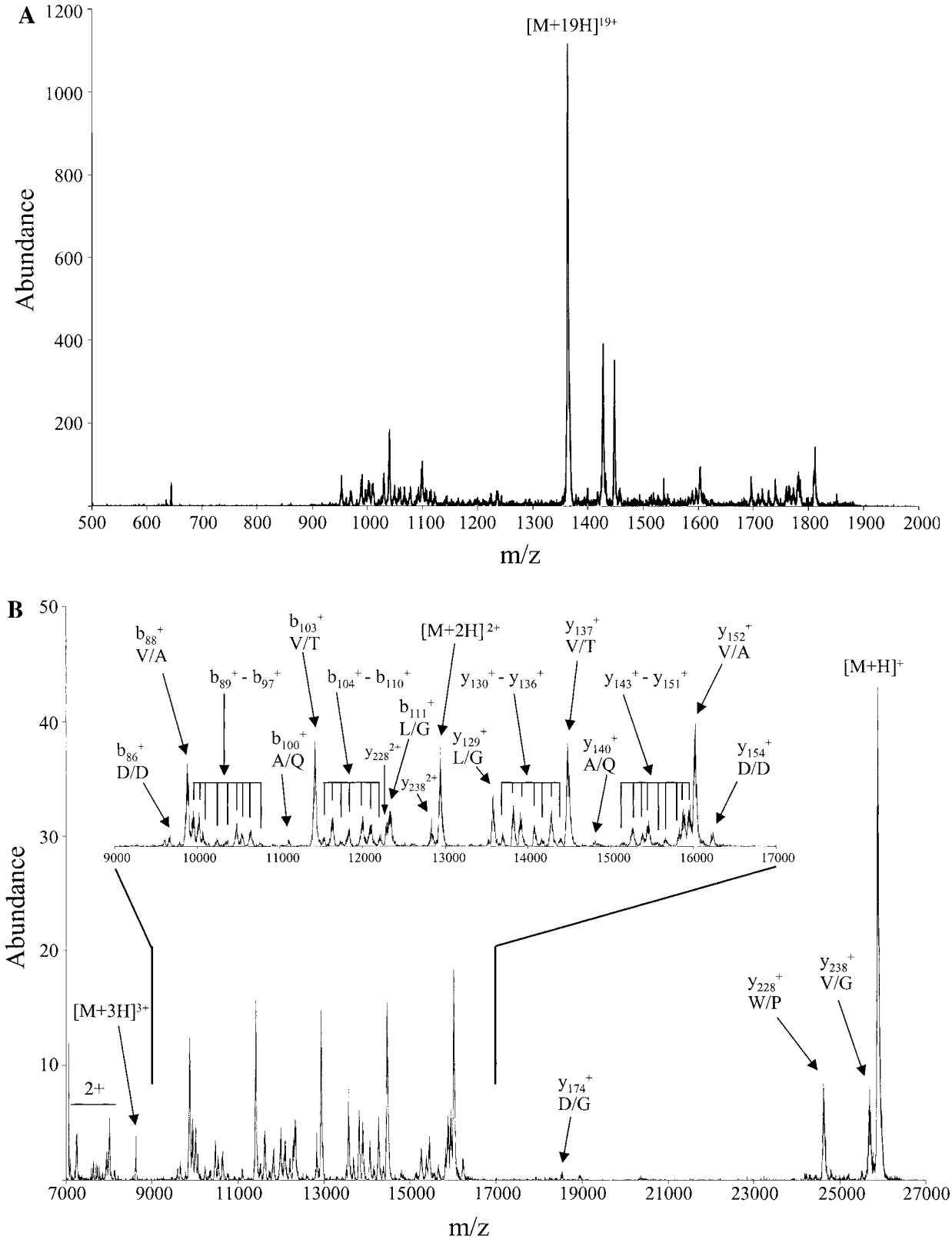


FIGURE 25. MS/MS product ion spectra of $[M + 19H]^{19+}$ porcine elastase (**A**) pre-ion/ion reactions and (**B**) post-ion/ion reactions. Hogan JM, McLuckey SA, J Mass Spectrom 2003, 38, 245–256. Reproduced with permission from John Wiley & Sons Limited. Copyright 2003.

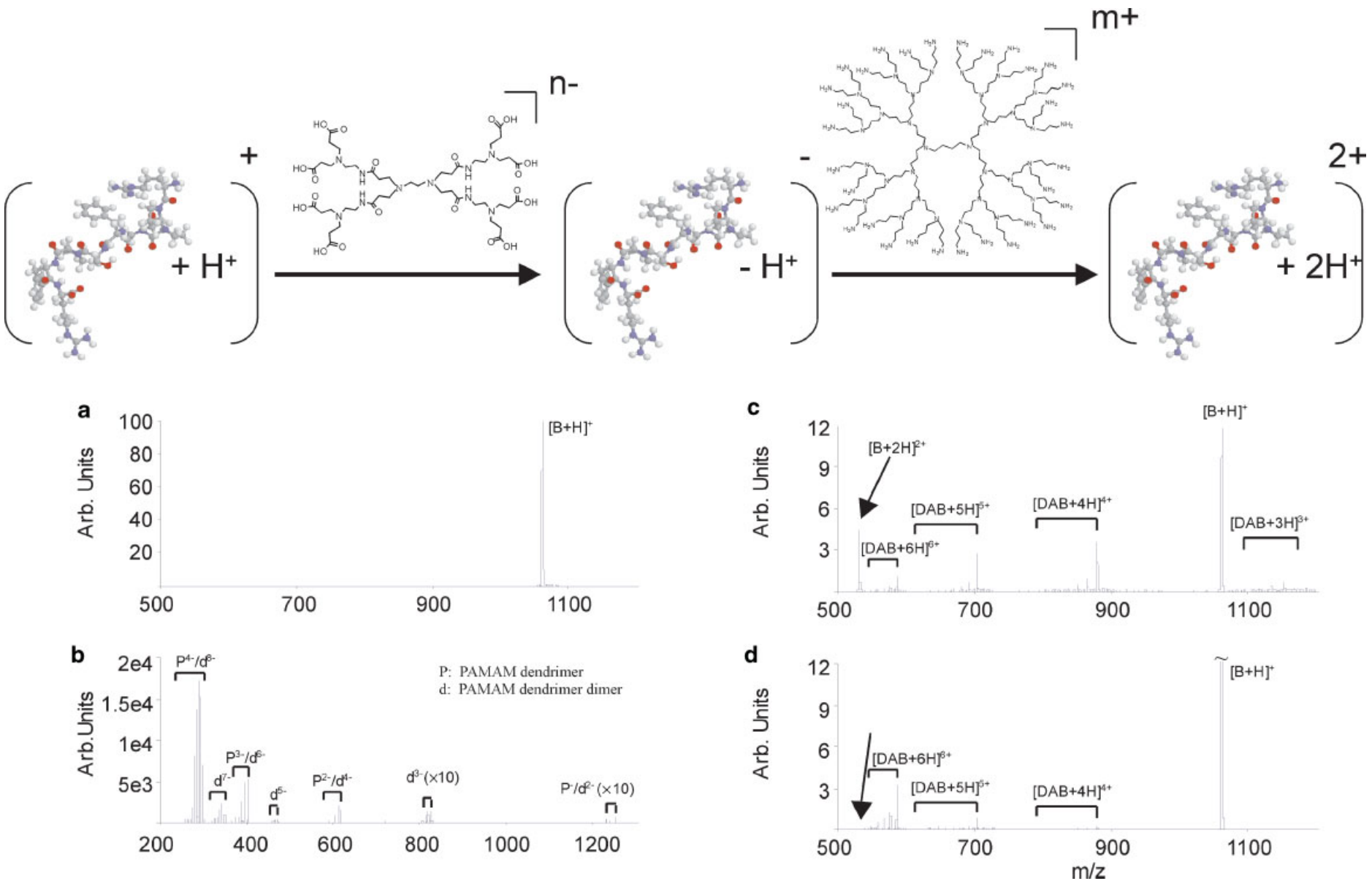


FIGURE 26. (**a**) Positive mass spectrum of isolated $[M+H]^+$ bradykinin. (**b**) Negative mass spectrum of PAMAM dendrimer anions used for the first step of charge inversion. (**c**) Positive mass spectrum for the charge inversion of $[M+H]^+$ bradykinin via reaction with PAMAM dendrimer anions followed by charge inversion of bradykinin $[M-H]^-$ via reaction with DAB cations. (**d**) Positive mass spectrum from the same processes as (c), without the anions admitted into the ion trap. The abundance scales for anions and cations in these spectra are not directly comparable. Adapted from He & McLuckey, J Am Chem Soc 2003, 125, 7756–7757. Copyright 2003 American Chemical Society.

Reaction 17 shows the conversion of a singly protonated peptide ion to a singly deprotonated peptide ion. Reaction 18 shows the conversion of the singly deprotonated peptide to a doubly protonated peptide. An example of such a series of reactions to yield doubly protonated bradykinin from singly protonated bradykinin is shown in Figure 26. A positive ion electrospray mass spectrum is shown of isolated bradykinin $[B+H]^+$ in Figure 26a. A negative ion electrospray mass spectrum of carboxylate-terminated polyamidoamine (PAMAM) dendrimer anions (used as the reactants in this experiment) is shown in Figure 26b. Figure 26c shows a positive ion mass spectrum resulting from the reaction of singly protonated bradykinin cations with PAMAM dendrimer anions (which converts singly protonated bradykinin to singly deprotonated bradykinin) followed by reactions of bradykinin $[B-H]^-$ with DAB dendrimer cations. The net result shows the formation of some doubly charged bradykinin cations $[B+2H]^{2+}$. Figure 26d shows a control experiment where the conditions were the same as those leading to Figure 26c except that no anions were admitted into the ion trap (hence the absence of $[B+2H]^{2+}$) (He & McLuckey, 2003). A similar experiment has also been recently reported to form a doubly deprotonated oligonucleotide from a singly deprotonated oligonucleotide in two subsequent ion/ion reactions (He & McLuckey, 2004a).

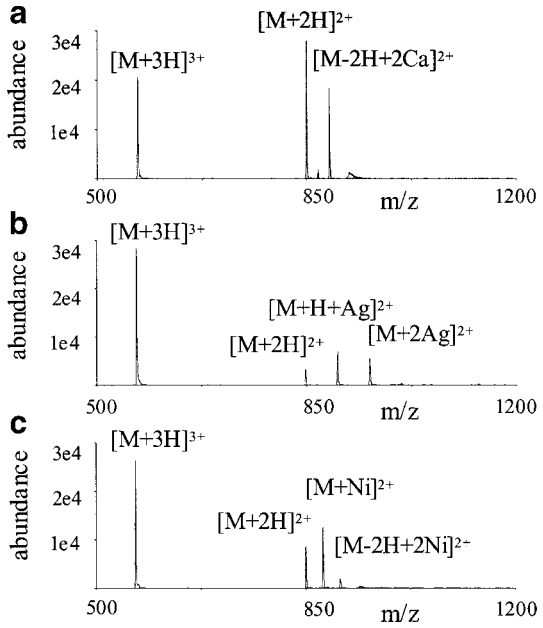


FIGURE 27. Ion/ion reaction spectra between $[M+3H]^{3+}$ *Trp*-11 neurotensin and (**a**) calcium acetate anions, (**b**) silver nitrate anions, and (**c**) nickel acetate anions. Reproduced from Newton & McLuckey with permission from J Am Chem Soc 2003, 125, 12404–12405. Copyright 2003 American Chemical Society.

C. Metal Ion Transfer

One potential ion/ion application involving metal containing ions involved reactions between metal containing salts and protonated peptides/proteins. Metal-cationized peptides/proteins have been generated in the gas phase from multiply protonated species using such reactions. Figure 27a–c shows the product ion mass spectra for cation switching reactions between a triply protonated peptide, *Trp*-11 neutrotensin, and calcium acetate, silver nitrate, and nickel acetate anions, respectively. These spectra show that by reacting the multiply protonated peptide with various metal containing anions, one or more of the peptide's protons can be switched for a metal (Newton & McLuckey, 2003). Similar experiments have shown that the reaction of multiply charged ubiquitin cations with copper nitrate anions can yield the insertion of copper into a gas-phase protein (Newton & McLuckey, 2003). The potential utility of these findings derives from the ability to insert a metal cationizing agent into a peptide independent of the ionization process. This is a desirable capability, for example, when metal cationized species fragment to give complementary information to that obtained from protonated peptides. For example, sodiated peptides have been shown to fragment adjacent to the C-terminal residue, whereas protonated peptides fragment all along the peptide backbone (Lin, Payne, & Glish, 2001). The insertion of sodium into a peptide ion has been demonstrated (Newton & McLuckey, 2003). The ability to switch the cationizing agent in macro-molecules could lead to the ability to obtain complementary fragmentation information by the insertion of various metals without having to resort to use of a variety of peptide ionization conditions.

Another potential application of ion/ion reactions involving metal containing ions involves the selective removal of various cations from gas-phase polypeptide ions. Ion/ion reactions between bradykinin $[M+Na+H]^{2+}$ and PF_6^- anions are shown in Figure 8. In this example, the reaction yields predominantly attachment of the PF_6^- anions, some removal of the sodium cation (the $[M+H]^+$ peak), and a very small amount of removal of the proton (the $[M+Na]^+$ peak). In contrast, Figure 28 shows the product ion spectra from ion/ion reactions between bradykinin $[M+Na+H]^{2+}$ and PDCH anions. Figure 28c shows that the primary reaction product is $[M+Na]^+$ which indicates the net removal of a proton. These experiments suggest that the selection of appropriate anion reagents can lead to the selective removal of a cationizing species (Newton et al., 2004). The selective removal of cations could have similar potential applications as previously discussed with the insertion of cations.

VI. CONCLUSIONS

The dimensionality associated with the study of ion/ion reactions is very large considering the range of species from which gaseous ions can be formed. Viewed in this context, it is clear that only a very small subset of reactant ion combinations has thus far been examined. Nevertheless, it is already apparent that many interesting and potentially useful reaction types occur. A number of new developments have taken place since the most recent review of this area. These have involved new developments in instrumentation, the observation of new reaction phenomena, and expansions in applications. In terms of instrumentation, new ion

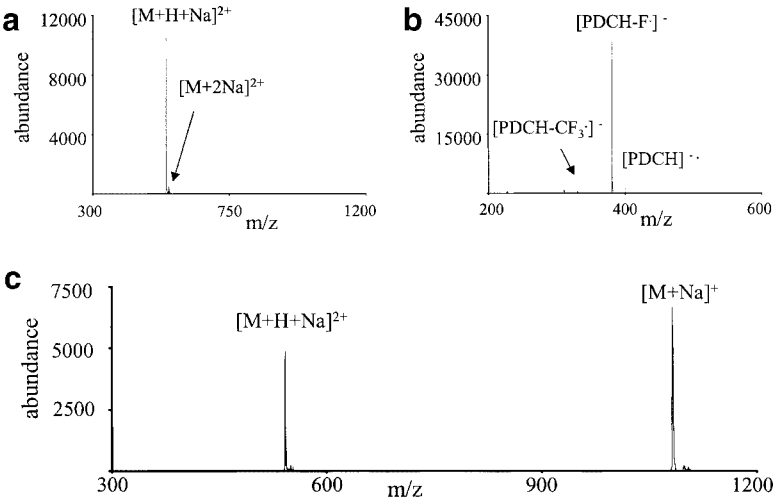


FIGURE 28. (**a**) Mass spectrum of isolated $[M + H + Na]^{2+}$ bradykinin. (**b**) Mass spectrum of PDCH anions. (**c**) Ion/ion reaction product spectrum of ions from (a) and (b). From Newton et al., Phys Chem Chem Phys 2004, 6, 2710–2717. Reproduced by permission of the PCCP Owner Societies, Copyright 2004.

source geometries for effecting ion/ion reactions at atmospheric pressure have been described. Also, new variations of ion inlets have been adapted to quadrupole ion trap mass spectrometers. Several new techniques have been described that include trapping by proxy, a means for storing high mass ions by the electric field of lower mass ions in a quadrupole ion trap, and ion parking, a technique for the selective inhibition of ion/ion reaction rates. The ion parking process is useful for concentrating ions initially dispersed among several charge states into a single charge state and for forming a charge state purified parent ion population when mixtures of macro-molecules are present. The ion parking technique, in conjunction with the use of ion/ion reactions for product ion charge state manipulation, have been applied both for the study of the dissociation of model proteins and in top-down proteomics applications.

A major recent development has been the initiation of studies involving multiply charged cations in reactions with multiply charged anions. The phenomenology associated with these reactions has led to new insights into the dynamics of the reactions by virtue of the fact that charged products of each of the reactants can be analyzed. It is also interesting that complexes of macromolecules can be assembled in the gas phase via ion/ion reactions. Much has yet to be learned about the nature of such complexes and how this capability might find use. Nevertheless, it is clear that ion/ion reactions can be effective in gas phase synthesis strategies. It has also become straightforward to study ion/ion charge inversion reactions. While studies of this type are at an early stage, it is already clear that sequential charge inversion reactions can lead to an increase in the net charge of an ion. Several useful applications for such a capability are readily apparent. Likewise, the ability to insert metal ions into polypeptides and, to some degree, remove metals selectively from ions with mixed cationizing reactions has interesting potential application.

It is anticipated that the study and use of ion/ion reactions involving high mass multiply charged ions will become increasingly widespread. For example, the recent description of electron transfer reactions that give rise to c/z-type fragments from polypeptide ions expands ion/ion chemistry application to direct structural analysis of polypeptides. This adds significantly to the already clear utility of ion/ion reactions for charge state manipulation, including charge inversion, and the potential utility of metal ion transfer and complex formation reactions. These new examples of reaction phenomenologies highlight the high degree of dimensionality associated with ion/ion chemistry and suggest that prospects are good for the discovery of new reaction types. Furthermore, recent descriptions of ion/ion reactions effected in linear ion traps are highly significant insofar as such forms of electrodynamic ion traps are expected to become widely used both as stand alone instruments and as part of hybrid systems. Hence, MS^n studies involving ion/ion reactions need no longer be restricted to three dimensional quadrupole ion trap instrumentation.

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