Univerzita Karlova v Praze

Přírodovědecká fakulta

Studijní program: Fyzikální chemie

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Studium úlohy proteinů 14-3-3 v regulaci G-proteinové signalizace

Role of the 14-3-3 proteins in the regulation of G-protein signaling

Disertační práce

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Abstract

The 14-3-3 family of phosphoserine/phosphothreonine-binding proteins dynamically regulates the activity of their binding partners in various signaling pathways that control diverse physiological and pathological processes such as signal transduction, metabolic pathways, cell cycle and apoptosis. More than 300 different cellular proteins from diverse eukaryotic organisms have been described as binding partners for the 14-3-3 proteins.

During my Ph.D., I was particularly interested in the role of 14-3-3 proteins in the regulation of G protein signaling pathway. The 14-3-3 proteins affect the G protein signaling via the interaction with negative regulators of G protein cascade – the RGS proteins and phosducin. I employed both biochemical and biophysical approaches to understand how the activity and function of RGS3/14-3-3 and phosducin/14-3-3 complexes are regulated.

I solved the low-resolution solution structure of RGS3/14-3-3 protein complex that shows the RGS domain of RGS3 bound to the 14-3-3 dimer in a unique manner by interacting with less-conserved regions on the outer surface of the 14-3-3 dimer outside its central channel. This was the first experimental evidence showing that the 14-3-3 protein directly interacts with its binding partner using regions other than that of the central channel. The involvement of such less-conserved regions may provide a general explanation for the observed isoform-specific interactions between 14-3-3 and their ligands. The structure of the RGS3/14-3-3 complex also provides an explanation for the 14-3-3-dependent inhibition of RGS3 function. It shows that the 14-3-3 protein, besides binding to the phosphorylated N terminal 14-3-3 binding motif, interacts with the C terminal RGS domain in close proximity to the Gα-binding interface, and hence can sterically occlude it. In addition, the time resolved fluorescence measurements indicate that the 14-3-3 protein binding causes significant conformational changes of the RGS domain structure within its Gα-interacting portion.

To gain insight into the role of 14-3-3 in the regulation of phosducin function, I studied structural changes of fosducin induced by phosporylation and the 14-3-3 protein binding using time-resolved fluorescence spectroscopy. I provided the structural explanation for the 14-3-3-dependent inhibition of phosducin function.