Abstract

PPM1D protein phosphatase is a negative regulator of the cellular response to DNA damage. The tumor suppressor protein p53 plays an irreplaceable role in these processes. PPM1D phosphatase is one of its main negative regulators and expression of PPM1D is also regulated by p53. Physiologically, PPM1D contributes to the restoration of cellular homeostasis by inactivating p53. However, overexpression of PPM1D is associated with the development and progression of several types of tumors. Understanding the mechanisms by which PPM1D is regulated could therefore be an important step towards the fight against cancer.

The structure of PPM1D is characterized not only by a compact phosphatase domain but also by the presence of several flexible and unstructured regions. Therefore, the preparation of this protein is a challenging task, which significantly complicates its characterization *in vitro*. For this reason, a truncated protein $PPM1D_{1\text{-}397}$ (PPM1D NTD) was used in this thesis. In order to obtain a stable protein with high level of purity, the expression and purification of PPM1D NTD were extensively optimized. SAXS data suggest that the proline-rich loop (30–99), which is specific to PPM1D, adopts an extended and likely flexible conformation in solution. The long-term goal of the project is to study the protein-protein interactions of PPM1D phosphatase. One of the interaction partners of PPM1D is LZAP protein. Phosphatase assays results in the presence of LZAP demonstrate a negative effect of LZAP on PPM1D activity. These findings contradict previous studies on the effect of LZAP on PPM1D activity. This thesis also discusses the possible interaction of PPM1D with 14-3-3 proteins. These proteins modulate the activity and structure of their binding partners through binding to phosphorylated motifs. PPM1D contains several sequence motifs that are recognized by 14-3-3. Two of these sites (Ser46, Ser97) are also physiologically phosphorylated and are located in the unstructured proline-rich loop. For the purpose of studying this interaction, *in vitro* phosphorylation protocol for PPM1D was optimized using protein kinase A.

[IN CZECH]

Key words: recombinant proteins, phosphatase, signalization, inhibition, regulation, p53