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REVIEW

Electron capture dissociation mass spectrometry in characterization of peptides and proteins

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Abstract Electron capture dissociation (ECD) represents one of the most recent and significant advancements in tandem mass spectrometry (MS/MS) for the identification and characterization of polypeptides. In comparison with the conventional fragmentation techniques, such as collisionally activated dissociation (CAD), ECD provides more extensive sequence fragments, while allowing the labile modifications to remain intact during backbone fragmentation—an important attribute for characterizing post-translational modifications. Herein, we present a brief overview of the ECD technique as well as selected applications in characterization of peptides and proteins. Case studies including characterization and localization of amino acid glycosylation, methionine oxidation, acylation, and ''top–down'' protein mass spectrometry using ECD will be presented. A recent technique, coined as electron transfer dissociation (ETD), will be also discussed briefly.

Keywords Electron capture dissociation · Electron transfer dissociation · Electrospray ionization · Fourier transform mass spectrometry · Post-translational modifications

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Introduction

In biotechnology industry, the development and manufacturing of therapeutic peptides and proteins (e.g., insulin, growth hormone, interferons, interleukins, colony stimulating factors, erythropoietin, tissue plasminogen activator, and vaccines) requires the routine application of a battery of reliable and efficient analytical methods. Generally, throughout the production process, analytical determinations are made to closely monitor the lot-to-lot consistency of the therapeutic products. The resulting quality assessments are often utilized for regulatory filing and routine evaluation of product purity and stability (Rouse et al. 2005). Moreover, proteins produced using bacterial and yeast expression systems, as well as insect and mammalian cell culture, are subjected to rigorous quality control scrutiny (Fuchs 2002; Janis and Davis 1994; Jones et al. 2005; Murano 1997; Roy and Gupta 2004; Wurn 2004).

Besides in vitro systems, site-specific covalent modifications of biotechnology-derived products have been used to alter pharmacokinetic property, improve solubility, shield immunogenic epitopes, and/or improve potency (Lundblad and Bradshaw 1997; Tang et al. 2004; Frokjaer and Otzen 2005). Some of the site-specific protein modifications/chemical conjugations include pegylation, dextranation, succinylation, monnosylation, acylation, immunoconjugation, and fusion to other proteins (e.g., albumin, immunoglobulin domains), just to name a few (Frokjaer and Otzen 2005; Haag and Kratz 2006; Molineux 2003, 2004; Sheffield 2001; Smith et al. 1993; Roberts et al. 2002). In this regard, the multiplicity of potential reactive sites in a target therapeutic protein warrants the application of definitive analytical techniques to carefully determine reaction stoichiometry and the site(s) of modification. Indeed, batch variations could potentially lead to lack of efficacy of the drug, and/or onset of undesirable physiological side effects (Wang 1999; Schellekens 2005).

Lastly, stabilization of diagnostic and therapeutic proteins against oxidation in aqueous formulation is another key objective that necessitates routine quality control tests. For example, the non-specific sulfur oxidation such as *S*-thiolation, methionine oxidation (sulfoxide and sulfone), and free cysteine residues (sulfinic and sulfonic acids) are among some of the susceptible sites that could compromise product's stability and potency (Wang 1999). Clearly, the development of rapid and accurate monitoring systems is important for ensuring the quality of the protein products.

To this end, advancements in soft ionization techniques, namely, electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), have ameliorated many limitations associated with the traditional MS, allowing MS to directly analyze large intact biomolecules (e.g., >100,000 Da) of all types (Ryan and Patterson 2002; Aebersold and Mann 2003; Yates 2004). While a single stage MS experiment determines the molecular weight (MW) of an analyte, tandem MS (MS/MS) can provide information on its structure, such as the sequences and modifications of proteins and DNA. In this regard, one of the most significant developments in tandem mass spectrometry of peptides and proteins is electron capture dissociation (ECD).

Tandem mass spectrometry

Tandem mass spectrometry or MS/MS is one of the most widely used techniques to improve specificity and analyze individual components in complex mixtures. In a typical MS/MS experiment, the molecular ions (also referred to as precursor ions) of polypeptides are dissociated in the gas-phase and their modifications can be revealed from the resulting product ion fragments. The most common method for ion dissociation involves the collision of the peptide (or protein) ions of interest with neutral, non-reactive, gas-phase target atoms or molecules such as argon, helium, or molecular nitrogen. These collisions result in excitation of the analyte ions and ensuing fragmentation or dissociation.

The nomenclature used for assignment of product ions in MS/MS spectra of peptides and proteins is depicted in Fig. 1. When a peptide is cleaved (i.e., unimolecular dissociation in vacuo) and the charge is retained by the N-terminal fragment, the descriptors a_n, b_n, and c_n are used, where n represents the position of the fragmentation in the amino acid chain. Likewise, if the charge is retained by the C-terminal portion of

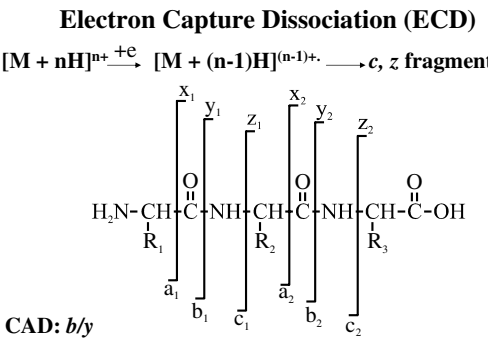

Fig. 1 A simplified representation of the ECD process and the nomenclature used for assignment of product ions in the CID and ECD spectra of peptides and proteins. In mass spectrometry, the information about the structure of an analyte (precursor ion) is derived from its corresponding fragmentation pathways (product ions). The terms CID and CAD are interchangeably used throughout the manuscript

the molecule, x_n, y_n, and z_n designations are assigned to the fragment ions (Fig. 1). Additionally, a superscript denotes the charge state of each fragment ion. Therefore, since the product ions are a direct consequence of dissociation of the parent or precursor ion upon collision with a target gas, this process is referred to as the collision-induced dissociation (CID) or collisionally-activated dissociation (CAD) (McLuckey 1992; Sleno and Volmer 2004). Nowadays, sophisticated custom-designed or commercially available software packages can dramatically ease the interpretation of peptide-based MS/MS spectra (Fischer et al. 2005; Savitski et al. 2005; Strupat 2005; Wysocki et al. 2005; Tang et al. 2005).

Despite its widespread use and popularity, there are two notable caveats associated with CID, which limits its effectiveness as a tool for probing site-specific covalent modifications. First, CID often selectively cleaves certain inter-residue bonds, leaving amino acid sequence gaps, which can be highly predominant for larger polypeptides (Senko et al. 1994; Kelleher 2004; Nemeth-Cawley et al. 2003; Reid and McLuckey 2002). Second, the labile covalent modifications often have the propensity to undergo elimination *prior* to the peptide backbone fragmentation. This attribute can pose a significant challenge when attempts are made to determine the site(s) of covalent modification in a protein (e.g., changes due to amino acid oxidation, conjugation of excipients, Maillard reactions, post-translational modifications, etc.).

Electron capture dissociation (ECD) introduced by the elegant work of McLafferty and co-workers in 1998 (Zubarev et al. 1998; Zubarev 2004), largely overcame these limitations by providing more extensive peptide sequence fragments, while permitting the labile modifications to remain intact during the peptide backbone dissociation. In the remaining part of this manuscript, we will present a brief and general overview of ECD as well as selected examples of its utility in the characterization of polypeptide covalent modifications. Based on the examples discussed below, we envisage that a combination of up-front single or multi-dimensional separation techniques with ECD mass spectrometry can offer a myriad of opportunities in specific screening of polypeptide covalent modifications.

Electron capture dissociation mass spectrometry

In an ECD experiment, the multiply charged ions produced by ESI, are trapped within the confines of a combination of magnetic and electrostatic fields of a Fourier transform mass spectrometer (FTMS) or also referred to as a Fourier transform ion cyclotron resonance mass spectrometer (FTICR/MS) instrument, and are irradiated with a beam of relatively low energy (≤0.2 eV) electrons generated by an electron gun (McLafferty et al. 2001; Breuker et al. 2004). Figure 2 depicts the schematic of a FTICR/MS instrument, coupled with an ESI source, originally developed by McLafferty and co-workers at Cornell University (Beu et al. 1993). In order to yield high-resolution mass spectra, the FTICR/MS must be operated under high vacuum (typically $< 10^{-8}$ torr), which is realized by a differential pumping system that consists of multiple mechanical, diffusion, and cryogenic pumps. The up-front quadrupole lenses are utilized to collimate and focus the gas-phase ion beam during transport to the center of the permanent magnetic field (usually 3–12 Tesla), where ions are trapped, excited, and detected in an ICR cell (Marshall et al. 1998, 2002).

The partial neutralization of multiply-protonated ions with low energy electrons typically renders extensive inter-residue backbone cleavage to yield c and $z^{\bullet}$ fragment ions (Fig. 1). This unique c/z fragmentation is in sharp contrast to the b/y fragmentation by low energy MS/MS. While y and b ions originate from the dissociation of the amide bond, the c and $z^{\bullet}$ fragments result from the cleavage of the N–C_{α} amine backbone bond; this unique c/z dissociation pathway was first observed in the experiment of 193 nm photodissociation of polypeptides (Guan et al. 1996), and was the result of recombination of multiply charged polypeptide ions with the low-energy photoelectrons ejected from the FTICR/MS cell walls. More intriguingly, even labile modifications remained intact during backbone fragmentation, which made ECD particularly amenable to detection and localization of post-translational modifications (e.g., glycosylation, ubiquitination, amidation, lipidation, sulfation, oxidation, and phosphorylation) (Sze et al. 2002; Shi et al. 2001;

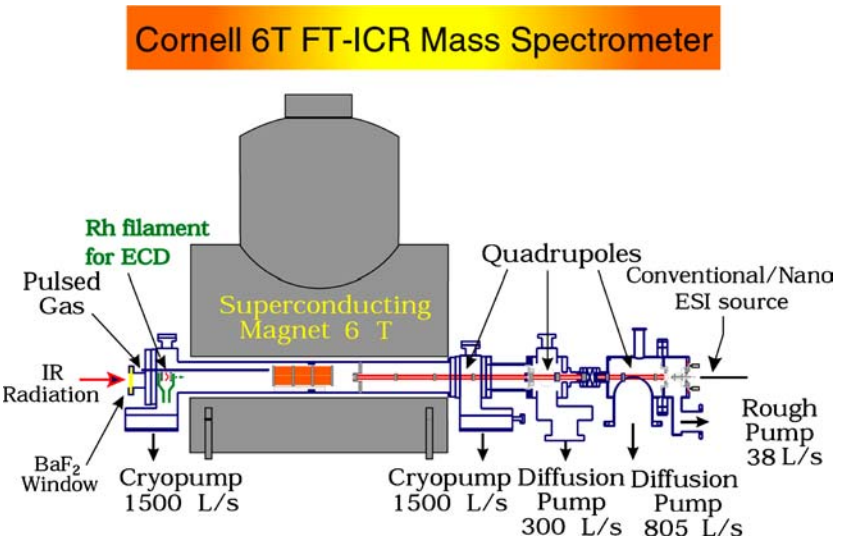

Fig. 2 A simplified instrument schematic of a typical FTMS (also referred to as FTICR/MS) with ECD capability (courtesy of Professor F.W. McLafferty, Department of Chemistry & Biochemistry, Cornell University, Ithaca, New York, USA)

Cooper et al. 2004; Pesavento et al. 2004; Walsh et al. 2005).

ECD has been proposed as a ''non-ergodic'' fragmentation process, in which the intra-molecular energy randomization is slower than the ECD cleavages (Zubarev 2003; Cooper et al. 2005). This unique non-ergodic feature has been exemplified by the ability of ECD in preserving the labile side chain modifications groups on the peptide backbone fragments. This is in contrast to CID and other conventional fragmentation techniques, which typically eject the labile modifications prior to the peptide backbone dissociation (Zubarev 2003).

Selected applications of ECD-MS

Case I. O-linked glycosylation

From a biological standpoint, protein glycosylation is one of the major post-translational modifications with significant effects on protein folding, conformation distribution, stability, and activity. Carbohydrates in the form of asparagine-linked (*N*-linked) or serine/threonine (*O*-linked) oligosaccharides are major structural components of many cell surfaces and secreted proteins. In contrast to the *N*-linked glycosylation (occurring at asparagine residues in an Asn-*Xxx*-Ser/Thr, in which *Xxx* could be any amino acid except proline), there is no sequence consensus for the *O*-linked glycosylation. From a biotechnology perspective, glycosylation can have a dramatic impact on protein stability, solubility, immunogenicity, activity, and ultimately clinical efficacy (Hermeling et al. 2004; Sinclair and Elliott 2005; Jefferis 2005). For example, the presence of glycans is obligatory in achieving the biological modulating function of human recombinant gonadotrophins formed in CHO (Chinese hamster ovary) cell lines. In this regard, investigation of batch-to-batch uniformity of several gonadotrophins using MALDI- and ESI-MS was reported recently (Gervais et al. 2003).

Interestingly, the characterization of *O*-linked glycosylation by tandem mass spectrometry has been particularly difficult (Peter-Katalinic 2005), because the conventional MS/MS methods (e.g., CID) yield weak or no signal for glycan-carrying ions. This is mainly due to the higher labile nature of glycosidic bonds relative to peptide bonds. Mirgorodskaya et al. (1999) first demonstrated that ECD could be applicable to the analysis of *O*-glycosylated peptides. The backbone fragmentation, which resulted from the ECD experiment led to unequivocal determination of five GalNAc sites in a 3 kDa glycopeptide isolated from a mixture of glycoforms. Haselmann et al. (2001) demonstrated that four of six GalNAc monosaccharides could be mapped in a 6.8 kDa

peptide. Additionally, characterization of a 25–residue peptide containing up to five sialic acid and six GalNAc modifications was achieved.

Case II. Methionine oxidation

Methionine (Met) has a sulfur-containing side chain that is susceptible to oxidation via enzymatic or non-enzymatic pathways. In human biology, the Met oxidation in proteins plays a prominent role in aging and age-related degenerative diseases (Stadtman et al. 2005). From a biotechnology standpoint, protein inactivation by unwanted oxidation has to be inhibited or minimized (Chu et al. 2004). Oxidation of labile amino acids (e.g., Met to Met-sulfoxide and Met-sulfone) can compromise the product's quality and possibly elicit immunogenic reactions. For instance, identification of oxidation of Met^{111} residue in therapeutic protein, interferon-alpha-2b bulk solution, was reported using MS-based peptide mapping (Gitlin et al. 1996).

Recently, Guan and co-workers demonstrated that the combined data from CID and ECD spectra can be used to rapidly detect and localize the Met-oxide (Met(O)) residues in a series of peptides, including one with an internal disulfide bond (Guan et al. 2003). A distinct fragmentation pathway in CID of Met(O)-containing peptides is the neutral loss of 64 Da from the precursor and/or product ions. The characteristic neutral loss of 64 Da corresponds to the ejection of methanesulfenic acid (CH_3SOH, 64 Da) from the side chain of Met(O). The loss of 64 Da is unique, in proteins and peptides, to Met(O), and is useful in differentiating between Met(O) and phenylalanine (both residues have the same nominal mass of 147 Da) in MS/MS peptide sequencing (Schey and Finley 2000). However, this low-energy pathway can further inhibit the sequence fragmentation by CID, especially for larger peptides. In fact, the complete MS/MS characterization of Met(O) using CID has so far been limited to small peptides, such as those from enzymatic digestion (Schey and Finley 2000).

CID of the Met-oxidized human growth hormone-releasing factor (GHRF) only yields limited backbone fragmentation (Fig. 3, top-panel), with 12 out of total 43 inter-residue bonds being cleaved. The most abundant product ions arise from the cleavages at the C-terminal sides of asparic acid and glutamic acid residues, the favorable cleavage sites in CID. The peaks corresponding to the loss of CH_3SOH (64 Da) from $[M + 7H]^{7+}$ and three product ions (y_{19}^{4+}, y_{42}^{6+}, b_{37}^{4+}) are observed. In contrast, ECD offers far more extensive backbone fragmentation (Fig. 3, bottom-panel), with 41 of the total 43 inter-residue bonds being cleaved. Of the c, $z^{\bullet}$ fragment ions, 32 are complementary pairs whose masses sum to that of the molecular ion (no loss of 64 Da is observed). The position of Met(O)-27 can be unequivocally determined by the two complementary pairs of ECD product ions ($c_{26}^{3+}/z_{18}^{2+\bullet}$ and $c_{27}^{3+}/z_{17}^{2+\bullet}$). The product maps of CID and ECD are summarized in Fig. 3, which provide compelling evidence in support of the complementary nature of the two dissociation techniques. The characteristic elimination of CH_3SOH (64 Da) in CID serves as a signature tag for the presence of Met(O) in peptides. ECD then offers extensive backbone fragmentation without detaching the labile side chain, to allow for the direct localization of the Met(O) residues.

The human atrial natriuretic peptide (ANP) is a 28 amino acid peptide with a 17 amino acid ring closed by a disulfide bond between Cys-7 and Cys-23. The predominant fragmentation pathway in CID of ANP(O) is the loss of CH_3SOH (64 Da) from the molecular ion $[M + 5H]^{5+}$ (Fig. 4). No appreciable backbone fragmentation or disulfide bond cleavage is observed in the CID spectrum. It is well known that disulfide bonds are generally resistant to cleavage by low-energy CID (Loo et al. 1990); thus, reduction of the disulfide bonds with dithiothreitol followed by –SH alkylation is an obligatory step prior to MS/MS analysis. ECD of ANP, however, resulted in 14 backbone cleavages, including six from cleavages within the cyclic structure. The product ions from within the cyclic structure arise from ECD cleavage of two bonds: a backbone amine bond and a disulfide bond (Fig. 4). None of the Met(O)-containing products (c_{12}, c_{16}, c_{18}, and c_{19}) is accompanied by the loss of CH_3SOH (64 Da). The ability to cleave disulfide bonds is a distinctive feature of ECD. In fact, the disulfide bond cleavage is favored over backbone bond cleavage

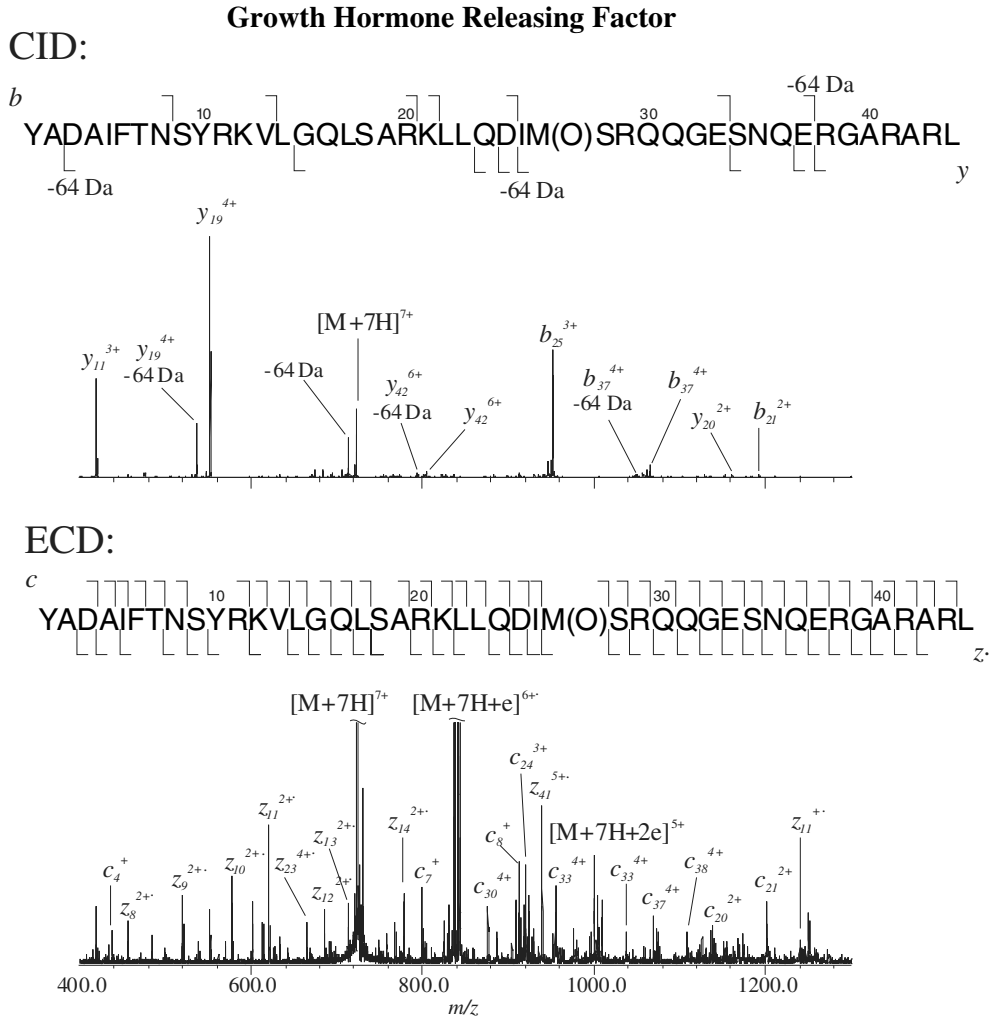

Fig. 3 Product maps indicating the cleavage sites in CID and ECD of the human growth hormone-releasing factor (GHRF) with their corresponding spectra

in ECD (Zubarev et al. 1999; Kleinnijenhuis et al. 2003). It has been suggested by McLafferty and co-workers that disulfide bond cleavage is likely due to the high affinity of the -S-S- site for the $H^{\bullet}$ atom (from neutralization of a proton by electron capture), to form a hypervalent intermediate that rapidly leads to the dissociation of the disulfide bond.

Case III. Acylation

Therapeutic polypeptides are often, but not exclusively, given intravenously to alleviate problems associated with their poor gastrointestinal absorption and pre-systemic degradation. In this regard, chemical acylation using fatty acids (e.g., palmitic and myristic) to enhance drug's binding to serum albumin, yielding higher blood circulation time, has been documented. Fatty acylation has also been known to increase interferon-alpha delivery into human skin (Foldvari et al. 1998, 1999).

From a biological standpoint, studies indicate that acylation plays an important role in cell's metabolism and regulatory network (Resh 2004). For example, ghrelin is a post-translationally acylated peptide hormone that has an unusual C8:0 acylation (octanoic acid modification) at

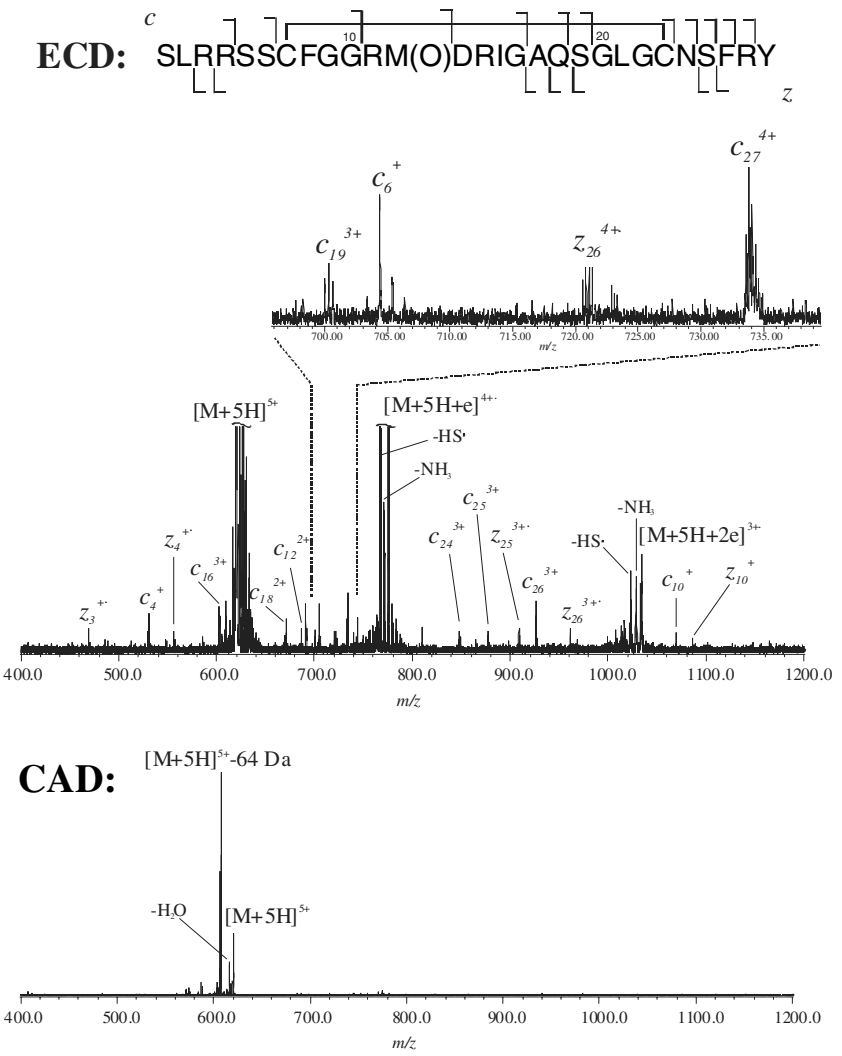

Fig. 4 CID and ECD spectra of $[M + 5H]^{5+}$ of ANP(O) with its corresponding product ion map. The isotopic resolution as shown in the zoom view of the ECD spectrum is crucial in assignment of the charge states and identification the product ions

Ser-3 residue. The ester-linked C8:0 fatty acyl moiety is essential for the activities of ghrelin, which include growth hormone secretion, feeding regulation, and energy homeostasis (Smith et al. 2005).

CID of ghrelin $[M + 5H]^{5+}$ yielded two *b*- and 12 *y*-fragments (Fig. 5, top-panel), cleaving 12 inter-residue bonds, far fewer than the 21 inter-residue bonds cleaved by ECD. The comparison of backbone cleavages effected by ECD and CID of ghrelin $[M + 5H]^{5+}$ is shown in Fig. 5. The major CID product ions of ghrelin and des-acyl ghrelin are produced from fragmentations near the N-terminal region (Guan 2002). In comparison, the backbone fragmentation by ECD is far less selective; variations in ECD product ion abundance are much less significant than those in the CID spectrum. Many of the CID fragments are accompanied by structurally uninformative H_2O losses, which are absent in the ECD spectra. These neutral losses (H_2O and the C8:0 fatty acid) from the backbone fragments are undesirable, and severely complicate MS/MS data interpretation. Among the three backbone fragments (b_5, b_{17}, and y_{26}) that contain Ser-3, the ester-linked octanoyl group is retained only on y_{26}, but completely lost from b_5 and b_{17} ions.

Figure 5 (bottom-panel) is the ECD spectrum of $[M + 5H]^{5+}$ of ghrelin. The observed 17 *c* and 17 $z^{\bullet}$ ions correspond to the cleavage of 21 out of 23 possible backbone amide bonds. The N-terminal sides of four prolines are not considered

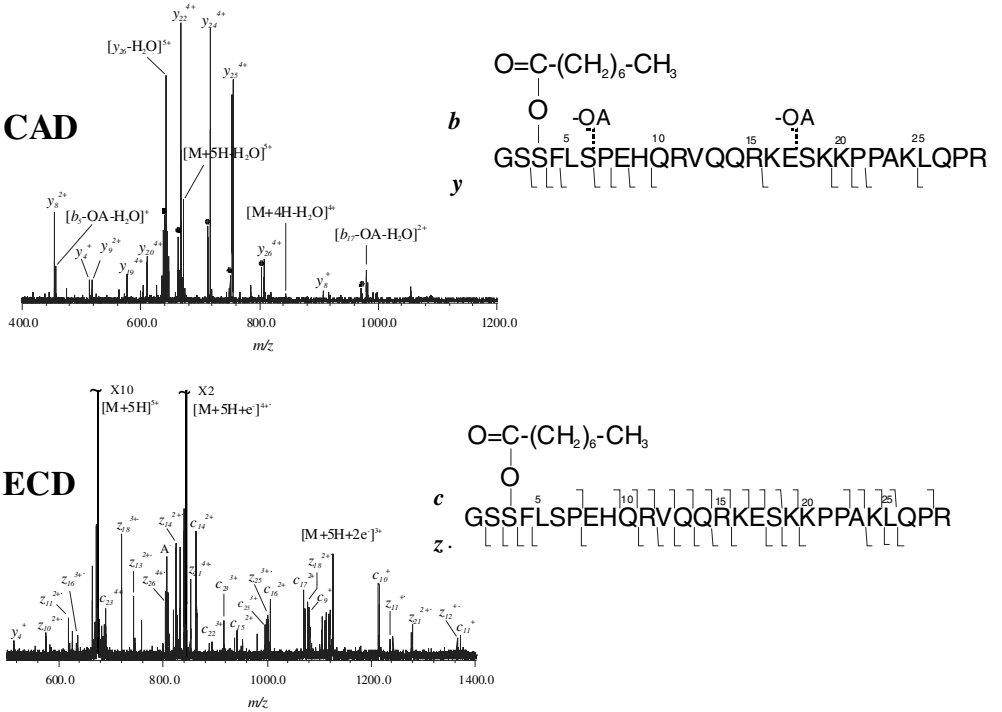

Fig. 5 CID and ECD spectra of $[M + 5H]^{5+}$ of ghrelin with its corresponding product ion map. The dots in the top-panel denote signals corresponding to loss of a water molecule. OA is the abbreviation for octanoic acid

because they are not susceptible to cleavage by ECD due to the cyclic structure of proline (i.e., the peptide backbone would still be linked via the proline side chain even if the backbone imide-N to C bond is cleaved). The mass difference of 213 Da between *m/z* 999.5 and *m/z* 803.2 corresponds to the mass of a C8:0 acylated serine, which permits the localization of the acylation site at Ser-3. The ester-linked C8:0 fatty acyl group is retained on all backbone fragment ions (*c* and $z^{\bullet}$) that originally contained the acylated Ser-3, with each of these fragments having a mass shift of 126 Da from the corresponding fragment produced from des-octanoyl ghrelin (data not shown). The retention of the ester-linked C8:0 acyl group on the backbone fragments during the ECD process supports the non-ergodic mechanism, and is in agreement with previous ECD results on other labile post-translational modifications. The ECD spectra of ghrelin and des-octanoyl ghrelin are very similar with respect to the backbone fragmentation, perhaps implying that there is little interaction between the peptide backbone and the hydrophobic C8:0 acyl chain. ECD-FTICR/MS provides sufficient structural information for identifying the chemical nature and determining the site of the fatty acid modification in ghrelin. ECD offers more extensive sequence fragmentation than CAD, and preserves the C8:0 fatty acyl group on the backbone sequence fragment ions (*c* and $z^{\bullet}$ ions) allowing for direct localization of the acylation site.

Case IV. ECD for ''top–down'' MS of large intact proteins

Arguably, the impact of ECD has been most significant in the ''top–down'' analysis of large proteins. The ''top–down'' approach refers to subjecting intact proteins and their gas-phase fragments directly to tandem mass spectrometry for protein identification and characterization. It is in contrast with the ''bottom–up'' approach for protein identification, which is based on mass spectrometric analysis of peptides derived from proteolytic digestion (Pandey and Mann 2000; Peterman et al. 2005), usually with trypsin. Although the ''bottom–up'' approach has been

widely used in protein identification, in particular, by reverse phase capillary LC/MS/MS using instruments with relatively modest mass resolution, it does not reveal the MW information of intact proteins, and rarely provides the complete sequence coverage. The ''bottom–up'' approach is especially problematic when analyzing labile covalent adducts that degrade under the protein digestion protocols and co-elution of some peptides in complex mixtures, yielding possible ion suppression and presenting difficulties in detection of low abundance-low recovery peptides.

The emerging popularity of ''top–down'' approach in recent years results from the combination of high resolving power of FTICR/MS with the extensive fragmentation of large proteins by ECD. The high resolving power of FTICR/MS is important in the unequivocal assignment of the charge state, thus the masses, of multiply charged product ions generated by ECD. The power of ECD-FTICR/MS has been demonstrated by the determination of multiple modification sites in intact proteins. For example, McLafferty and co-workers identified five deamidation sites in RNase A (Zabrouskov et al. 2006) despite the only 1 Da difference in the molecular mass change (i.e., conversion of $-NH_2 \rightarrow -OH$).

Figure 6 shows the ECD-FTICR/MS spectrum of viral prolyl 4-hydroxylase (P4H, ~26 kDa) which was over-expressed in *E. coli* for 12 h leading to 151 unique masses (Ge et al. 2002). This approach was successful in identification of unexpected modifications (e.g., DNA-derived sequence errors) at three amino acid residues and removal of the amino terminal Met. While each of the two approaches, ''bottom–up'' and ''top–down'', offer specific advantages and drawbacks, the combination of the two methodologies can be an extremely powerful tool in protein analysis (Kelleher et al. 1999). This complementary attribute has been elegantly applied in obtaining high sequence coverage, intact MW measurement, and/or site-specific localization of covalent modifications. The combined ''top–down'' and ''bottom–up'' FTICR/MS was used to characterize a host of biomarkers, as large as 8 kDa or higher, in

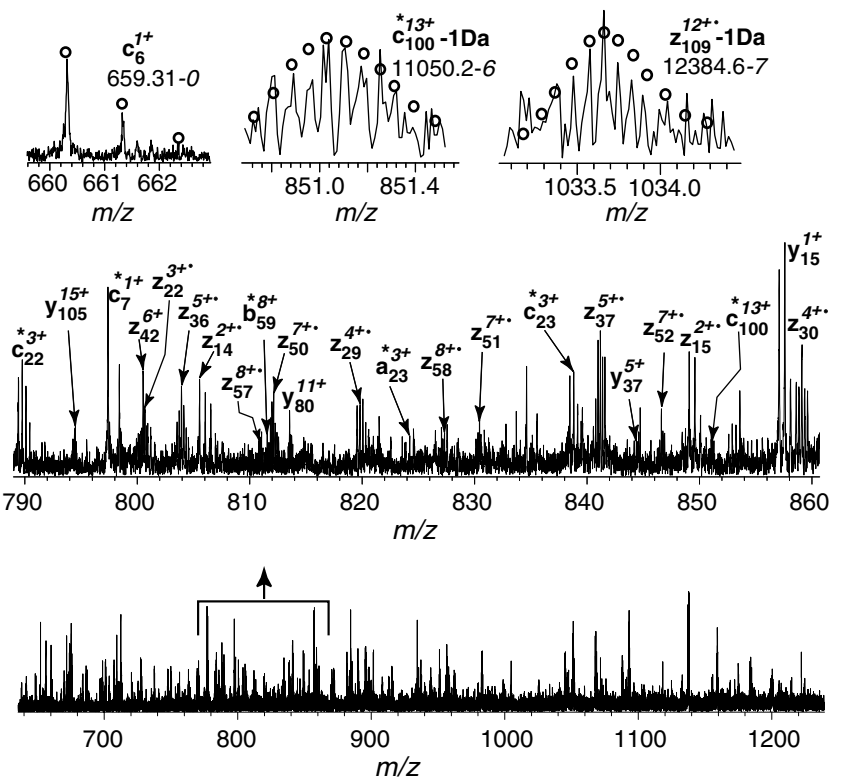

Fig. 6 ECD-FTICR/MS spectrum of viral prolyl 4-hydroxylase (P4H; ~26 kDa) over-expressed for 12 h in *E. coli* (Ge et al. 2002). ECD spectrum contained 277 isotopic clusters representing 151 distinctive masses (courtesy of Professor F.W. McLafferty and Dr. Y. Ge, Department of Chemistry & Biochemistry, Cornell University, Ithaca, New York)

urine obtained from patients with renal diseases (Chalmers et al. 2005). A similar approach was utilized in identification of a phosphorylation site in *Drosophilia* stem-loop-binding proteins that play a role in high-affinity RNA binding (Borchers et al. 2006). In spite of the ease of use and dramatic advancements in commercially available ECD-FTMS; front-end sample preparation, chromatography, and down stream data handling still remain to be critical in achieving meaningful results (Cooper et al. 2005; Medzihradszky et al. 2004; Savitski et al. 2005).

Electron transfer dissociation mass spectrometry (ETD-MS)

Despite the advantages of ECD-MS described above, ECD is still a technique that can only be implemented on FTICR/MS, the most expensive type of MS instrumentation. Recently, Hunt and coworkers developed an ECD-like technique, electron transfer dissociation (ETD), which can be used with low-cost, widely accessible quadrupole ion trap instruments (Syka et al. 2004; Coon et al. 2005). In ETD, anions (e.g., anthracene anions) are used as vehicles for delivering electrons to multiply charged polypeptides. The transfer of electrons from the anions to protonated peptides results in fragmentation patterns similar to those by ECD. Among a number of successful applications, ETD in conjunction with on-line liquid chromatography has been employed in analysis of post-translational modifications in paxillin (Schroeder et al. 2005) and differentiation of aspartic and isoaspartic residues in peptides (O'Connor et al. 2006).

Conclusions

Electron capture dissociation is rapidly becoming a powerful and complementary fragmentation technique in tandem mass spectrometry of peptides and proteins. The detection and localization of covalent modifications on polypeptides using a combination of high resolution mass spectrometry and ECD have ameliorated a number of limitations associated with the conventional MS/MS techniques (Meng et al. 2005). Other future applications could be in the area of characterizing drug–protein complex (e.g., tumor-targeting drug conjugates, in which proteins are covalently linked with small cytotoxic drugs) (Jaracz et al. 2005; Wu and Senter 2005). For instance, high-resolution mass spectrometry can be used to first determine the drug-to-protein stoichiometry followed by ECD-MS to rapidly pinpoint the site(s) of modification. We certainly hope that this brief account of ECD will cultivate additional thoughts and broaden the use of this powerful technique.

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