Cytoplasmic Domain Structures of Kir2.1 and Kir3.1 Show Sites for Modulating Gating and Rectification

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The family of inwardly-rectifying potassium (Kir) channels of eukaryotic cells are unique because they conduct K^+ ions better in the inward than outward direction. In native tissues, the small outward K^+ current through Kir channels influences the resting membrane potential and membrane excitability. The major structural mechanism underlying inward rectification involves a physical occlusion of the pore by polyamines and Mg^{2+} from the cytoplasmic side of the channel[1,2]. In addition to the property of inward rectification, Kir channels respond to a variety of intracellular messengers, including G proteins (Kir3 channels), ATP (Kir6 channels) and pH (Kir1 channels)[3]. The aberrant activity of Kir channels has been linked to a variety of endocrine, cardiac and neurological disorders. For instance, the loss of Kir3 channels leads to hyperexcitability and seizures in the brain[4], cardiac abnormalities[5] and hyperactivity and reduced anxiety. Mutations in Kir1 and Kir2.1 channels have been implicated for Bartter's syndrome[6] and Andersen's syndrome[7], respectively. The high resolution structures of Kir 3.1 and Kir 2.1, elucidated with data collected at SSRL 9-1 and ALS respectively, yielded insight into the gating, inward rectification, and causes of Andersen's Syndrome.

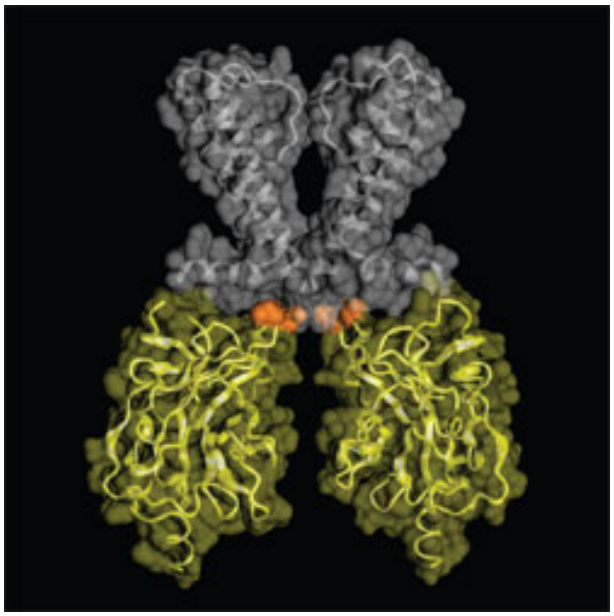

Figure 1. Kir2.1 cytoplasmic domain's backbone and surface are highlighted in yellow merged with KirBac1.1 transmembrane region's backbone and surface in gray. The gate forming tip of the G-loop, amino acids 304-306, is highlighted in red.

By comparing the Kir3.1 and Kir2.1 structures, a high degree of flexibility was observed at the narrowest region of the channel's tetrameric pore, the G-loop (Figure 1). The G-loop contains several small or hydrophobic residues and is anchored by glycine. In the Kir3.1 structure solved at SSRL, the distance at the narrowest point of the G-loop was 9.0 Å between the atomic centers of diagonally-positioned subunits, which differed from the 5.7 Å distance observed for equivalent positions of A306 in the Kir2.1 structure. Thus, the physical opening formed by four opposing hydrophobic G-loops is too narrow to accommodate a hydrated potassium ion to pass and leads us to conclude that the Kir3.1 and Kir2.1 structures are of a closed state. Mutations, based on the structure and studied by eletrophysiology, dramatically reduced the flexibility of the G-loop. Bulky sidechains in the G-loop chains inhibited channel current. These results reinforce the role of the G-loop to form the closed state.

The elucidated structures not only showed insight into the gating of the Kir family of channels but also lead to a better understanding of the inward rectification properties of this family of channels. By studying the electro-potential surfaces of the Kir3.1 and Kir2.1 structures, the Kir2.1 structure shows a remarkably high degree of electronegative surface potential as compared to that of Kir3.1. Interestingly, a recent structure of KirBac1.1's cytoplasmic pore exhibits less electronegative surface than Kir3.1. Previously, the strong rectification of Kir2.1 has been attributed to two principal electronegative regions; D172 in the M2 domain[8] and E224/E299 in the cytoplasmic domains[9,10]. Using the structure of Kir2.1 as a guide and electrophysiology experiments to confirm our findings, we identified that D255 and D259 are linked to Kir2.1's strong rectification properties unlike other members in the Kir family.

The Kir2.1 structure allowed the first structural understanding of Andersen's Syndrome. Out of the eighteen positions in the Kir2.1, ten were visualized with eight located on the top surface of the cytoplasmic structure (R189, T192, R218, G300, V302, E303, R312, Δ314-315), which may be near the punitive PIP_2 (phosphatidylinositol-4,5-bisphosphate)-binding site, and the other two buried in the protein interface (G215D, N216H). Some of these residues are interestingly close to the G-loop region and generally result in a loss of function via dominant negative interactions and heteromeric assembly[7]. For all but one mutation, G300V, the resulting mutant protein was aggregated, pointing to folding and tetramerization defects as the main reason for the disease. To validate the point, one of the mutations known to disrupt a charged pair interaction, R218Q, was rescued from the folding defect by a compensating mutation to R/K at T309 as predicted by the Kir2.1 structure.

The elucidated structures of the Kir2.1 and Kir3.1 cytoplasmic domains have provided us with a broader understanding of how this channel gates and rectifies itself. Furthermore, the electrophysiology experiment of the Andersen's Syndrome mutates coupled with the structural information has allowed for the first time to provide an explanation of how these mutations could interfere with the folding and gating of the Kir2.1 channel. Our better understanding may lead to therapeutic treatments for the disease.

Primary Citation

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