

Transient receptor potential channel vanilloid receptor subunit 1 (TRPV1) is a thermosensitive cation channel activated by noxious heat as well as a wide range of chemical stimuli. Although ATP by itself does not directly activate TRPV1, it was shown that intracellular ATP increases its activity by directly interacting with the Walker A motif residing on the C-terminus of TRPV1. In order to identify the amino acid residues that are essential for the binding of ATP to the TRPV1 channel, we performed the following point mutations of the Walker A motif: P732A, D733A, G734A, K735A, D736A, and D737A. Employing bulk fluorescence measurements, namely a TNP-ATP competition assay and FITC labeling and quenching experiments, we identified the key role of the K735 residue in the binding of the nucleotide. Experimental data was interpreted according to our molecular modelling simulations.

Calmodulin (CaM) is known to play an important role in the regulation of TRP channels activity. Although it has been reported that CaM binds to the Cterminus of TRPV1 (TRPV1-CT), no classic CaM-binding motif was found in this region. In this work, we explored this unusual TRPV1 CaM-binding motif in detail and found that five residues from a putative CaM-binding motif are important for TRPV1-CT's binding to CaM, with arginine R785 being the most essential residue. The homology modelling suggests that a CaM-binding motif of TRPV1-CT forms an alpha helix that docks into the central cavity of CaM.