

Abstract

Some proteins require presence of their specific ligand, cofactor or prosthetic group for their activity. Binding of this specific molecule can cause conformational changes which permit to perform their function. In some occasions the identification of conformational changes could be really challenging task. In this thesis we describe the novel approach for monitoring structural changes in proteins using chemical cross-linking and high resolution mass spectrometry and its application on model calmodulin system. It is demonstrated that analysis using isotope-labelled cross-linking agents enabled us to get insight into the structural rearrangement caused by presence or absence of the protein ligand. However, it is shown that the method has potential drawback due to limited enzymatic proteolysis.

The novel approach that also makes it possible to quantify the changes in protein structure was used together with other methods for characterization of the neutral trehalase Nth1 in complex with Bmh1 protein (yeast isoform of protein 14-3-3). The results revealed that Bmh1 induce structural rearrangement of Nth1 molecule with changes within the EF-hand like motif which is essential for the activation process.