pH dependence of the Ca^{2+} release activated Ca^{2+} (CRAC) channel

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CRAC channels activated by the depletion of intracellular Ca^{2+} stores provide a major pathway for Ca^{2+} entry in many cell types. Characteristic properties of CRAC channels include high selectivity for Ca^{2+} over monovalent cations, feedback inhibition by permeating Ca^{2+}, known as fast Ca^{2+} dependent inactivation (FCDI), and block by low external pH (Malayev & Nelson, 1995). The functional CRAC channels are composed of a tetramer of the Orai1 proteins, which forms the channel pore, and a protein called stromal interaction molecule 1 (STIM1), a Ca^{2+} binding protein that plays the role of Ca^{2+} sensor in the endoplasmic reticulum (Soboloff *et al*., 2006). The glutamate 106 residue (E106) in a predicted transmembrane domain of Orai1 has been reported to act as the selectivity filter and to play a role in FCDI of CRAC channels (Yamashita *et al*., 2008). In this work we show that glutamate 106 is also a protonation site responsible for I_{CRAC} block at low pH.

STIM1 and Orai1 were previously subcloned into pCMV-Sport6 and the GFP co-expressing vector pAdTrack-CMV (Scrimgeour *et al.*, 2009). The Orai1 E106D mutation was generated using pCMV-Sport6-Orai1 as a template according to the protocol specified by the QuikChange II site-directed mutagenesis kit (Stratagene). Whole-cell patch clamping was performed at room temperature using a computer based patch-clamp amplifier (EPC-9, HEKA Elektronik) and PULSE software (HEKA Elektronik).

I_{CRAC} mediated by heterologously expressed Orai1 and STIM1 was inhibited by low pH reaching virtually complete block at pH 5.5. The apparent pKa of CRAC channel pH dependence was 7.8±0.1 (n=4). The E106D Orai1 mutant, which has higher selectivity for Na^+ over Ca^{2+} and is blocked by Ca^{2+} in time and voltage dependent manner (Yamashita *et al*., 2008), showed no such dependence on pH. In contrast, lowering pH from 7.4 to 6.3 or below increased the amplitude of the current and reduced the extent of inactivation at negative potentials suggesting that the Ca^{2+} block of Na^+ current was reduced. The apparent pKa of the block of Na^+ conductance through E106D mutant by Ca^{2+} was 6.1±0.1 (n=4). Investigation of Ca^{2+} currents mediated by this mutant in the absence of all permeable monovalent cations in the external solution revealed that FCDI of E106D is much faster than that of WT Orai1 and is progressively reduced at lower external pH.

Overall, these results suggest that ring of negative charges at position 106 in the Orai1 pore controls not only the selectivity of the channel, but also contributes to a complex mechanism of FCDI and accounts for the pH dependence of CRAC channel.

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