

Protein chemistry and mass spectrometry in biochemical research

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Ph. D. Thesis (2006)

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Abstract

Mass spectrometry is a fast and growing analytical approach frequently used not only for detection of small molecules, but also for detection and characterization of macromolecules such as proteins and peptides. The presented PhD thesis summarizes several mass spectrometric applications for identification and characterization of proteins in complex biological matrices, applications for determination of protein structures and characterization of posttranslational modifications. Analytical methods are described in different chapters and include: separation of protein/peptides by two dimensional SDS electrophoresis, separation of proteins/peptides by high performance liquid chromatography, protein sequencing by Edman degradation, etc. As an example of the usage of mass spectrometry for identification of proteins, a method for enrichment followed by characterization of proteins associated with membrane microdomains is shown. For determination of proteins structure by mass spectrometry, chemical cross-linking is usually used. The length of spacer arm of the cross-linker determines the distance constrains between modified amino acids. The information of distances between selected amino acids help to better define protein structure, especially in proteins, which do not crystalized or their tree dimensional structure information is not complete. As an example of the usage of mass spectrometry for determination of posttranslational modifications, analysis of disulphide bonds in enzyme beta N-Acetylhexosaminidase and elucidation of glycans structures is selected. Papers, on which the author of the thesis participated and describing different applications of mass spectrometry in biochemical research, are incorporated.