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

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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 cations for 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 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

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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-nitrosylation, 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