Modelling and Simulation Studies of the Intracellular Domains of the Inwardly Rectifying K^+ Channels

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1 Introduction.

Cells communicate with their extracellular environment through a diverse range of proteins that are present in the membrane. Membrane proteins account for the ~25% of the genes in most genomes. Ion channels are membrane proteins that have a ubiquitous presence in cells and reflecting their roles in cellular physiology. Potassium (K^+) channels form a large and diverse family responsible for a range of functions in various cell types and tissues, including control of cell electrical excitability in the nervous and cardiovascular systems. K^+ channels that are responsible to maintain and stabilize the resting membrane potential are the inwardly rectifying potassium channels (Kir). ATP-sensitive K^+ channels (K_{ATP}) belong to this group and are responsible in coupling the membrane electrical activity to energy metabolism. In the beta cells of the pancreas, they couple the rate of insulin release to the blood glucose levels and it has been well established that type II diabetes results from defective metabolic regulation of K_{ATP} channels. Despite the recent advances in the understanding of the K^+ channel structure and function, the molecular mechanisms underlying the ATP-dependent inhibition of Kir channels still remain elusive.

2 Results.

The crystal structure of the C-terminal intracellular domain of the inwardly rectifying K^+ channel Kir3.1 has recently been published at 1.8Å [1]. In the present study, molecular dynamics simulations were performed to investigate the conformational dynamics of both monomeric and homotetrameric forms of the protein. The structure of the Kir3.1 domain also provides a template for homology modeling of the equivalent domain of other Kir channels, and thus offers the prospect understanding their mechanisms of inhibition and gating. Models of the monomeric and tetrameric forms of the intracellular domains of the mammalian Kir6.2 channels were built based on the x-ray structure of the C-terminal domain of the Kir3.1 channel. Automated docking was employed to identify the residues and the binding site involved in the inhibition of Kir6.2 channels by ATP [2]. Extensive molecular dynamics simulations were performed to investigate the stability of these structures, with and without bound ATP in both the monomeric and tetrameric forms. Simulations were carried out for a total of 10 ns using GROMACS. The overall RMSD (root mean square deviation) of the Cα atoms for the simulations are described in Table 1. The loop regions form the most flexible part of the monomeric structure. They are stabilised in the tetramer by interactions with the adjacent subunits. The RMSD for the core region (i.e. excluding the loops) is low, suggesting the stability of the core structure. It has been experimentally observed that the mutation of Lys185 to Asp/Glu reduces ATP sensitivity considerably [2]. The distance between Lys185 and

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the ß-phosphate from ATP averaged over the entire trajectory for the structures was between 3.1-3.5A suggesting that Lys185 interacts with ß-phosphate of ATP. These simulations help in studying the nature of residues that contribute to the binding of ATP to the channel and thus could be involved in the structural and functional properties. Furthermore, such computational studies aid in understanding and interpreting the mutation data and relate the structure to the physiological function at an atomic level.

3 Figures and tables.

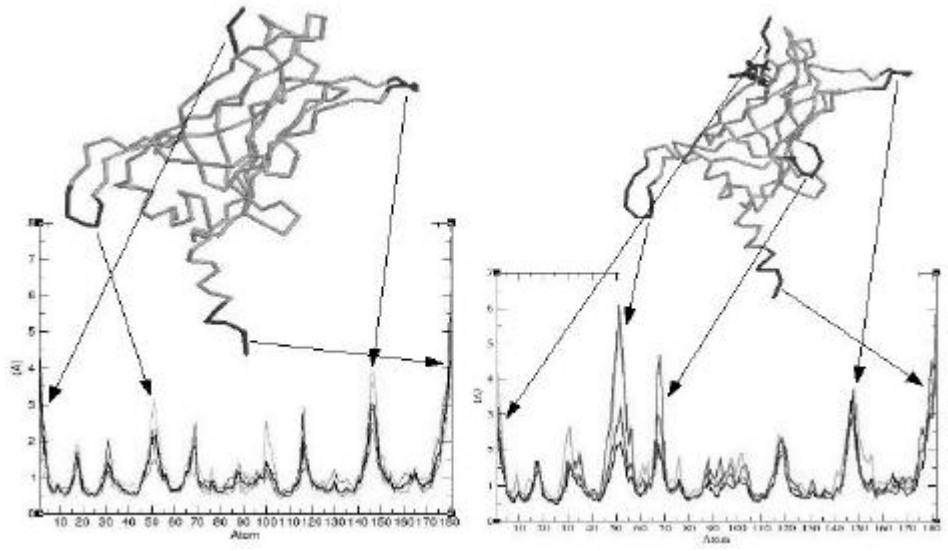

Figure 1: RMSF (root mean square fluctuation) of residues in each subunit of the intracelleular domain from (a) Kir3.1 and (b) Kir6.2. The RMSF peaks correspond to loops in the structure and the corresponding regions are indicated using arrows.

Protein	Ligand	Time (ns)	RMSD (Overall, Å)	RMSD (cutoff at 2.5 Å)
Kir3.1 monomer	-	5	2.8	2.2
Kir3.1 tetramer	-	5	2.6	1.9
Kir6.2 monomer	-	5	3.8	2.7
Kir6.2 monomer	ATP	5	3.2	2.0
Kir6.2 tetramer	-	5	3.2	2.9
Kir6.2 tetramer	ATP	5	3.7	2.4

Table 1: Summary of simulations.

4 References and bibliography.

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