ABSTRACT

The mitogen-activated protein kinase (MAPK) cascade is an essential member of the cell defense system against stressors. The capability and efficiency of the cell reactions to different stress signals depend on signal transduction pathway, where signals from MAPK kinase kinase (MAP3K) are transferred through phosphorylation to downstream MAPK kinase (MAP2K) and finally to MAPK. Apoptosis signal-regulating kinase 1 (ASK1) is a member of a MAP3K family and its activation and inhibition has a significant participation in a regulation of cell response to stress stimuli. The regulation of ASK1 has a strong influence in pathogenesis of several diseases, the excessive activation of human ASK1 or failure in the control of its function are associated with cardiovascular diseases, neurodegenerative disorders, inflammatory diseases, infectious diseases, tumorigenesis, asthma, diabetes and ageing. The activity of ASK1 is regulated by its interaction with several proteins, the attention is focused on two physiological inhibitors, mammalian thioredoxin (TRX) and the 14-3-3 protein. ASK1 in its inactive form is inhibited by bonds formation with TRX and 14-3-3, however the explicit mechanism of this interaction is unclear due to the absence of structural data.

This work is a part of an extensive research about human ASK1, focusing on the structural characterization of the interaction between ASK1 and TRX. We believe that the understanding of ASK1 structure is critical for any intervention into its regulation and that the controlled inhibition of ASK1 molecule might be important for a treatment of many different diseases. The aim of this work is the structural characterization of the TRX binding domain of N-terminal human ASK1 (ASK1-TBD) and the ASK1:TRX complex using various biophysical approaches: native gel electrophoresis for the test of the interaction between ASK1 and TRX; dynamic light scattering measurements for a study of polydispersity and aggregation behavior of protein samples; circular dichroism measurements for a study of the protein secondary structure and small angle X-ray scattering measurements for the low resolution structure determination.

Results showed that ASK1-TBD is a rigid and monomeric domain and it forms with TRX a stable complex through a large binding interface. The catalytic motif of TRX is necessary for the ASK1-binding and the interaction is not accompanied by the formation of intermolecular disulfide bridges. ASK1-TBD has a compact and slightly asymmetric shape and it interacts with TRX without inducing any dramatic conformational change.