Modern Chemistry & Applications
ISSN: 2329-6798

Quan and Liu, Mod Chem appl 2013, 1:1
DOI: 10.4172/2329-6798.1000e102

Editorial Open Access

CID, ETD and HCD Fragmentation to Study Protein Post-Translational Modifications

Lingdong Quan[1] and Miao Liu[2*]

[1]Arthritis and Tissue Degeneration Program, Hospital for Special Surgery, USA
[2]Department of Pathology and Microbiology, University of Nebraska Medical Center, Emile Street, Omaha, Nebraska, 68198, USA

Gas phase biomolecule-ion chemistry has played a crucial role in mass spectrometry (MS) based proteomics study. The key step generating the structure information of a protein or a peptide is by ion dissociation or transformation to the characteristic tandem mass spectrometry spectra (MS2) fragmentation patterns. Collision-induced dissociation (CID) is the most widely applied fragmentation method for proteome identification and quantification analysis. Under CID condition, the peptide/protein precursor ion undergoes one or more collisions by interactions with neutral gas molecules, contributing to vibrational energy which will redistribute over the peptide/protein ion. The vibrational energy can result in ion dissociation occurring at amide bonds along the peptide backbone, generating b- and y-type fragment ions or leading to losses of small neutral molecules, such as water and/or ammonia or other fragments derived from side chains [1].

In spite of the prevalence of CID, it is well-known that there are biases regarding the length, the amino acid components and the charge state of a peptide/protein ion. In general, CID is more effective for small, low-charged peptides. The presence of basic residues in a peptide sequence may also prevent dissociation and generating few sequence ions. In addition, CID is not suitable for fragmentation of intact proteins, and peptides with labile post-translational modifications, such as phosphorylation and S-nitrosylation [2]. Complementary to CID fragmentation, electron-capture dissociation (ECD) that generates radical cationsfor a multiply protonated protein/peptide,or electron-transfer dissociation (ETD) that transfers electron to a multiply protonated peptide/protein, could lead to the cleavage of the N-Cα backbone bonds and to generate c- and z-type fragment ions [1]. Different ion types can provide complementary information for the structural characterization of a certain peptide. Another important feature of ETD fragmentation is that it can identify CID-labile post-translational modifications (PTMs). Ideally, for peptides with PTMs, ETD can provide both the sequence information and the localization of the modification sites. Another alternative type of fragmentation method is the beam-type CID or high-energy collision dissociation (HCD). The fragmentation pattern of HCD is featured with higher activation energy and shorter activation time comparing the traditional ion trap CID. HCD also generates b- and y-type fragment ions. While the higher energy for HCD leads to a predominance of y-ions; b-ions can be further fragmented to a-ions or smaller species [3]. Without the low mass cut-off restriction and with high mass accuracy MS2 spectra, HCD has been successfully applied for de nonopeptide sequencing, providing more informative ion series [4]. As for PTMs studies, certain diagnostic ions specific for HCD could be recognized for PTMs identification.

It has been suggested that ETD tends to perform better than CID or HCD on higher charge states but yielding the lowest number of total identifications due to its slower scan rate [5]. Using CID, ETD, or HCD alone, alternating acquisition or intelligent acquisition (decision tree mode) all has been applied for protein PTMs analysis.

PTMs serve as one of the most important regulatory mechanisms for fine-tuning protein activity, protein localization, and protein interactions. MS based proteomics analysis holds great potential for the analysis of protein PTMs that occurs on the amino acid side chains or the amine and carboxyl terminal of the protein. Precise identification of the modification type and the modification site can be very challenging regarding the stability of the modification and the gas phase dissociation behavior of the modified peptide precursor. Direct detection of protein S-nitrosylation is tremendously difficult due to the labile nature of NO attachment to the specific protein cysteine suppressive peptide backbone fragmentation has been observed due to the neutral loss of NO group under CID or ETD fragmentation mode [6]. Most of the identification strategies for protein S-nitrosylation are indirect methods by transforming the NO to another detectable tag before the MS analysis [7].

Protein phosphorylation is another type of reversible modification that plays pivotal roles in virtually all cellular processes. Deregulation of phosphorylation has been implicated in a variety of disease states. Characterization of phosphorylation site by MS relies on the structural informative ions. Under CID condition, a neutral loss of phosphoric acid is often observed. This sort of "nonsequence" ionprovides diagnostic information regarding the presence of phosphorylation. While suppressive peptide backbone fragmentation could be observed along with the neutral loss, more sequence information can be obtained under sequential MS3 fragmentation. However, ECD is more preferred when dealing with multiply phosphorylated peptide, because it can cause efficient peptide backbone fragmentation while leaving the modification intact [8]. In addition a back-to-back evaluation of the HCD and CID for phosphoproteomics analysis showed the great potential for HCD to provide richer fragment ion spectra for phosphopeptides [9]. Faster scan rate for HCD can be achieved [10], and the optimized alternating acquisition method is expected to improve the identification coverage and accurate site localization for phosphoproteomics analysis.

The complementary fragmentation modes arealso suitable for PTMs such as glycosylation and ubiquitination [5]. It has been reported that CID/HCD enabled the identification of glycan structure and peptide backbone, allowing glycopeptide identification, whereas ETD enabled the elucidation of glycosylation sites by maintaining the glycan-peptide linkage [11]. Intelligent acquisition control has been designed accordingly, that ETD spectra can only be acquired when glycan oxonium ions from MS2 HCD are detected [12]. For protein ubiquitination analysis, ETD can better preserve the gly-gly mass tag, and it is more sensitive for higher charged peptides, thus provides significant alternative fragmentation information that complements

***Corresponding author:** Miao Liu, Department of Pathology and Microbiology, University of Nebraska Medical Center, Emile Street, Omaha, Nebraska, 68198, USA, E-mail: mli1@unmc.edu

Received November 15, 2012; **Accepted** November 19, 2012; **Published** November 21, 2012

Citation: Quan L, Liu M (2013) CID, ETD and HCD Fragmentation to Study Protein Post-Translational Modifications. Mod Chem appl 1:e102. doi:10.4172/mca.1000e102

CID-derived data to improve the coverage when mapping ubiquitination sites in proteins [13].

In general, CID/HCD works well for most stable modifications such as acetylation and methylation. While for other types of modifications such as phosphorylation, glycosylation and ubiquitination, alternative fragmentation or intelligent acquisition often times will provide complementary information for both peptide identification and modification site localization. While for extremely labile modification such as S-nitrosyaltion, an indirect method would be considered.

Compared to CID, HCD and ETD require more careful instrument maintenance and tuning. Longer acquisition time remains a disadvantage of ETD. With the fast development of the MS instruments, the scan rate tradeoff for HCD and ETD will be significantly improved. In the meanwhile, more robust software will be developed to support the alternative fragmentation/intelligent acquisition.

References

1. Huang TY, McLuckey SA (2010) Gas-phase chemistry of multiply charged bioions in analytical mass spectrometry. Annu Rev Anal Chem (Palo Alto Calif) 3: 365-385.
2. Elviri L (2012) ETD and ECD Mass Spectrometry Fragmentation for the Characterization of Protein Post Translational Modifications. In Tandem Mass Spectrometry - Applications and Principles 161-178.
3. Frese CK, Altelaar AF, Hennrich ML, Nolting D, Zeller M, et al. (2011) Improved peptide identification by targeted fragmentation using CID, HCD and ETD on an LTQ-Orbitrap Velos. J Proteome Res 10: 2377-2388.
4. Chi H, Sun RX, Yang B, Song CQ, Wang LH, et al. (2010) pNovo: de novo peptide sequencing and identification using HCD spectra. J Proteome Res 9: 2713-2724.
5. Guthals A, Bandeira N (2012) Peptide identification by tandem mass spectrometry with alternate fragmentation modes. Mol Cell Proteomics 11: 550-557.
6. Cook SL, Jackson GP (2011) Characterization of tyrosine nitration and cysteine nitrosylation modifications by metastable atom-activation dissociation mass spectrometry. J Am Soc Mass Spectrom 22: 221-232.
7. Liu M, Talmadge JE, Ding SJ (2012) Development and application of site-specific proteomic approach for study protein S-nitrosylation. Amino Acids 42: 1541-1551.
8. Palumbo AM, Tepe JJ, Reid GE (2008) Mechanistic insights into the multistage gas-phase fragmentation behavior of phosphoserine- and phosphothreonine-containing peptides. J Proteome Res 7: 771-779.
9. Jedrychowski MP, Huttlin EL, Haas W, Sowa ME, Rad R, et al. (2011) Evaluation of HCD- and CID-type fragmentation within their respective detection platforms for murine phosphoproteomics. Mol Cell Proteomics 10: M111 009910.
10. Nagaraj N, D'Souza RC, Cox J, Olsen JV, Mann M (2010) Feasibility of large-scale phosphoproteomics with higher energy collisional dissociation fragmentation. J Proteome Res 9: 6786-6794.
11. Scott NE, Parker BL, Connolly AM, Paulech J, Edwards AV, et al. (2011) Simultaneous glycan-peptide characterization using hydrophilic interaction chromatography and parallel fragmentation by CID, higher energy collisional dissociation, and electron transfer dissociation MS applied to the N-linked glycoproteome of Campylobacter jejuni. Mol Cell Proteomics 10: M000031-MCP201.
12. Saba J, Dutta S, Hemenway E, Viner R (2012) Increasing the productivity of glycopeptides analysis by using higher-energy collision dissociation-accurate mass-product-dependent electron transfer dissociation. Int J Proteomics 2012: 560391.
13. Sobott F, Watt SJ, Smith J, Edelmann MJ, Kramer HB, et al. (2009) Comparison of CID versus ETD based MS/MS fragmentation for the analysis of protein ubiquitination. J Am Soc Mass Spectrom 20: 1652-1659.