

Abstrakt EN

Protein-protein interactions have an exceptional position among other mechanisms in the regulation of signal transduction. Their systematic investigation is very important and logical step in the process of understanding to the transduction and its mechanisms at a molecular level.

During my Ph.D. I was particularly interested in three important processes. ASK1 kinase is well-known initiator of the apoptosis. Under physiological conditions it is maintained in an inactive state by its two interaction partners the 14-3-3 protein and TRX1. These two proteins dissociate in the presence of reactive oxygen species by unclear mechanism and the kinase is therefore activated. The next process is an interaction between the 14-3-3 protein and phosducin and investigation of their role in the G protein signalling especially important in the biochemistry of vision. The third process is an activation of protein Nth1 through the interaction with Bmh1, yeast analog of the 14-3-3 protein, and calcium cations. I employed various biophysical method, particularly analytical ultracentrifugation, in order to explain molecular mechanisms of described processes. These techniques were used to solve the low-resolution structures of complexes TRX1 and the 14-3-3 protein with corresponding binding domains of ASK1. These structures confirmed binding stoichiometries acquired from sedimentation velocity analysis. This analysis also provided binding affinity in terms of K_d and suggested an interaction interface between binding partners. In the case of the ASK1:TRX1 complex the main amino acid residues responsible for the interaction were identified together with the fact that TRX1 binds in a close proximity of ASK1 dimerization interface. In the case of phosducin, I studied the influence of different parts of the protein on the interaction with the 14-3-3 protein. I discovered that the N-terminal part of phosducin is fully responsible for the stability of the complex where 14-3-3 sterically occludes the binding interface for another binding partners. In the case of Nth1 and Bmh1, I studied the binding affinity between those proteins depending of the presence of calcium ions. Results suggested that there is no influence of calcium ions on this interaction.