Abstract EN

The aim of this study was to investigate the regulatory mechanisms of two important signaling proteinkinases and promising therapeutic targets, ASK1 and CaMKK2. ASK1 kinase is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family that activates c-JNK kinase and p38 MAP kinase pathways in response to various stress stimuli, including oxidative stress. The function of ASK1 is associated with the activation of apoptosis and thus plays a key role in the pathogenesis of multiple diseases including cancer, neurodegeneration or cardiovascular diseases. The natural inhibitor of ASK1 is a ubiquitous oxidoreductase, thioredoxin, which is probably bound to N-terminus of ASK1, thus preventing a homophilic interaction and subsequent ASK1 activation. It has been suggested, that upon oxidative stress and oxidation of thioredoxin active site, thioredoxin dissociates from ASK1, but the structural basis of this interaction remains unclear.

Calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) is a member of CaM kinase pathway that activates CaMKI, CaMKIV and AMPK involved in gene expression regulation or apoptosis activation. Function of this protein is often associated with neuropathology, carcinogenesis and obesity. CaM kinases are activated via binding Ca^{2+} sensor protein calmodulin (CaM). Structural studies of CaMKK2 revealed distinct structural features, suggesting a potentially different activation mechanism. CaMKK2 is also regulated and inhibited through phosphorylation. It has been identified two potential phosphorylation sites, Ser^{100} and Ser^{511}, for 14-3-3 protein binding. These universal dimers regulate their multiple phosphorylated substrates through conformational modulation or masking of signaling sequences. However, the role of 14-3-3 proteins in CaMKK2 regulation is not sufficiently understood.

Biophysical and structural characterization of the complexes allowed us to describe the interaction interface, to estimate biding affinity, stoichiometry and complex dynamics, thus contributing to elucidation of these regulatory mechanims.