

## Abstract

Through binding interactions with more than 300 binding partners, 14-3-3 proteins regulate large amount of biologically relevant processes, such as apoptosis, cell cycle progression, signal transduction or metabolic pathways. The research discussed in this dissertation thesis was focussed on investigating the role of 14-3-3 proteins in the regulation of two selected protein kinases ASK1 and CaMKK2. The main goal was to elucidate the mechanisms by which phosphorylation and 14-3-3 binding regulate functions of these protein kinases using various biochemical and biophysical methods, such as site-directed mutagenesis, enzyme activity measurements, analytical ultracentrifugation, small-angle X-ray scattering, chemical crosslinking, nuclear magnetic resonance and fluorescence spectroscopy.

A structural model of the complex between the catalytic domain of protein kinase ASK1 with 14-3-3 $\zeta$ , which was calculated using the small-angle X-ray scattering and chemical crosslinking data, suggested that this complex is conformationally heterogeneous in solution. This structural model together with data from time-resolved fluorescence and nuclear magnetic resonance suggested that the 14-3-3 $\zeta$  protein interacts with the catalytic domain of ASK1 in the close vicinity of its active site, thus indicating that the complex formation affects the structure and/or the accessibility of the active site. Another important result of this thesis was the finding that the oxidation-induced disruption of interaction between ASK1 and thioredoxin 1, which is also a physiological inhibitor of ASK1, is mainly due to the disulfide bond formation between residues Cys<sup>32</sup> and Cys<sup>35</sup> in the catalytic site of thioredoxin.

To understand the role of 14-3-3 proteins in the regulation of protein kinase CaMKK2, a structural model of the complex between CaMKK2 and 14-3-3 $\gamma$  was calculated based on small-angle X-ray scattering data. The model revealed that although the catalytic domain of CaMKK2 is located outside the central channel of the 14-3-3 $\gamma$  dimer, the 14-3-3 $\gamma$  protein directly interacts with this domain and affects its structure outside regions containing 14-3-3 binding motifs.

The results of this thesis confirmed that 14-3-3 proteins are important allosteric modulators of protein kinases ASK1 and CaMKK2.