Abstract

This dissertation is focused on 30 kDa protein phosducin (Pdc) and on the regulation of its function through the interaction with 28 kDa adaptor protein 14-3-3. These two proteins participate in G-protein signal transduction pathways, especially in the process of light signal transduction. It is assumed that Pdc binds to the $G_t\beta\gamma$ complex of G-protein called transducin and through this interaction it inhibits the reassociation of $G_t\beta\gamma$ with $G_t\alpha$ thus reducing the visual signal transfer. This process is thought to participate in a long-term light adaptation. The regulation of Pdc function is further regulated by its phosphorylation and subsequent binding to the 14-3-3 protein. It has been speculated that the 14-3-3 binding plays a key role in the inhibition of the interaction between phosphorylated Pdc (Pdc-PP) and $G_t\beta\gamma$. The formation of the 14-3-3/Pdc-PP complex leads to the reassociation of $G_t\beta\gamma$ with $G_t\alpha$ and consequently to the amplification of visual signal transfer. Nevertheless, the mechanism by which the 14-3-3 protein binding inhibits the interaction between Pdc and $G_t\beta\gamma$ remains elusive.

The main aims of this dissertation were: (i) to investigate the structure of Pdc in its apo-state (in the absence of the binding partner) and in the complex with 14-3-3, and (ii) to suggest the mechanism of the 14-3-3-mediated regulation of Pdc function. The structure of Pdc and the 14-3-3/Pdc-PP complex was studied using various biophysical methods including small-angle X-ray scattering (SAXS), NMR, H/D exchange coupled to mass spectrometry (HDX-MS) and fluorescence techniques. Our data suggested that the N-terminal domain of Pdc (Pdc-ND) is intrinsically disordered protein. The phosphorylation of Pdc affects conformation of both its domains - unstructured Pdc-ND as well as structured C-terminal domain (Pdc-CD). The 14-3-3 protein binds Pdc-PP with the binding affinity in micromolar range and the complex formation affects the conformation of Pdc especially within Pdc-ND. Both phosphorylation sites are essential for the complex formation. The majority of Pdc-ND, which accounts for the most of the $G_t\beta$ binding surface, is located either in the central channel of the 14-3-3 dimer or in the close vicinity of the 14-3-3 outer surface. Therefore, our data suggest that the 14-3-3 binding masks the majority of the $G_t\beta$ binding surface of Pdc and thus inhibits its interaction with $G_t\beta$. In addition, NMR and HDX-MS measurements revealed that Pdc-PP remains highly flexible after the 14-3-3 binding, which indicates the formation of a “fuzzy” complex. Our data also showed that the 14-3-3 binding slows down dephosphorylation of Pdc-PP *in vitro*.