Abstract

Heme-sensing proteins are heme proteins to which heme serves as a signalling molecule. Association or dissociation of heme moiety and heme-sensing proteins influences various physiological functions, such as enzyme activity or gene expression regulated by these heme-sensing proteins.

The main object of this thesis is heme-sensing protein Bach1 and its interaction partner, transcription factor MafK. Bach1 refers to the group of transcription factors involved in repression of gene expression. The target genes of Bach1 regulation are hemeoxygenase genes. Hemeoxygenase controls the excess free heme degradation. Due to the excess of free heme in the cell, Bach1-heme interaction inactivates Bach1 controlled repression of hemeoxygenase resulting in the free heme degradation. In the state of physiological free heme concentration, Bach1-heme interaction does not occur and activated Bach1 represses the hemeoxygenase expression via binding to the target gene enhancers. Bach1 is incapable of making efficient Bach1-DNA bonding by itself, therefore the transcription factor MafK is essential. Protein MafK modulates the Bach1-DNA binding by making the heterodimer formation Bach1-MafK, which binds to DNA.

The first aim of this thesis is to summarize the recent knowledge about transcriptional factors Bach1 and MafK, their interaction, DNA binding activity and heme influence on their function, published in scientific journals. Experimental part of this thesis focuses on the transcription factor MafK. The first aim of the experimental part was to prepare the suitable plasmid involving MafK gene with His-tag, amplify this plasmid and isolate it. The second aim was the expression of transcription factor MafK in a prokaryotic system followed by the preliminary isolation of this protein. The small amount of MafK protein was isolated and its authenticity was verified by the immunochemical method using antibodies against His-tag. The last step of the experimental part refers to the preliminary characterization of isolated MafK protein preparate. On the ground of experimental process and achieved results, we proposed the isolation process optimalization.

Key words: heme, heme-containing sensor proteins, prokaryotic expression, protein isolation