Abstract

Protein lipidation occupies an important place in the post-translational modification group. It plays an important role in the processes of cell differentiation or synaptic transmission. Changes in the presence of lipidated proteins may in some cases indicate the appearance of human diseases such as Huntington's disease, schizophrenia, or cancer. For this reason, their analysis has become desirable, but also problematic due to its specific characteristics.

This work is focused on the development of conditions for measurement of lipopeptides produced by cleavage of proteins containing lipomodification. Two separation procedures were tested, in which the aim of work was to enrich the lipopeptides and remove as many other molecules as possible from the samples. The first test method - phase interface separation, using water and chloroform, was able to concentrate the lipopeptides on the phase interface and separate some of the added unmodified peptides from the mixture. The second method of separation was a solid phase using a C18 stationary phase. As a more suitable solid phase extraction variant, elution with solutions containing acetonitrile with trifluoroacetic acid was chosen as compared to solutions containing isopropyl alcohol. With the modified elution procedure, it was possible to separate unmodified peptides from the lipopeptides without major losses.

Thus optimized procedures were applied to samples obtained by tryptic digests of the mouse mammary tumor virus protein, which contains lipomodification in its structure. The obtained samples were analyzed by LC-MS. The results showed that both methods - phase interface separation and solid-phase extraction - are capable to separate lipopopeptides from unmodified peptides at a loss of up to 10 % in complex mixtures. At the same time, lipopeptides are concentrated and it is possible to analyze and subsequently identify them by searching in databases such as ProteinPilot (SCIEX).

Keywords: lipoproteins, lipopeptides, separation, extraction, SPE, LLE, mass spectrometry, liquid chromatography