

Homocysteine is a plasmatic, sulphur-containing amino acid derived from methionin. It is an amino acid not used in protein synthesis and it's role is to serve as an intermediate in numerous metabolic pathways. Elevated concentrations of homocysteine in blood are associated with many pathologies, as cardiovascular and neurodegenerative diseases, diabetes or osteoporosis, thus homocysteine has been a common subject of many biochemical experiments in last two decades.

For this bachelor thesis, the proteomic comparison of proteins secreted by primary human hepatocytes under 2mM homocysteine and primary human hepatocytes with no homocysteine added to the incubation medium was made, using large-gel two-dimensional electrophoresis.

Hepatocytes were incubated in serum free medium for 48 hours. Proteins secreted to medium were precipitated by acetonitrile. Two dimensional electrophoresis was made. Protein maps were analysed by PDQuest Advanced 8.0.1 2D Gel Analysis Software (Bio-Rad, USA). Search criteria for differentially secreted proteins were three-fold change at the signicance level 95 %, Student's t-test. These proteins were cut off the preparative gels and identified by tandem mass spectrometry.

In total, there were 17 spots equal to 15 proteins found as differentially exprimed proteins. Among these proteins, there were secreted proteins as well as intracellular proteins (cytosolar, mitochondrial). In many cases, a connection between the protein and metabolism of homocysteine could be found.

(In Czech)