ABSTRACT

Calmodulin kinase cascade is a signaling pathway which is involved in the response to the increasing intracellular calcium levels. Ca^{2+} is a ubiquitous second messenger which promotes wide-range of cellular signaling events. Many of these signaling pathways start with the binding of Ca^{2+} to its primary intracellular receptor calmodulin. Calmodulin in turn binds to its downstream targets in the Ca^{2+}/calmodulin signaling cascade. One of the most important enzymes of this cascade is a Ca^{2+}/calmodulin-dependent protein kinase kinase 2 (CaMKK2).

CaMKK2 is a serine/threonine protein kinase which regulates for example gene transcription or energy homeostasis by phosphorylation of its downstream targets. Catalytic domain (which provides kinase activity) is located in the middle part of the protein and possesses structure typical for kinases. CaMKK2 consists of 588 amino acids but the secondary structure is known only for the region of the kinase domain (298 residues). The rest of the protein is assumed to be unstructured as long as CaMKK2 is not bound to any interaction partner.

The aim of this study was to prepare several constructs of human isoform of CaMKK2 for the further structural and activity studies. It is believed that CaMKK2 is regulated by site-specific phosphorylation. Phosphorylation of some particular residues should enhance the activity of the kinase whereas other phosphorylated residues promote inhibition. Putative regulation of CaMKK2 also involves interaction with the scaffolding protein 14-3-3.

Results of this thesis showed that 14-3-3 binding to CaMKK2 is a phosphorylation-dependent interaction. Four different constructs of human CaMKK2 (which differ in number or location of specific phosphorylation sites) were prepared. Some of these phosphorylation sites also correspond to the putative 14-3-3 binding sites. All prepared CaMKK2 constructs were successfully expressed and purified. The activity of prepared enzymes was confirmed by kinase assay. The interaction between phosphorylated CaMKK2 and 14-3-3 protein was verified by native electrophoresis and analytical ultracentrifugation.