Abstract

Some proteins and enzymes require presence of their specific ligand, cofaktor or prosthetic group for their activity. Binding of this specific molecule causes conformational changes, which permits to perform their function. In some occasions the identification of conformational changes is difficult. Using chemical cross-linking coupled with mass spectrometry perform complex tool for searching and low resolution visualization of this changes.

The aim of this thesis is study of conformational changes induced by binding of calcium ion to calmodulin protein molecule. Calmodulin is a secondary intermediate messenger, which can interact with various proteins. This feature associates with wide dynamical range of calmodulin. Thus calmodulin is the suitable target for identifying conformational changes. After reaction of protein with chemical cross-linkers with different arm length (DSG and DSS) were products of reaction digested by trypsine. Formed linked peptides were separated by high-performance liquid chromatography and analysed followed mass spectrometry. Seven unique intramolecular cross-links were identified. Using isotope unlabeled cross-link reagents in the presence of Ca^{2+} in combination with using isotope labeled reagents in calcium free conditions we quantified formed lysine-lysine cross-links. (in Czech)