Abstract

Ca^{2+}/calmodulin-dependent protein kinase kinase 2 (CaMKK2) belongs to the serine/ threonine protein kinase family, which is involved in the calcium signaling pathway. The increase of intracellular calcium concentration induces the activation of calmodulin (CaM), which then activates its binding partners including CaMKII, CaMKIII, CaMKK1 and CaMKK2. CaMKK2 activates CaMKI, CaMKIV and AMP-dependent kinase, AMPK, by phosphorylation.

CaMKK2 is naturally present in cells in an autoinhibited state, which is caused by the steric hindrance of the active site by the autoinhibitory domain. When calmodulin binds to the calmodulin-binding domain, the autoinhibitory domain is removed and the active site becomes accessible. Upon activation, CaMKK2 undergoes autophosphorylation, which increases its enzyme activity. Negative regulation of CaMKK2 is mediated by cAMP-dependent protein kinase A (PKA)- and GSK3-dependent phosphorylation. Sites phosphorylated by PKA have been identified for both CaMKK1 and CaMKK2. Two of them are also motifs recognized by scaffolding 14-3-3 proteins. Previous studies have shown that the 14-3-3 protein binding maintains phosphorylated CaMKK2 in an inhibited state by blocking the dephosphorylation of S495, which prevents the binding to calmodulin. However, it is unclear if it is the only role of the 14-3-3 protein in the regulation of CaMKK2.

The main goal of this master thesis was to optimize the protocol for the phosphorylation of human CaMKK2 (residues 93-517), which contains four phosphorylation sites (Ser100, Thr145, Ser495 and Ser511) and to characterize the CaMKK2:14-3-3γΔC protein complex by analytical ultracentrifugation-sedimentation velocity method, chemical crosslinking coupled to MS, hydrogen-deuterium exchange coupled to MS and small angle X-ray scattering.

The apparent dissociation constant of the CaMKK2:14-3-3γΔC complex was determined by analytical ultracentrifugation, chemical crosslinking provided information about the mutual orientation of both proteins. The hydrogen-deuterium exchange provided information about areas which are affected by the complex formation, and the small-angle X-ray scattering together with computer modeling provided the basic structural parameters of the complex, its molecular envelope, and an approximate model consistent with previous experiments.