Abstract

Ca\(^{2+}\)/calmodulin-dependent kinases are members of CaMK family, which is involved in CaMK cascade. One of CaMK family members is Ca\(^{2+}\)/calmodulin-dependent kinase 2 (CaMKK2), which is activated by Ca\(^{2+}\)/CaM-binding. There are some structural differences between CaMKK2 and other protein kinases, one of them is a structure near αE-helix and autoinhibitory domain.

Due to the overlap of autoinhibitory domain and Ca\(^{2+}\)/CaM-binding domain it can be supposed that Ca\(^{2+}\)/CaM-binding induces structural changes near autoinhibitory domain and thus can affect the accessibility of this region. CaMKK2 W445F mutant, which contains only one tryptophane residue Trp\(^{374}\) close to the αE-helix, was expressed and purified. Structural changes in this region were monitored using tryptophan fluorescence intensity quenching experiments, which can provide information about the accessibility of region surrounding tryptophan residue. The fluorescence of Trp\(^{374}\) was quenched using acrylamide. Comparison of fluorescence quenching experiments performed in the presence and absence of calmodulin suggests that the complex formation induces structural change in the region surrounding Trp\(^{374}\).