Abstract

The aim of this diploma thesis is a biophysical characterization of the protein complex that consists of two regulatory proteins, the phosducin (Pd) and the 14-3-3 protein. These proteins are involved in the regulation of a signal cascade in vertebrate eye´s retina. Pd is a 33kDa protein located in photoreceptor cells in retina, but it has been found in other tissues as well. In retina, phosducin affects transfer of light signaling from eye to brain, by binding $G_{t\beta\gamma}$ subunit of transducin that is the main part of G-protein signaling. In light-adapted retina, unphosphorylated phosducin down-regulates the light response by binding to $G_{t\beta\gamma}$. This process is important for protecting retina in eyes in response to very intense light. It has also been found that phosducin affects hypertension. Phosducin reduces blood pressure of human and mice, especially during sleep. The function of phosducin is regulated in dark-adapted retina by 14-3-3. 14-3-3 is a 28kDa protein that has been found in many eukaryotic tissues, e.g. brain, and is involved in many processes, e.g. apoptosis. The 14-3-3 protein binds phosphorylated Pd and keeps him in a rod inner segment. For 14-3-3 to Pd binding, two sites on Pd must be phosphorylated, Ser54 and Ser73. This interaction, hinders Pd binding to $G_{t\beta\gamma}$, and hence enables the formation of heterotrimeric transducin. Transducin is then prepared to transfer a new signal. It is also possible that 14-3-3 protects phosducin against *proteases* and *phosphatases*.

Pd wt, PdQ52K, PdQ52KS73A, 14-3-3ζ wt a 3-3ζnoW have been produced in *E. coli* BL21(DE3) cells and purified. Several biophysical techniques were used to study the 14-3-3/Pd complex. Native electrophoresis showed the formation of the complex with doubly phosphorylated Pd (Ser54, Ser73). Limited proteolysis revealed structural changes of Pd in response to 14-3-3 binding. Analytical ultracentrifugation revealed the stoichiometry of the 14-3-3/Pd complex (2:1), where two monomers of 14-3-3 bind one Pd. The dissociation constant was estimated to be 5μM. Time-resolved tryptophan fluorescence measurements were used to study the flexibility of Pd upon the 14-3-3 protein binding. No significant structural change of Pd was observed in response to phosphorylation.