Abstract

The aim of this diploma thesis was to study the role of posttranslational modifications of phosducin and their role in the interaction with the 14-3-3 protein as well as the influence of the complex formation on these modifications.

Phosducin is a 33kDa protein commonly present in photoreceptor cells of the retina as well as other tissues. Despite many experiments, its physiological functions are still elusive. It has been speculated that fosducin plays an important regulatory role in visual phototransduction pathway, regulation of blood pressure and expression of G-proteins. The phosducin function is regulated through binding to the 14-3-3 protein, a regulatory protein involved in many biochemical processes. Phosducins binding to 14-3-3 protein requires phosphorylation of two serine residues Ser-54 and Ser-73 within the N-terminal domain of phosducin. However, the role of the 14-3-3 protein binding in the regulation of phosducin function is still unclear.

In this diploma thesis proteins 14-3-3ζΔC and phosducin (mutation Q52K) were successfully expressed and purified. The effect of the complex formation on phosducin posttranslational modifications was investigated using limited proteolysis and dephosphorylation. These experiments revealed that the complex formation significantly slowed down both the dephosphorylation of pSer-54 and pSer-73 and the proteolytic degradation of phosducin. This indicates that the complex formation might have a protective function against dephosphorylation and proteolytic cleavage. The importance of the two 14-3-3ζΔC protein binding motifs containing pSer-54 and pSer-73 for the interaction between phosducin and 14-3-3ζΔC protein was investigated using steady-state fluorescence anisotropy measurements with synthetic phosphopeptides. These measurements revealed similar binding affinities for both studied motifs thus suggesting their similar importance for the interaction between phosducin and 14-3-3ζΔC protein.