

ABSTRACT

This dissertation concerns with characterization of binding sites for calcium binding protein S100A1 and phosphatidylinositol phosphates on intracellular regions of transient receptor potential channels (TRPs), particular from canonical (TRPC), vanilloid (TRPV) and melastatin (TRPM) families. TRPs represent superfamily of important mediators that play critical roles in sensory physiology: contributions to taste, olfaction, vision, hearing, touch and thermo- and osmo- sensation. They serve as non-selective and nociceptive membrane receptors responsible for the modulation of driving force for cations entry into the cell. TRPs are composed from six transmembrane domains and N- and C- termini intracellular regions. Overall four monomer units form a characteristic assembly of functional channel. It was demonstrated that most of this almost thirty-member family transporters are activated by a variety of different stimuli and function as signal integrators. The most examined intracellular TRPs modulators are cytosolic calcium binding proteins and membrane anchored phosphatidylinositol phosphates (PIPs). These signal integrators bind specific domains in intracellular termini of TRPs, thereby change their structure and activate or inhibit the transportation function of receptor. To identify a novel ligand binding sites in intracellular tails of TRPs we primarily utilized biophysical tools. As first, we have identified positive charged amino acid residues involved in the interaction with S100A1 using steady-state anisotropy fluorescence measurements. This binding site was confirmed as multiple calcium binding proteins interaction site, because calmodulin binds the same region as was described previously. The second binding site for S100A1 in the C-terminus of TRPV1 we have characterized by steady-state anisotropy fluorescence and surface plasmon resonance measurements. The selected TRPV1 binding domain also represents multiple binding site whereas the calmodulin and phosphatidylinositol-4, 5 bisphosphate binding sites were characterized within the same region in the past. Strict calcium dependence of the TRPV1/S100A1 interaction was also confirmed. In TRPs melastatin family, where we focused on TRPM1 and TRPM4, it was identified PIPs binding sites in the proximal and distal parts of the N-termini using surface plasmon resonance measurements. The important arginine and lysine residues participating in the TRPM/PIPs complexes formations were determined. Altogether, our study demonstrated the identification and characterization of novel S100A1 and PIPs binding sites in the TRPC6, TRPV1, TRPM1 and TRPM4 receptors. The findings in the dissertation raising awareness about the potential modulating of these receptors, which can lead to the future modifications of cation transport and thereby possible treatment of human diseases linked with disorders in TRPs.