ABSTRACT

All living organisms are exposed to various forms of stress during their lifetime. This is probably the reason why an evolutionally well conserved the mitogen-activated protein kinase (MAPK) system of signaling cascades was formed to regulate cellular stress response. Those signaling pathways consist of three consecutive classes of protein kinases: MAP3K, MAP2K and MAPK. The signal is then transmitted from MAPK to another protein kinases and transcription factors.

Apoptosis signal-regulating kinase 1 (ASK1) is a member of MAPK pathway, more specifically is classified as a member of the mitogen-activated protein kinase kinase kinase family (MAP3K). Human ASK1 consists of 1374 amino acids which are folded into several domains and sequence motives. The N-terminal coil-coiled domain (NCC), the serine/threonine kinase domain and the C-terminal coil-coiled domain (CCC) are three main domains. In addition, several regions responsible for the interaction between ASK1 and their binding partners have also been identified. The activity of ASK1 is regulated by various factors including thioredoxin and the 14-3-3 proteins, which function as inhibitors, and TNF receptor associated factors (TRAFs), which function as activators.

The aim of this study was the preparation of six different expression constructs of the N-terminal sequence of human ASK1. This fragment should be responsible for the formation of the complex between ASK1 and thioredoxin. The C-terminus of the sequence was selected very carefully with the effort not to disrupt the predicted secondary structure elements. Then a new expression construct of the thioredoxin containing a single point mutation C73S which prevents the formation of dimers was prepared. This construct also allows the cleavage of the His-tag after the purification of the fusion protein to obtain nearly native form of the protein.

(In Czech)