Abstract

Elucidating the structure of proteins in living organisms is an important step in describing their role. One method of structural biology is chemical cross-linking in combination with mass spectrometry, which is a commonly used tool for characterisation of the tertiary structure of proteins or protein-protein interactions. This method is based on the reaction of a cross-linking agent consisting of two reactive groups linked by an arm of a defined length to form a covalent bond. The main aim of the project was to verify whether chemical cross-linking using istopically labeled cross-linking agents can be used for quantification of two different structural states. The reagents used in this work were DSPU and an isotopically labeled form of DSPUx. Both the DSPU and DSPUx reagents contain a labile urea molecule in their structure, which is cleaved into characteristic fragments during collision-induced dissociation, allowing the identification of cross-linked peptides.

Using top-down and bottom-up approaches, it was found on selected proteins (insulin, human carbonic anhydrase, and bovine serum albumin) that DSPU and DSPUx reagents interact with selected peptides and proteins and are suitable for quantification of structural changes. Subsequently, structural differences in the presence (holoform) and absence (apoform) of heme were investigated in a myoglobin model. Finally, the reagents were used to study the structural changes of 5'-adenosine monophosphate-activated protein kinase in muscle cells from patients with myotonic dystrophy type 1 and in cells from healthy subjects.

Key words

proteins, protein quantification, cross-linking reactions, mass spectrometry, isotope covalent labeling

[IN CZECH]