

Abstract

Neutral trehalase 1 (nth1) is a yeast enzyme from the family of hydrolases, which catalyzes hydrolysis of trehalose to two glucose molecules. Trehalose is a non-reducing disaccharide, which serves as a carbon source in a yeast cells as well as a stress metabolite. When a cell is under stress conditions it accumulates trehalose and through the recovery process the trehalose is hydrolyzed 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 Ca²⁺ ions (Franco, A., *et al.*, 2003). 14-3-3 proteins are family of acid regulatory proteins, which participate 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. 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 the 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.