The pacemaker channels HCN2 and HCN4 have been identified in cardiac sino-atrial node cells. These channels differ considerably in several kinetic properties including the activation time constant (tau act), which is fast for HCN2 (144 ms at -140 mV) and slow for HCN4 (461 ms at -140 mV). Here, by analyzing HCN2/4 chimeras and mutants we identified single amino acid residues in transmembrane segments 1 and 2 and the connecting loop between S1 and S2 that are major determinants of this difference. Replacement of leucine 272 in S1 of HCN4 by the corresponding phenylalanine present in HCN2 decreased tau act of HCN4 to 149 ms. Conversely, activation of the fast channel HCN2 was decreased 3-fold upon the corresponding mutation of F221L in the S1 segment. Mutation of N291T and T293A in the linker between S1 and S2 of HCN4 shifted tau act to 275 ms. While residues 272, 291, and 293 of HCN4 affected the activation speed at basal conditions they had no obvious influence on the cAMP-dependent acceleration of activation kinetics. In contrast, mutation of I308M in S2 of HCN4 abolished the cAMP-dependent decrease in tau act. Surprisingly, this mutation also prevented the acceleration of channel activation observed after deletion of the C-terminal cAMP binding site. Taken together our results indicate that the speed of activation of the HCN4 channel is determined by structural elements present in the S1, S1-S2.
linker, and the S2 segment.