10. Summary

Neutral trehalase 1 is a yeast enzyme from the family of hydrolases, which catalyzes hydrolysis of trehalose to two glucose molecules. Trehalose is a non-reducing disacharide, which serves as a carbon source in a yeast cells as well as a stress metabolite. When a cell is under stress conditions it accumulate trehalose and through the recovery process the trehalose is hydrolyses by trehalases.

The main subject of our study was Nth1 from *S. cerevisiae*. It was published earlier (*Panni, S., et al., 2008*), that Nth1 must be phosphorylated by PKA and in the presence of 14-3-3 protein to be active. The activity of Nth1 also slightly increases in the presence of Ca^{2+} ions (*Franco, A., et al., 2003*). 14-3-3 proteins are family of acid regulatory proteins, which participates in variety of processes in the cells, like regulation of the cell-cycle, cell metabolism, transcription, apoptosis etc. They have more than 400 known binding partners, which include transcription factors, signalling molecules, enzymes and others. They control the regulation of their binding partners through phosphorylated motives in sequence by changing conformation of the binding partner, revealing or masking specific sequence or by mediation of protein-protein interactions. There are many isoforms of 14-3-3 proteins through all eukaryotic organisms; there are two isoforms in yeast, called Bmh1 and Bmh2 (*Obsil, T., Obsilova, V., 2011)*.

To investigate details of activation of Nth1 by Bmh the whole spectrum of biochemical and biophysical methods was used. By site-directed mutagenesis, sedimentation analysis and enzyme activity assay was found, that Nth1 forms a stable complex with Bmh, with stoichiometry 1:2 and, that phosphorylation sites, responsible for interaction with 14-3-3 protein, are Ser60 and Ser83, located on the disordered N-terminus. That was confirmed by limited proteolysis and peptide analysis on the MALDI-TOF mass spectrometer.

To determine conformational changes of both proteins pNth1 and Bmh1 upon teh complex formation, the HDX coupled with mass spectrometry and CD spectroscopy was used. Then a homology model of Nth1 and Bmh1 was created, using the measured data, and distance constraints obtained by chemical cross-linking coupled with mass spectrometry were used to refine these models. Significant part of Bmh1 is affected after

complex formation (not only the binding groove). The interaction with Bmh1 affects mostly the sequence 100-200 AA in the Nth1 molecule. That probably reflects the conformational change of Nth1, which leads to revealing the activation site, which is normally buried inside the protein structure, and its better accessibility to the substrate.

As a conclusion, we suggest, that for 14-3-3 dependent activation of Nth1 are essential phosphorylation sites Ser60 and Ser83. Bmh1 binding to the pNth1 leads to the conformational changes on the Nth1 molecule, which consequently leads to the revealing of the active site and activation of the enzyme.