Abstract

Protein kinase ASK1 (apoptosis signal-regulating kinase 1) is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family and plays a crucial role in immune and stress responses. Since the increased activity of ASK1 has been linked to the development of several diseases including cancer, cardiovascular and neurodegenerative diseases, this enzyme is a promising target for therapeutical intervention in these pathologies. The molecule of ASK1 consists of 1374 amino acid residues, but catalytic activity possesses only a kinase domain located approximately in the middle of the molecule. The activity of ASK1 is regulated by interactions with various proteins including the 14-3-3 protein. This protein recognizes a phosphorylated motif around Ser966 at the C-terminus of the catalytic domain of ASK1. This binding interaction inhibits ASK1 through unknown mechanism. ASK1 under stress conditions, such as oxidative stress, is dephosphorylated at Ser966 and the 14-3-3 protein dissociates. This dissociation is then one of the factors that lead to the activation of ASK1.

The aim of this diploma thesis was to prepare a complex of the catalytic domain of ASK1 with the 14-3-3ζ protein for subsequent structural studies. Both proteins were expressed in *E. coli* cells and successfully purified. In addition, several mutant forms of both proteins were prepared as well. The prepared kinase domain was shown to be catalytically active. The catalytic domain of ASK1 was then phosphorylated by protein kinase A at Ser966 and the result of the phosphorylation reaction was verified using mass spectrometry. The interaction between the phosphorylated catalytic domain of ASK1 and the 14-3-3ζ protein was studied using native electrophoresis and analytical ultracentrifugation. The results show that the phosphorylated catalytic domain of ASK1 forms a stable complex with the 14-3-3ζ protein, which can be used for following structural studies.