Involvement of a Novel C-Terminal Kinase Domain of Kir6.2 in the K-ATP Channel Rundown Reactivation

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Abstract Rundown is a generally encountered problem while recording K_{ATP} channel activity with inside-out patches. No assigned structural fragment related to this mechanism could be derived from any of the proceeded functional analyses as yet. Therefore, based on a combined sequence and secondary structure alignment against known crystal structure of segments from closely related proteins, we propose here the three-dimensional structure model of an intracellular C-terminal domain of the Kir6.2 subunit in K_{ATP} channels. An *E. coli* CMP-kinase was suggested as template for the model building. The subdomain arrangement of this novel kinase domain and the structural correlation for UDP-docking were described. With structural-functional interpretation, we conclude that the reactivation of K_{ATP} channel rundown by MgATP or UDP is very possibly regulated by this intracellular kinase domain at the C-terminus of Kir6.2 subunit in K_{ATP} channels.

Keywords: Channel gating, 3-D homology modelling, Kinase domain.

Introduction

The ATP-sensitive K^+-channels (K_{ATP}-channels or K-ATP channels) are distributed in

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a wide variety of tissues, including brain nerve cells, cardiac and skeletal muscles, and pancreatic β-cells [2,3,12]. They play a pivotal role in coupling membrane excitation to cellular metabolism. The molecular architecture of K_{ATP} channels is an octameric complex of two structurally unrelated subunits that assemble with 4:4 stoichiometry [10,11,21]. The ion-pore subunits are members of the inwardly rectifying potassium channel family (Kir), which have two membrane-spanning domains (M1 and M2) flanking the K^+-selective ion-pore region (H5) and cytoplasmic amino (N-) and carboxyl (C-) termini [13]. The regulatory subunits are sulfonylurea receptors (SUR), members of the ATP-binding cassette superfamily which have two intracellular nucleotide binding folds, NBF1 and NBF2 [1].

The regulation of K_{ATP} channel activity by cytosolic constituents is extremely complex and is still not fully understood. In addition to the ATP-dependent channel closure at physiological conditions, some cytosolic agents are required to maintain the ability of the channel to enter the open state, since after formation of an inside-out patch the activity of the channel declines with time. This phenomenon has been described as rundown for K_{ATP} channels [8,17,18,23,24]. Providing that channel activity has not completely vanished, it can be partially restored by brief exposure of the patch to MgATP or UDP [6,15,17,18,23,24,25]. It has been proposed that this reactivation may involve protein phosphorylation on residues other than serine/threonine in Kir6.2 and that the hydrolysis energy of MgATP appears to be utilized for such reactivation [7,8,9,14,17]. However, the ability of MgATP to reactivate the channel activity is variable, declines with time and is ineffective after complete rundown: consequently it is also suggested that an endogenous kinase responsible for phosphorylation may exist and is gradually inactivated or lost from the patch membrane with time [18]. Moreover, trypsin digestion of Kir6.2 leads to the prevention of rundown, indicating an exposed binding fold responsible for the

deduced conformational change and the rundown mechanism [18,19]. On the other hand, UDP can also reactivate the K_{ATP} channel rundown, which are presented in different kinetic styles between Kir6.1 and Kir6.2 [24]. Whether a common structural motif or a similar pathway and/or mechanism involved in the reactivation by MgATP and UDP remains controversial [24]. However, at present stage, no direct structural evidence indicating the K_{ATP} channel rundown or its reactivation mechanism is available. Therefore, based on a combined sequence and secondary structure alignment against known crystal structure of segments from closely related proteins, we propose here the three-dimensional (3-D) structure model of the intracellular part of the Kir6.2 subunit of K_{ATP} channels. A kinase domain was suggested for the region of C-terminal residues. For further investigation on the molecular catalytic behaviour of this kinase domain, nucleotide-docking simulation was accomplished. The residues required for binding contacts with nucleotides in our structure model are also described in this paper.

Methods

Search for templates

BLAST algorithm was employed to search in PDB the protein segments whose sequences are similar to that of mouse Kir6.2 (Genbank accession number S68403) and whose structures can serve as viable structural templates. The crystal structures of *E coli* CMP-kinase (CKE, 6137462) (free and in complexed forms) [4,5] were chosen for the determination of structural conserved regions (SCRs). The Kir6.2 residues used for model building are according to their paired sequence (see below) compared to CKE sequence. In addition, immediately after the primary sequence comparison with BLAST, residues before 228 were eliminated according to the results.

Paired sequence alignment

GCG program was used to determine the equivalent residues. Residue regions of CKE represented as continuous lines dominantly observed were employed as appropriate template regions and the corresponding fragments in Kir6.2 were chosen for alignment. The amino acid sequences of these Kir6.2 fragments were then included in the multiple sequence alignment of the appropriate CKE regions to specify the residue numbers for model building [22].

Model building and docking simulation for nucleotides

Modelling by homology was performed essentially following the procedures described by Siezen [22]. Briefly, the residue fragments of Kir6.2 were chosen according to the results from GCG paired sequence alignment. They were then superimposed onto the crystal coordinates of Cα atoms of the corresponding SCRs from CKE structure. This generated the secondary structure and relative position of the definite secondary structural elements in the chosen residue fragments of Kir6.2. Junctions between the secondary structural elements were individually regularized by energy minimization to give reasonable geometry.

UDP and CDP molecules were created from small molecule units in databank via modification with cvff forcefield. Upon docking, total energy and van der Waal contacts between the complexes of CDP- and UDP-binding to Kir6.2 model were compared. All the calculations and structure manipulations were performed with the Discover/Insight II molecular simulation and modelling programmes (from Molecular Simulation Inc., San Diego, CA; 950 release) on Silicon Graphics Octane/SSE workstation.

Results and discussion

Paired sequence and structural alignment

From BLAST results, the crystal structures of *E coli* CMP-kinase (CKE, 6137462) (free and in complexed forms) [4,5] were chosen as template protein and for the determination of structural conserved regions (SCRs). Sequence comparison and the residue identities between Kir6.2 and CKE are shown in Figure 1. Two residue fragments of Kir6.2 were chosen according to the results from GCG paired sequence alignment, and then used for structural alignment. These are residues 229 - 281 and 338 - 385 (from N- to C-termini of Kir6.2), for which residues 3 - 54 and 62 - 113, respectively, of CKE are applied to create coordinates (Figures 1 & 2). This modelled part of the molecule is located at the intracellular C-terminus of Kir6.2.

Overall structural features and comparison with template

Structural information from our model suggests a novel kinase domain at the intracellular C-terminus of Kir6.2 subunit with the subdomain arrangement that is very similar to those observed in the crystal structure of *E. coli* CMP-kinase (CKE) and of other NMP kinases [4,5]. Figure 2 shows the comparison of the folding patterns between CKE and this novel kinase domain of Kir6.2 C-terminus. A binding cleft for nucleotides can be observed in the upper middle part of the structure in both molecules (Figure 2).

Nucleotide binding

For further investigation on the molecular catalytic behaviour of this kinase domain, or, to confirm the role this novel deduced kinase domain may play in Kir6.2, the nucleotide-docking simulation was accomplished. Energy minimization gave very stable conformation for both UDP and CDP molecules upon binding to this kinase domain (CDP/UDP: -625.77/-621.45 (Kcal/mol) for total energy; 253.42/253.28 for

van der Waal's contact; rmsd=0.110/0.103). Figure 3 illustrates the binding of UDP with our structure model. The UDP molecule is located and properly oriented in the binding cleft flanked by several α-helices that protrude their residue side chains towards UDP to form binding contacts. The details for the side chain contacts are shown in Figure 4.

Because structure models do not give sufficient details to allow us to measure the hydrogen bonding distances, we can merely depict here what we have observed from the structure in Figure 4 regarding the interaction between the corresponding atoms from residue side chains of Kir6.2 and from UDP molecule. These residues are: Arg-365, Arg-367, Lys-373 on one side of UDP and Glu-241 on the other side. They form putative hydrogen bonds between UDP and Kir6.2 in appropriate orientation and reasonable spatial range of distance. This may imply and emphasize the utilization of UDP by this kinase domain on Kir6.2.

It is interesting to note the following: (i) The two parts of Kir6.2 residues forming H-bonds with UDP are coming from the two separate residue fragments mentioned previously in the structural alignment section. (ii) They are all charged residues. Such facts or observations indicate that they are indeed appropriate to contribute to the construction of the binding moiety and form H-bonds with nucleotides upon docking. Such information may, therefore, allow us to make decisions on selecting reasonable candidates for site-directed mutagenesis and then further to verify the role of this kinase domain in the reactivation of K_{ATP} channel rundown for the forthcoming investigation in the future.

Structural-functional interpretation

The most important structural-functional interpretation provided by this 3-D structure model is with respect to the reactivation of K_{ATP} channel rundown. As it has been

described in the introduction section, providing the channel activity has not completely vanished, it can be partially restored by brief exposure of the patch to MgATP and UDP [9,17,18,24]. Such reactivation may involve protein phosphorylation on residues other than serine/threonine in Kir6.2 and the hydrolysis energy of MgATP appears to be utilized. In addition, the ability of MgATP to reactivate the channel activity is declines with time and is ineffective after complete rundown. All this suggested that an endogenous kinase responsible for phosphorylation may exist and is gradually inactivated or lost from the patch membrane with time [18].

One explanation for these results is that the K_{ATP} channel complex exists in an active, phosphorylated state and an inactivated, dephosphorylated state. Dephosphorylation by membrane-associated phosphatases causes the channel to enter a closed state from which it can only exit on phosphorylation by an endogenous kinase. Thus in the absence of MgATP, channel activity will slowly rundown as more channels enter the dephosphorylated closed state.

We would propose that such phosphorylation should occur via this novel kinase domain at C-terminus of Kir6.2. The following statements may clarify and support such hypothesis. One should not forget that the rundown of K_{ATP} channels can be also reactivated by exposure of the patch to UDP. This kinase domain provides an obvious link between the MgATP- and UDP-reactivation of channel rundown. The template protein, CMP-kinase, was proposed to be CMP-specific in prokaryotes and CMP/UMP-dispensable in eukaryotes [4]. If we look into the reversible reaction of this kinase, a spectrum of agents required for channel gating could be observed. This kinase uses MgATP to produce UDP and MgADP when UMP is present. Vice verse, when UDP is applied, MgADP can be used to generate MgATP. This explains at least in part why MgATP and UDP can both reactivate the channel rundown, and, as a

consequence, describes the utilization of the hydrolysis energy of MgATP for channel phosphorylation through an endogenous pathway. However, some aspects still remain to be clarified. For instance, where is the phosphorylation site to allow channels to enter the activatable state after MgATP hydrolysis? Is some other membrane-associated component involved in the contribution to phosphorylation simultaneously, instead of this kinase domain alone for reactivation? And how these components work mutually? What contributes to the rest part of the binding moiety for nucleotides since structure model has its own limitation to provide sufficient details? For the last question, N-terminal residues of Kir6.2 seems to be good candidate upon considering the tetrameric assembling structural characteristics of these K-channel family members [16,20].

Conclusion

Our model has been for the first time describing the three-dimensional structural information for subdomain arrangement of the residues at the intracellular C-terminus of Kir6.2 and the structural correlation for nucleotide-binding, as well as the functional implication such putative kinase domain can infer. Due to the limitation of predictive structure model, only the structural correlation derived from the reported functional results related to rundown reactivation was discussed in this paper. Nevertheless, such information provides directions for further functional assays and the appropriate candidates for site-directed mutagenesis before the crystal structure of Kir6.2 is determined.

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