Abstract

Modification of proteins by lipid structure is relatively common post-translational modification that affects the properties of proteins directly and has a forthright effect on the binding of modified proteins to cell membranes. Some lipoproteins play key role in various pathological processes. Before the proteomic analysis of these proteins, the sample of interest is digested using a protease. The resulting hydrolysate contains both unmodified peptides and peptides bearing a lipid modification. During the subsequent chromatographic separation, the lipopeptides differ significantly from the unmodified peptides. For this reason, the analysis of lipopeptides, lipoproteins respectively, is problematic in terms of separation and detection.

The subject of the study of this diploma thesis was the optimization of the method of lipoprotein analysis using liquid chromatography and mass spectrometry. The procedures were tested on lipoprotein Cya A (bifunctional adenylate-cyclase toxin from *Bordetella pertussis*) and MMTV (matrix protein of mouse mammary tumour virus).

First, various sample preparation procedures involving proteolytic cleavage were tested. When enzymatic digestion using trypsin on filter (eFASP) was used, the lipid modification was detected with high degree of reliability. In the next step, the gradient elution setting for the chromatographic method was tested. A gradient of 70 minutes was chosen (0-5 min 5 % B, do 25. min 25 % B, do 50. min 100 % B, 50-55 min 100 % B, 56-70 min 5 % B), 100% ACN with 0,1% FA was used as an organic mobile phase. Subsequently, the mass detection method was optimized. Standard solutions of synthesized lipopeptides were fragmented in order to find optimal conditions for fragmentation. HCD fragmentation with a collision energy of 30 and 45 eV was chosen to obtain structurally rich MS/MS spectra. Using this method recorded MS/MS spectra obtained information both on peptide structure and N-terminal myristoylation indicator ions. Furthermore, a script for peptide spectra evaluation targeting N-terminal myristoylation was proposed in collaboration with Kamila Clarova, M.Sc.

The complete optimized procedure was applied to sample containing protein MAPPhis (matrix protein of Mason-Pfizer monkey virus). The aim of this experiment was to verify the functionality of the system and to detect the N-terminal myristoylation of this protein. The results showed that the proposed procedure is able to detect N- terminal myristoylation of proteins.