ABSTRACT

Identification of the binding sites on transient receptor potential cation channel TRPC6 for Calmodulin and S100A1

The TRP (transient receptor potential) group of ion channels represents a large subset of membrane receptors. A part of this supergroup are canonical TRPC channels with a sequence homology analogical to TRP receptor first discovered at fruit fly (*Drosophila melanogaster*). These membrane channels are involved in a variety of physiological functions in different cell types and tissues. TRPC6 is a non-selective cation channel that modulates the calcium level in eukaryotic cells (including sensory receptor cells) in response to external signals. TRPC6 channel contains binding domain CIBR (Calmodulin inositol binding region), which is also able to adapt to calcium binding protein S100A1. Characterisation of the integrative binding site for calmodulin (CaM) and S100A1 at the C-tail of TRPC6 is presented in this work. Using site-directed mutagenesis, soluble protein fragments TRPC6 CT (801-787) were prepared with intentional changes in amino acid sequence. Several positively charged amino acid residues (Arg852, Lys856, Lys859, Arg860 and Arg864) were determined by measurement of fluorescence anisotropy influence and their participation in the calcium-dependent binding of CaM and/or S100A1 to the TRPC6 termini. Both interactions are influenced by presence of Ca^{2+} ionts. Homology model of complex TRPC6-CaM revealed a 1-5-10 recognition motif suitable for CaM. However, results indicate a unique involvement of overlapping binding site for S100A1 on the C tail of TRPC6. The triple mutation Arg852/Lys859/Arg860 exhibited significant disruption of the S100A1-TRPC6 complex.

Keywords: anisotropy fluorescence, Ca^{2+} binding proteins, calmodulin, ion channel, S100A1, TRPC receptor, TRPC6

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