Abstract

The main goal of this bachelor thesis is to study the interaction between phosducin and the 14-3-3 protein which are involved in the regulation of visual signal transduction in vertebrate retina. This process is mediated by G-protein signaling pathway and is regulated by several proteins including phosducin depending on light intensity.

Phosducin, a 33kDa protein, is expressed in many tissues mainly in photoreceptor cells of the retina. The visual signal transduction and its amplification are regulated through inhibition of the G-protein (transducin) function. The signal transduction involves a dissociation of heterotrimeric transducin ($G_t\alpha\beta\gamma$) to the α subunit ($G_t\alpha$) and the βγ complex ($G_t\beta\gamma$). The signalling is terminated by their reassociation back to the $G_t\alpha\beta\gamma$ complex. The phosducin binds to $G_t\beta\gamma$ and thus prevents the formation of the $G_t\alpha\beta\gamma$ heterotrimer. This decreases the amount of functional $G_t\alpha\beta\gamma$ complexes and suppresses the signal transduction. If the signal transduction needs to be amplified (e.g. during the night) then phosducin is phosphorylated and this inhibits its interaction with $G_t\beta\gamma$. The phosphorylated phosducin is bound to the 14-3-3 protein. However, the role of the 14-3-3 protein in the regulation of phosducin is still unclear.

The 14-3-3 protein is a 28kDa protein which is expressed in all eukaryotic cells. It has a rigid structure and can bind more than three hundred proteins. Thereby, it participates in many biochemical processes. In photoreceptor cells it binds phosducin phosphorylated at Ser-54 and Ser-73. This binding interaction further inhibits phosducin binding to $G_t\beta\gamma$ complex and thus the 14-3-3 protein also participates in the regulation of signal transduction. In addition, the 14-3-3 protein has also been suggested to protect the phosphorylated phosducin against the activity of proteases and phosphatases.

During this bachelor work a mutant of phosducin (PdQ52K) and a mutant of the 14-3-3ζ protein (14-3-3ζnoW) were expressed in *E. coli* BL21(DE3) cells and successfully purified. Next, the interaction between the N-terminal part of phosducin and the 14-3-3 protein was investigated using acrylamide quenching of tryptophan fluorescence. The obtained values of Stern-Volmer quenching constants for PdQ52K both in the absence and the presence of 14-3-3ζnoW suggest that the complex formation has no significant effect on N-terminal part of PdQ52K molecule containing residue Trp-29.