Euro analytica 2020 Mass spectrometry- based proteomics: Past, present and future - M. V. Jagannadham - CSIR Centre for Cellular and Molecular Biology.

Abstract:

Protein purification, characterization and functional analysis play a crucial role in understanding the biology of the cells. Earlier, sequencing of proteins by Edmann degradation method has been used for the identification of proteins for a long time. However, the complexities associated with these time-consuming processes necessitated the development of new technologies that would be users-friendly and require less time. With the development of soft ionization techniques in mass spectrometry, protein identification, quantification and detection of the post - translational modifications became a routine practice in several laboratories. Detection of false discoveries in protein identification, and discrepancies among different quantification methods led to the continuous evolution of Mass spectrometry methods. The use of different ionization methods viz, Collision Induced Dissociation (CID), High Energy Collision (HCD), Electron Transfer Dissociation (ETD) and Electron Capture Dissociation (ECD) is advantageous for sequencing of the peptides/proteins. In addition to this, chemical modification of peptides followed by mass spectrometry helps in improving the sequencing efficiency of peptides. A comparative study among different quantification methods, and problems associated with both database and *de novo* sequencing methods will be discussed. Both top-down and bottom up approaches will be explained with relevant examples. The future prospects in the technological developments will also be highlighted.Sequence determination of peptides using mass spectrometry plays a crucial role in the bottom-up approaches for the identification of proteins. It is important to minimise false detection and validate sequence of the peptides in order to correctly identify a protein. The MS/MS spectra obtained are often with incomplete fragmentation and poor spectral quality. Chemical modification of peptides followed by mass spectrometry is another method for improving the spectral quality. *In silico* derived tryptic peptides with different N-terminal amino acids were designed from human proteins, synthesized and analysed using LC coupled ESI-MS/MS. The effect of acetylation on the fragmentation of peptides was also studied. N-terminal acetylation of the tryptic peptides was shown to form b_1-ions, improve the abundance and occurrence of b-ions. In some cases, the intensity and occurrence of some y-ions also varied. Thus, acetylation was found to be a fragmentation directed chemical modification that improves the efficiency of sequence determination of peptides. Acetylation is a simple reaction that can be carried out on a mixture of peptides as is required in proteomics. The acetylation method was extended to tryptic peptides generated from the proteome of an Antarctic bacterium *Pseudomonas syringae* Lz4W using the proteomics work flow and mass spectra of the peptides were analysed. Comparison of the MS/MS spectra of the acetylated and unacetylated peptides revealed that acetylation helped in improving the spectral quality and validated the peptide sequences. Using this method, 673 proteins of the 1070 proteins identified were validated. It is important to minimise false detection and validate sequence of the peptides in order to correctly identify a protein.

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