Abstract

MAP kinase signaling cascade plays an important role in the cellular response to various stress stimuli from the external environment. This signaling cascade is divided into three levels: MAP kinase kinase kinases (MAP3K) phosphorylate and thus activate MAP kinase kinases (MAP2K) and those subsequently phosphorylate and thus activate MAP kinase (MAPK) pathway, which regulates many cellular functions such as apoptosis, cell differentiation and morphogenesis. One of the important MAP3K is protein kinase ASK1 (Apoptosis signal-regulating kinase 1), which is an important regulator of cellular immune and stress responses. Given that the increased activity of ASK1 is related to the development of serious diseases such as cancer, cardiovascular and neurodegenerative diseases, ASK1 is an interesting target in the pharmacy in the development of new drugs. Human ASK1 consists of 1374 amino acids and is divided into three domains: a central Ser/Thr catalytic domain and two coiled-coil domains, of which the first is located at the N- and the second at the C-terminus of the molecule of this protein kinase. ASK1 is regulated by its binding partners, which include a small cellular redox protein thioredoxin (Trx-1), which binds to the N-terminal part of ASK1. Trx-1 is a potent antioxidant and so it protects cells against toxic stimuli from the environment. The mechanism of the regulation of ASK1 activity using Trx-1 is one of the most studied, and although it is not completely solved, it appears that it is related to the redox reactions which take place within the Trx-1 molecule. Interaction with ASK1 is possible only while Trx-1 is reduced, its oxidized form dissociates from ASK1 molecule immediately.

Aim of this study was following: (i) preparation of Trx-1 and four fragments of N-terminal part of ASK1 with different lenghts; (ii) basic biophysical characterization of prepared proteins; and (iii) the study of the interactions between fragments of ASK1 and Trx-1 to specify the position of the binding site for Trx-1. Results showed that only two of the four prepared fragments of ASK1 are soluble (sequences 46-302 and 88-302). Furthermore, it was found out that both fragments interact with Trx-1 under reducing conditions with the same binding affinity. At the end it can be said that the binding site for Trx-1 in ASK1 molecule is located within the sequence of 88-302 and that this area creates a domain that can be separately prepared and used as a model for study of the interactions between ASK1 and Trx-1.

(In Czech)