Research Paper

Structural Diversity in the Cytoplasmic Region of G Protein-Gated Inward Rectifier K⁺ Channels

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ABSTRACT

Inward rectifier K⁺ (Kir) channels can be functionally categorized into two groups: those that are constitutively active and those that are constitutively inactive, with examples such as Kir2.x and Kir3.x, respectively. Their cytoplasmic regions are thought to be critical for control of channel gating, but a structural basis for this hypothesis is not known. In this study, we report a structure for the cytoplasmic region of G protein-gated Kir channel, Kir3.2, and compare it with those of Kir3.1 and Kir2.1 channels. The isolated cytoplasmic region of Kir3.2 forms a tetrameric assembly in solution and also in the crystal. While the secondary structure arrangement and the subunit interface of the Kir3.2 crystal structure are found to be nearly identical to those of Kir3.1 and Kir2.1, it is quite different at and around loops between βC- and βD-strands and between βH- and βI-strands. These structural elements are located at the interface with the plasma membrane. Therefore, these structural elements could associate with the Kir channel transmembrane helices and be involved in the regulation of Kir channel gating.

INTRODUCTION

The members of the inwardly rectifying K⁺ (Kir) channel family can be functionally classified into two groups: constitutively active and constitutively inactive Kir channels.¹ G protein-gated Kir (Kᵢᵣ) channels that consist of subunits Kir3.1-Kir3.4 belong to the latter group.² Under physiological conditions, the Kᵢᵣ channel is activated by G protein βγ subunits (Gᵦbg).³,⁵ in the presence of phosphatidylinositol-4,5-bisphosphate (PIP₂).⁶,⁷ These activators associate with N- and C-termini of the channel in the cytoplasm. A crystal structure of Kir3.1 suggests that both termini interact with each other and form a tetrameric assembly with a cytoplasmic pore in the fourfold axis.⁸ Since this particular structure of Kir3.1 was obtained in the absence of activators, it is considered to reflect the closed state of the channel. However, the cytoplasmic region of the constitutively active Kir2.1 channel shares a strong structural resemblance to that of Kir3.1.⁹ Therefore it is not clear which structural elements of the cytoplasmic region of Kᵢᵣ channels are responsible for channel gating.

A crystal structure is used to capture a single conformational state among a number of possible protein conformations. The multiple conformations of the same protein in different crystallization conditions or of homologues help to evaluate the pliability and diversity in the structure, and thus to understand the relationship between structure and function. In this study we examined a crystal structure of the cytoplasmic region of the Kᵢᵣ channel Kir3.2 and compared it with those of Kir3.1 and Kir2.1.⁸,¹¹ Whereas most of the structural elements in the Kir3.2 cytoplasmic region were comparable to those of Kir3.1 and Kir2.1,¹¹,¹⁵ significant differences were identified in the loops at the surface of the protein which is oriented toward the transmembrane domain.¹⁰ In Kᵢᵣ channels this area includes the residues crucial for Na⁺-dependent activation of Kir3.2 and Kir3.4,¹²,¹³ and PIP₂ sensitivity in Kir3.4.¹⁴ In the constitutively active Kir2.1 channels genetic heterogeneity of this area is responsible for polymorphic ventricular tachycardia¹⁵ and it is also thought to be a gating element.⁹ Thus, this local structural diversity may reflect the functional difference in the interaction between cytoplasmic and transmembrane domains of Kir channels, and thus be involved in the control of channel gating.
**Experimental Procedures**

Protein expression and purification of the cytoplasmic region of Kir3.2. An amino terminus (amino residues: 53-74) of the longest isoform of mouse Kir3.2 (Kir3.2c) was directly concatenated to a carboxyl terminus (amino residues: 200-381) and subcloned into NdeI and XhoI sites of pET28a vector (Novagen, WI). Protein was expressed in Rosetta2 (DE3) cells (Novagen) with the induction of 0.03 mM isopropyl-1-thio-β-D-galactopyranoside at 18˚C for 12 hours. The cells were lysed by sonication with 40 mM Tris-HCl (pH 8.6), 150 mM KCl, 10% (w/v) sucrose, 4 mM β-mercaptoethanol, 100 μg/ml lysozyme, 1 mM phenylmethylsulfonyl fluoride and 10 μg/ml each of aprotinin, leupeptin, chymostatin and antipain A. Supernatant obtained following centrifugation at 40,000 rpm for 30 min with a 45Ti rotor (Beckman Coulter, CA) was incubated with Talon resin (Clontech, CA) at 4˚C for 1 hour. After washing with 20 mM imidazole, polyhistidine-tagged Kir3.2 was eluted with 250 mM imidazole. Overnight incubation with thrombin (1 U/mg protein, Novagen) was employed to cut the tag off. The cytoplasmic Kir3.2 protein was subjected to Superdex 200 (Amersham Biosciences, Sweden). Protein in the peak fraction was concentrated to 7.5 mg/ml and dialyzed against 10 mM Tris-HCl (pH 8.0), 150 mM KCl, 10% sucrose and 2 mM β-mercaptoethanol at 4˚C overnight. Purified protein contained four residues (glycine-serine-histidine-methionine) at its amino terminus as a cloning artifact.

Crystallization of the cytoplasmic region of Kir3.2. Crystallization conditions were examined with commercially available screening kits: Crystal Screen Kit 1 and 2 (Hampton Research, CA), and Wizard I, II and III (Emerald BioSystems, WA). Crystals were grown by vapor diffusion by mixing equal volumes of protein solution (7.5 mg/ml) and reservoir solution with 100 mM HEPES-NaOH (pH 7.0), 0.2 M MgCl2 and 30% (v/v) isopropanol. Crystals were obtained in the presence of either isopropanol or ethanol, but their appearance and quality varied suggesting that additional conditions should be fixed for further crystallographic experiments. A tetragonal plate was visible after 2–3 days of incubation at 4˚C and reached its maximum size of about 120 x 120 x 30 μm.

Prior to data collection, crystals were transferred to cryoprotectant solution supplemented with 20% (v/v) glycerol and frozen by immersing directly in liquid nitrogen. The data were collected at
Crystal Structure of Kir3.2 Cytoplasmic Region

| Table 1  | Summary of X-ray diffraction data for the cytoplasmic domain of Kir3.2 |
|----------|--------------------------------------------------------------------------------|
| **Data Collection Statistics**                      |                                                                                  |
| Space Group                                       | *P*42,2                                                                         |
| Unit Cell Dimensions                               | a = b = 77.143 Å, c = 87.307 Å, α = β = γ = 90°                               |
| No. per a.u.                                       | 1                                                                               |
| Lambda (Å)                                        | 0.9                                                                             |
| d min (Å)                                         | 2.3                                                                             |
| Total Observation                                  | 127,686                                                                         |
| Unique Observation                                 | 11,612                                                                          |
| Rmerge (%)                                        | 12.1 (46.6)                                                                     |
| Completeness                                      | 99.8 (99.7)                                                                     |
| I/σ (I)                                           | 15.5 (4.93)                                                                     |
| Redundancy                                         | 9.4 (9.0)                                                                       |
| **Refinement Statistics**                          |                                                                                  |
| Resolution (Å)                                    | 10–2.3                                                                          |
| Rmerge (%)                                        | 24.4                                                                            |
| Rfree (%)                                          | 27.9                                                                            |
| No. of protein atoms                               | 1486                                                                            |
| No. of waters                                      | 109                                                                             |
| Average B value                                    | 44.8                                                                            |
| Overall                                           | 42.3                                                                            |
| Main                                              | 47.1                                                                            |
| Side chain/water                                   | 47.1                                                                            |
| Rms deviation                                      |                                                                                 |
| Bonds (Å)                                         | 0.008                                                                           |
| Angles (Å)                                        | 1.4                                                                             |
| Ramachandran plot                                 | 89.8/10.2/0.0/0.0                                                               |

*Number of protein molecules per asymmetric unit (a.u.).

Size exclusion chromatography. The cytoplasmic region of Kir3.2 for crystallization was dialyzed against 150 mM KCl, 10 mM Tris-HCl (pH 8.0) and either 10% sucrose or 20% glycerol by changing the solution twice for 24 hours. As an experimental control, N- and C-termini of mouse Kir3.1 (amino acid residues: 41-62 and 191-387, respectively) were connected with di-peptide (Gly-Ser), subcloned into NdeI and XhoI sites of pET28a vector, purified through Talon resin, and then analyzed by a size fractionation column. Prior to the separation with the Superdex 200, the protein solution was spun down to remove the aggregation and then loaded onto the column. The elution profile of the protein was monitored with absorbance at 280 nm.

RESULTS AND DISCUSSION

Preparation of Kir3.2 channel cytoplasmic region. We prepared a direct concatemer of cytoplasmic N- and C-termini of Kir3.2 (respectively amino acid residues: 53-74 and 200-381 in the longest isoform), with reference to the successful production of a compact domain of equivalent regions of Kir3.1 (Fig. 1A). Whereby isoforms of Kir3.2 differ at their distal N- or C-terminal ends, the concatemer corresponds to the core region which is common to all of the variants. Most of the protein expressed in bacteria was recovered in the soluble fraction. Whereas the amino acid sequence of the Kir3.2 cytoplasmic region shares 59% identity to the equivalent region of Kir3.1 (Fig. 1B) the protein started to form irreversible precipitates in the purification buffer which had been used for the preparation of Kir3.1. A glycerol (20% (v/v)) supplement in the preparation buffer partly improved the protein stability, but the addition of sucrose (10% (w/v)) did so dramatically (Fig. 1C). Pegan et al. (2005) reported that the Kir3.2 cytoplasmic region behaves as a dimer in solution. In this study it was eluted at a tetramer position in size exclusion column chromatography. This discrepancy is probably due to the difference in the buffer composition and/or in the sequence of the construct at either terminus.

Crystal structure of Kir3.2 channel cytoplasmic region. One of the crystals diffracted around 2.3 Å resolution. The structure was solved by the molecular replacement method with Kir3.1 coordinates (1N9P) as a search model. The solution was brought to crystallographic refinement. Data collection and refinement statistics are presented in Table 1. The structure of the cytoplasmic regions of Kir3.2 is composed of 13 β-strands and 2 α-helices (Fig. 2Aa). A short β-strand (βA) on the N-terminus forms a β-sheet with the βM- and βL-strands on the C-terminus (β-sheet 1). Three additional β-sheets composed of the following strands, βD, βE, βH and βI (β-sheet 2), βB, βC, βG and βJ (β-sheet 3), and βK and βN (β-sheet 4), form the core region of the Kir3.2 structure. The organization of the secondary structure of Kir3.2 shows a strong similarity to that of Kir3.1 (Fig. 2Ab). When comparing the Kir3.2 core region with those of Kir3.1 (1N9P) and the classical inward rectifier Kir2.1 (1U4F), root mean squares for the Cα atom positions are 0.216 Å and 0.254 Å, respectively. Thus the overall structural elements of the cytoplasmic region seem highly conserved in the Kir channel family.

Four monomers assemble to form a biological unit with a fourfold axis at the center of the tetramer along a crystallographic axis (Fig. 2B). The manner of tetrameric assembly and orientation of the secondary structure of Kir3.2 are comparable to those of Kir3.1 and Kir2.1 reported previously. The β-sheets 1 and 2 create a dent on one subunit and interact with the β-sheet 2 on the neighboring subunit. The N-terminal region of each monomer is located at

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**Figure Legends**

- **Figure 1A**: Structure of Kir3.2 cytoplasmic region.
- **Figure 1B**: A glycerol (20% (v/v)) supplement in the preparation buffer partly improved the protein stability.
- **Figure 1C**: Pegan et al. (2005) reported that the Kir3.2 cytoplasmic region behaves as a dimer in solution.
- **Figure 2Aa**: The structure of the cytoplasmic regions of Kir3.2 is composed of 13 β-strands and 2 α-helices.
- **Figure 2Ab**: When comparing the Kir3.2 core region with those of Kir3.1 (1N9P) and the classical inward rectifier Kir2.1 (1U4F), root mean squares for the Cα atom positions are 0.216 Å and 0.254 Å, respectively.
the interface of the C-termini of two adjacent subunits (Fig. 2C). The configuration of each terminus appears to be crucial for the oligomerization of Kir channels, which is consistent with previous observations that either terminus was unstable when expressed in bacteria alone.\(^{25,26}\) The KirBac1.1 structure shows the N-terminus of one subunit disposed counterclockwise in the neighboring subunit as seen in the Kir3.2 structure from the membrane side along the fourfold axis (Fig. 2C).\(^{10}\) According to this domain orientation, the N-terminus (indicated by a dotted line in Fig. 2C) has an interaction surface of 1,087 Å\(^2\) with the same subunit (blue) and 621 Å\(^2\) with the neighboring subunit (yellow), while the N-terminus connects to C-terminus colored yellow in the current model. The interaction between two C-termini of neighboring subunits buries a total of 1,318 Å\(^2\) of solvent-accessible surface area with 745 Å\(^2\) of non-polar and 574 Å\(^2\) of polar contacts. In addition to this direct interaction, water molecules penetrate into the interface, especially between C-termini, creating the hydrogen-bonding network to unite neighboring subunits.

It was shown that the potency of G\(_{\beta\gamma}\) for the activation of K\(_C\) channel was greatly reduced by the mutations introduced at Leu333 in Kir3.1 and the corresponding residue Leu339 in Kir3.4.\(^{27}\) The residue is located on a loop between βL- and βM-strands (LM loop) in the cytoplasmic region of K\(_C\) channels.\(^{8,9}\) Kir3.2 also possesses the equivalent amino acid at the 344 position and has been reported to attenuate G\(_{\beta\gamma}\)-dependent activation of the channel when mutated to Glu.\(^{28,29}\) Not only Leu344, but also the LM loop where Leu344 is located, seemed to participate in the G\(_{\beta\gamma}\)-dependent activation of the Kir3.2.\(^{28}\) Because, in the current model, the electron density at Leu344 was weak and at the tip of LM loop (corresponding to the 345 and 346 positions) was missing, it was difficult to obtain the structural bases for the function of the LM loop in G\(_{\beta\gamma}\)-dependent channel activation. However, the LM loop distribution at the interface between N- and C-termini in the Kir3.2 is in reasonable agreement with the previous observations that G\(_{\beta\gamma}\)-binding sites distribute at both N- and C-termini of K\(_C\) channels.\(^{25,30}\) Therefore, the LM loop possibly participates in the G\(_{\beta\gamma}\)-recognition leading to the channel gating.

**Ion pathway in the cytoplasmic region.** To detect structural differences between cytoplasmic segments of Kir3.2, Kir3.1 and Kir2.1, we compared their surface traces viewed from the inner leaflet of the membrane (Fig. 3A). Positively charged residues in Kir3.2 tend to concentrate more at the face toward the membrane than negatively charged residues (Fig. 3Aa). This feature appears to be common to the other Kir channels and it is probably important for the association with PIP\(_2\) and transmembrane helices (Figs. 3Ab and c). Along the fourfold symmetry axis, there is a pore in the Kir3.2 cytoplasmic region. Its narrowest portion is created by the side chain of Tyr266 on each of the subunits distributed diagonally in the tetramer (Fig. 3Ba). The area surrounded by Tyr266 is smaller than the estimated size of K\(^+\) ion with a hydration shell (9 Å in diameter). On the other hand, in the Kir3.1 (1N9P) structure there is a pore with a 10.1 Å gap in the corresponding region. In the Kir2.1 mutant structure, a water-caged K\(^+\) ion is located at the center of the ring of Asp255, whose corresponding residue in Kir3.2 is Tyr267.\(^{11}\) Two glycine residues (Gly265 and Gly269), which are conserved in all Kir2.x and Kir3.x subunits sandwich Tyr266. This arrangement of amino acids seems to confer flexibility at this position in Kir channels to change inner diameter without altering the entire conformation of the cytoplasmic region.

Kir3.2 clearly possesses a wider opening at the entrance of cytoplasmic pore close to the membrane, compared with Kir3.1 and
Kir2.1 (Fig. 3B). A loop between the BH- and β-strands (HI loop) in Kir2.1 is reported to be the gating element, and Ala306 on the loop generates the narrowest constriction in the Kir2.1 crystal structure (Fig. 3Bc).\(^9\) However the corresponding residue in Kir3.2, Gly318 shows a distance of 12.9 Å between the carbonyl oxygen atoms on subunits located diagonally in the tetramer. This distance is enough to allow the permeation of hydrated K\(^+\) ion. Pegan et al (2005) also suggested that the HI loop was flexible due to the distribution of residues with small side chains at the base.\(^9\) Flexibility in the loops at Tyr266 and HI will probably give rise to different conformations in different crystallization conditions. Thus, two flexible loops along the ion pathway in the cytoplasmic structure of Kir channels have the potential to modulate K\(^+\) ion flow.

In the Kir3.2 structure, the top of the βD-strands (Val235 and Glu236) extensively interact with the BH-strand and it is bent to be exposed toward the ion pathway. This is not the case in the Kir3.1 structure (circles in Fig. 3B). Thus, structural variation of the HI loop between different Kir channel structures appears to be due not only to the intrinsic profile of the HI loop, but also to its relation with the βD-strand and a loop between βC- and βD-strands (CD loop).

Figure 3. The center of the Kir3.2 cytoplasmic region. (A) A comparison of surface traces of different Kir channels. Surfaces of Kir3.2 (a), Kir3.1 (1N9P, b) and Kir2.1 (c) are viewed from the membrane side along the fourfold axis. Positively charged residues, Arg, Lys and His, are colored in blue, and negatively charged ones, Glu and Asp, are in red. (B) Amino acids in the center of the Kir channels cytoplasmic regions. The center of the Kir channel cytoplasmic region is enlarged and viewed from the side perpendicular to the fourfold axis. Two molecules have been omitted from the side view for clarity. Side chains of amino residues on a loop between βH- and βI-strands (the HI loop) are shown as spheres (yellow: carbon, blue: nitrogen, red: oxygen, orange: sulfur). A circle in each panel indicates the top of βD-strand where a structural difference between three channels is observed. The narrowest point along the pore in the Kir3.2 cytoplasmic region (a) is shaped by phenyl groups of Tyr266 on individual subunits. This Tyr266 is sandwiched between two glycines (Gly265 and Gly269) whose Cα positions are shown as green circles. These amino residues are conserved in Kir3.1 (b) and Kir2.1 (c). Residues corresponding to Tyr266, Gly265 and Gly269 in Kir3.2 are Phe255, Gly254 and Gly258 in Kir3.1, and Phe254, Gly253 and Gly257 in Kir2.1.

Local conformational deviation in K\(^+\) channels. HI and CD loops are located in the area facing the plasma membrane, which suggests the significance for this area in the regulation of Kir channel gating by the cytoplasmic region. To further understand structural deviation in the cytoplasmic regions of Kir channels, we tried to identify amino acid residues in the CD loop. Whereas electron density was clear at the top of the βD-strand with bending toward the cytoplasmic pore at Val235 and Glu236, electron density corresponding to the proximal three amino residues at the tip of the CD loop is missing (Fig. 4A). A comparison of these parts of βD-strands and CD loops in three Kir channel structures shows the flexibility of this loop (Fig. 4B). This flexibility seems to originate in two small amino acids, Gly227 following the βC-strand and Ala237 at the bend in the βD-strand, which are conserved in Kir3.1-Kir3.3, Kir2.1-Kir2.4, and Kir6.1 and Kir6.2. Asp228 in Kir3.2 and the corresponding residue in Kir3.4 have been reported to be the residues responsible for Na\(^+\)-dependent activation of their Kir channels. However, there was no visible electron density around Asp228 on the CD loop in this Kir3.2 structure (Fig. 4A). In our crystallization conditions, there was 45 mM Na\(^+\) ion. This is close to the half-maximal effective concentration for Na\(^+\) activation of Kir3.2 homomeric channels. If Asp228 is actually involved in Na\(^+\) binding, flexibility at the CD loop could be due to structural heterogeneity reflecting the different Na\(^+\)-binding states of this loop.

To examine the disposition of the flexible region the cytoplasmic region of Kir3.2 was superimposed onto that of KirBac1.1 (Fig. 4C-F).\(^9\) The distribution of the helices of KirBac1.1 indicate that the CD and HI loops are close enough to associate with the slide and the M2 helices, respectively (Fig. 4E and F). This suggests another possibility; the heterogeneity in the CD loop may be intrinsic due to the lack of other elements to be structured. The separate zones of flexibility at the CD and HI loops suggest that the membrane interface of the cytoplasmic region of Kir channels associates with either transmembrane channel domains or the membrane itself.

The area in the cytoplasmic region of Kir channels, which contains the CD and HI loops, has also been suggested to be a region crucial for the regulation of channel gating. The CD loop where Asp228 is located includes an arginine, which is conserved in the Kir channel family. Mutations at the corresponding residue in Kir2.1 (R218W and R218Q) attenuate apparent PIP\(_2\) affinity and lead to reduced activity and Andersen syndrome.\(^2\)\(^,\)\(^1\)\(^5\)\)\(^3\)\(^1\)\(^3\) Ile229 in Kir3.4 was reported to be the PIP\(_2\)-sensitive residue by substituting with leucine.\(^1\)\(^4\) Though how this hydrophobic residue is involved in PIP\(_2\)-Kir channel interaction is still unclear as the isomers only differ by the position of a methyl group. It is possible that the alteration at...
the side chain of Ile234 causes a steric interference in the flexibility of the loop, leading to modification of PIP₂-Kir channel interaction in the mutant. Glu236 is one of the two residues on the top of the βD-strand which is known to influence inward rectification in Kir2.1 and possibly affect K⁺ ion permeation.³² Thus, this area that is restricted to the interface to the membrane may mediate regulation of Kir channel gating by the cytoplasmic region.

CONCLUSION

To identify structural elements involved in the regulation of KC₃ channel gating by the cytoplasmic N- and C-termini, we examined the crystal structure of the cytoplasmic region of Kir3.2. Its overall conformation is almost equivalent to those of other Kir channels which have been reported previously.⁸⁻¹¹ However, a comparison of these different structures revealed a flexibility in the loops between β-strands. Significant differences were found in the CD and HI loops and on top of the βD-strand. This is where the cytoplasmic region of the channel may interact with the transmembrane domain, and the slide and the M2 helices. Amino acids that are crucial for the regulation of Kir channel function showed a clumped distribution in these loops. These results suggest that the interface between cytoplasmic and transmembrane domains plays a pivotal role in the regulation of Kir channel gating. To elucidate how the interaction between these two domains controls channel gating, the entire structure of a mammalian KC₃ channel needs to be elucidated.

References
1. Hille B. Ion channels of excitable membranes. IIIrd ed. Sinauer 2001.
2. Yamada M, Inanobe A, Kurachi Y. G protein regulation of potassium ion channels. Pharmacol Rev 1998; 50:723-60.
3. Ito H, Tung RT, Sugimoto T, Kobayashi I, Takahashi K, Katada T, Uti M, Kurachi Y. On the mechanism of G protein βγ subunit activation of the muscarinic K⁺ channel in guinea pig atrial cell membrane. Comparison with the ATP-sensitive K⁺ channel. J Gen Physiol 1992; 99:961-83.
4. Logothetis DE, Kurachi Y, Galper J, Nee RJ, Clapham DE. The βγsubunits of GTP-binding proteins activate the muscarinic K⁺ channel in heart. Nature 1987; 325:321-6.
5. Reuveny E, Slesinger PA, Inglese J, Morales JM, Iniguez-Lluhi JA, Lefkowitz RJ, Bourne HR, Jan NY, Jan LY. Activation of the cloned muscarinic potassium channel by G protein βγ subunits. Nature 1994; 370:143-6.
6. Huang CL, Feng S, Hilgemann DW. Direct activation of inward rectifier potassium channels by PIP₂, and its stabilization by Gβγ. Nature 1998; 391:803-6.
7. Sui JI, Petri-Jacques L, Logothetis DE. Activation of the atrial Kᵦᵨᵦ channel by the βγ subunits of G proteins or intracellular Na⁺ ions depends on the presence of phosphatidylcholinositol phosphates. Proc Natl Acad Sci USA 1998; 95:1307-12.
8. Nishida M, MacKinnon R. Structural basis of inward rectification: Cytoplasmic pore of the G protein-gated inward rectifier GIRK1 at 1.8 angstrom resolution. Cell 2002; 111:957-65.
9. Pegan S, Arrabah C, Zhou W, Kwiatkowski W, Collins A, Slesinger PA, Choe S. Cytoplasmic domain structures of Kir2.1 and Kir3.1 show sites for modulating gating and rectification. Nat Neurosci 2005; 8:279-87.
10. Aoki S, Ueda K, Inagaki T, Inoue M, Takahashi K, Kurachi Y. The effect of phosphatidylserine on the activity of the cloned muscarinic K⁺ channel. J Gen Physiol 1992; 101:161-73.
11. Pegan S, Arrabah C, Zhou W, Kwiatkowski W, Collins A, Slesinger PA, Choe S. Andersen’s syndrome mutation effects on potassium channel function. J Biol Chem 1999; 274:8639-48.
13. Sui JL, Chan KW, Logothetis DE. Na⁺ activation of the muscarinic K⁺ channel by a G-protein-independent mechanism. J Gen Physiol 1996; 108:381-91.
14. Zhang H, He C, Yan X, Mirshahi T, Logothetis DE. Activation of inwardly rectifying K⁺ channels by distinct PtdIns(4,5)P₂ interactions. Nat Cell Biol 1999; 1:183-8.
15. Platter NM, Tawl R, Tristani-Firouzi M, Canun S, Bendahhou S, Tsunoda A, Donaldson MR, Ianaccone ST, Brunt E, Barohn R, Clark J, Deymeer F, George JAL, Fish FA, Hahn A, Nitu A, Ozdemir C, Serdaroglu P, Subramony SH, Wolfe G, Fu YH, Pracek IJ. Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen’s syndrome. Cell 2001; 105:531-9.
16. Orwinowski Z, Mino W. Processing of X-ray diffraction data collected in oscillation mode. Methods Enzymol 1997; 276:307-26.
17. Vagin A, Teplyakov A. MOLREP: An automated program for molecular replacement. J Appl Cryst 1997; 30:1022-5.
18. Brunger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, Read RJ, Rice LM, Simonson T, Warren GL. Crystallography and NMR System: A new software suite for macromolecular structure determination. Acta Crystallogr D Biol Crystallogr 1998; 54:905-21.
19. Emseley P, Cowtan K. CoM: Model-building tools for molecular graphics. Acta Crystallogr D Biol Crystallogr 2004; 60.
20. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: A program to check the stereochemical quality of protein structures. J Appl Crystallogr 1993; 26:283-91.
21. Fraternali F, Cavallo L. Parameter optimized surfaces (POPS): Analysis of key interactions and conformational changes in the ribosome. Nucleic Acids Research 2002; 30:2950-60.
22. DeLano WL. The PyMOL Molecular Graphics System. San Carlos, CA: DeLano Scientific, 2002.
23. Notredame C, Higgins D, Heringa J. T-Coffee: A novel method for multiple sequence alignments. 2000; 302:205-17.
24. Inanobe A, Horio Y, Fujita A, Tanemoto M, Hibino H, Inageda K, Kurachi Y. Molecular cloning and characterization of a novel splicing variant of the Kir3.2 subunit predominantly expressed in mouse testis. J Physiol 1999; 521:19-30.
25. Huang CL, Slesinger PA, Casey PJ, Jan YN, Jan LY. Evidence that direct binding of Gbg to the GIRK1 G protein-gated inwardly rectifying K⁺ channel is important for channel activation. Neuron 1995; 15:1133-43.
26. Inanobe A, Morishige KI, Takahashi N, Ito H, Yamada M, Takumi T, Nishina H, Takahashi K, Kaneko Y, Katao T, Kurachi Y. Gbg directly binds to the carboxyl terminus of the G protein-gated muscarinic K⁺ channel, GIRK1. Biochem Biophys Res Commun 1995; 212:1022-8.
27. He C, Zhang H, Mirshahi T, Logothetis DE. Identification of a potassium channel site that interacts with G protein bg subunits to mediate agonist-induced signaling. J Biol Chem 1999; 274:12517-24.
28. Finley M, Arrabit C, Fowler C, Suen KF, Slesinger PA. BL-JLM loop in the C-terminal domain of G protein-activated inwardly rectifying K⁺ channels is important for Gbg subunit activation. J Physiol 2004; 555:643-57.
29. Ivanina T, Rush J, Varon D, Miu Ilme C, Frohnhovers-Steinecke B, Schreibmayer W, Csernauer CW, Dascal N. Mapping the Gbg-binding sites in GIRK1 and GIRK2 subunits of the G protein-activated K⁺ channel. J Biol Chem 2003; 278:29174-83.
30. Huang C, Jan YN, Jan LY. Binding of the G protein bg subunit to multiple regions of G protein-gated inward-rectifying K⁺ channels. FEBS Lett 1997; 405:291-8.
31. Lopes CBM, Zhang H, Rohaci T, Jin T, Yang J, Logothetis DE. Alterations in conserved Kir channel-PIP interactions underlie channelopathies. Neuron 2002; 34:933-44.
32. Yang J, Jan YN, Jan LY. Control of rectification and permeation by residues in two distinct domains in an inward rectifier K⁺ channel. Neuron 1995; 14:1047-54.