Abstract

Ca^{2+}/calmodulin-dependent protein kinase kinases (CaMKK) are serine/threonine kinases involved in the calcium signaling pathway. Two CaMKK isoforms were described in mammals: CaMKK1 and CaMKK2. The increase in calcium concentrations induces Ca^{2+}/CaM binding to the C-terminal segment of CaMKK, thus relieving autoinhibition by disrupting the interaction between the autoinhibitory segment and the kinase domain. Active CaMKK then phosphorylate and activate their downstream kinases CaMK1 and CaMK4, and in the case of CaMKK2 also AMPK.

The activity of CaMKK is also regulated by phosphorylation mediated by cAMP-dependent protein kinase A (PKA). This phosphorylation creates two binding motifs recognized by the regulatory 14-3-3 proteins. Previous studies have suggested that the 14-3-3 protein keeps phosphorylated CaMKK1 in the inhibited state by blocking the dephosphorylation of the inhibitory phosphorylation site and it has been speculated that CaMKK2 is regulated in a similar manner. However, the role of 14-3-3 protein in the regulation of CaMKK2 is unclear. In order to study this protein complex, it is necessary to prepare recombinant CaMKK2 fully phosphorylated at both 14-3-3 binding motifs.

The main aim of this bachelor thesis was to optimize the protocol for the phosphorylation of human CaMKK2 (residues 93-517) containing only two phosphorylation sites, Ser100 and Ser511, and characterize the CaMKK2:14-3-3γΔC and CaMKK2: CaM: protein 14-3 -3γΔC complexes by analytical ultracentrifugation.

Mass spectrometry and phos-tag SDS-PAGE were used to optimize the phosphorylation of CaMKK2-S100, S511 by PKA. Analytical ultracentrifugation revealed that doubly phosphorylated CaMKK2 forms with the 14-3-3 protein complex with molar stoichiometry 1:2 and dissociation constant in micromolar range. The CaMKK2:CaM:14-3-3 protein complex is formed with molar stoichiometry 1:1:2.