

## 11. Summary

The 14-3-3 proteins are dimeric molecules with a characteristic shape and molecular mass about 30 kDa found in all eukaryotes. They are playing a key role in a variety of biological processes such as signal transduction, cell differentiation and apoptosis. The C-terminal segment of human 14-3-3 $\zeta$  plays an important role as an autoinhibitor which can occupy the ligand binding groove in the absence of binding partner and blocks the binding of inappropriate ligand. The C-terminal segment structure has not been identified for any of the known crystallographic structures. Unlike the helical region  $\alpha 1$ - $\alpha 9$ , the C-terminal segment shows the highest sequence variability. It is believed that the C-terminal segment is the most flexible region and can exist in a lot of conformations.

The yeast isoforms of the 14-3-3 proteins Bmh1 and Bmh2 possess a distinctly variant C-terminal segment which is longer and contains a polyglutamine stretch of unknown function. The role of this C-terminal part has been studied with many of different biophysical methods. Dynamic light scattering, sedimentation velocity, time resolved fluorescence anisotropy decay, and size exclusion chromatography measurements showed that an apparent size of the molecules Bmh1 and Bmh2 is significantly bigger compared to the 14-3-3 isoforms. Therefore, it is reasonable to speculate that the C-terminal segment of the Bmh protein either induces formation of bigger oligomers than expected dimers. On the other hand analysis of the SE data revealed that Bmh1 and Bmh2 form stable dimers. Time resolved tryptophan fluorescence experiments revealed no dramatic structural changes of the C-terminal segment upon the ligand binding. The removal of the C-terminal segment does not have any significant effect on binding affinity of Bmh proteins which was studied by fluorescence anisotropy-based binding assay.

In 2008 it was found out that yeast 14-3-3 proteins Bmh1 and Bmh2 can interact with an enzyme neutral trehalase 1 (Nth1). Nth1 is responsible for degradation of nonreducing disaccharide trehalose into two molecules of glucose. The enzymatic activity of Nth1 in yeast is enhanced by 14-3-3 protein binding in a phosphorylation dependent manner. Native gel electrophoresis, sedimentation analysis, and enzyme kinetics measurement were used to study the interaction between Nth1 and yeast 14-3-3 isoforms Bmh1 and Bmh2. We determined four serine residues that are phosphorylated by PKA in vitro (20, 21, 60 and 83), which all are located in a disordered N-terminal segment of Nth1. Sedimentation analysis and enzyme kinetics measurements show that both yeast 14-3-3 isoforms form a stable complex with

phosphorylated Nth1 and significantly enhance its enzymatic activity. The 14-3-3 dependent activation of Nth1 is significantly more potent compared to calcium dependent activation. Kinetic studies of mutant forms *in vitro* and *in vivo* suggest that phosphorylation sites Ser60 and Ser83 are primarily responsible for PKA-dependent and 14-3-3-mediated activation of Nth1.

Taken together, the C-terminal part of Bmh proteins adopts an open and extended conformation, thus increasing the apparent molecular size and thus the C-terminal segment can hardly function as an autoinhibitor.