The influence of a C-terminal basic residue on peptide fragmentation pathways

Ross Chawner [a], Claire E. Eyers [a,*], Simon J. Gaskell [b,*]

[a] Michael Barber Centre for Mass Spectrometry, School of Chemistry, University of Manchester, UK
[b] Queen Mary University of London, Mile End Road, London, E1 4NS, UK

ARTICLE INFO

ABSTRACT

A typical 'bottom up' proteomic workflow uses tandem mass spectrometric data to infer product ion sequence and hence identity of the protein from which they derive. Such analysis is typically performed following proteolysis with the endoproteases trypsin or Lys-C; peptides produced therefore terminate in the basic residues arginine or lysine. Removal of these C-terminal basic residues using the exopeptidase, carboxypeptidase B, generates peptides whose analysis by tandem MS yields evidence of substantially different fragmentation properties. The decompositions of peptide ions both prior to and following treatment with carboxypeptidase B have been examined using collision-induced dissociation and electron transfer dissociation. Changes in properties following secondary enzyme treatment are attributed primarily to removal of a strongly basic site, with a consequent effect both on the propensity to retain charge and the stability of the fragment ions. The data suggest a complementary value in proteome analyses for MS/MS of tryptic/Lys-C peptides with and without subsequent carboxypeptidase B treatment.

© 2012 Elsevier B.V. All rights reserved.

Tandem Mass Spectrometric (MS/MS) analysis of peptides with a C-terminal basic residue is typically performed during a standard 'bottom up' proteomics workflow [1,2]. Peptides terminating in the basic residues arginine and lysine are produced from digestion of a protein using the endoprotease trypsin. Such peptides are ideal for study by MS/MS as they appear in the optimal mass range for analysis and are commonly observed as doubly protonated species following electrospray ionisation [3]. The highly basic residue at the carboxyl-terminus sequesters one of the available protons, while the other can become 'mobile' upon activation, thus facilitating fragmentation by Collision-Induced Dissociation (CID) [4–6]. The more recent implementation of the endoprotease Lys-C (cleaving C-terminal to Lys residues) has triggered a growing interest in peptide ion fragmentation by Electron Transfer Dissociation (ETD) [7]. Proteolysis using Lys-C generates larger peptides which may contain internal arginine residues, thereby enhancing their observation as highly charged species which exhibit excellent fragmentation efficiency by ETD [8]. Carboxypeptidase B (CBPB) is an exopeptidase which selectively cleaves the C-terminal basic residues, arginine and lysine, from a polypeptide. Accordingly, the enzyme may be particularly useful following tryptic/Lys-C digestion of a protein, as removal of the C-terminal basic group is expected to have a marked effect on peptide fragmentation chemistry. Tandem MS analyses of the products of trypsin or Lys-C digestion, before and after CBPB treatment may therefore yield data of complementary value.

The mechanistic principles of CID are well understood and have been comprehensively reviewed by Paizs and Suhai [9]. The dominant species in CID MS/MS spectra are the products of amide bond cleavage; migration of a mobile proton to the nitrogen atom of the amide group weakens the peptide bond [10] and dissociation into diagnostic b/y-ion fragments results. The N-terminal b-ion series is believed to include cyclic structures whereas the C-terminal y-ion series constitutes a protonated linear truncated peptide (y_n) or amino acid (y_1) [11–13]. The fragment ions most likely to be observed in a given CID MS/MS analysis are determined by the amino acid composition (and hence gas-phase ion chemistry) of the peptide in question and the instrument upon which analysis is performed. Boyd and co-workers [14] have shown that amino acid composition, in particular the number and location of basic residues, plays a critical role in obtaining informative low energy CID MS/MS spectra for multiply charged peptides and the type of ions observed. Intramolecular coulombic repulsion between protons is not sufficient to result in effective peptide fragmentation and proton mobilisation is required to initiate charge directed, structurally informative decompositions. Such mobilisation is however not readily achieved under low energy CID conditions if protons are sequestered by highly basic residues. It is known that b-ion fragments produced from protonated peptides are under-represented following CID in a quadrupole type collision cell (such as in a quadrupole time-of-flight (QToF) mass analyser) when compared to a quadrupole ion trap (QIT) [15]. During MS/MS analysis in a QToF instrument, peptide ions are subjected to multiple collisions with the inert buffer gas and b-ions, which are often less stable than their y-ion counterparts, undergo secondary fragmentation to lower members of the ion series. Equivalent analysis with a

* Corresponding authors.
 E-mail addresses: claire.eyers@manchester.ac.uk (C.E. Eyers),
principal@qmul.ac.uk (S.J. Gaskell).

quadrupole ion trap (QIT) instrument results in the observation of higher members of the b-ion series. Following the initial fragmentation event the product ions are no longer resonant with the excitation voltage and therefore do not experience multiple ion-neutral collisions with the buffer gas [15].

Electron Transfer Dissociation results in peptide backbone fragmentation and is particularly useful for more highly charged species ($z \geq 3$) or when preservation of labile side-chains/post translational modifications (PTMs) is required. Unlike ETD, CID often results in preferential loss of labile groups such as phosphate, which in turn makes the assessment of modification sites difficult [16]. ETD along with Electron Capture Dissociation (ECD) shows a unique propensity for random cleavage of backbone N–C_α bonds. The process appears to proceed with little dependence upon peptide length or amino acid composition; albeit with a higher efficiency of fragmentation being observed for multiply charged sequences. The products of ETD may remain bound together in a non-covalent dissociation complex post fragmentation, similar to that described by McLafferty and co-workers in early ECD studies [17]. This effect is expected to be particularly pronounced during ECD/ETD analysis of doubly charged analytes (yielding a singly charged precursor to fragmentation). A low energy collisional activation, termed 'supplemental activation' following ETD can be employed to help break this complex [18].

The decomposition chemistry observed for CID and ETD of doubly charged peptide ions is determined by the nature of the immediate precursors to fragmentation – doubly charged even-electron ions and singly charged radical cations respectively. Thus for CID and ETD regimes, cleavage results in the formation of a pair of singly charged ions (in most cases) and an ion/neutral pair, respectively. We have previously shown [15] that singly charged N-terminal ions from tryptic peptides fragment more readily than their C-terminal counterparts, an effect attributable to the extent of proton mobilisation in product ions. This leads to an under-representation of N-terminal fragments in product ion spectra that is more pronounced in spectra recorded on QToF instruments than on QITs (because of differing extents of promotion of subsequent fragmentation in the different experimental regimes as stated above). For the case of fragmentation of singly charged radical cations, formed by electron transfer to doubly charged precursors, the observation of product ions will reflect the gas-phase basicities of the complementary fragments. Thus, for both CID and ETD of doubly charged peptides, fragmentation is expected to be significantly affected by the removal of the C-terminal basic residue.

The objective of the work presented here was to investigate the influence of the C-terminal basic residue present in peptides produced by either tryptic or Lys-C proteolysis on the fragmentation products generated by either CID or ETD. Dissociation of trypsin/Lys-C peptides by ETD typically results in the preferential generation of z-ion fragments over their c-ion counterparts. This is a direct consequence of the presence of the basic residue at the C-terminus, meaning that the z-ion series is more likely to retain the available charge post fragmentation and is therefore more highly represented in ETD generated MS/MS spectra. CBPB treatment of such peptides is expected to alter the observed bias in z-ion formation as the C-terminal basic residue is no longer available for charge retention. We hypothesised that an effect would also be observed upon the relative distribution of b- and y- ions in CID MS/MS spectra post-CBPB treatment. The C-terminal arginine/lysine residue would normally sequester one of the available protons with an effect on the stability of the y-series fragments, as discussed above. Removal of this site of high gas-phase basicity will influence charge site distribution in C-terminal fragment ions, with a consequent change in the relative stabilities of b- and y- series ions.

1. Experimental

1.1. Protein digestion

Bovine serum albumin (BSA) in 50 mM ammonium bicarbonate was incubated with 4 mM dithiothreitol (DTT) at 60 °C for 45 min to reduce cystine residues. Following this, the sample was cooled to room temperature and iodoacetamide (14 mM) added prior to 1 h incubation in the dark. The DTT concentration was then increased to 7 mM to quench the alkylation reaction prior to an 18 h incubation, 37 °C with either porcine trypsin or Lys-C (both from Sigma, Poole, Dorset, UK) at a 2:100 (w:w) enzyme:substrate ratio.

1.2. Secondary digestion with carboxypeptidase B

An aliquot of the initial trypsin and Lys-C digestions was removed and incubated with carboxypeptidase B (Worthington Biochemical, New Jersey, USA) at a ratio of 12 units of enzyme per milligram of protein for 2 h at room temperature.

1.3. Nano-flow LC–MS (QIT) analysis

Digested material (500 fmol) was injected and desalted in-line using an Acclaim PepMap 100 trapping column (75 μm × 2 cm; 5 μm particle size; 100 Å pore size) prior to separation by reversed phase chromatography with an Acclaim PepMap C18 column (75 μm × 15 cm; 2 μm particle size; 100 Å pore size) both purchased from LC Packings Dionex (Surrey, UK). A flow rate of 300 nL/min was used for the LC separation on an EASY n-LC system (Proxeon, Odense, Denmark) coupled to an amaZon ETD ion trap mass spectrometer (Bruker, Bremen, Germany). The column was equilibrated with 0.2% (v/v) formic acid (Solvent A) and developed with 90% (v/v) acetonitrile containing 0.2% (v/v) formic acid (Solvent B); 0–55% over 55 min, 55–90% over 35 min and 90–100% over 5 min.

The 3 most abundant precursor ions from each scan were selected for MS/MS analysis with the CID fragmentation amplitude set at 1.2 V and ramped between 30% and 300% of this value. ETD MS/MS analysis was performed with a reaction time of 150 ms and the 'Smart Decomp' option set to auto, thereby adjusting the amplitude of supplemental activation according to charge state.

1.4. Nano-flow LC–MS (QToF) analysis

Reversed phase chromatography on a nanoACQUITY UPLC (Waters, Manchester, UK) was used to separate 100 fmol of digested material. The sample was desalted in-line using a Symmetry C18 trapping column (180 μm × 20 mm; 5 μm particle size; 100 Å pore size) and chromatographic separation performed with a BEH130 C18 column (75 μm × 100 mm; 1.7 μm particle size; 130 Å pore size). Both columns were from Waters, Manchester, UK. A flow rate of 300 nL/min was employed and the column temperature maintained at 35 °C. Solvent A was 0.1% (v/v) formic acid and Solvent B 100% acetonitrile; solvent B was ramped from 1 to 40% over 30 min, 40 to 85% over 5 min and then the column re-equilibrated with 1% Solvent B for 25 min. The chromatography system was coupled to a Synapt G2 HDMS mass spectrometer (Waters, Manchester, UK) set to transmit all species between 50 and 2000 m/z. The 3 most intense ions observed in a given scan were subjected to CID with the collision offset potential ramped between 5 and 40 V.

1.5. QIT data analysis

Peaklists from both the CID and ETD MS/MS spectra were extracted using Data Analysis software (Bruker) and the generated
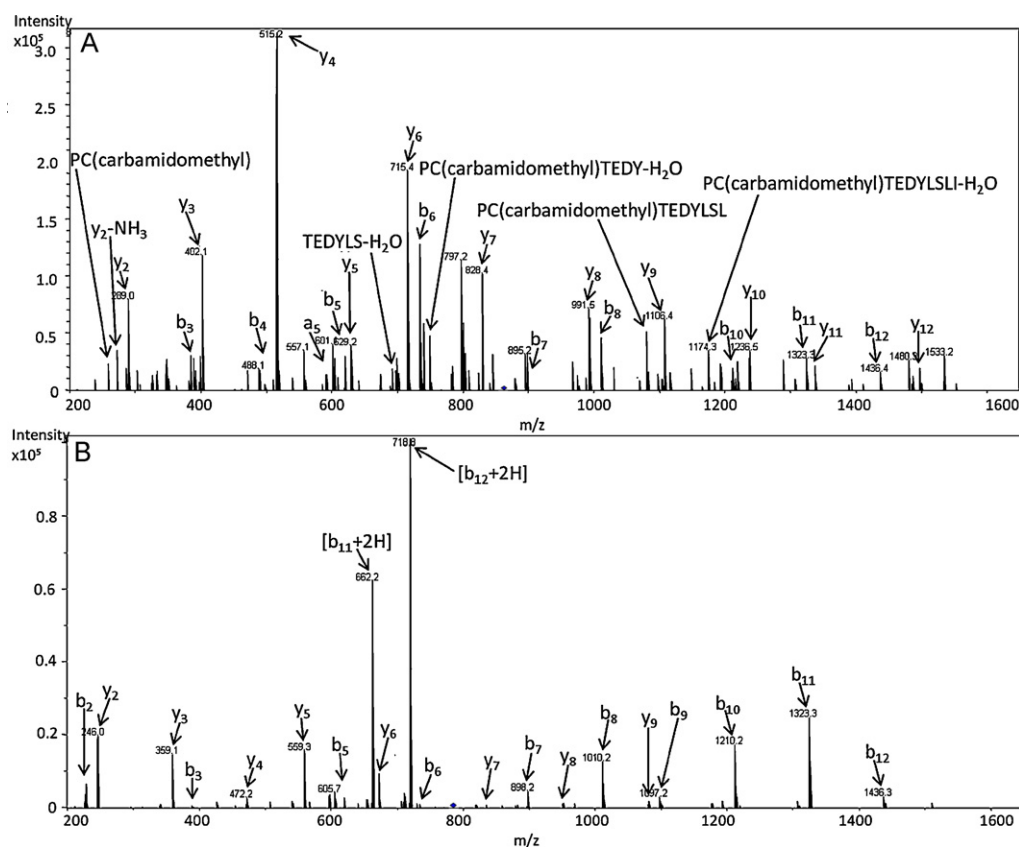
Fig. 1. Comparison of QIT product ion spectra resulting from (A) CID of doubly charged tryptic peptide MPC(carbamidomethyl)TEDYLSLILNR at m/z 863.4 and (B) CID of doubly charged carboxypeptidase B treated analogue MPC(carbamidomethyl)TEDYLSLILN at m/z 784.8.

.mgf files searched using Batch-Tag Web in Protein Prospector version 5.9.4 [19]. The precursor charge state range was set to consider +1 to +4 species with a precursor mass tolerance of 0.4 Da and a fragment mass tolerance of 0.8 Da. A script written in house at the University of California which has been described in detail previously [20] was then used to extract all product ions which match the theoretical fragment masses of the observed precursors. For CID spectra the script considers the sequence ions (b, a and y) along with potential neutral losses ($-H_2O$, $-NH_3$, $-SOCH_4$) from these products and the precursor from which they derive. The potential sequence ions (b, c − 1•, c, y, z• and z + 1) are considered for ETD spectra. Data from each individual peptide were then collated, combined and analysed using Excel.

2. Results and discussion

Peptides observed as doubly charged species both prior to and following treatment with CBPB have been extracted from the datasets produced by n-LC MS/MS analysis on both the QIT and QToF instruments and can be seen in Figs. 1–3. These data are presented to illustrate the general effect upon CID and ETD MS/MS spectra following removal of the C-terminal basic residue. Fig. 1 shows that following treatment of a tryptic peptide with CBPB there is a difference in the observed fragmentation pathways of the truncated precursor. Amongst the fragment ions observed for the peptide MPC(carbamidomethyl)TEDYLSLILN are b_2 and b_9 fragments (Fig. 1B) which are not observed for the tryptic analogue (Fig. 1A). The formation of the b_2 product is unexpected as cleavage C-terminal to proline is required [21,22]. It had previously been thought that the steric strain implicit in formation of a bicyclic oxazolone structure (with the proline residue incorporated) disfavours b_2 ion formation via this mechanism [4,23]. However a

study by Hopkinson and coworkers [24] has found this destabilisation to be negligible. Instead, they report that the presence of a prolyl residue at position 2 in the tripeptide Gly–Pro–Gly slightly increases the barrier to b_2 ion formation while simultaneously decreasing the barrier to y_2 ion formation when compared to the tripeptide Gly–Phe–Gly. It is therefore possible that either an alternative mechanism for b_2 ion formation from the CBPB treated analogue is induced in this case or simply that the energy barrier toward b_2 ion formation is reduced. The normally favoured fragmentation N-terminal to proline is also not expected as a b_1 ion would result and this is not predicted via the oxazolone pathway. Our observations are supported by Hopkinson and coworkers [21] who have reported that various tripeptides containing a prolyl residue in the central position fragment to form both the b_2 and y_2 products. This observation is attributed to the higher basicity of the secondary amide incorporating the prolyl nitrogen. Protonation at the N-terminal bond is competitive, but the $C–N^+$ bond of the secondary amide is sterically crowded when compared to that of a primary amide; the bond is therefore weaker and cleavage N-terminal to the prolyl residue is observed. Extrapolation of these observations in relatively small tripeptide systems to the larger sequences being examined in our present study must however be performed with caution. A study conducted in the Wysocki laboratory [25] investigated the corresponding bond cleavage C-terminal to proline in larger tryptic peptides and found evidence suggesting that proline containing b_2 ions may be formed via the diketopiperazine pathway rather than the more commonly expected oxazolone pathway [13,26]. The study was performed following the observation of unusual fragmentation behaviour for the peptides VPDPR and VPAPR, where prevalent bond cleavage C-terminal to proline and N-terminal to aspartic acid was seen to give complementary b_2/y_3 ions. Various approaches to elucidate the underlying
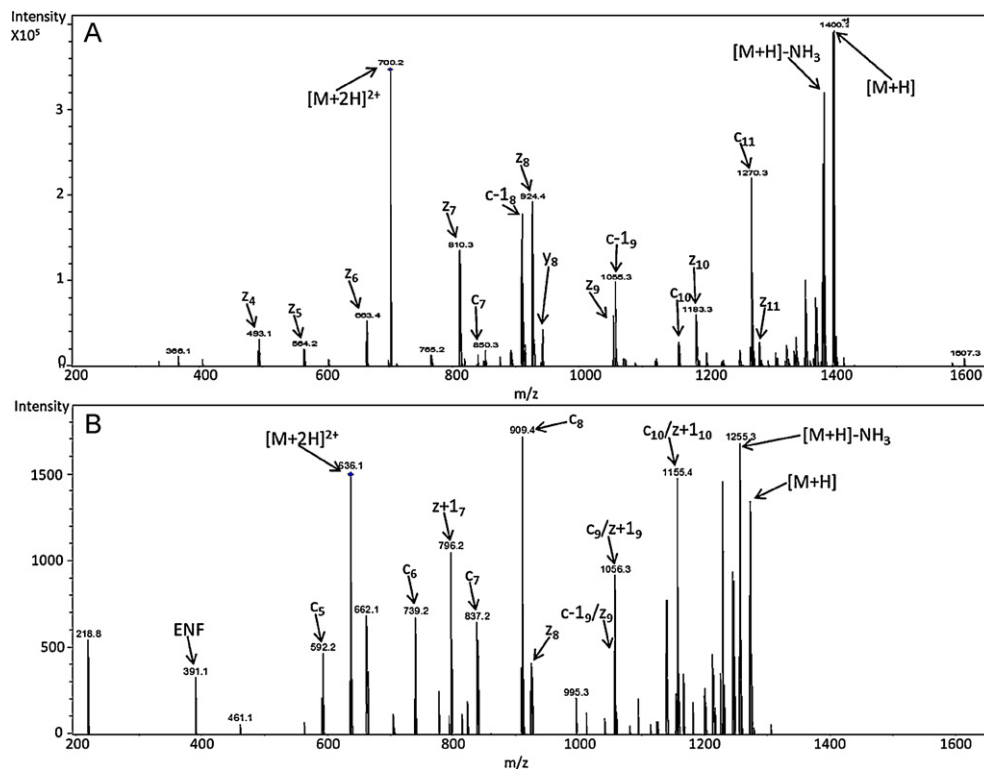
Fig. 2. Comparison of QIT MS/MS spectra resulting from (A) ETD of the doubly charged tryptic peptide TVMENFVAFVDK at m/z 700.3 and (B) ETD of the doubly charged CBPB treated analogue TVMENFVAFVD at m/z 636.2.

mechanism of this bond cleavage were undertaken, with a notable observation being that N-terminal acetylation of the peptide VPAPR prevented formation of the b_2 fragment ion that was generated from the unmodified precursor. This finding is consistent with the b_2 ion being formed by the diketopiperazine pathway, whereby attack of the carbonyl carbon at the site of protonation by the lone pair of electrons on the N-terminal amino-nitrogen is required. Modification of the N-terminus prevents this attack and accounts for the absence of the VP b_2 ion. If the ion were formed via the oxazolone pathway N-terminal modification would not prevent b_2 ion formation as the required attack of the carbonyl carbon by the N-terminal carbonyl oxygen could still occur. It is possible that in our present study removal of the arginine residue may result not only in 'mobilisation' of the sequestered proton but could also facilitate a change in the gas-phase ion chemistry, enabling the diketopiperazine fragmentation pathway to become accessible.

CID of the peptide MPC(carbamidomethyl)TEDYLSLILN exhibits an unusual propensity for formation of doubly charged b-ions (Fig. 1B). These species are not generated by CID of the tryptic analogue MPC(carbamidomethyl)TEDYLSLILNR (Fig. 1A) and this can be attributed to the presence of the arginine residue which sequesters one of the available protons within the y-ion series. It is known that the observed product ions of low energy CID are determined by the relative stabilities of the potential ionised fragments: competition for retention of the available proton takes place between the fragments which exist in a proton bound dimer [27,28]. Following removal of the C-terminal basic residue, the proton affinity of the C-terminal fragments is reduced and any available protons are more likely to be retained by the b-ion series. Interestingly, notable doubly charged b-ions are only observed for sequences incorporating 10 or more amino acid residues, with the two most intense ions in the spectrum being those attributed to the doubly charged b_{11} and b_{12} ions (the b_{12} species being the base peak). Associated signal intensity of these doubly charged fragment ions rapidly increases in correlation with the number of amino

acid residues, presumably due to decreased coulombic repulsion between the charges accommodated within the b-ion structure and the increased gas-phase proton affinity relative to the y-ion series now lacking a highly basic residue as discussed above. The fragmentation products of peptides following CBPB treatment, observed in both the CID and ETD spectra (Figs. 1 and 2 respectively), demonstrate an expected phenomenon. Prior to removal of the C-terminal basic residue the corresponding y- and z- ion series dominate the CID and ETD product ion spectra, respectively, both in terms of their frequency of observation and in the majority of cases, signal intensity. After the secondary enzymatic treatment, the respective b- and c-ion series become more prominent. Each is observed both with greater frequency and in several cases with higher relative signal intensity than the analogous ions in the initial tryptic MS/MS product ion spectra. This phenomenon is particularly prevalent when considering ETD spectra of doubly protonated precursor ions. The domination of the MS/MS spectra of tryptic peptides (Fig. 2A) by C-terminal fragment ions can be attributed to the proton affinity of the arginine/lysine side chain present within the z-ion series. Competition for retention of the available proton within the dissociation complex takes place, with the z-ion series being that most likely to remain charged. Removal of the C-terminal residue is expected, depending on amino acid sequence, to result in at least a partial switch in the dominant ion series, with the N-terminus of the peptide now being the site with highest gas-phase proton affinity. The observation that the location of basic residues within a peptide backbone can dictate the ion series most likely to be seen has been previously reported both for singly charged peptides in CID and for multiply charged species in ETD [9,20]. The switch in preferred ion current from carboxy- to amino-terminally derived product ions is particularly striking in the QToF CID spectra of the doubly charged peptide LKPDPNTLC(carbamidomethyl)DEFK derived from Lys-C digestion, and its CBPB-treated analogue (Fig. 3). The sequence contains an internal lysine residue close to the N-terminus and the presence of this highly basic amino acid appears to assist the
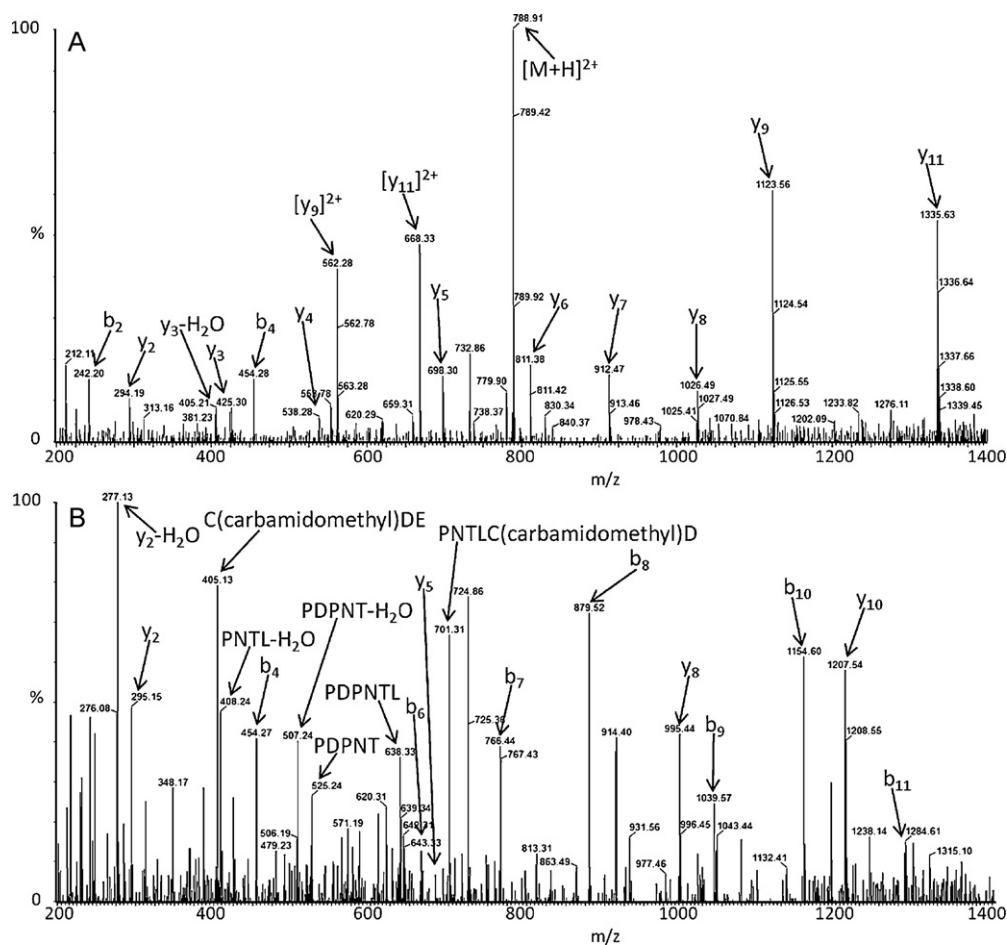
Fig. 3. Comparison of QToF MS/MS spectra resulting from (A) CID of doubly charged tryptic peptide LKPDPNTLC(carbamidomethyl)DEFK at m/z 788.8 and (B) CID of doubly charged carboxypeptidase B treated analogue LKPDPNTLC(carbamidomethyl)DEF at m/z 724.8.

apparent switch in bias of observed product ions following removal of the C-terminal residue. The original lysine terminating product ion spectrum shown in Fig. 3A contains two b-ion fragments and nine y-ion species whereas that of the truncated CBPB analogue (Fig. 3B) exemplifies the switch in dominant product ion series with a total of seven N-terminal b-ions and just four C-terminal y-ion fragments.

To enable an evaluation of the validity of generalisations comparing fragmentation behaviour, data from multiple tryptic and Lys-C peptides and their CBPB-treated analogues have been collated (Tables 1 and 2). These tables show the percentage contributions of each ion type toward the total product ion current from n-LC QIT MS/MS analysis of the proteolytic cleavage products of BSA. The CID datasets show very similar trends for both the tryptic and Lys-C digestions with an approximate 10% increase in the proportion of b-ion and b-ion derived fragments observed following CBPB treatment and a corresponding decrease of around 6% in the proportion of y-ion and derived fragments. CBPB treatment of Lys-C peptides gives a 7% increase in the proportion of c-ion fragments generated by ETD. However, the corresponding increase in the c-ion series from tryptic precursor ions is negligible (1.5%). We postulate that this is a result of the small number of tryptic peptides which remain as doubly charged (or greater) species following CBPB treatment making them amenable to study by ETD. The charge density of these CBPB treated tryptic precursor ions is extremely low and poor fragmentation efficiency is thus often observed by ETD. The ion statistics are therefore based upon far fewer ions than the other datasets and it is difficult to make conclusions

regarding the global effect of CBPB treatment toward ETD of tryptic peptides.

An interesting observation from the ETD derived ion statistics is that the propensity for formation of hydrogen atom transfer products differs greatly in the tryptic/Lys-C peptides when compared to their CBPB truncated analogues. Removal of the C-terminal basic residue sees an increase from 16% to 29.0% of the ion current being attributed to $c - 1^\bullet$ ions for Lys-C peptides with a corresponding increase from 16% to 43% observed for tryptic peptides. The increased observation of $c - 1^\bullet$ ions is accompanied by a decrease in the formation of $z + 1$ species with the associated ion current falling from 15% to 8% (Lys-C peptides) and 13% to 5% (tryptic peptides), contrary to the expectation of a concomitant increase in $z + 1$ ion formation. This effect is illustrated by the spectra in Fig. 4 where the apparent isotope distributions (reflecting the distorting effect of hydrogen migrations accompanying fragmentation) of product ions resulting from ETD of the Lys-C peptide EC(carbamidomethyl)C(carbamidomethyl)HGDLLEC(carbamidomethyl)ADDRADLAK and its CBPB treated analogue are shown. In this instance the Lys-C derived precursor is observed as a quadruply charged species and the CBPB truncated precursor is observed as a triply charged ion. Fig. 4A shows the apparent isotope distribution of the c_9 product ion (1131.4 mono isotopic m/z) and no evidence of hydrogen atom transfer is visible. However, the corresponding apparent isotope distribution of the c_9 ion resulting from ETD of the CBPB treated precursor (Fig. 4B) incorporates a significant peak indicating that hydrogen transfer has taken place. This change

Table 1
Summary of CID fragmentation behaviour following several digestion regimes, with analysis by ESI-QIT MS/MS. The values shown are the percentage of total product ion current from all precursors observed both prior to and following CBPB treatment.

Ion type	Lys-C	Lys-C CBPB	Trypsin	Trypsin CBPB
$MH-SOCH_4$	0.1	0.3	0.1	0.0
$MH-H_2O$	0.3	0.3	0.0	0.0
$MH-NH_3$	0.3	0.3	0.0	0.0
b	19.8	33.0	22.2	32.6
a	6.3	4.9	5.3	6.8
y	37.8	31.6	35.6	29.4
$b-H_2O$	11.4	11.0	10.5	10.7
$b-NH_3$	4.3	4.0	5.3	5.7
$y-H_2O$	10.4	9.9	10.5	11.8
$y-NH_3$	8.6	4.1	9.9	2.9
$y-SOCH_4$	0.6	0.3	0.4	0.0
$a-SOCH_4$	0.0	0.3	0.0	0.0
Total precursor ions	29	14	30	12
Precursor charge states	$25 \times 2+/4 \times 3+$	$12 \times 2+/2 \times 3+$	$21 \times 2+/9 \times 3+$	$11 \times 2+/1 \times 3+$
Total fragment ions	780	328	902	279

N.B. Ion types whose percentage ion current does not exceed 0.2% in any analysis have been omitted to allow for random matching.

Table 2
Summary of ETD fragmentation behaviour following several digestion regimes, with analysis by ESI-QIT MS/MS. The values shown are the percentage of total product ion current from all precursors observed both prior to and following CBPB treatment.

Ion type	Lys-C	Lys-C CBPB	Trypsin	Trypsin CBPB
MH-17	0.4	0.0	0.0	0.0
c	25.0	32.1	28.5	30.0
$z^\bullet$	31.2	21.0	31.9	12.5
$z+1$	15.4	8.0	13.2	5.0
$c-1^\bullet$	15.8	29.0	15.6	42.5
b	4.0	4.9	2.0	0.0
y	8.0	4.9	8.4	7.5
Total precursor ions	25	9	32	3
Precursor charge states	$8 \times 2+/10 \times 3+/7 \times 4+$	$6 \times 2+/3 \times 3+$	$11 \times 2+/16 \times 3+/5 \times 4+$	$3 \times 2+$
Total fragment ions	696	162	813	40

N.B. Ion types whose percentage ion current does not exceed 0.2% in any analysis have been omitted to allow for random matching. In addition, ion types attributed to charge reduction but no fragmentation have also been removed for clarity.

in propensity for hydrogen atom transfer is further highlighted by the spectra in Fig. 4C and D, relating to the z_{12} and z_{11} ions from the Lys-C and CBPB precursors respectively. These ions are both produced from the same bond breakage (between two leucine residues) and the apparent isotope distribution in

Fig. 4C indicates significant $z+1$ ion formation whereas that in Fig. 4D does not.

Further studies are required to better understand these changes in the propensity for hydrogen atom transfer. It is possible that the observed increase in proton abstraction of c-ions may be related to
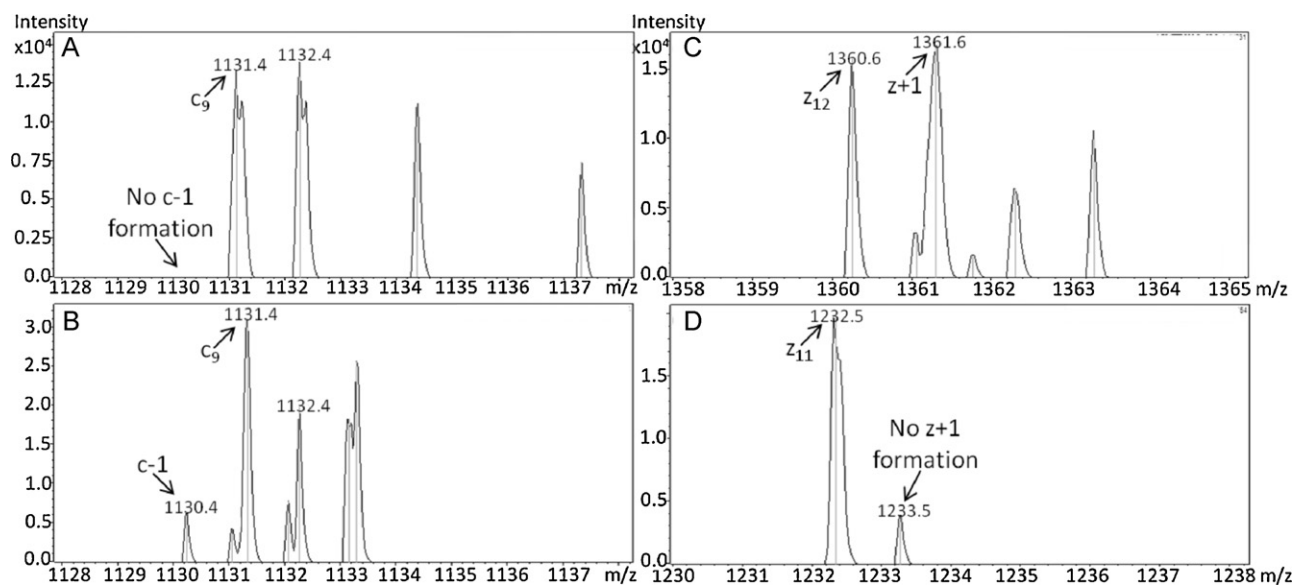

Fig. 4. Comparison of product ion apparent isotope distributions resulting from ETD of the Lys-C derived peptide EC(carbamidomethyl)C(carbamidomethyl)HGDLLEC-(carbamidomethyl)ADDRADLAK and the cognate CBPB treated analogue. A and B show the apparent isotope distributions of the c_9 product ion from the Lys-C and CBPB peptides respectively, with the CBPB treated analogue exhibiting increased $c-1$ ion formation. C and D show the corresponding apparent isotope distributions of the Lys-C peptide z_{12} product ion and CBPB peptide z_{11} product ion (relating to the same bond cleavage) with the Lys-C peptide exhibiting evidence of $z+1$ ion formation.

the increased propensity of doubly protonated CBPB treated peptides to contain internal amino acids with basic side chains (His, Lys, Arg), necessary to yield multiply charged precursor ions for ETD fragmentation. A recent study by Nishikaze and Takayama [29] found that hydrogen atom transfer is highly dependent upon precursor charge state and amino acid composition, in particular the presence of basic sites. They report that doubly protonated species exhibit extensive hydrogen atom transfer to give $c^{+\cdot}$ ions (described in this present study as $c - 1^\bullet$ ions) whereas a triply protonated form of the same sequence shows no hydrogen transfer. Crucially, highly basic doubly charged peptides are found to exhibit prevalent hydrogen transfer due to an extended lifetime of the dissociation complex formed between separating c- and $z^\bullet$-ion fragments post $N–C_\alpha$ bond cleavage. The precursor ion has a fairly compact structure as coulombic repulsion only exists between two protonation sites and in addition internal hydrogen bonding toward the basic residue helps to stabilise the intermediate complex and hence facilitate the hydrogen transfer process. The absence of hydrogen transfer for triply charged precursor ions is attributed to the open structure of such species resulting from coulombic repulsion between three charges accommodated along the backbone. The dissociation complex is therefore unstable due to this repulsion and consequently the fragments separate before hydrogen transfer can occur. In the context of our study this accounts for the increased observation of $c - 1^\bullet$ ions following CBPB treatment for both tryptic and Lys-C peptide ions. The data shown in Tables 1 and 2 are derived from a mixture of precursor ion charge states. CBPB treatment of certain sequences reduces in their charge state due to removal of the C-terminal basic residue. We postulate that the increased $c - 1^\bullet$ ion formation following CBPB treatment is due to the reduced charge density of these species. An extended lifetime of the intermediate dissociation complex therefore results, hydrogen transfer becomes more prevalent and the N-terminal fragment ion series is more likely to retain the available charge as the C-terminal series is now deficient of a highly basic residue.

3. Conclusions

The majority of bottom-up proteomic studies involve MS/MS analysis of proteins that have undergone proteolytic digestion using either trypsin or Lys-C; resultant peptides in each case terminate in the basic residues arginine or lysine. Current understanding of peptide fragmentation trends and mechanistic principles recognises the critical influence of this basic C-terminal amino acid. Here we have examined a range of product ion spectra generated by CID and ETD of peptide ions produced by electrospray ionisation both prior to and following the removal of this C-terminal basic residue. The data suggest that the observed dominant ion series from both fragmentation techniques is at least in part determined by the site within the polypeptide backbone with the highest gas phase basicity. In addition, fragmentation at sites along the backbone that were not observed prior to removal of the basic C-terminal amino acid occurs in the CBPB truncated analogue. For peptide sequences containing internal arginine/lysine (and to a certain extent histidine) residues the position of these amino acids within the sequence also influences the degree to which a given ion series dominates an MS/MS spectra. Studies such as those presented here further our understanding of gas phase peptide fragmentation chemistry and assist the development of more sophisticated spectral interpretation algorithms helping to reduce the current level of 'redundant' information that exists in peptide-based MS/MS studies. Furthermore, the data substantiate the notion that the simple sequential application of trypsin or Lys-C and CBPB digestion yields data of complementary value to the use of trypsin/Lys-C alone, suggesting applications in large-scale proteomic studies.

References

[1] J.K. Eng, A.L. Mccormack, J.R. Yates, An approach to correlate tandem mass-spectral data of peptides with amino-acid-sequences in a protein database, Journal of the American Society for Mass Spectrometry 5 (11) (1994) 976–989.

[2] R.G. Sadygov, D. Cociorva, J.R. Yates, Large-scale database searching using tandem mass spectra: looking up the answer in the back of the book, Nature Methods 1 (3) (2004) 195–202.

[3] H. Steen, M. Mann, The ABC's (and XYZ's) of peptide sequencing, Nature Reviews Molecular Cell Biology 5 (9) (2004) 699–711.

[4] V.H. Wysocki, G. Tsaprailis, L.L. Smith, L.A. Breci, Mobile and localized protons: a framework for understanding peptide dissociation, Journal of Mass Spectrometry 35 (12) (2000) 1399–1406.

[5] S.G. Summerfield, S.J. Gaskell, Fragmentation efficiencies of peptide ions following low energy collisional activation, International Journal of Mass Spectrometry 165 (1997) 509–521.

[6] G. Tsaprailis, et al., Influence of secondary structure on the fragmentation of protonated peptides, Journal of the American Chemical Society 121 (22) (1999) 5142–5154.

[7] H. Molina, et al., Global proteomic profiling of phosphopeptides using electron transfer dissociation tandem mass spectrometry, Proceedings of the National Academy of Sciences of the United States of America 104 (7) (2007) 2199–2204.

[8] D.M. Good, et al., Performance characteristics of electron transfer dissociation mass spectrometry, Molecular & Cellular Proteomics 6 (11) (2007) 1942–1951.

[9] B. Paizs, S. Suhai, Fragmentation pathways of protonated peptides, Mass Spectrometry Reviews 24 (4) (2005) 508–548.

[10] A. Somogyi, V.H. Wysocki, I. Mayer, The effect of protonation site on bond strengths in simple peptides – application of ab-initio and modified neglect of differential-overlap bond orders and modified neglect of differential-overlap energy partitioning, Journal of the American Society for Mass Spectrometry 5 (8) (1994) 704–717.

[11] T. Yalcin, et al., Why are B ions stable species in peptide spectra? Journal of the American Society for Mass Spectrometry 6 (12) (1995) 1165–1174.

[12] D.R. Mueller, M. Eckersley, W.J. Richter, Hydrogen transfer-reactions in the formation of Y+2 sequence ions from protonated peptides, Organic Mass Spectrometry 23 (3) (1988) 217–222.

[13] M.M. Cordero, J.J. Houser, C. Wesdemiotis, The neutral products formed during backbone fragmentations of protonated peptides in tandem mass-spectrometry, Analytical Chemistry 65 (11) (1993) 1594–1601.

[14] X.J. Tang, P. Thibault, R.K. Boyd, Fragmentation reactions of multiply-protonated peptides and implications for sequencing by tandem mass-spectrometry with low-energy collision-induced dissociation, Analytical Chemistry 65 (20) (1993) 2824–2834.

[15] K.W. Lau, et al., Observations on the detection of b- and y-type ions in the collisionally activated decomposition spectra of protonated peptides, Rapid Communications in Mass Spectrometry 23 (10) (2009) 1508–1514.

[16] D.T. McLachlin, B.T. Chait, Analysis of phosphorylated proteins and peptides by mass spectrometry, Current Opinion in Chemical Biology 5 (5) (2001) 591–602.

[17] D.M. Horn, Y. Ge, F.W. McLafferty, Activated ion electron capture dissociation for mass spectral sequencing of larger (42 kDa) proteins, Analytical Chemistry 72 (20) (2000) 4778–4784.

[18] D.L. Swaney, et al., Supplemental activation method for high-efficiency electron-transfer dissociation of doubly protonated peptide precursors, Analytical Chemistry 79 (2) (2007) 477–485.

[19] K.R. Clauser, P. Baker, A.L. Burlingame, Role of accurate mass measurement (± 10 ppm) in protein identification strategies employing MS or MS–MS and database searching, Analytical Chemistry 71 (14) (1999) 2871–2882.

[20] R.J. Chalkley, et al., Statistical analysis of peptide electron transfer dissociation fragmentation mass spectrometry, Analytical Chemistry 82 (2) (2010) 579–584.

[21] D.L. Tabb, et al., Statistical characterization of ion trap tandem mass spectra from doubly charged tryptic peptides, Analytical Chemistry 75 (5) (2003) 1155–1163.

[22] L.A. Breci, et al., Cleavage N-terminal to proline: analysis of a database of peptide tandem mass spectra, Analytical Chemistry 75 (9) (2003) 1963–1971.

[23] T. Vaisar, J. Urban, Probing the proline effect in CID of protonated peptides, Journal of Mass Spectrometry 31 (10) (1996) 1185–1187.

[24] R.N. Grewal, et al., Fragmentation of protonated tripeptides: the proline effect revisited, Journal of Physical Chemistry B 108 (15) (2004) 4899–4908.

[25] L.L. Smith, K.A. Herrmann, V.H. Wysocki, Investigation of gas phase ion structure for proline-containing b(2) ion, Journal of the American Society for Mass Spectrometry 17 (1) (2006) 20–28.

[26] M.J. Nold, et al., Amide bond dissociation in protonated peptides. Structures of the N-terminal ionic and neutral fragments, International Journal of Mass Spectrometry and Ion Processes 164 (1–2) (1997) 137–153.

[27] B. Paizs, S. Suhai, Towards understanding the tandem mass spectra of protonated oligopeptides. 1: Mechanism of amide bond cleavage, Journal of the American Society for Mass Spectrometry 15 (1) (2004) 103–113.

[28] B. Paizs, S. Suhai, Towards understanding some ion intensity relationships for the tandem mass spectra of protonated peptides, Rapid Communications in Mass Spectrometry 16 (17) (2002) 1699–1702.

[29] T. Nishikaze, M. Takayama, Influence of charge state and amino acid composition on hydrogen transfer in electron capture dissociation of peptides, Journal of the American Society for Mass Spectrometry 21 (12) (2010) 1979–1988.