# Abstract

The yeast enzyme neutral trehalase (Nth1, EC 3.2.1.28) from the *Saccharomyces cerevisiae* helps these organisms to survive adverse living conditions. Nth1 hydrolyses a storage and protective disaccharide trehalose into two molecules of glucose. The activity of this enzyme is regulated by PKA phosphorylation, $Ca^{2+}$ binding and the yeast 14-3-3 protein (Bmh1) binding. $Ca^{2+}$ binds to the Ca-binding domain located within N-terminus of Nth1 and contains so called EF-hand motif ($D^{114}$TDKNYQITIED$^{125}$) which is highly conserved among many Ca-binding proteins.

The main aim of this project was to reveal the structural basis of the Bmh1- and calcium-dependent activation of Nth1. Other goals were to solve the structure of Nth1 itself and the structure of its complex with Bmh1. To reveal how the calcium regulates the Nth1 activity we prepared twelve mutant forms of Nth1 using site directed mutagenesis. These mutations were located within the region of EF-hand motif and its close vicinity. We estimated the enzymatic activity of all these mutants in the presence of Bmh1 and/or $Ca^{2+}$. The ability of Nth1 to form stable complexes with Bmh1 was verified using the native polyacrylamide gel electrophoresis and analytical ultracentrifugation. The impact of mutations on the structure and properties of Nth1 was tested using CD spectroscopy and differential scanning fluorimetry. To investigate the structure of Bmh1, Nth1 and their complex we used hydrogen/deuterium (H/D) exchange and chemical crosslinking coupled with mass spectrometry and small angle X-ray scattering (SAXS). In parallel with these methods were carried out first crystallographic experiments with Nth1 itself and also with the pNth1:Bmh1 complex.

Results of these studies revealed that the Bmh1 binding induces significant structural changes of several pNth1 regions including the N-terminal segment where Bmh1 protein binds and also the Ca-binding domain and the catalytic trehalase domain of pNth1. We suggest that changes of the catalytic domain of pNth1 allow a better access for its substrate and thus activate this enzyme. The binding surface between pNth1 and Bmh1 includes not only the ligand binding groove of Bmh1 where the phosphorylated N-terminal part of pNth1 binds but also regions located outside the central channel of the Bmh1 dimer. The low resolution structure revealed that the EF-hand motif forms separate Ca-binding domain which interacts not only with the 14-3-3 protein but also with the catalytic trehalase domain of pNth1. The integrity of EF-hand motif is essential for Bmh1 dependent activation of Nth1 and for $Ca^{2+}$ binding. The Bmh1-dependent activation of pNth1 in the presence of $Ca^{2+}$ is more effective compared to the absence of $Ca^{2+}$. We assume that calcium binding, although not being crucial for its activation, enhances the whole activation process of pNth1 by affecting its structure. Our data also suggest that the Ca-binding domain containing the EF-hand motif functions as the intermediary through which Bmh1 modulates the function of the catalytic domain of Nth1.

Our study of the yeast 14-3-3 protein complex with fully active enzyme pNth1 provides a unique structural view of a key regulatory mechanism of this enzyme. Moreover, it is also the first detailed structural study of the neutral trehalase from a eukaryotic organism. These results may not only contribute to a better understanding of the trehalose metabolism in the yeast *Saccharomyces cerevisiae*, but also the role of 14-3-3 proteins in the regulation of other proteins functions.