Published in final edited form as:

Anal Chem. 2006 June 1; 78(11): 3788–3793. doi:10.1021/ac060164j.

Phosphopeptide Anion Characterization via Sequential Charge Inversion and Electron Transfer Dissociation

Harsha P. Gunawardena, Joshua F. Emory, and Scott A. McLuckey^{*}

Department of Chemistry, Purdue University, West Lafayette, IN 47907-2084

Abstract

Sequential ion/ion reactions have been used to characterize phosphopeptides present in relatively simple peptide mixtures, including one generated from the tryptic digestion of α -casein. The phosphopeptides in these mixtures gave rise to either low or no signals via positive ion electrospray ionization. Strong signals, however, were generated in the negative ion mode. An initial ion/ion reaction that employed multiply-protonated amino-terminated dendrimers converted phosphopeptide anions to the doubly protonated species. The doubly charged cations were then subjected to ion/ion electron transfer to induce dissociation. Electron transfer dissociation of doubly positive charged phosphopeptides yields characteristic c- and z-type fragment ions by dissociation of the N-C α bond along the peptide backbone while preserving the labile post-translational modifications. These results illustrate the ability to alter ion charge after ion formation and prior to structural interrogation. Phosphopeptides provide an example where it can be difficult to form strong doubly charged cation signals directly when they are present in mixtures, which, as a result, precludes the use of electron transfer dissociation as a structural probe. The sequential ion/ion reaction process described here, therefore, can provide a new capability for structural interrogation in phosphoproteomics.

Keywords

charge inversion; electron transfer dissociation; phosphopeptide anions; ion/ion reaction

INTRODUCTION

Protein phosphorylation is known to play key regulatory roles in many cellular processes and is among the most widely studied post-translational modifications.¹ A variety of techniques have been applied to the study of protein phosphorylation, including classical methods, such as Edman degradation and ³²P labeling, and instrumental methods, such as mass spectrometry. Several characteristics of phosphopeptides and phosphoproteins present challenges for the study of protein phosphorylation. A principal issue stems from the fact that phosphorylation is often sub-stoichiometric causing phosphoproteins to often be present in lower abundances than many other components in the sample. As a consequence, methods that rely on protein digestion give rise to phosphopeptides of low relative concentration. Techniques designed to separate and/or concentrate the components of interest, therefore, are usually employed. Commonly used techniques include high performance liquid chromatography (HPLC), reversed phase beads², and selective enrichment of phosphopeptides using immobilized metal affinity chromatography (IMAC).³ In addition to IMAC, phosphoprotein isotope-coded affinity tag (PhIAT) and phosphoprotein isotope-coded solid-phase tag (PhIST) can be used to selectively enrich the phosphopeptides in the sample. The PhIAT/PhIST tags replace the

Address reprint requests to: Scott A. McLuckey, Department of Chemistry, Purdue University, West Lafayette, IN, USA 47907-2084. Phone: (765) 494-5270, Fax: (765) 494-0239, E-mail: mcluckey@purdue.edu.

labile phosphate group with heavy and light isotope tags which do not dissociate as readily as a phosphate group during MS/MS analysis enabling the phosphorylation site to be identified by MS/MS analysis of the PhIAT or PhIST tagged peptides.⁴

Recent advances in peptide and protein mass spectrometry have made it a very attractive alternative for studying protein phosphorylation⁵, usually in conjunction with separation/concentration techniques. There are, however, phosphorylation analysis issues specific to mass spectrometry, such as those associated with the ionization and dissociation stages in a typical tandem mass spectrometry experiment.⁶ For example, mass spectrometric responses of phosphopeptides in the positive ion mode are often suppressed relative to unmodified peptides present in a mixture due to the reduction in isoelectric point arising from phosphorylation.⁷ For this reason, screening for phosphopeptides is often done in the negative ion mode, facilitated by parent ion scans.^{8, 9} The propensity for formation of phosphate related anions upon collisional activation of negatively charged phosphopeptides makes such screening approaches possible. However, it is frequently difficult to determine the site of modification in the negative ion mode via conventional ion activation methods.

Phosphopeptide structure analysis by tandem mass spectrometry is usually done with positively charged ions.¹⁰ However, cleavage of the phosphate group is often the dominant process when collisional activation or infra-red multi-photon dissociation activation methods are employed.^{11, 12} Alternatively, electron capture dissociation (ECD) and electron transfer dissociation (ETD) of multiply-charged cations show extensive cleavage of the peptide backbone with preservation of labile bonds, such as phosphate bonds and glycosidic bonds.^{13, 14} Analysis of multiply-charged negative peptides has been recently demonstrated via electron detachment dissociation (EDD)¹⁵ and by electron transfer dissociation (ETD)¹⁶, although the generality of these methods for phosphopeptide analysis is less well established than ECD and phosphopeptide cation ETD. In any case, electron transfer, electron capture, or electron detachment techniques are promising for phosphopeptide structural determination, provided multiply charged precursor ions are available.

Given that a phosphopeptide may ionize more readily in the negative ion mode, whereas structural determination may be more readily accomplished with positive ions, we have explored the possibility of employing ion/ion charge inversion reactions to enable a strategy that relies on forming ions in the negative ion mode and analyzing the ions in the positive ion mode. If multiply charged cations can be formed via charge inversion, a subsequent electron transfer ion/ion reaction can be used for structural determination. Several ion/ion reaction steps within a single MSⁿ sequence have already been demonstrated in several analytical applications. For example, sequential ion parking steps¹⁷, each of which involves a proton transfer ion/ion reaction, have been used to concentrate and charge-state-purify protein ions for subsequent ion activation.¹⁸ Ion parking has also been used prior to a collisional activation step followed by ion/ion proton transfer reactions to simplify interpretation of the product ion spectra.¹⁹ Recently, sequential electron transfer ion/ion reactions and proton transfer ion/ion reactions have been used to characterize large polypeptide and protein cations.²⁰ Sequential charge inversion ion/ion reactions have been demonstrated to be capable of increasing the charge of an ion in either the positive²¹ or negative²² mode. Charge inversion reactions allow for the decoupling of the ionization polarity from the polarity of ions eventually subjected to structural interrogation. In this work, we demonstrate the proof-of-principle for formation of singly charged phosphopeptide anions with structural interrogation of doubly charged cations via ETD. An intervening charge inversion step enables the overall process. The charge inversion-ETD of the phosphopeptides presented here enables identification of a phosphopeptide and the localization of the phosphorylation site without previously enriching the sample for phosphopeptides by other methods.

EXPERIMENTAL SECTION

All experiments were performed using a modified Finnigan Ion Trap Mass Spectrometer (ITMS) that consists of four ion sources (two electrospray ionization sources, and two atmospheric sampling glow discharge ionization sources) as described in detail elsewhere.²³ In brief, a DC turning quadrupole, the potentials applied to which are under software control, allows sequential injection of ions from three ion sources through an ion trap end-cap electrode. The fourth source and associated ion optics directly inject ions through a hole in the ion trap ring electrode. The injection and timing of all sources are controlled by the ITMS software. In this report, all three sources on the front end of the DC turning quadrupole were used for multiple ion/ion reactions while a few experiments involved the use of the fourth source. Two of the three front end sources were nano-electrospray ionization sources used for independently generating peptide anions and amino terminated dendrimer cations, while a third ASGDI source was used for producing azobenzene anions. Nanoelectrospray is performed using borosilicate glass capillaries (0.86 mm i.d., 1.5 mm o.d.) that were pulled using a P-87 Flaming/Brown micropipette puller (Sutter Instruments, Novato, CA) to form nano-electrospray emitters. A stainless steel wire, attached to an electrode holder (Warner Instruments, Hamden, CT), was inserted into the capillary and a potential of 1–2 kV was applied to the wire to induce electrospray.²⁴ The ASGDI source for these studies consisted of two metal half-plates mounted within the ion source, as described in detail elsewhere.²⁵ The voltage and current necessary to create a discharge was produced by applying ~ -400 V on one half-plate via a PVX-4150 high voltage pulser (Directed Energy Inc, Fort Collins, CO), while the other plate was grounded. Azobenzene was introduced using a heated inlet system (with variable temperature controller). During operation, the pressure of the glow discharge source was maintained at $\sim 0.8 - 1.6$ torr. The experimental sequence typically consisted of the following steps: dendrimer cation accumulation and isolation, peptide anion accumulation and isolation, mutual storage of oppositely charged ions to effect ion/ion reactions, isolation of a charge inversion product ion, and accumulation of azobenzene reagent anions followed by mutual storage of oppositely charged ions to induce electron transfer dissociation. A few experiments also involved the use of a second ASGDI source for introducing proton transfer reagents for charge reducing CID products. Ion isolation steps were performed by rf ion isolation ramps tuned to eject ions from selected ranges of mass-to-charge ratio.²⁶ Mass analysis was effected via resonance ejection.²⁷

Neurotensin, bradykinin, bombesin, angiotensin, α -casein, ammonium bicarbonate, diaminobutane (DAB) core dendrimer generation 4 (g4), perfluoro-1,3-dimethylcyclohexane (PDCH), and azobenzene were purchased from Sigma-Aldrich (St. Louis, MO). TPCK-treated trypsin was purchased from Worthington Biochemical Cooperation (Lakewood, NJ). Synthetic peptides KGAILKGAILR and RKRARKE were custom synthesized by SynPep (Dublin, CA). LKRApYLG-NH₂ was purchased from AnaSpec (San Jose, CA). α -Casein was digested using TPCK-treated trypsin with a 1:100 enzyme/protein ratio in 20 mM ammonium bicarbonate buffer, pH ~ 8 , for 4 hours at 37 °C. The reaction was terminated by freezing the sample for a few minutes followed by desalting using a gel filtration column from Amersham Biosciences (Pittsburgh, PA). The tryptic mixture was dried and portions were resuspended separately in aqueous solutions of 1% acetic acid and 2% ammonium hydroxide, respectively. All standard peptides were used without further purification. Working peptide aqueous solutions of ~ 1 μ M in 1% acetic acid and 2% ammonium hydroxide were prepared from a stock solution of 1 mg/mL. A solution of 0.1–0.2 mg/mL of DAB-g4 dendrimer was produced in pure water (conductance of 18 M Ω). Head space vapors from PDCH were sampled from the room temperature liquid while azobenzene vapors in air were generated by heating the sample above its melting temperature.

RESULTS AND DISCUSSION

Sequential Proton Transfer Charge Inversion and Electron Transfer Ion/Ion Reactions

The overall approach outlined here assumes the formation of phosphopeptide anions and is not tied to any particular ionization method for the analyte ions. The charge state of the anion is not critical but it is advantageous that the anion be singly charged for the first step of the overall process (i.e., the charge inversion step). This is because there are fewer possible proton transfer reaction channels available to the reactants when the anion is singly charged. The simplest case, in terms of the number of possible reaction channels, involves the reaction of a singly charged analyte anion and a triply protonated reagent cation, as illustrated in Scheme 1.

Previous studies have suggested that the rate determining step for ion/ion reactions in an electrodynamic ion trap is the formation of an orbiting pair²⁸, as reflected by k_{pair} . The trajectories of the ions can lead them to form an intimate collision complex, represented by k_c , from which one (k_{dis1}), two (k_{dis2}), or three (k_{dis3}) proton transfers can occur. Alternatively, the complex can be stabilized (k_{cool}) via the cooling mechanisms in effect in the ion trap.²⁹ A competitive process within the orbiting ion pair is proton transfer at a distance, represented by k_{hop} . For the present application, it is desirable that k_{hop} be minimal, because it results in neutralization of the analyte. Furthermore, it is also desirable that complex formation, single proton transfer, and double proton transfer be minimal. The sequence leading to $(M+2H)^{2+}$ ions (or, perhaps, more highly charged analyte ions in the case of more highly charged reagents) is most desirable. The nature of the reagent cation is the major available experimental variable that can be used to influence which reaction channels dominate. While research into ion/ion charge inversion reactions is still in an early stage, it has been noted that reagent species formed from dendrimers show a number of desirable characteristics. For example, the relatively large collision cross-sections of the molecules tend to favor formation of the intermediate intimate collision complex, which is expected to be key to charge inversion. Furthermore, very little adduct ion formation has been noted. Also, the relatively wide range of charge states that can be accessed provides a degree of influence over the charge states of the charge inversion products. For these reasons, DAB-g4 dendrimer cations were selected for the charge inversion step.

Electron transfer dissociation, which constitutes the second step of the process, requires that the analyte ion carry at least two positive charges. The major possible reaction channels for a doubly protonated analyte species in reaction with an anion radical is illustrated in Scheme 2.

This scheme shows the formation of an orbiting ion pair that can either lead to intimate complex formation, proton transfer, or electron transfer. The intimate complex can also break up leading to net proton transfer or electron transfer. In this application, electron transfer that leads to dissociation to c- and z-types ions is preferred. The nature of the reagent anion plays a major role in determining the relative propensities for proton transfer versus electron transfer.³⁰ For this work, anions derived from azobenzene were chosen as the reagent species for ETD because they were shown previously to lead to relatively efficient ETD with a model triply protonated peptide.²⁹ Hence, based on current understanding of ion/ion charge inversion and ETD, this work utilized an optimized set of reagent ions.

Electrospray Ionization of Peptide Mixtures containing Phosphopeptides

The sequential ion/ion reaction approach was applied first to a synthetic mixture with components of known relative concentration and then to a mixture derived from tryptic digestion of α -casein. Figure 1 shows electrospray mass spectra derived from a synthetic peptide mixture in both positive and negative ionization modes. In positive ESI mode, ionization of the phosphopeptide LKRApYLG-NH₂ was minimal relative to the generally

much more basic peptides. When the same set of peptides was subjected to negative ESI, singly charged ions of the phosphopeptide represent the base peak of the spectrum.

Electrospray response tends to be maximized when the analyte is present in solution as an ion of the same polarity as the electrospray and when it is present on the surface of the charged droplets.^{31,32} While the addition of acid to the solution subjected to positive electrospray tends to favor protonation of all peptide components in solution, it is clear that the available charge is not partitioned into the phosphopeptide in relation to its concentration in solution. Likewise, under the basic conditions used in the negative ion mode, several of the peptides are not well represented, although the phosphopeptide is.

Figure 2 compares the positive and negative ion electrospray mass spectra of the tryptic digest of α -casein.

The positive mode ESI spectrum (Figure 2a) consists of tryptic peptides that are mostly singly and doubly charged species. Larger peptides having more than two charge sites are also observed due to missed cleavages. It is also noteworthy that no signals due to tryptic phosphopeptides are clearly apparent in this m/z window. Figure 2b, which shows the negative mode ESI spectrum of the tryptic digest, clearly shows anions assigned to the phosphopeptide indicated as C in the figure insert. The mass of this tryptic fragment is consistent with three-fold phosphorylation. This peptide is also comprised of three glutamic acid residues. Including the C-terminus, there are seven acidic sites in this peptide. Therefore it is not surprising that ESI response is greater for this peptide in the negative ion mode than in the positive ion mode.

Negative Ion to Positive Ion Charge Inversion of Phosphopeptides

Figure 3 shows the spectrum resulting from the mutual storage of $(R+7H)^{7+}$ reagent ions derived from the DAB-g4 dendrimer with singly charged anions of LKRApYLG-NH₂. Both singly and doubly protonated species are formed from this peptide, although no triply protonated peptides are formed with this reagent. The relative abundance of the doubly protonated species as well as the appearance of higher charge states is expected to be related to the size and charge state of the dendrimer reagent as well as the composition and potential sites of protonation of the peptide. Higher generation dendrimers of higher charge, for example, might lead to higher relative abundances of doubly charged ions. However, this particular peptide is relatively small and might not be expected to form triply protonated ions due to the high electrostatic repulsion that would result.

Figure 4 shows the charge inversion products formed from the candidate phosphopeptide (C) present in the tryptic digest of α -casein. Like the synthetic phosphopeptide, singly and doubly protonated ions were formed with no evidence for formation of a triply charged ion. The $(M+2H)^{2+}/(M+H)^+$ ratio for the tryptic peptide is somewhat higher than that for the synthetic peptide, perhaps due to the fact that the tryptic peptide is somewhat larger. However, there are only three nominally strong basic sites in the tryptic peptide, the N-terminus and two adjacent lysine residues at the C-terminus. The proximity of the lysine residues minimizes the likelihood that all three sites will be protonated. However, tryptic peptides are generally characterized by the presence of a least two basic sites. Hence, the formation of doubly charged tryptic peptides as the most highly charge species via charge inversion is likely to be a common result.

The key result from both Figure 3 and Figure 4 is that doubly protonated phosphopeptides were formed by charge inversion despite the fact that they were not formed directly via positive ion electrospray. It is difficult to determine precisely the efficiency with which charge inversion to the doubly protonated species takes place due to the significant differences in detector responses for positive and negative ions. However, an upper limit to efficiency can be estimated from the relative abundances of the dendrimer cation product ion charge states following a

relatively short ion/ion reaction time. For example, in the case of the $(R+7H)^{7+}$ reagent ion, if it is assumed that the $(R+6H)^{6+}$, $(R+5H)^{5+}$, and $(R+4H)^{4+}$ ions arise from the transfer of one, two, and three protons, respectively, in a single ion/ion encounter, the relative propensities for neutralization of the phosphopeptide (i.e., from one proton transfer), formation of the $(M+H)^+$, and $(M+2H)^{2+}$ ions can be estimated from the abundances of the cation products. When such a comparison was made for the $(R+7H)^{7+}$ ion of R = diaminobutane core generation 4 dendrimer (DAB-g4) and the $(M-H)^-$ ions of LKRApYLG-NH₂, it was estimated that roughly 20% of the anions were neutralized and that the rest underwent charge inversion with the majority resulting in the $(M+H)^+$ charge state, as observed experimentally. This estimate is based on the assumption that the reagent ion charge states of $(R+5H)^{5+}$ and $(R+4H)^{4+}$ arise from multiple proton transfers in a single ion/ion encounter. However, some of these ions can also be formed via sequential ion/ion single proton transfer reactions. Hence, a fractional neutralization of 20% is regarded as a minimum value in this case. However, the extent of sequential ion/ion reactions is minimized at relatively short reaction times so that charge inversion efficiencies of a few tens of percent are likely to apply to these reactions. This is consistent with efficiencies noted for two-step ion/ion charge inversion reactions that have been demonstrated to be capable of increasing the net charge state of an ion^{21,22}.

Electron Transfer Dissociation of Phosphopeptide Cations formed via Charge Inversion

Electron transfer from reagent anions to doubly charged peptide cations can give rise to characteristic c- and z-type fragments, fragments characteristic of some side chains³³, and singly charged survivors that appear along with proton transfer products (see Scheme 2). The extent of ETD of doubly protonated peptides is generally low relative to more highly charged peptides and also appears to vary with peptide size, charge location, and site of protonation.³⁴ It has been noted that the use of elevated bath gas temperatures can provide more extensive sequence information for some doubly protonated peptides.³⁵ Unfortunately, the instrument used to collect these data does not have ability to heat the bath gas, which would have determined if elevated bath gas temperatures would be useful for the phosphopeptide ions studied here.

Figure 5a shows the results obtained from electron transfer to doubly protonated LKRApYLG-NH₂, formed via charge inversion. In this case, evidence for fragmentation at every inter-residue bond is noted. Localization of the phosphate group is achieved by z-type fragment ions N-terminal to tyrosine and c-type fragment ions C-terminal to tyrosine that are 79 Da higher than the corresponding unmodified peptide. No evidence for loss of the modification is evident from the c- and z-type ions. Losses of HPO₃ and H₃PO₄ presumably arise from collisional activation of unreacted doubly charged ions upon resonance ejection, a phenomenon noted previously.³³ For comparison, Figure 5b shows a product ion spectrum derived from collision induced dissociation of the charge inverted phosphopeptide, followed by proton transfer ion/ion reactions with 1,3-perfluorodimethyl cyclohexane (PDCH) anions to reduce product ion charges largely to +1. As is often noted in the collision-induced dissociation of phosphopeptide cations, losses of H₂O, HPO₃, and H₃PO₄ are prominent, as well as sequential cleavages that follow one or more of these losses. As a result, unambiguous localization of the phosphate group is precluded.

Figure 6a shows data highlighting the fragmentation resulting from electron transfer to the doubly protonated tryptic phosphopeptide EQLpSTpSEENpSKK, formed via charge inversion of the deprotonated species (see Figure 2 and Figure 4). Characteristic c- and z-type fragment ions are observed with the cleavages concentrated at the termini. Similar behavior has been noted previously for the ETD of other doubly charged peptides.³³ In this case, two of the phosphate groups could be localized from the identities of the fragment ions, despite the fact that the complete sequence of the peptide is not reflected in the spectrum. Figure 6b shows the

ion trap collision-induced dissociation data for the doubly protonated species formed via charge inversion for comparison. Small molecule losses dominate the spectrum, although cleavages c-terminal to the glutamic acid residues giving rise to the complementary ion pairs b_4/y_8 and b_5/y_7 are both noted.

In this particular case, the CID results provide additional sequence information, although it is restricted to one additional residue. Nevertheless, it illustrates the possibility for complementary structural information arising from subjecting charge inversion products both to CID and ETD.

CONCLUSIONS

Ion/ion charge inversion reactions allow for ionization of phosphopeptides in the negative ion mode and, in the same experimental sequence, structural interrogation via electron transfer to doubly protonated versions of the ions. This approach provides a new option in the analysis of phosphopeptides and is expected to be most useful when little or no signal due to multiply charged species can be generated in the positive ion mode. Such a scenario is commonplace in the positive ion electrospray of peptide mixtures in which acidic phosphopeptides are present. The strategy was illustrated here with a relatively small model peptide and with a somewhat larger phosphopeptide with few strongly basic sites. Nevertheless, sufficient numbers of doubly protonated species could be generated via charge inversion for subsequent electron transfer dissociation. While the formation of doubly protonated species is a minimal condition for ETD, it is desirable to be able to form triply protonated species for better ETD efficiency. This might be achieved by use of more highly charged reagents for the charge inversion step. The use of enzymes that yield larger peptide fragments, such as Glu-C or Lys-C, might also be expected to facilitate formation of more highly charged positive ions in the charge inversion step.

Acknowledgements

The authors acknowledge support from the National Institutes of Health, Grant GM 45372.

REFERENCES

1. Hunter T. *Cell* 2000;100:112–127.
2. Neubauer G, Mann M. *Anal. Chem* 1999;71:235–242. [PubMed: 9921130]
3. McLachlin DT, Chait BT. *Curr. Opin. Chem. Biol* 2001;5:591–602. [PubMed: 11578935]
4. Qian W-J, Goshe MB, Camp DG II, Yu L-R, Tang K, Smith RD. *Anal. Chem* 2003;75:5411–5450.
5. Aebersold R, Goodlett DR. *Chem Rev* 2001;101:269–295. [PubMed: 11712248]
6. Reinders J, Sickmann A. *Proteomics* 2005;5:4052–4061. [PubMed: 16196093]
7. Janek K, Wenschuh H, Bienert M, Krause E. *Rapid Commun. Mass Spectrom* 2001;15:1593–1599. [PubMed: 11544598]
8. Wilm M, Neubauer G, Mann M. *Anal. Chem* 1996;68:527–533. [PubMed: 8712361]
9. Schlosser A, Pipkorn R, Bossemeyer D, Lehmann WD. *Anal. Chem* 2001;73:170–176. [PubMed: 11199962]
10. Huddleston AJ, Annan RS, Bean MF, Carr SA. *J. Am. Chem. Soc. Mass Spectrom* 1993;7:774–779.
11. Annan RS, Carr SA. *Anal. Chem* 1996;68:3413–3421. [PubMed: 8843139]
12. Flora JW, Muddiman DC. *Anal. Chem* 2001;73:3305–3331. [PubMed: 11476230]
13. Coon JJ, Syka JEP, Shabanowitz J, Hunt DF. *Int. J. Mass Spectrom* 2004;236:33–42.
14. Stone DH, Hemling ME, Carr SA, Horn DM, Lindh I, McLafferty FW. *Anal. Chem* 2001;73:19–22. [PubMed: 11195502]
15. Budnik BA, Haselmann KM, Zubarev RA. *Chem. Phys. Lett* 2001;342:299–302.

16. Coon JJ, Shabanowitz J, Hunt DF, Syka JEP. *J. Am. Chem. Soc. Mass Spectrom* 2005;16:880–882.
17. McLuckey SA, Reid GE, Wells JM. *Anal. Chem* 2002;74:336–346. [PubMed: 11811406]
18. Reid GE, Shang H, Hogan JM, Lee GU, McLuckey SA. *J. Am. Chem. Soc* 2002;124:7353–7362. [PubMed: 12071744]
19. Amunugama R, Hogan JM, Newton KA, McLuckey SA. *Anal. Chem* 2004;76:720–727. [PubMed: 14750868]
20. Coon JJ, Ueberheide B, Syka JEP, Dryhurst DD, Ausio J, Shabanowitz J, Hunt DF. *Proc. Nat. Acad. Sci* 2005;102:9463–9468. [PubMed: 15983376]
21. He M, McLuckey SA. *J. Am. Chem. Soc* 2003;125:7756–7757. [PubMed: 12822966]
22. He M, McLuckey SA. *Anal. Chem* 2004;76:4189–4192. [PubMed: 15253662]
23. Badman ER, Chrisman PA, McLuckey SA. *Anal. Chem* 2002;74:6237–6243. [PubMed: 12510744]
24. Van Berkel GJ, Asano KG, Schnier PD. *J. Am. Soc. Mass Spectrom* 2001;12:853–862. [PubMed: 11444609]
25. McLuckey SA, Glish GL, Asano KG, Grant BC. *Anal. Chem* 1988;60:2220–2228.
26. McLuckey SA, Goeringer DE, Glish GL. *J. Am. Soc. Mass Spectrom* 1991;2:11–21.
27. Kaiser RE, Cooks RG, Stafford GC, Syka JEP, Hemberger PH. *Int. J. Mass Spectrom. Ion Processes* 1991;106:79–115.
28. Wells JM, Chrisman PA, McLuckey SA. *J. Am. Chem. Soc* 2003;125:7238–7249. [PubMed: 12797797]
29. Goeringer DE, McLuckey SA. *Int. J. Mass Spectrom* 1998;177:163–174.
30. Gunawardena HP, He M, Chrisman PA, Pitteri SJ, Hogan JM, Hodges BDM, McLuckey. *J. Am. Chem. Soc* 2005;127:12627–12639. [PubMed: 16144411]
31. Cech NB, Enke CG. *Mass Spectrom. Rev* 2001;20:362–387. [PubMed: 11997944]
32. Pan P, Gunawardena HP, Xia Y, McLuckey SA. *Anal. Chem* 2004;76:1165–1174. [PubMed: 14961751]
33. Cooper HJ, Hudgins RR, Hakansson K, Marshall AG. *J. Am. Soc. Mass Spectrom* 2002;13:241–249. [PubMed: 11908804]
34. Pitteri SJ, Chrisman PA, Hogan JM, McLuckey SA. *Anal. Chem* 2005;77:1831–1839. [PubMed: 15762593]
35. Pitteri SJ, Chrisman PA, McLuckey SA. *Anal. Chem* 2005;77:5662–5669. [PubMed: 16131079]

Figure 1.

Electrospray mass spectra of an equimolar mixture of synthetic peptides A–G (~1 μ M each)
(a) Positive ion mode nano-electrospray from 1% acetic acid aqueous solution (b) Negative ion mode nano-electrospray from 2% ammonium hydroxide aqueous solution.

Figure 2.

Electrospray mass spectra of an α -casein tryptic digest. (a) Positive ion mode nanoelectrospray in 1% acetic acid aqueous solution. (b) Negative ion mode nanoelectrospray in 2% ammonium hydroxide aqueous solution. Peptide assignments are based on matching observed masses with expected masses for tryptic digestion and were not confirmed with MS/MS.

Figure 3.

Mass spectrum resulting from ion/ion reactions between (R+7H)⁷⁺ ion of R = diaminobutane core generation 4 dendrimer (DAB-g4) with (M-H)⁻ ions of LKRApYLG-NH₂. (Note that the abundance scale of Figure 3 cannot be compared directly with that of Figure 1(b), in part due to different detector responses for negative ions and positive ions.)

Figure 4.

Spectrum resulting from the ion/ion reaction between the singly deprotonated phosphopeptide EQLpSTpSEENpSKK and the $(\text{R}+7\text{H})^{7+}$ ion of $\text{R} = \text{DAB-g4}$ dendrimer.

Figure 5.

MS³ product ion spectra of phosphopeptides (a) Product ion spectrum resulting from ion/ion reactions of LKRApYLG-NH₂ (2+) and azobenzene (-1) (b) Product ion spectrum resulting from CID of LKRApYLG-NH₂ (2+) followed by ion/ion reactions with PDCH anions.

Figure 6. Spectra derived from (a) ETD of the doubly protonated tryptic phosphopeptide EQLpSTpSEENpSKK formed via charge inversion of the singly deprotonated species and (b) CID of the doubly protonated species formed via charge inversion.

Scheme 1.

Ion/ion proton transfer reaction scheme for a singly deprotonated analyte species and a triply protonated reagent ion.

Scheme 2.

Ion/ion reaction scheme for a doubly protonated analyte species and a singly deprotonated reagent ion.