

Advancing proteomics with ion/ion chemistry

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Mass spectrometers, instruments that use electric and/or magnetic fields to measure a gas-phase ion's mass-to-charge ratio (m/z), are used in a wide variety of applications—with the field having a reputation for providing good sensitivity and high-informing power. Protein analysis (proteomics) is a relatively recent affair for the field and was enabled in the late 1980s with the advent of biomolecule ionization methods such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI). Today, the area of protein analysis garners considerable attention from many in the mass spectrometry (MS) field; given the myriad of possible protein forms and their broad dynamic range (abundance) in the cell, the analytical challenge is paramount. Here we discuss a developing technology—ion/ion chemical reactions—that promises to transform how we think about and conduct protein sequence analysis via MS.

MASS SPECTROMETRY-BASED PROTEOMICS OVERVIEW

Perhaps the most common and effective mass spectrometry-based protein sequencing methods have been those based on the bottom-up strategy (1–3). Here a protein or an entire proteome is enzymatically digested with trypsin (approximately 40 peptides/protein), chromatographically separated, and interrogated with tandem mass spectrometry [MS/MS; the process of ion fragmentation with subsequent mass-to-charge ratio (m/z) measurement]. Electrospray ionization (ESI) is the typical interface and is used to convert condensed phase ions eluting from the high-performance liquid chromatography (HPLC) column to multiply protonated molecules in the gas phase. The mass spectrometer first records the m/z of each peptide ion and then selects the ions individually to obtain sequence information via MS/MS. Collision of the peptide cation with rare gas atoms is the most widely used method to induce peptide fragmentation (collisional-activated dissociation; CAD). If the deposited energy is distributed randomly, a homologous series of backbone fragment m/z peaks is produced. Peptide identification, however, can

only be achieved if a complete or nearly complete distribution of backbone cleavages is present. CAD often fails in this regard when the peptide contains: (i) certain amino acids, particularly those that inhibit random protonation along the peptide backbone; (ii) a posttranslational modification (PTM) that dissociates by a lower energy pathway (than that involved in cleavage of the amide linkage); or (iii) more than approximately 15 amino acids (i.e., large peptides) (4). Note that CAD has been used to characterize peptides with PTMs and even whole proteins; however, the amount of information obtained from CAD fragmentation, and the probability of success, falls off rapidly as a function of these parameters.

In a very real sense, these limitations have narrowly defined the field of MS-based protein measurement [i.e., mandating the use of trypsin to render a collection of peptides of suitable size and sequence (no internal basic residues) for successful MS/MS]. This requirement poses three significant limitations. First, making an already complex mixture even more complex is a considerable disadvantage. Second, the view that one gene equals one protein is simply not accurate for the study of higher eukaryotes; the

majority (up to three-fourths) of human proteins are alternatively spliced, hence the detection of tryptic peptides will rarely reveal this diversity (5–7). This is mainly because a detected peptide could come from any form of the protein, spliced or nonspliced. And third, after transcription, messenger RNA (mRNA) editing, and translation, a further level of complexity is added in the form of protein PTM.

Provided that bottom-up analysis does actually generate a suitable MS/MS spectrum for the identification of a PTM-containing peptide, assigning biological meaning and relevance can be difficult. First, PTMs on multi-domain proteins and among components of protein-protein machines work in concert; to determine their biological relevance, these patterns must be detected within the context of one another (across the whole protein). Second, analysis of short peptide sequences is unlikely to reveal isoform identity (8). Different variants perform different functions and will be modified in distinct ways. New technologies that are capable of sequencing large peptides and whole proteins will be required to acknowledge this diversity in addition to these important, yet unmonitored (at the protein level), biological events.

LARGE-MOLECULE MS

For the reasons presented above, large peptide (i.e., 3–10 kDa) and whole protein sequencing recently has become an area of interest in the field of proteomics. Kelleher, McLafferty, and others have pursued direct dissociation of intact proteins either by CAD or electron capture dissociation (ECD) (9). Most often this experiment utilizes the high resolving power of a Fourier transform-ion cyclotron resonance-MS (FTICR-MS) but not always. Whatever the case, the intact protein mass is measured while the product ions (MS/MS) are used for sequencing and locating sites of modification. When the process is implemented successfully, the entire protein is characterized—a feat rarely achieved with the bottom-up strategy. Unfortunately, mainstream implementation of this top-down type analysis has been challenging for the following reasons: (i) ESI-charge envelopes reduce sensitivity; (ii) precursor m/z peaks can overlap and result in mixed MS/MS spectra (in complex mixtures); (iii) tandem mass spectral complexity can be high, even for the highest resolving power instrument; (iv) CAD is less effective with increasing precursor mass; and (v) coupling ECD with chromatography has been challenging. Doubtless, the ability to routinely characterize large molecules—either whole proteins or large peptides—via MS would significantly increase the informing power of a proteomics experiment (vide supra). Evolution in this field, however, will require the development of new tools and technologies. Here, we discuss a developing area—ion/ion chemical reactions, that, when compiled into sequences, show great promise to propel evolution in the emergent field of large-molecule MS.

ION/ION CHEMICAL REACTIONS

Peptides and proteins ionized with ESI are generally multiply charged. These charges (z) come in the form of protons, where the magnitude of z is proportional to the number of basic residues contained in the peptide's sequence. For mass spectrometric molecular weight determination, the peptide cation must have at least

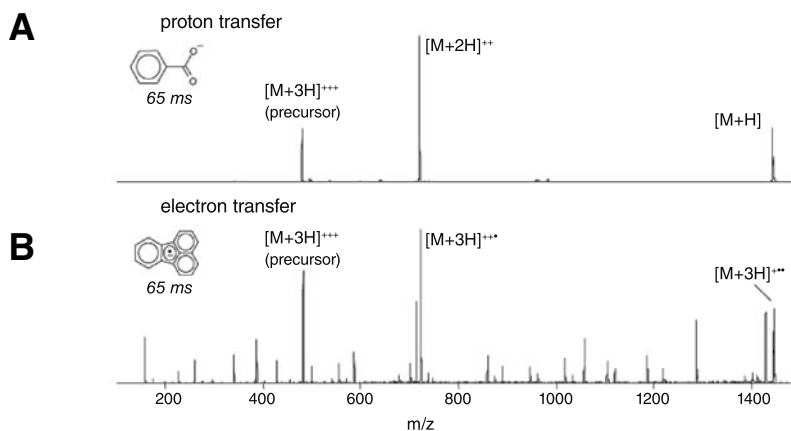


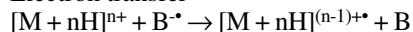
Figure 1. Proton and electron transfer ion/ion reactions following exposure of a triply protonated peptide to anions of either (A) benzoic acid or (B) fluoranthene for 65 ms. m/z , mass-to-charge ratio.

one charge (i.e., $z \geq 1$). But since ESI generates peptides with multiple charges, peptide cations of interest can be reacted with ions of the opposite polarity (anions, ion/ion reactions) to generate products whose m/z is measurable. This field is relatively new, having only become practical since the advent of ESI. Ion/ion reactions of multiply protonated peptide cations with singly charged anions can be broadly classified as one of three types: (i) proton transfer, (ii) electron transfer, or (iii) anion attachment (10,11).

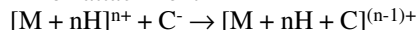
Proton transfer



Electron transfer



Anion attachment



Anion composition is the primary factor that determines which of these pathways is followed (12,13). Most anionic reagents proceed exclusively via the proton transfer pathway (proton transfer reaction; PTR). Until recently, PTR was considered the only viable reaction pathway for multiply protonated peptide cations with singly charged anions. Radical anions of polyaromatic hydrocarbons are one subset of anions that show the ability to transfer electrons to peptide cations (12–15). Anion attachment is relatively rare, but phosphorus hexafluoride, F^- , and certain metal-containing anions can form long-lived complexes

with peptide cations (16–19). These complexes are, however, primarily intermediates of the proton transfer reaction. Again, with anionic composition being the main driver of the chemistry, continued exploration will almost certainly reveal new classes of anions that form even more tightly bound complexes. For now, proton and electron transfer reactions are the key components of the ion/ion reaction toolbox, with each having its own analytical utility.

Figure 1A shows the products of a 65-ms reaction of a triply protonated peptide with the benzoate anion of benzoic acid. The reaction simply reduces the charge of the peptide precursor cation to generate the +1 and +2 form, where proportioning between the two is determined by the reaction duration. Ion/ion reactions proceed at a rate proportional to the square of the precursor charge (z); therefore, a triply charged precursor cation reacts at a rate nine times faster than a singly charged product ion (20). Because of this, PTRs have many uses: (i) charge state reduction of peptide/protein cations, (ii) simplification of product ion spectra (following MS/MS), and (iii) concentration and purification of peptide/protein cation charge envelopes (10,11). Further, McLuckey et al. have reported charge inversion reactions by use of multiply deprotonated anionic reagent species (21). This work represents an exciting new approach for precursor manipulation, but its detailed discussion is beyond the scope of this short review. Figure 2A displays the mass analysis

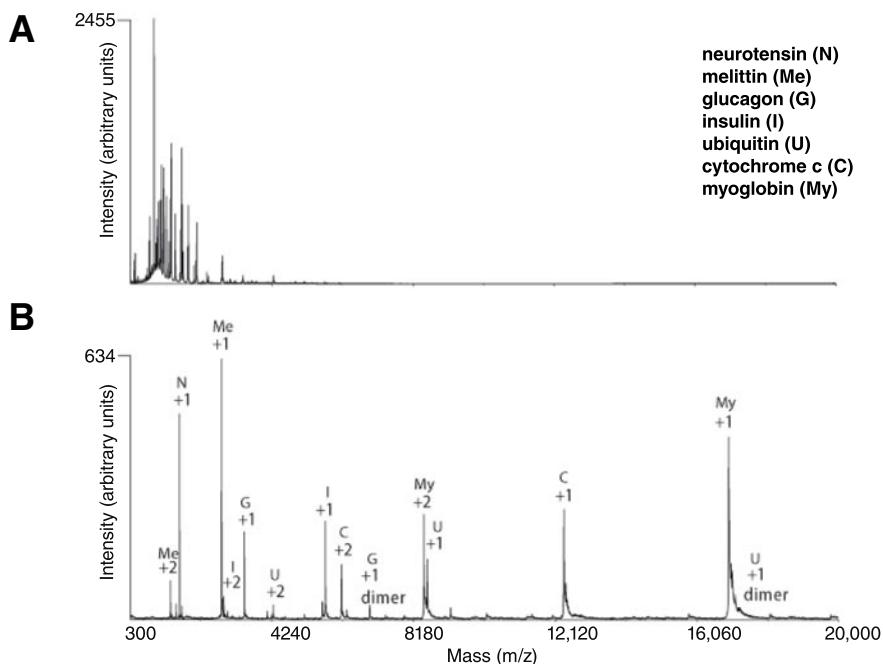


Figure 2. Mass spectra of an equimolar seven-component protein mixture (A) without proton transfer reaction (PTR) and (B) with PTR. *m/z*, mass-to-charge ratio. Adapted with permission from Reference 22.

of a seven-component peptide/protein mixture directly following ESI; numerous *m/z* peaks are all present and confined within a small *m/z* range from approximately 600–1500. The PTR charge reduction of this mixture, prior to *m/z* analysis, generates the highly simplified spectrum shown in Figure 2B (22). Here, all peptide/protein cations exist in only one charge state and are spread out over a much wider *m/z* range (i.e., better resolved from one another).

The PTR is relatively straightforward, but highly multifunctional (vide infra).

Electron transfer reactions are much newer, and their development was inspired by the unique peptide/protein fragmentation technique, ECD (12,14,23). Figure 1B displays the result following a 65-ms reaction of the radical anion of fluoranthene with the same triply protonated peptide shown in Figure 1A. By using the radical polyaromatic hydrocarbon anion, the electron

transfer pathway is initiated and results in extensive peptide backbone fragmentation, or electron transfer dissociation (ETD). Figure 3 displays the result of an ETD reaction of a triply protonated phosphopeptide and demonstrates the utility of ETD for protein sequence analysis. This peptide has nine amino acids. Therefore, it can break in any of the eight positions between each of the nine residues. If, for example, the peptide breaks between the I (isoleucine) and L (leucine) residues, two fragment ions would be observed—the first having an *m/z* of 582 (the signal labeled c_4), and the second with a recorded *m/z* of 637 (z_5). By subtracting consecutive signals in the spectrum, the mass (and thus identity) of the next residue can be determined. Extension of this process will reveal the sequence of the entire peptide/protein and the location of any modifications, including phosphorylation. ETD is the ion/ion analog of ECD, and it brings all of the benefits of ECD to lower-cost benchtop instrumentation. In a qualitative sense, there appears to be no significant differences between the two methods. ETD is indifferent to peptide length or the presence of PTMs, performed on a time-scale that permits coupling with chromatography, and can be coupled with other ion/ion reactions (i.e., proton transfer) (12–15,24–29). ETD has become recognized as a new avenue for large-scale PTM identification, but it also offers new bioinformatic and large-molecule analysis possibilities (vide infra).

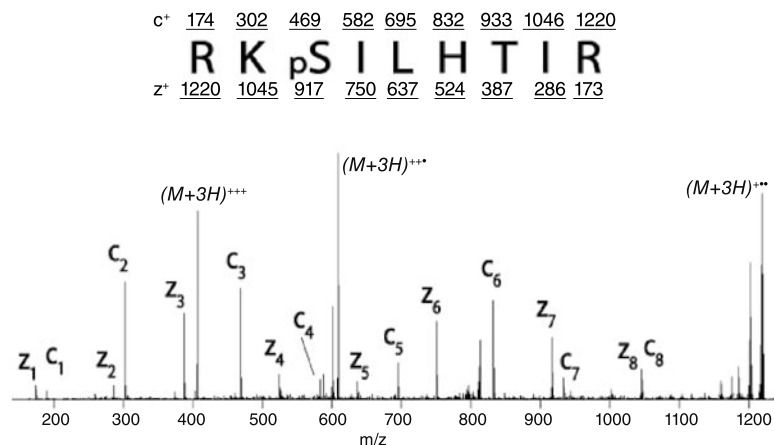


Figure 3. Electron transfer dissociation (ETD) fragmentation of a short, phosphorylated peptide. The presence of every possible product ion allows for straightforward sequence identification and posttranslational modification (PTM) site location. *m/z*, mass-to-charge ratio. Adapted with permission from Reference 15.

INSTRUMENTATION FOR PERFORMING ION/ION REACTIONS

Two general types of instrumentation for conducting ion/ion reactions have been described. The first reacts peptide cations with anionic reagents prior to admission into the mass spectrometer, while the second uses quadrupole-ion trapping devices as reaction chambers (10–12,30,31). The first approach has the primary advantage of being adaptable to virtually any type of mass spectrometer with relative simplicity. Its major limitations are the inability to generate multiple anionic reagents simultaneously (e.g., ETD- and PTR-

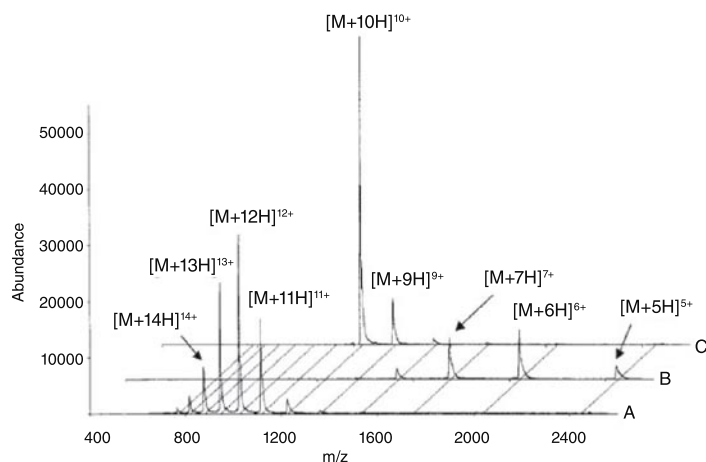


Figure 4. Mass spectra of bovine cytochrome c ions. Panel A displays the electrospray ionization (ESI) charge state distribution; note that the protein cation exists in seven charge states. Panels B and C display the results following a 150-ms proton transfer reaction (PTR) ion/ion reaction of these cations (C) with and (B) without ion parking. *m/z*, mass-to-charge ratio. Adapted with permission from Reference 33.

type anions) and difficulty controlling the reactions. Quadrupole radio frequency (RF) ion trap mass spectrometers come in two varieties, three-dimensional (QIT) and linear (QLT); both of which can confine ions of opposite polarity in the same space (13). Of the two, QLT instruments have several unique advantages, including greater ion capacity (approximately 30-fold) and higher ion-injection efficiency (approximately 10- to 30-fold). The Finnigan LTQ (Thermo Electron, San Jose, CA, USA) is one such instrument and has the added benefit of a segmented linear trap (three sections) (32). Manipulation of the DC bias potentials, applied to these segments and end lenses, allows for axial segregation of precursor cations and reagent anions during anion injection and isolation. This means that a selected peptide cation is reacted with a selected reagent anion and no prior mixing takes place. Initiation and termination of the ion/ion reaction is also controlled by adjustment of these DC bias potentials (14).

A useful feature for performing any chemical reaction is the ability to have real-time control. Ion trap mass spectrometers (QIT or QLT) become miniature reaction vessels during ion/ion reactions, but retain their innate *m/z* sorting abilities. An ion that undergoes a reaction immediately experiences a change in *m/z* value and a concurrent shift in frequency of ion motion. By application of a supplementary AC voltage (across the electrodes of an

ion trap), one can inhibit the reaction of either a selected ion/ion product or a wide range of ion/ion products. Supplemental excitation increases the targeted ion's kinetic energy and renders it less susceptible to further reaction (i.e., ion parking). McLuckey et al. have pioneered this approach and described its utility for concentrating ESI protein charge envelopes into a single charge state and for protecting *c*- and *z*-type fragment ions generated via ETD (33–35). No matter which type of ion/ion reaction is performed, the ability to control the reaction is advantageous; below we outline two circumstances.

ESI generates charge envelopes, which distribute analyte signal in many channels. Increasing peptide length generally correlates with an increase in the number of charge states (e.g., a 12-residue peptide may have three charge states, a 70-kDa protein may have dozens). To obtain the greatest sensitivity and to maximize product ion yields upon dissociation, every peptide/protein cation would ideally exist in one charge state. ESI of bovine cytochrome *c* generates ions with charge states ranging from +16 to +10, but only one of these can be selected for subsequent dissociation (Figure 4A). However, application of a supplementary AC waveform (targeting the *m/z* of the +10 ion) during a 150-ms PTR causes the entire ion population to be concentrated into the +10 charge state (Figure 4C) (33).

Reaction control is likewise advantageous during electron transfer

reactions, where secondary electron transfer events can result in the production of internal fragments and the consumption of first-generation *c/z*-type product species (signal loss). For example, a c_{50} fragment, produced following a single electron transfer to a whole protein cation, could subsequently receive an electron, cleave, and form two product ions (e.g., c_{25} and z'_{25}). The N-terminal fragment, c_{25} , is still recognizable within the context of the original precursor protein; however, the z'_{25} product contains neither the amino nor carboxy terminus of the original precursor and appears, with a variety of other similar products, as elevated noise. McLuckey has recently described a broadband version of the targeted ion parking approach to excite all *m/z* ratios in the trap, except those of the reactants (peptide precursor cation and reagent anion) (34). By preserving *c/z*-type product ion signal and concomitantly reducing the chemical noise generated by secondary electron transfer, this reaction control technology holds great promise to increase ETD efficiencies.

SEQUENTIAL ION/ION REACTIONS FOR LARGE-MOLECULE MS

Electron and proton transfer reactions deliver orthogonal functions and are easily, and ideally, coupled in discrete reaction sequences. The value of doing this becomes readily apparent during large peptide and whole protein analysis. PTRs provide opportunities for an initial precursor ion screening step; here an eluting abundant *m/z* can be isolated and subjected to a brief PTR followed by rapid *m/z* analysis. The charge state and relative purity of the precursor cation can be determined from this step (36). Next, an ion parking experiment can be engaged to concentrate all of the precursor cation charge states into one selected *m/z* value. From here the concentrated precursor *m/z* can be dissociated via ETD. ETD has been used successfully to dissociate large peptides and even whole proteins. Direct dissociation of such species can generate highly complicated and convoluted spectra;

however, Figure 5A displays the result of a 15-ms ETD reaction of the +13 cation of ubiquitin with radical anions of fluoranthene (24). In this experiment, product ions have charges ranging from +1 to +12. Ion traps in routine operation mode do not have sufficient resolution to determine z for $z \geq 3$. The entire mixture however, can be simplified by using a subsequent PTR simplification step. In this case, all generated c/z -type fragment ions were stored in the front section of the QLT, while anions of benzoic acid were injected, purified, and finally mixed with the c/z -type products (for 50, 100, and 150 ms). The higher charged fragments are preferentially concentrated to lower charge states as the PTR duration is extended (to predominately singly charged products in the case of the 150-ms reaction) (see Figure 5D). This effect can be observed by following the small expanded region of each spectrum plotted in Figure 5. The result is an easily interpreted spectrum where one can simply read N and C termini of the protein (c - and z -type ion series, respectively) (see Figure 5D). Mass range limits the ability to read past the first and last approximate 20 residues, but hybrid instrumentation should remove this limitation (*vide infra*). We have recently demonstrated the utility of this approach to characterize whole proteins (up to about 70 kDa) as they elute from a chromatographic column in a rapid, data-dependent fashion (1–3 s/protein) (24). Of course, any of these ion/ion reactions can be performed in any order with CAD. Prior to the introduction of ETD, McLuckey and colleagues used PTR with CAD for whole protein and large peptide characterization (37).

FUTURE DIRECTIONS

The ability to monitor transcriptional editing and posttranslational modification is a major motivation driving the development of large peptide and whole protein characterization technologies. Also, since most of these processes are unknown, conventional data interpretation methods (*i.e.*, protein database searching) are unlikely to identify

perhaps the most interesting and important subset of data—that derived from modified or spliced protein forms. Detection of these events will require the ability to deduce whole or partial sequence information directly from tandem mass spectra (*de novo*). Numerous *de novo* algorithms have been developed, although none have had widespread success. These works, however, have primarily relied on CAD-type fragmentation. The ion/ion processes described here generate higher-quality data and are likely to make automated *de novo* sequencing a much more tractable proposition. Zubarev *et al.* have recently reported the use of ECD-type fragmentation, combined with CAD, for high-throughput *de novo* sequencing (38). Those results demonstrate that electron-based fragmentation methods (ECD and ETD) do indeed hold great promise to bring new bioinformatic possibilities.

Ion trap systems are ubiquitous and are easily modified to perform ion/ion reaction sequences, thus these technologies have potential for a rapid, large-scale impact on proteomic research. PTR can effectively counter spectral complexity, overlapping precursor m/z peaks (precursor purification) and ESI-charge envelopes (precursor concen-

tration). ETD is the ion/ion analog of ECD, but offers certain advantages—routine compatibility with chromatography and other ion/ion technologies being the most important ones. Further, RF QLT devices are common components of many hybrid mass spectrometers (*i.e.*, QLT-FTICR or QLT-orbitrap), and these instruments could be adapted to accommodate ion/ion reactions. Pairing the ion processing abilities of ion/ion chemistry with high resolving power and high mass accuracy instrumentation is of obvious utility. This instrumentation would: (*i*) more effectively handle larger species (increase sequence coverage, better locate PTMs, etc.) and (*ii*) drive new bioinformatics opportunities (*i.e.*, *de novo*, etc.).

To date, all ion/ion work has been performed using in-house modified mass spectrometers—that is, no commercial ion/ion-enabled mass spectrometer system is available. Obviously, this has limited its widespread implementation. However, no less than three major mass spectrometer instrument manufacturers have begun to develop commercial systems capable of performing ETD. Whether they will accommodate other ion/ion reactions and/or technologies

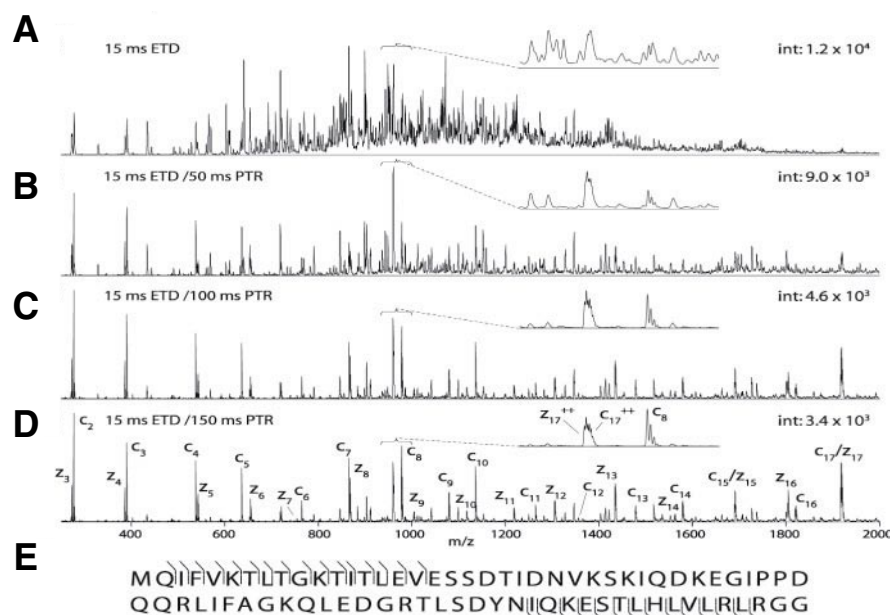


Figure 5. Whole protein dissociation (ubiquitin +13, m/z 659). (A) The initial result of a 15-ms ion/ion reaction with the radical anion of fluoranthene. (B–D) The subsequent reaction of these products with even-electron anions of benzoic acid for 50, 100, and 150 ms, respectively (each spectrum is the average of approximately 50 spectra). ETD, electron transfer dissociation; PTR, proton transfer reaction; m/z , mass-to-charge ratio. Adapted with permission from Reference 24.

is unclear, but that the field is about to undergo rapid expansion is certain.

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COMPETING INTERESTS STATEMENT

J.J.C. is an inventor on a pending patent for electron transfer dissociation that has been licensed to ThermoElectron.

REFERENCES

- Fenn, J.B., M. Mann, C.K. Meng, S.F. Wong, and C.M. Whitehouse. 1989. Electrospray ionization for mass spectrometry of large biomolecules. *Science* 246:64-71.
- Karas, M. and F. Hillenkamp. 1988. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. *Anal. Chem.* 60:2299-2301.
- Delahunty, C. and J.R. Yates. 2005. Protein identification using 2D-LC-MS/MS. *Methods* 35:248-255.
- Huang, Y., J.M. Triscari, G.C. Tseng, L. Pasa-Tolic, M.S. Lipton, R.D. Smith, and V.H. Wysocki. 2005. Statistical characterization of the charge state and residue dependence of low-energy CID peptide dissociation patterns. *Anal. Chem.* 77:5800-5813.
- Resch, A., Y. Xing, B. Modrek, M. Gorlick, R. Riley, and C. Lee. 2004. Assessing the impact of alternative splicing on domain interactions in the human proteome. *J. Proteome Res.* 3:76-83.
- Ast, G. 2005. The alternative genome. *Sci. Am.* 292:40-47.
- Godovac-Zimmermann, J., O. Kleiner, L.R. Brown, and A.K. Drukier. 2005. Perspectives in splicing up proteomics with splicing. *Proteomics* 5:699-709.
- Jenuwein, T. and C.D. Allis. 2001. Translating the histone code. *Science* 293:1074-1080.
- Kelleher, N.L. 2004. Top-down proteomics. *Anal. Chem.* 76:197A-203A.
- McLuckey, S.A. and J.L. Stephenson. 1998. Ion ion chemistry of high-mass multiply charged ions. *Mass Spectrom. Rev.* 17:369-407.
- Pitteri, S.J. and S.A. McLuckey. 2005. Recent developments in the ion/ion chemistry of high-mass multiply charged ions. *Mass Spectrom. Rev.* 24:931-958.
- Coon, J.J., J.E.P. Syka, J.C. Schwartz, J. Shabanowitz, and D.F. Hunt. 2004. Anion dependence in the partitioning between proton and electron transfer in ion/ion reactions. *Int. J. Mass Spectrom.* 236:33-42.
- Gunawardena, H.P., M. He, P.A. Chrisman, S.J. Pitteri, J.M. Hogan, B.D.M. Hodges, and S.A. McLuckey. 2005. Electron transfer versus proton transfer in gas-phase ion/ion reactions of polyprotonated peptides. *J. Am. Chem. Soc.* 127:12627-12639.
- Syka, J.E.P., J.J. Coon, M.J. Schroeder, J. Shabanowitz, and D.F. Hunt. 2004. Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. *Proc. Natl. Acad. Sci. USA* 101:9528-9533.
- Coon, J.J., J.E.P. Syka, J. Shabanowitz, and D.F. Hunt. 2005. Tandem mass spectrometry for peptide and protein sequence analysis. *BioTechniques* 38:5-19.
- Stephenson, J.L. and S.A. McLuckey. 1997. Gaseous protein cations are amphoteric. *J. Am. Chem. Soc.* 119:1688-1696.
- Newton, K.A., M. He, R. Amunugama, and S.A. McLuckey. 2004. Selective cation removal from gaseous polypeptide ions: proton vs. sodium ion abstraction via ion/ion reactions. *Phys. Chem. Chem. Phys.* 6:2710-2717.
- Newton, K.A., R. Amunugama, and S.A. McLuckey. 2005. Gas-phase ion/ion reactions of multiply protonated polypeptides with metal containing anions. *J. Phys. Chem. A Mol. Spectrosc. Kinet. Environ. Gen. Theory* 109:3608-3616.
- Payne, A.H. and G.L. Glish. 2001. Gas-phase ion/ion interactions between peptides or proteins and iron ions in a quadrupole ion trap. *Int. J. Mass Spectrom.* 204:47-54.
- McLuckey, S.A., J.L. Stephenson, and K.G. Asano. 1998. Ion/ion proton-transfer kinetics: implications for analysis of ions derived from electrospray of protein mixtures. *Anal. Chem.* 70:1198-1202.
- He, M., J.F. Emory, and S.A. McLuckey. 2005. Reagent anions for charge inversion of polypeptide/protein cations in the gas phase. *Anal. Chem.* 77:3173-3182.
- Ebeling, D.D., M.S. Westphall, M. Scaif, and L.M. Smith. 2000. Corena discharge in charge reduction electrospray mass spectrometry. *Anal. Chem.* 72:5158-5161.
- Zubarev, R.A., N.L. Kelleher, and F.W. McLafferty. 1998. Electron capture dissociation of multiply charged protein cations. A nonergodic process. *J. Am. Chem. Soc.* 120:3265-3266.
- Coon, J.J., B. Ueberheide, J.E.P. Syka, D.D. Dryhurst, J. Ausio, J. Shabanowitz, and D.F. Hunt. 2005. Protein identification using sequential ion/ion reactions and tandem mass spectrometry. *Proc. Natl. Acad. Sci. USA* 102:9463-9468.
- Pitteri, S.J., P.A. Chrisman, J.M. Hogan, and S.A. McLuckey. 2005. Electron transfer ion/ion reactions in a three-dimensional quadrupole ion trap: reactions of doubly and triply protonated peptides with SO₂⁻. *Anal. Chem.* 77:1831-1839.
- Hogan, J.M., S.J. Pitteri, P.A. Chrisman, and S.A. McLuckey. 2005. Complementary structural information from a tryptic N-linked glycopeptide via electron transfer ion/ion reactions and collision-induced dissociation. *J. Proteome Res.* 4:628-632.
- Pitteri, S.J., P.A. Chrisman, and S.A. McLuckey. 2005. Electron-transfer ion/ion reactions of doubly protonated peptides: effect of elevated bath gas temperature. *Anal. Chem.* 77:5662-5669.
- Chrisman, P.A., S.J. Pitteri, J.M. Hogan, and S.A. McLuckey. 2005. SO₂⁻ electron transfer ion/ion reactions with disulfide linked polypeptide ions. *J. Am. Soc. Mass Spectrom.* 16:1020-1030.
- O'Connor, P.B., J.J. Cournoyer, S.J. Pitteri, P.A. Chrisman, and S.A. McLuckey. 2006. Differentiation of aspartic and isoaspartic acids using electron transfer dissociation. *J. Am. Soc. Mass Spectrom.* 17:15-19.
- Scaif, M., Westphall, M. S., J. Krause, S. L. Kaufman, and L. M. Smith. 1999. Controlling charge states of large ions. *Science* 283:194-197.
- Loo, R.R.O., H.R. Udseth, and R.D. Smith. 1992. A new approach for the study of gas-phase ion-ion reactions using electrospray ionization. *J. Am. Soc. Mass Spectrom.* 3:695-705.
- Schwartz, J.C., M.W. Senko, and J.E.P. Syka. 2002. A two-dimensional quadrupole ion trap mass spectrometer. *J. Am. Soc. Mass Spectrom.* 13:659-669.
- McLuckey, S.A., G.E. Reid, and J.M. Wells. 2002. Ion parking during ion/ion reactions in electrodynamic ion traps. *Anal. Chem.* 74:336-346.
- Chrisman, P.A., S.J. Pitteri, and S.A. McLuckey. 2005. Parallel ion parking: improving conversion of parents to first-generation products in electron transfer dissociation. *Anal. Chem.* 77:3411-3414.
- Chrisman, P.A., S.J. Pitteri, and S.A. McLuckey. 2006. Parallel ion parking of protein mixtures. *Anal. Chem.* 78:310-316.
- Reid, G.E., H. Shang, J.M. Hogan, G.U. Lee, and S.A. McLuckey. 2002. Gas-phase concentration, purification, and identification of whole proteins from complex mixtures. *J. Am. Chem. Soc.* 124:7353-7362.
- Stephenson, J.L., S.A. McLuckey, G.E. Reid, J.M. Wells, and J.L. Bundy. 2002. Ion/ion chemistry as a top-down approach for protein analysis. *Curr. Opin. Biotechnol.* 13:57-64.
- Savitski, M. M., M. L. Nielsen, F. Kjeldsen, and R. A. Zubarev. 2005. Proteomics-grade de novo sequencing approach. *J. Proteome Res.* 4:2348-2354.

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