Abstract

Mass spectrometry (MS) is a rapid, specific and very sensitive analytical method with a broad spectrum of proteomic applications such as protein identification and sequencing, 3D protein structure characterization or study of protein-protein interaction. The introduction of two ionization techniques in late 1980's that are able to ionize the large biomolecules such as proteins, oligosaccharides or nucleic acids with no or low fragmentation has started the rapidly expanding field of MS-based proteomics.

The presented thesis was aimed at the application of mass spectrometric approaches to answer several proteomic questions. Firstly we have employed the chemical cross-linking in combination with MS analysis to solve the 3D structure and protein-protein interactions of three model systems: (1) homodimeric human regulatory protein 14-3-3ζ, (2) model of 14-3-3ζ and regulatory domain of tyrosine hydroxylase, and (3) system of two membrane proteins, cytochrome P450 2B4 and cytochrome b_5, involved in xenobiotics biotransformation. This approach works in aqueous solutions under physiological conditions and thus preserves native structure of the investigated proteins.

The second part of the thesis was focused on MS identification of proteins/peptides in fungal spores of *Aspergillus* and *Pseudallescheria* strains, namely on discovery and characterization of new biomarkers for early stage detection of serious invasive mycoses caused by these molds.

The last part was devoted to the elucidation of particular problems in proteomic and lipidomic projects such as (1) localization of double bond in peptides or lipids by MS, (2) identification of new wheat flour allergens, and (3) determination of proteome changes in leukemic cells during apoptosis induced by medical treatment (including changes in the expression of 14-3-3 proteins).