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ORIGINAL ARTICLE

Characterization of calmodulin binding domains in TRPV2 and TRPV5 C-tails

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Abstract The transient receptor potential channels TRPV2 and TRPV5 belong to the vanilloid TRP subfamily. TRPV2 is highly similar to TRPV1 and shares many common properties with it. TRPV5 (and also its homolog TRPV6) is a rather distinct member of the TRPV subfamily. It is distant for being strictly Ca^{2+}-selective and features quite different properties from the rest of the TRPV subfamily. It is known that TRP channels are regulated by calmodulin in a calcium-dependent manner. In our study we identified a calmodulin binding site on the C-termini of TRPV2 (654–683) and TRPV5 (587–616) corresponding to the consensus CaM binding motif 1-5-10. The R679 and K681 single mutants of TRPV2 caused a 50% decrease in binding affinity and a double mutation of K661/K664 of the same peptide lowered the binding affinity by up to 75%. A double mutation of R606/K607 and triple mutation of R594/R606/R610 in TRPV5 C-terminal peptide resulted in the total loss of binding affinity to calmodulin. These results demonstrate that the TRPV2 C-tail and TRPV5 C-tail contain calmodulin binding sites and that the basic residues are strongly involved in TRP channel binding to calmodulin.

Keywords TRP channel · Steady-state fluorescence anisotropy · Binding site · Calmodulin · Calcium

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Introduction

Transient receptor potential (TRP) channels are a wide family of non-selective ion channels responsible for monovalent and divalent cation influx into the cells. Members of this family are involved in many sensory processes such as invertebrate vision and hearing, mammalian temperature-, mechano- and chemo-sensation (Clapham 2003; Clapham et al. 2001; Ramsey et al. 2006). The TRP channels discovered so far can be divided into seven subfamilies according to their primary structure: TRPV, TRPC, TRPA, TRPM, TRPP, TRPML and TRPN (Moiseenkova-Bell and Wensel 2009). All are predicted to have six transmembrane helices (S1–S6) and a pore-forming loop between S5 and S6, with varying sizes of intracellular amino and carboxy termini, and are thought to form tetrameric assemblies (Clapham 2003; Venkatachalam and Montell 2007). Both the N- and C-terminal intracellular domains are comprised of many different domains that are responsible for binding different compounds that can regulate the channels (Clapham 2003).

Although all TRPs are non-selective cation channels, their role in Ca^{2+} influx represents one of their most common physiological roles. Negative feedback by permeating Ca^{2+} ions is a critical mechanism of TRP channel regulation for maintaining Ca^{2+} homeostasis in the cells. TRPV, TRPC and TRPM have all been confirmed to show Ca^{2+}-dependent inhibition, inactivation or desensitization (Gordon-Shaag et al. 2008). For TRP channels, Ca^{2+} has been proposed to produce desensitization via kinases, phosphatases, phospholipases and calmodulin (CaM) (Gordon-Shaag et al. 2008).

CaM is a small cytoplasmic protein that is highly conserved throughout eukaryotic evolution. It is capable of regulating the biological activities of many cellular

proteins and transmembrane ion transporters, mainly in a Ca^{2+}-dependent manner. CaM has four EF-hand motifs that change conformation upon binding calcium ions. Each EF-hand motif contains two alpha helices connected by a 12-residue loop (Saimi and Kung 2002). The calcium ion binds to the loop region and changes the relative positions of the alpha helices. This Ca^{2+}–calmodulin complex binds the target proteins, which typically contain basic amphiphilic alpha-helix domain (BAA), and consequently initiates various signaling cascades (Yap et al. 2000). The BAA domain can be classified into motifs based upon variations in positions of conserved hydrophobic residues 1–10, 1–14 or 1–16 in the sequence (Rhoads and Friedberg 1997).

So far, a large number of Ca^{2+}–calmodulin binding sites (CBS) have been identified on either the N- (NT) or C-terminus (CT) of different TRPs. Each sequence conforms to at least one of the known consensus CaM-recognition motifs 1-8-14 or 1-5-10 (Rhoads and Friedberg 1997; Zhu 2005). In several cases, the 1-8-14 or 1-5-10 motifs contain Gln, Gly, Pro, Ser and Thr at the hydrophobic positions, indicating that these sites may be atypical (Friedlova et al. 2009; Grycova et al. 2008; Zhu 2005).

In our study we focused our attention on two members of the TRPV subfamily, TRPV2 and TRPV5, and their interactions with CaM. TRPV2 is 50% identical to TRPV1 but in contrast is insensitive to capsaicin. It has been proposed to mediate high threshold (>52°C) noxious heat sensation, but its presence in non-sensory tissue indicates additional functions (Clapham et al. 2001). On the other hand, TRPV5 and its 75% similar, sequence homology TRPV6 share only 23% sequence similarity with the rest of the TRPV subfamily and their functional properties also differ significantly. They are strictly Ca^{2+} selective and are believed to function as transcellular Ca^{2+} transporters in the kidney and small intestine (Nijenhuis et al. 2005). Evidence shows that there have been several CBS identified so far within either the N- or C-tail of TRPV1 (Numazaki et al. 2003; Rosenbaum et al. 2004), TRPV4 (Strotmann et al. 2003), TRPV5 and TRPV6 (Lambers et al. 2004). In TRPV5, binding sites for CaM were identified in the N-terminus in the 1–327 region and in the C-terminus in the 578–730 region. These binding sites were confirmed by whole-cell patch clamp analysis and CaM binding studies using CaM-coupled agarose beads (Lambers et al. 2004). However, there has been no detailed characterization of these binding sites. TRPV2 is suspected to contain CBS as well, but no evidence has been shown to date. We therefore decided to search for CaM binding motifs in the C-termini of TRPV2 and TRPV5 and focus on their detailed characterization.

Materials and methods

TRPV2 and TRPV5 peptide synthesis and labeling

C-terminal sequences of human TRPV2 and TRPV5 were searched for potential CaM binding motifs using the CaM target database (Yap et al. 2000). Thirty-amino-acid-long peptides of the human TRPV2 C-terminus (654–683) and human TRPV5 C-terminus (587–616) were designed. R679A, K681A and K661A/K664A mutants of TRPV2 and R610A, R606A/K607A and R594A/R606A/R610A of TRPV5 were created. All peptides were synthesized and labeled with fluoresceinisothiocyanate (FITC) on their N-terminus by GenScript USA Incorporated, New Jersey, USA. The lyophilized peptides were diluted in 20 mM Tris–HCl buffer (pH 7.5) to a concentration of 100 nM and further used for steady-state fluorescence anisotropy measurement.

CaM expression and purification

CaM was expressed from the pET3a vector in BL21 *Escherichia coli* cells. Protein expression was induced by isopropyl-1-thio-β-D-galactopyranoside (Roth) for 12 h at 25°C. The cells were pelleted by centrifugation and resuspended in 50 mM Tris–HCl buffer (pH 7.5) containing 2 mM EDTA and 0.2 mM PMSF. The cells were disrupted by sonication and centrifuged. $CaCl_2$ was added (final concentration 5 mM) to the supernatant. The protein was purified using affinity chromatography on Phenyl Sepharose CL4B (Amersham Biosciences) where 50 mM Tris–HCl buffer (pH 7.5) containing 1.5 mM EDTA and 100 mM NaCl was used for elution. Gel permeation chromatography on Superdex 75 column (Amersham Pharmacia Biotech) was used as a final purification step. The protein was eluted by 10 mM HEPES buffer (pH 7.0) containing 25 mM NaCl and 1 mM $CaCl_2$. Protein samples were concentrated using spin columns for protein concentration (Millipore). Protein concentration was assessed by the measurement of absorption at 280 nm. The purity was verified using 15% SDS-polyacrylamide gel electrophoresis (PAGE).

TRPV2 and TRPV5–CaM binding assays

Steady-state fluorescence anisotropy measurements were performed on a ISS PC1Photon^{TM} Counting Spectrofluorimeter at room temperature (RT) in a buffer containing 20 mM Tris–HCl (pH 7.5), 10 μM $CaCl_2$ with 100 nM peptide probes labeled with FITC on their N-terminus. Such samples were titrated with increasing amounts of 100 μM CaM in Tris–HCl buffer (pH 7.5). At each CaM

concentration, the steady-state fluorescence anisotropy of FITC was recorded (excitation at 480 nm, emission at 520 nm). The fraction of bound CaM (F_B) was calculated from Eq. 1 (Lakowicz 2006), where Q represents the quantum yield ratio of the bound to the free form and it was estimated by the ratio of the intensities of the bound to the free fluorophore. Parameter r_{max} is the anisotropy at saturation, r_{obs} is the observed anisotropy for any CaM concentration, and r_{min} is the minimum observed anisotropy for the free TRPV2 or TRPV5 peptide. F_B was plotted against CaM concentration and fitted using Eq. 2 (Lakowicz 2006) to determine the equilibrium dissociation constant (K_D) for CaM/TRPV2 and CaM/TRPV5 peptide complex formation. [P_1] stands for the TRPV2 peptide–FITC or TRPV5 peptide–FITC concentration, [P_2] is the CaM concentration. Non-linear data fitting was performed using the SigmaPlot 10.0 program. All experiments were carried out in at least triplicate.

$$F_B = (r_{obs} - r_{min})/[(r_{max} - r_{obs})Q + (r_{obs} - r_{min})] \quad (1)$$

$$F_B = \frac{K_D + [P_1] + [P_2] - \sqrt{(K_D + [P_1] + [P_2])^2 - 4[P_1][P_2]}}{2[P_1]} \quad (2)$$

Effect of calcium on TRPV2-CT and TRPV5-CT binding to CaM

To assess the role of Ca^{2+} on the binding of TRPV2-CT and TRPV5-CT to CaM, we buffered Ca^{2+} present in buffer after CaM purification with 2 mM EDTA. Then we performed the binding assays for wild types of TRPV2-CT and TRPV5-CT as described above.

Circular dichroism spectroscopy

To confirm that the peptides adopt α-helical topology in presence of CaM, circular dichroism (CD) spectroscopy experiments were performed. The experiments were carried out on Jasco J-810 spectropolarimeter (Japan). The ECD spectra were measured from 200 to 350 nm at a scanning speed of 100 nm/min in a quartz optical cell with a path length of 0.1 cm (Starna, USA), a response time was 1 s and sensitivity 1,000 mdegree. All spectra were recorded in 0.5 nm wavelength increments with a 1.0 nm bandwidth. The final spectra were an average of 3 scans recorded at room temperature. The protein concentration was 0.2 mg ml^{-1} in 20 mM Tris–HCl (pH 7.5), 250 mM NaCl and 1 mM $CaCl_2$. The data were converted to mean residue ellipticity (MRE) according to the Eq. 3, where θ_{obs} is the observed ellipticity in degrees, c is protein concentration in milligrams per milliliter, l is the path length in centimeters, M_w is the protein molecular weight and N_R is the number of amino acids in the protein. The data were analyzed using CDNN software (Bohm et al. 1992).

$$\text{MRE} = \frac{\theta_{obs} \times 100 \times M_w}{clN_R} \quad (3)$$

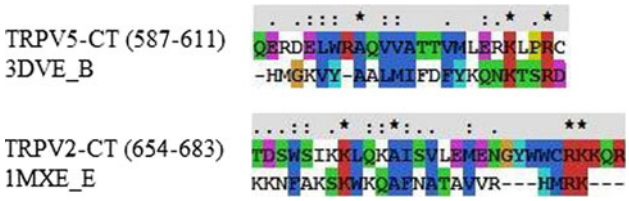

Fig. 1 The sequence alignment of the TRPV5 C-terminus CBS (587–611) with the voltage-dependent N-type calcium channel subunit alpha-1B (pdb code 3DVE_B) and TRPV2 C-terminus CBS (654–683) with the CBS of target sequence of rat calmodulin-dependent protein kinase I (pdb code 1MXE_E); identical amino acids are marked with an *asterisk*, similar amino acids with the more important groups are indicated with a *colon*, *dots* indicate similar amino acids of the less important groups that are less likely to influence the protein structure

Computer homology modeling

The sequences of human TRPV2-CT (654–683) and human TRPV5-CT (587–611) were modeled via homology modeling using the program Modeller 9v5 (Eswar et al. 2006). Although there is no known structure of any CBS among the TRP channels, we used structures of other CaM binding peptides complexed with Ca^{2+}–CaM. The peptides contained CaM binding motif with crucial hydrophobic amino acid residues at positions 1-5-10 and had high degree of sequence similarity. For the TRPV5 C-tail homology model, a structure of the voltage-dependent N-type calcium channel subunit alpha-1B (pdb code 3DVE_B) complexed with Ca^{2+}–CaM (Kim et al. 2008), was used as a template having 58% sequence similarity. For the TRPV2 C-tail homology model, structure of target sequence of rat calmodulin-dependent protein kinase I (pdb code 1MXE_E) complexed with Ca^{2+}–CaM (Clapperton et al. 2002), was used as template possessing 57% sequence similarity. The sequences were aligned using CLUSTALX 2.0.10 (Larkin et al. 2007) (Fig. 1). The models were optimized by energy minimization using Swiss-PdbViewer (Guex and Peitsch 1997) with the GROMOS96 parameters set (Guex and Peitsch 1997) and checked with ProSA-web (Sippl 1993; Wiederstein and Sippl 2007) for recognizing errors in the 3D protein structure. The models were completed and validated in Wincoot 0.5.2 (Emsley and Cowtan 2004).

Results and discussion

The TRP family of cation channels has been shown to be regulated by calcium via CaM binding. In most cases, the

regulation occurs via direct binding of CaM to the cytoplasmic part of the protein. Multiple CBS have been identified so far and they differ significantly in their affinities to CaM and Ca^{2+} and their conformations as well as the regions of CaM that are bound to these sites. CBS on C-termini of TRPC1-7 family members have been studied in detail by Tang et al. and the dissociation constants differ from 0.01 μM in case of TRPC2-CT (905–934)/Ca^{2+}–CaM complex to 0.6 μM in case of TRPC4-CT (787–812)/Ca^{2+}–CaM complex (Tang et al. 2001). Among members of the TRPV subfamily, Ca^{2+}–CaM was proved by Grycova et al. and Rosenbaum et al. (Grycova et al. 2008; Rosenbaum et al. 2004) to be involved in the desensitization of TRPV1 via the calcium-dependent binding of CaM to the C-terminus with the dissociation constant of the TRPV1-CT/Ca^{2+}–CaM complex 1.5 ± 0.4 μM (Grycova et al. 2008). Numazaki et al. (Numazaki et al. 2003) also showed that CaM binds to the N-terminus in a calcium-dependent manner. Further, Strotmann et al. (2003) and Zhu (2005) revealed several CBS in the N- and C-termini of TRPV4, but whether some of these sites are involved in Ca^{2+}-dependent channel inactivation remains to be investigated. Hoenderop et al. (2001) characterized TRPV5 and TRPV6 electrophysiologically, revealing that these channels display a Ca^{2+}-dependent feedback regulation of channel activity. Furthermore Lambers et al. (2004) confirmed a CaM binding domain in the N-terminus of TRPV5 between residues 1–327 and in the C-terminus between residues 578–730 by whole-cell patch clamp analysis and CaM binding studies. The aim of this study was to identify and characterize CaM binding domains at the C-termini of TRPV2 and TRPV5 ion channels using fluorescence anisotropy as a powerful tool for determining binding interactions between potential partners in vitro. We wanted to prove whether CaM binding motifs engaged in this binding differ or are the same as in previously described structures. Furthermore, we intended to determine the influence of some basic amino acids on this binding. We predicted from our computer models that the positively charged residues on TRPV5-CT and TRPV2-CT, respectively, could interact with negatively charged residues on CaM which is common for interactions of different cytoplasmic proteins, e.g., myristoylated alanine-rich C kinase substrate (MARCKS) (Yamauchi et al. 2003) or adducin (Matsuoka et al. 1996) and ion channels including TRPs and what we managed to prove in the past when studying the TRPV1 channel (Grycova et al. 2008).

We searched the CaM target database (Yap et al. 2000) and found a putative binding site location on human TRPV5-CT (Fig. 2) between residues 587–616 (QERDEL WRAQVVATTVMLERKLPRCLWPRS), corresponding to the consensus CaM binding motif 1-5-10 and also containing the so called TRP box; a conserved domain among TRPC, TRPM and TRPV family members (Birnbaumer et al. 2003). In addition the sequence also contains other CaM binding motifs such as 1-16 (Rhoads and Friedberg 1997) or 1-11 (Majava and Kursula 2009). TRP channel family members are known to contain multiple CaM binding motifs (Zhu 2005). We performed the sequence analysis which pointed out conserved basic amino acid residues among the TRPV channel family members possessing 1-5-10 CaM binding motif. The importance of these amino acid residues was then confirmed by the fluorescence anisotropy binding assay.

We designed peptides that corresponded to this putative binding site. We first performed CaM binding assays in solutions lacking calcium. We observed no increase in anisotropy, suggesting that the binding of TRPV5-CT to CaM could be calcium-dependent (Fig. 3b) and calcium was added to the solution. We then performed CaM binding assays with calcium present in the reaction mixture (Fig. 3a). The equilibrium dissociation constant of the

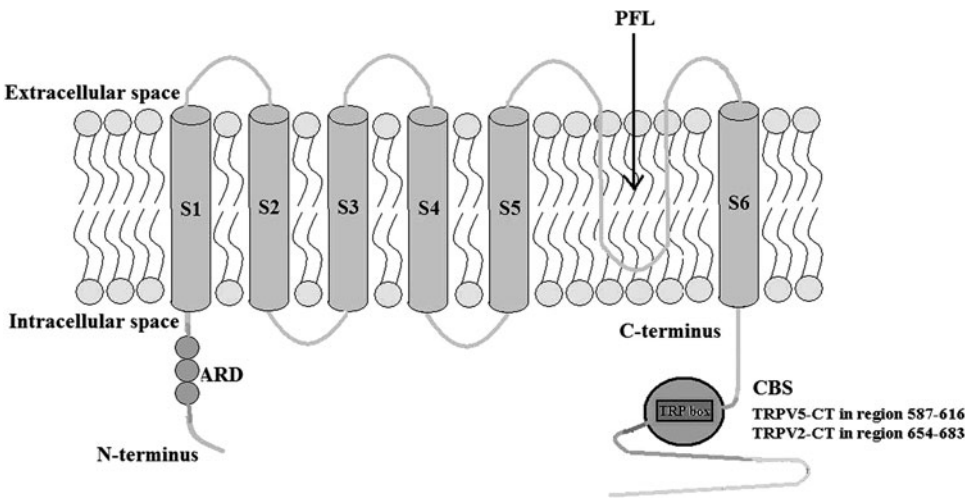

Fig. 2 Scheme of the predicted structure of TRPV ion channels; barrels *S1–S6* are six transmembrane domains, *black arrow* shows the pore forming loop (*PFL*), *circles* on the N-terminus represent ankyrin repeat domains (*ARD*) and *circle* on the C-terminus shows the position of the putative CBS including the TRP box on TRPV5-CT (587–616) and TRPV2-CT (654–683), respectively

TRPV5-CT construct	K_D [μM]
WT	0,306 ± 0,019
R610A	0,389 ± 0,078
R606A/K607A	>>
R594A/R606A/R610A	>>

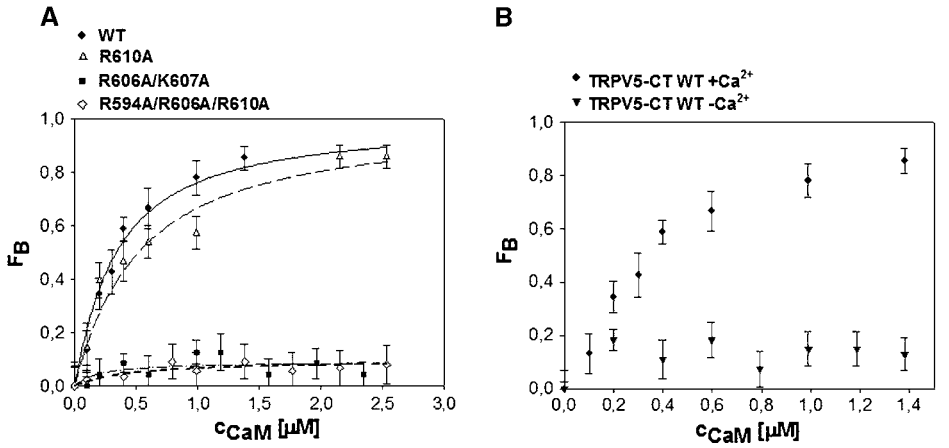

Fig. 3 Effect of Arg594, Arg606, Lys607 and Arg610 mutations on TRPV5-CT (587–616) on CaM binding and summary of dissociation constants (K_D) and their standard deviations of wild type and the mutants. **a** Fluorescence anisotropy binding isotherms of wild type and mutants of TRPV5 labeled with FITC on their N-terminus; *circles* wild type, *open triangles* mutant R610A, *squares* mutant R606A/K607A and *open diamonds* mutant R594A/R606A/R610A. **b** Fluorescence anisotropy binding isotherms of wild-type labeled with FITC in presence of calcium (*circles*) and without calcium (*triangles*)

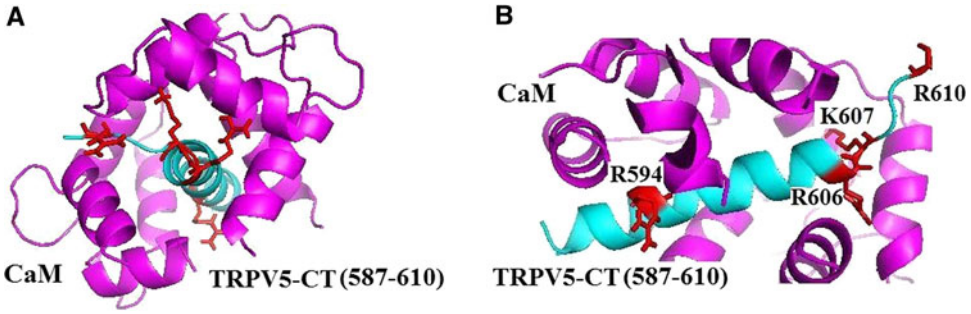

Fig. 4 Computer homology model of TRPV5-CT CBS (587–611) with Ca^{2+}–CaM. **a** The complete model of TRPV5-CT (587–611) in *blue* with Ca^{2+}–CaM in *magenta* and visualized mutated positively charged residues R594, R606, K607 and R610 on TRPV5-CT in *red*. **b** Detail of residues R594, R606, K607 and R610 that are thought to interact with CaM chains

complex TRPV5-CT/Ca^{2+}–CaM was estimated to be 0.306 ± 0.019 μM. In this motif there are six basic residues: R580, R594, R606, K607, R610 and R615. The Single R610A mutant showed no change in equilibrium dissociation constant compared to the wild type (WT). In contrast, the R606A/K607A double mutant and the R594A/R606A/R610A triple mutant seemed to completely lose their binding affinity to CaM (Fig. 3a). Based on these results, we concluded that the combination of basic residues R594, R606, K607 and R610 may participate in binding to CaM. According to the secondary structure prediction, the CaM binding region in TRPV5-CT forms an α-helix. In order to present a more detailed insight into the interactions of TRPV5-CT with CaM, a homology model of the binding site with Ca^{2+}–CaM was created (Fig. 4). As a template, the homologous voltage-dependent N-type calcium channel subunit alpha-1B (pdb code 3DVE_B) (Kim et al. 2008) containing the consensus CaM binding motif 1-5-10 was used (Rhoads and Friedberg 1997). The model suggests that the CaM-binding region of TRPV5-CT adopts an α-helical topology and it passes through the middle of CaM as observed for the majority of such sequences (Rhoads and Friedberg 1997).

All the replaced basic residues of TRPV5-CT are located in the helix sited inside the pore of CaM (Fig. 4) and according to our model these residues seem to interact with the negatively charged residues E54, E84, E87, E123 and E127 on CaM. This may be the cause of their severe affect on the binding of TRPV5-CT to CaM.

To our knowledge, there have been no CBS described for TRPV2 cytoplasmic regions to date. Hence we made a prediction of such regions and found one putative binding site located in the C-terminus of human TRPV2 (Fig. 2) between residues 654–683 (TDSWSIKKLQKAISVLEMENGYWWCRKKQR) corresponding to the potential CaM binding motif 1-5-10 and including TRP box (Birnbaumer et al. 2003). Interestingly, the hydrophobic residue in position 5 in this motif is exchanged for lysine. This kind of motif was also described for other CaM binding proteins such as inositol-1,4,5-triphosphate 3-kinase (Communi

TRPV2-CT construct	K_D [μM]
WT	0,191 ± 0,078
R679A	0,295 ± 0,042
K681A	0,289 ± 0,091
K661A/K664A	0,848 ± 0,129

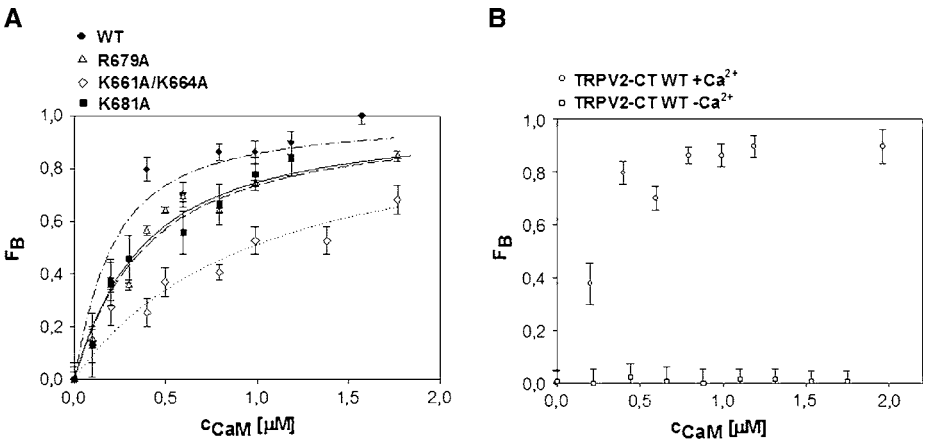

Fig. 5 Effect of Arg679, Lys681 and Lys661/Lys664 mutations on TRPV2-CT (654–683) on CaM binding and summary of dissociation constants (K_D) and their standard deviations of wild type and the mutants. **a** Fluorescence anisotropy binding isotherms of wild type and mutants of TRPV2 labeled with FITC on their N-terminus; *circles* wild type, *open triangles* mutant R679A, *squares* mutant K681A, *open diamonds* mutant K661A/K664A. **b** Fluorescence anisotropy binding isotherms of wild-type labeled with FITC in presence of calcium (*open circles*) and without calcium (*open squares*)

et al. 1995) or kinesin-like CaM binding protein (Reddy et al. 1996), where hydrophobic residues are at positions 1–10 and lysine is present in position 5. We designed a peptide matching this region and performed CaM binding assays (Fig. 5a) in the same manner as for TRPV5-CT (described above). The TRPV2-CT exhibited similar calcium dependence to TRPV5-CT (Fig. 5b). The equilibrium dissociation constant of the TRPV2-CT/Ca^{2+}–CaM complex was estimated to be 0.191 ± 0.078 μM. We mutated basic residues K661, K664, R679 and K681 in the sequence and performed binding assays with the mutants (Fig. 5a). According to the data obtained, basic residues seem to play an important role in the binding of TRPV2-CT to CaM. We observed a 30% decrease in binding affinity in mutants R679A and K681A and a 75% decrease in the K661A/K664A double mutant (Fig. 5). These results indicate that these basic amino acids participate in CaM binding. This points out that the regulation of the TRPV2 channel occurs via CaM binding to its C-terminus.

According to the obtained data, a homology model of the CBS on TRPV2-CT was created. Highly similar target sequence of rat calmodulin-dependent protein kinase I (pdb code 1MXE_E) (Clapperton et al. 2002) was used as template. The model (Fig. 6) shows that the putative CBS takes up the conformation of an α-helix. The model also suggests that the part of the α-helix that contains basic residues K661 and K664 (among others) runs through the pore. In contrast, the part of the α-helix comprising residues R679 and K681 is located outside the pore. This could explain why residues K661 and K664 have a greater impact on CaM binding than R679 and K681. The model proposes that the basic residues on TRPV2-CT could interact with the negatively charged residues E11, E14, E83, E84 and E87 on CaM but further investigation is necessary to prove this prediction.

In order to check effects of potential changes in secondary structure of the TRPV2-CT and TRPV5-CT upon the binding to CaM, we compared the CD spectra of the

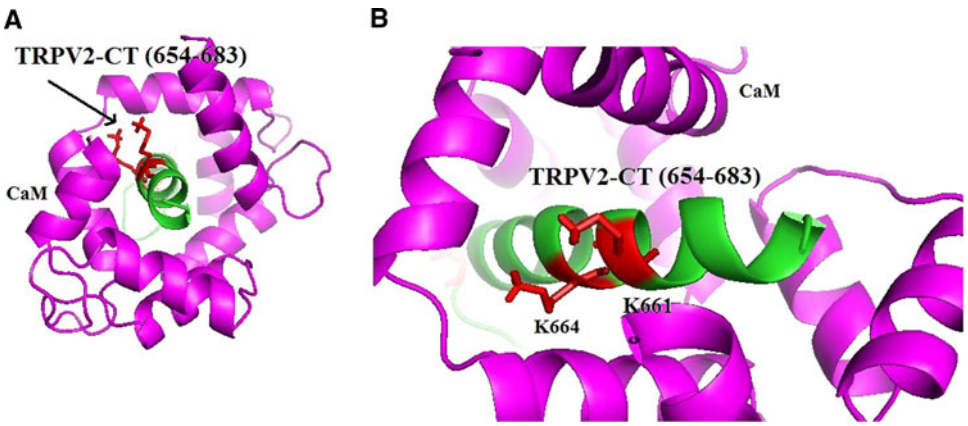

Fig. 6 **a** CBS of TRPV2 C-tail (654–683) in *green* with Ca^{2+}–CaM in *magenta*, mutated positively charged residues K661, K664, R679 and K681 in *red*; **b** detail of α-helix with residues K661 and K664 on TRPV2-CT that have greatest impact on binding of TRPV2-CT to CaM

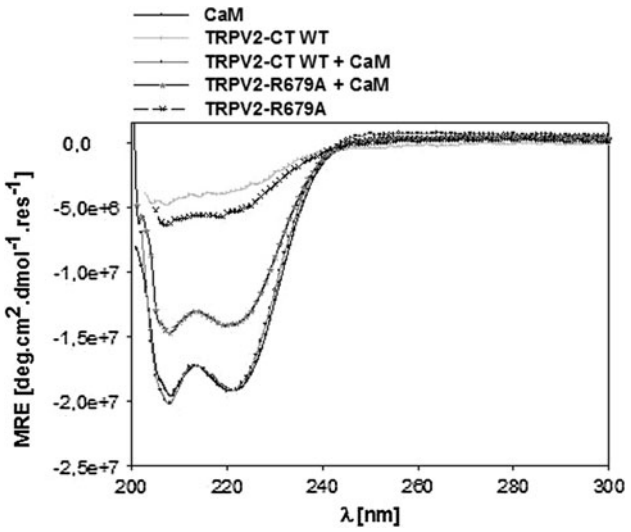

Fig. 7 Example of CD spectra of CaM (*black line and circles*), TRPV2-CT wild type (*light grey line and circles*), TRPV2-CT R679 mutant (*black dashed line and crosses*) and complexes of wild type (*dark grey line and circles*) and mutant with CaM (*black line and dark grey stars*)

mixture of the peptides and CaM (molar ratio 1:1) with the CD spectra of these proteins alone. According to the results the peptides adopt conformation of 10% helix, 28% antiparallel, 5% parallel, 20% beta-turn and 37% random coil. The secondary structure motifs in CaM are represented as follows: 59% helix, 4% antiparallel, 5% parallel, 15% beta-turn and 17% random coil. When the wild-type peptides are mixed with CaM in molar ratio 1:1, the ratio of secondary structure motifs remains unchanged as in case of CaM (Fig. 7). Contrary if the mutant peptides are mixed with CaM, the ratio of α-helix will decrease significantly suggesting that the exchange of basic amino acid residues for neutral affects the distribution of secondary structure elements in the peptide (Fig. 7).

In this study, it was demonstrated that the TRPV5 and TRPV2 C-termini contain CBS corresponding to the consensus CaM binding motif 1-5-10. However, with TRPV2 this motif is disrupted in position 5 by lysine. The basic residues R594, R606, K607 and R610 in TRPV5-CT have a severe impact on binding to CaM. Their mutation causes a total loss of the binding ability of TRPV5-CT to CaM. In TRPV2 we demonstrated for the first time the CBS in the intracellular domain. Moreover the mutation of K661 and K664 in this region causes a three-quarters decrease in binding ability to CaM and hence these residues seem to play important role in TRPV2-CT binding to CaM.

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