Abstract

Dissertation thesis The Study of selected TRP Channels and their Ligands deals with characterization of binding sites for Ca^{2+} binding proteins calmodulin and S100A1 and phosphatidylinositol-4,5-bisphosphate within the intracellular amino- and carboxy-termini of TRPV1, TRPV2, TRPV5 and TRPM3 subfamily members.

TRP channels comprise a family of more then 30 different ion channels that participate in many physiological processes like e.g. termosensation, mechanosensation, pH changes or cation homeostasis. They constitute of six transmembrane spanning domains, pore between domain five and six and intracellular N- and C-terminus. They are arranged into homo- or heterotetramers.

The function of TRP channels is regulated by many ligands (e.g. Ca^{2+} binding proteins, phosphatidylinositolphosphates, ATP), that bind to specialized binding domains present on intracellular termini.

Calmodulin (CaM) binding domains were suggested on C-termini of TRPV2 and TRPV5 by using bioinformatic tools. These domains are characterized by presence of conserved hydrophobic residues in particular positions. Steady-state anisotropy measurement was used to confirm that these domains bind CaM with high affinity, furthermore important basic residues having severe impact on the binding were determined using point mutagenesis experiment. The dependence of the binding on presence of calcium ions was confirmed as well.

Two independent CaM binding domains were identified on TRPM3 N-terminus. The rigidity and stability of the constructs was confirmed by mass spectroscopy and circular dichroism spectra measurement. Using steady-state fluorescence anisotropy measurement and surface plasmon resonance experiment high affinity of these constructs towards Ca^{2+} binding proteins CaM and S100A1 was determined and positively charged residues playing important role in the binding to these proteins were pointed out. The dependence of the binding on presence of kalcium ions was also determined.

Using steady-state fluorescence anisotropy measurement and surface plasmon resonance experiment three independent PIP_2 binding domains were identified on N- and C-termini of TRPV1 ion channel. Moreover one domain on the N-terminus and one domain on the C-terminus was revealed to overlap with previously identified CaM binding domains, which may probably play an important role in the regulation of the TRPV1 ion channel by these ligands.