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| Note        |                                                                                                             |
A Structural Determinant for the Control of PIP$_2$ Sensitivity in G Protein-gated Inward Rectifier K$^+$ Channels

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Inward rectifier K$^+$ (Kir) channels are activated by phosphatidylinositol-(4,5)-bisphosphate (PIP$_2$), but G protein-gated Kir (K$_G$) channels further require either G protein $\beta\gamma$ subunits (G$\beta\gamma$) or intracellular Na$^+$ for their activation. To reveal the mechanism(s) underlying this regulation, we compared the crystal structures of the cytoplasmic domain of K$_G$ channel subunits Kir3.2 obtained in the presence and the absence of Na$^+$.

The Na$^+$-free Kir3.2, but not the Na$^+$-plus Kir3.2, possessed an ionic bond connecting the N terminus and the CD loop of the C terminus. Functional analyses revealed that the ionic bond between His-69 on the N terminus and Asp-228 on the CD loop, which are known to be critically involved in G$\beta\gamma$- and Na$^+$-dependent activation, lowered PIP$_2$ sensitivity. The conservation of these residues within the K$_G$ channel family indicates that the ionic bond is a character that maintains the channels in a closed state by controlling the PIP$_2$ sensitivity.

The regulation of gating and ion conduction in inward rectifier K$^+$ (Kir) channels is fundamental to their physiological functions, such as the control of the deep resting membrane potential and cell excitability (1–3). The Kir channel is a tetramer with two structurally and functionally distinct transmembrane and cytoplasmic domains (4–6). The association of phosphatidylinositol-(4,5)-bisphosphate (PIP$_2$) with the cytoplasmic domain of Kir channels causes channel activation (7–9). However, the G protein-gated Kir (K$_G$) channels also require either G protein $\beta\gamma$ subunits (G$\beta\gamma$) (2, 10–12) or intracellular Na$^+$ (13, 14) for their activation. The binding of either G$\beta\gamma$ or Na$^+$ is thought to evoke conformational changes in the cytoplasmic domain of K$_G$ channels, which results in an increase in the channel sensitivity to PIP$_2$, leading to channel opening (7, 15, 16). Thus, in the absence of either G$\beta\gamma$ or Na$^+$ and in contrast with other Kir channels, K$_G$ channels are relatively insensitive to PIP$_2$.

The crystal structures of whole Kir channels (4–6, 17) and parts of Kir channels (18–20) have provided valuable insights into the distribution of amino acids essential for the interaction with PIP$_2$ (16, 21–23), G$\beta\gamma$ (24–26), and Na$^+$ (16, 27–29). Furthermore, a series of crystal structures of the bacterial homologue Kir channel, Kir Bac3.1, reveals that the channel undergoes subunit rearrangement at the cytoplasmic domain accompanied by the alteration in the interaction between the N and C termini (17). However, the mechanism(s) underlying the specific regulation of K$_G$ channel activity and the ligand-induced conformational changes remained to be clarified.

To address these questions, we determined the crystal structure of the cytoplasmic domain of the homotetrameric K$_G$ channel subunit Kir3.2 in the absence of Na$^+$ and performed the mutational analysis. The activator-free Kir3.2 possesses an ionic bond, which connects the N terminus (His-69) and the CD loop (Asp-228) of the C terminus. Because these residues are conserved within K$_G$ channel family subunits, this ionic bond seems to stabilize the closed state by preventing conformational changes in the cytoplasmic domain. The corresponding residues to His-69 and Asp-228 are known to be responsible for respective G$\beta\gamma$-dependent (25) and Na$^+$-dependent activation (16, 27, 28, 30). Furthermore, the CD loop of Kir channels has residues important for PIP$_2$-dependent activation (16, 21–23). Therefore, the disruption of the bond is a common mechanism for the different activators to trigger channel opening by allowing the cytoplasmic domain to change its conformation and promoting the association with PIP$_2$.

EXPERIMENTAL PROCEDURES

Crystallization, Data Collection, and Model Building of Cytoplasmic Domain of Kir3.2—The longest isoform of mouse Kir3.2 (Kir3.2c) is made up of 425 amino acids with two more amino acids on the N terminus compared with human Kir3.2. Therefore, His-69 and Asp-228 in mouse Kir3.2 correspond to His-67 and Asp-226 in human Kir3.2, respectively (27). The concatenation of cytoplasmic N and C termini of Kir3.2 (amino acid residues 53–74 and 200–381, respectively) was expressed in bacteria and purified without the addition of Na$^+$ as described previously (20). Crystals were grown by vapor diffusion at 4°C by mixing equal volumes of protein solution (5 mg/ml) and reservoir solution of 7.5–12.5% (v/v) ethanol, 0.1 M imidazole.
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HCl (pH 8.0), 100 mM MgCl₂, and 10–30 mM spermine. Crystals were immersed in reservoir solution supplemented with 25% (v/v) glycerol for cryoprotection. The data were collected at the BL41XU beam line at the SPring-8. Crystals belong to the space group I422 with cell dimensions a = b = 81.943 Å and c = 172.769 Å. The data were indexed and scaled using the HKL2000 suite (31). The initial phase was determined by molecular replacement using the coordinates of the cytoplasmic domain of Kir3.2 (Protein Data Bank code 2E4F) (20). Model refinement was carried out with programs in the CCP4i suite (32). The statistics of the crystallographic analyses are presented in Table 1. The illustrations of the structures were prepared using PyMOL (DeLano Scientific).

Electrophysiological Analyses—The cDNA of mouse Kir3.2d (33) were subcloned into the expression vector pcDNA3 (Invitrogen) and transfected with porcine m₂-muscarinic receptor into HEK293T cells with Lipofectamine (Invitrogen). Point mutations were introduced using a QuikChange site-directed mutagenesis kit (Stratagene), according to the manufacturer’s instructions. Mutations were confirmed with direct DNA sequencing. Inward Kir currents were recorded at a holding potential of −100 mV in excised inside-out patches using a patch clamp amplifier (Axopatch 200A, Axon Instruments). The current was low pass-filtered at 1 kHz and digitized at 10 kHz. Glass pipettes had resistances of 2.5–3.5 megohms when filled with pipette solution. The pipette solution contained 135 mM KCl, 1 mM CaCl₂, 1.6 mM MgCl₂, 5 mM HEPES, and 5 μM acetylcholine, and the bath solution was composed of 135 mM KCl, 5 mM EGTA, 2 mM MgCl₂, and 5 mM HEPES. PIP₂ (Sigma-Aldrich) dissolved in chloroform was stored at −20 °C. Before the experiment, an aliquot of PIP₂ solution was evaporated with a flow of argon gas and dissolved in the bath solution by sonication for 2–3 min. Solutions containing 2 mM K₂ATP were prepared freshly each day. The pH of all solutions was adjusted to 7.35 with KOH prior to the experiments. Because the perfusion of NaCl did not shift the zero current level recorded in the presence of 10 μM CdCl₂, NaCl was added to the bath solution without compensation for changes in the osmolarity and ionic strength. All experiments were performed at ambient temperature (23–25 °C). The data are expressed as means ± S.E. Statistics were performed using Student’s t test.

RESULTS

Differences in Conformation between Kir3.2 Structures Obtained in Presence and Absence of Na⁺—The concatenator of polypeptide segments of cytoplasmic N and C termini of Kir3.2 (Fig. 1A) was expressed and crystallized in the absence of Na⁺ (Na⁺-free Kir3.2). The structure was solved by the molecular replacement method with the Kir3.2 structure obtained in the absence of Na⁺ (Na⁺-plus Kir3.2) as a search model (20). The electron density was continuous enough to trace CD and LM loops, which were partially disordered in the Na⁺-plus structure. The solution was brought to crystallographic refinement. Data collection and refinement statistics are presented in Table 1.

The cytoplasmic domain of the Na⁺-free Kir3.2 is a β strand-rich immunoglobulin-like domain (Fig. 1B, left panel). The positions of backbone Ca atoms in the Na⁺-free Kir3.2 structure are similar to those in the Na⁺-plus Kir3.2 (root mean square deviation, 2.0 Å) (Fig. 1B, right panel). A large difference in the region connecting N and C termini and small differences in CD, DE, HI, and LM loops contribute to an increase (271 Å²) in the buried surface area between subunits in the
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In the Na⁺-free Kir3.2, a βA strand is missing, and an imidazole ring of His-68 approaches to within 2.9 Å of a carboxyl group of Asp-228 on the CD loop, possibly forming an ionic bond (Fig. 2B). In addition to this electrostatic interaction, a hydroxyl group of Tyr-353 interacts with His-68 and Asp-228 with distances of 3.4 and 2.7 Å, respectively. Therefore, the direct binding of the N terminus and the CD loop appears to influence the conformation at the interface between them.

Functional Implications of Role of Ionic Bond between N Terminus and CD Loop in Kir3.2 Channel Gating—Because neither Gβ7 nor Na⁺ was present in the crystallization conditions of the Na⁺-free Kir3.2 cytoplasmic domain, the ionic bond between His-68 and Asp-228 could be involved in the control of the sensitivity of the channel to PIP2. To test this possibility, we measured channel activity in inside-out membrane patches from HEK293T cells expressing either Kir3.2 WT or mutant channels (Fig. 3A). As noted elsewhere (27), the increase in current amplitude induced by PIP2 in Kir3.2 WT was small, and the CD loop resulted in a significant increase in the PIP2-induced current (39.8 ± 7.7%, n = 5), indicating that the D228N mutant is more sensitive to PIP2.

The amino acids corresponding to His-68 of Kir3.2 are not conserved among Kir channels (see Fig. 5B). However, although the substitution of a glutamine for His-68 (H68Q) produced a functional channel, mutants where His-68 were replaced by either glycine, cysteine, glutamate, tyrosine, or tryptophan dramatically reduced functional channel expression. The H68Q mutant showed a low basal activity associated with PIP2 (7.2 ± 1.6%, n = 7), which was as low as that observed in the WT (Fig. 3, A and C). This low sensitivity of the H68Q mutant to PIP2 in the absence of channel activators suggests that the contribution of His-68 to the control of the association with PIP2 is insignificant in the functional channel. When N termini of the two Kir3.2 structures were compared and an imidazole ring of His-69 in tetrameric assembly of the Na⁺-free Kir3.2. However, there was no apparent difference in the subunit interaction between the two Kir3.2 structures. The top of the cytoplasmic pore consists of HI loops from four subunits. This loop is positioned differently in different Kir channels, and it is thought to function as a part of the gate (5, 19). Both of the Kir3.2 crystal structures possess a wide opening at the entrance of cytoplasmic pore, enough to allow partially hydrated cations to pass through the pore.

A prominent difference between the two Kir3.2 structures is observed at the interface between the N and C termini (Fig. 1B, square). The side chain position of Asp-228 in the Na⁺-plus Kir3.2 is equivalent to that in other Kir channel structures (Fig. 2A) (4–6, 17–19, 34). However, the Cγ atom of the carboxyl group of Asp-228 in Na⁺-free Kir3.2 was rotated counterclockwise by ~70° compared with that in the Na⁺-plus Kir3.2. The positions of a carboxyl group of Asp-228 and a water molecule (wat2 in A) in the Na⁺-plus Kir3.2 were substituted by a water molecule (wat1 in B) and a carboxyl group of Asp-228, respectively, in the Na⁺-free Kir3.2. This unique side chain position of Asp-228 in the Na⁺-free Kir3.2 suggests that the pivoting movement of Asp-228 affects the conformation of the N terminus and the CD loop.

In the Na⁺-plus Kir3.2, an imidazole ring of His-69 contacts with the hydroxyl group of Tyr-353 with a distance of 3.0 Å (Fig. 2A). This interaction is involved in the hydrogen bond network with water molecules (wat2 and wat3 in A), partially contributing to the stabilization of the conformation of a β (βA) strand (amino acids 66–70), which is conserved among all Kir channel

**TABLE 1**

Summary of x-ray diffraction data for the cytoplasmic domain of Kir3.2 in the absence of Na⁺

| Data collection statistics |  |
|----------------------------|---|
| Space group                |  |
| a = b = 81.945 Å, c = 172.769 Å; α = β = γ = 90° |  |
| No. per a.u.               | 1 |
| Wavelength (Å)             | 1.00 |
| Resolution (Å)             | 2.2 |
| Total observation          | 145,544 |
| Unique observation         | 14,563 |
| Rmerge (%)                 | 6.3 (62.9) |
| Completeness (%)           | 99.4 (98.2) |
| Redundancy (%)             | 9.5 (9.1) |

| Refinement statistics      |  |
|----------------------------|---|
| Resolution (Å)             | 30.2 |
| Rwork (%)                  | 19.8 |
| Rfree (%)                  | 23.6 |
| No. of protein atoms       | 1574 |
| No. of water molecules     | 140 |
| Average B value            | 31.8 |
| Overall                    | 29.7 |
| Side chain/water r.m.s.d.  | 33.5 |
| Bonds (Å)                  | 0.012 |
| Angles                      | 1.27° |
| Ramachandran plot (%)      | 88.6 (11.4) |

* Value represents the number of protein molecules per asymmetric unit. r.m.s.d. values are root mean square deviation.

**FIGURE 2.** Enlarged view of the interface between N terminus and CD loop of Kir3.2 cytoplasmic domain. Ribbon models of the structural elements at the interface between the N terminus (N-ter) and the CD loop (C-ter) of Kir3.2 in the presence (A) or the absence (B) of Na⁺ are shown in blue and pink, respectively. The residues important in interactions are shown as stick models. Water (wat) molecules are indicated by small spheres. Large spheres indicate a Co atom at the terminal residue of the N terminus in each structure. The N termini in the two structures are shown in dense colors.
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the Na\(^{+}\)-plus Kir3.2 was found at the position equivalent to His-68 in the Na\(^{+}\)-free structure (Fig. 3B). In this case, it could be His-69 that geometrically made an ionic bond with Asp-228. The basal current amplitude associated with PIP2 of the mutant Kir3.2 which substituted a glutamine for His-69 (H69Q) was high (30.4 \(\pm\) 4.8%, \(n = 9\)). The introduction of a cysteine for His-69 also resulted in PIP2 increasing the basal current (30.6 \(\pm\) 8.4%, \(n = 3\)) (supplemental Fig. S1). Therefore, whereas His-68 binds to Asp-228 in the Na\(^{+}\)-free Kir3.2 crystal structure, in the functional channel it is His-69 that is more likely to be involved in the control of the interaction with PIP2, presumably by forming the ionic bond with Asp-228.

Functional Implications of Residues Forming Ionic Bond in Na\(^{+}\)-dependent Activation—Next, we examined the effect of the mutations on Na\(^{+}\)-dependent activation. In these experiments, we used ATP instead of PIP2 to produce PIP2 at the inner leaflet of patch membranes (14, 15, 27). Intracellular Na\(^{+}\) activated the Kir3.2 WT channel more effectively than GTP\(\gamma\)S, with a 130 (\(\pm\) 40)-fold increase with 200 mM Na\(^{+}\) (\(n = 5\)) (Fig. 4A) (27). The activation by Na\(^{+}\) was concentration-dependent with a Hill coefficient of 3.0 \(\pm\) 0.6 and a half-effective concentration (EC\(_{50}\)) value of 56.8 \(\pm\) 7.6 mM (Fig. 4E). The Kir3.2 D228N mutant was defective for Na\(^{+}\)-dependent activation (Fig. 4B). On the other hand, both H68Q and H69Q mutant channels were activated by Na\(^{+}\) in a concentration-dependent manner (Fig. 4C and D), but the EC\(_{50}\) values were increased at least 3-fold compared with the WT (Fig. 4E). Furthermore, the amplitude of channel currents supported by H68Q and H69Q mutants elicited by 200 mM Na\(^{+}\) were only 30-fold and 3-fold, respectively, greater than the current induced by 10 \(\mu\)M GTP\(\gamma\)S. In both cases, the currents elicited by GTP\(\gamma\)S were not significantly different from that of the WT. Thus, both mutations appear to reduce the sensitivity to Na\(^{+}\). Because H69Q and H69C mutants showed similar sensitivities to PIP2 and Na\(^{+}\) (supplemental Fig. S1), the effect of these point mutations at position 69 might be due to the elimination of the side chain of histidine, rather than to the particular characteristics of the introduced residues.

DISCUSSION

Role of Ionic Bond between N Terminal and CD Loop in Conformational Changes in Cytoplasmic Domain of Kir3.2—In crystal structures of full-length Kir channels (4–6, 17), the channels are presumed to be in the closed state, with the physical constraints for ion permeation in the transmembrane domain of the cytoplasmic domain in these complete structures is comparable with those of isolated cytoplasmic domains lacking the transmembrane domain (18–20). Thus, it could be speculated that, in the closed state, the attachment of the transmembrane domain had little effect upon the structure of the cytoplasmic domain. The association of G\(\beta\)\(\gamma\) and Na\(^{+}\) with the cytoplasmic domain activates K\(_{G}\) channels by increasing their PIP2 sensitivity and do not activate the channels by themselves (7–9). This mode of regulation implies that their conformational changes may be restricted to the cytoplasmic domain. The structure of the Na\(^{+}\)-free Kir3.2 would correspond to the closed state, and local structural differences between Na\(^{+}\)-free and Na\(^{+}\)-plus Kir3.2 structures would be consistent with this mode of regulation of the channel. Potentially, the structural difference results from the rotational movement of Asp-228, which couples to the movement of the CD loop (20). Gly-227 adjacent to Asp-228 tends to be conserved within the Kir channel family (Fig. 5B), thereby suggesting that Gly-227 underlies the conformational flexibility of the CD loop. Furthermore, the position of Asp-228 in the Na\(^{+}\)-free Kir3.2 would be stabilized by the positively charged histidine on the N terminus. Although His-68 is involved in the ionic bond with Asp-228 in the crystal structure (Fig. 2), His-69 appears to contact with Asp-228 in the full channel (Fig. 3). This discrepancy between which histidine acts as a partner for the ionic bond with Asp-228 can be attributed to the effect of the region...
linking cytoplasmic N and C termini. It is also possible that the precise position of Asp-228 in the Na\(^+\) free Kir3.2 is influenced by conformational changes in the N terminus and/or the linker induced by crystallization. In this case, the CD loop including Asp-228 and the N terminus are structural elements that can change their conformation. Although the absence of a structural model for the open state at the atomic level hampers any rigorous assessment of how the cytoplasmic domain changes its conformation during gating, it might link to the sequential conformational changes in the cytoplasmic domain to control the gate. This is consistent with the results from crystallographic and biochemical analyses of the bacterial Kir channel KirBac3.1 of which the N terminus and the CD loop alter their conformation during gating (17, 35). Thus, we propose a structural model of Kir3.2 in the closed state with an ionic bond between His-69 on the N terminus and Asp-228 on the CD loop (Fig. 5A). This ionic bond would be a crucial structural element for Kir3.2 channel activity as a constraint on the conformational changes in the cytoplasmic domain leading to channel opening.

In Na\(^+\)-sensitive Kir4.1/Kir5.1 channels, the aspartate that corresponds to Asp-228 in Kir3.2 was proposed to reduce PIP\(_2\) sensitivity by forming a bond with the conserved arginine in the same CD loop (Arg-230 in Kir3.2, Fig. 5B) rather than His-69 on the N terminus (30). This proposal could explain how Na\(^+\) increases PIP\(_2\) sensitivity in both the K\(_{Ca}\) channel and the Kir4.1-Kir5.1 complex. However, these two Kir channels are markedly different in their basal activity; the K\(_{Ca}\) channel is constitutively inactive and the Kir4.1/Kir5.1 channel is constitutively active.

**FIGURE 4.** Effects of amino acid substitution on Na\(^+\)-dependent channel activation. The effect of Na\(^+\) on currents of Kir3.2 WT (A), D228N (B), H68Q (C), and H69Q (D) mutant channels were examined. Experimental conditions were as presented in Fig. 3. ATP was applied to induce PIP\(_2\) at the inner leaflet of patch membrane. The Kir3.2 WT currents in the absence of channel activators (*) and in the presence of ATP alone (**) or ATP and GTP\(_7\) (***) are enlarged in the inset of A. The currents activated by various concentrations of Na\(^+\) were normalized against that activated by 10 \(\mu\)M GTP\(_7\) (E). N-ter, N terminus.

**FIGURE 5.** Model of cytoplasmic domain of Kir3.2 insensitive to PIP\(_2\) and comparison of amino acid sequences of the interface between the N and C terminus. A, structural model of cytoplasmic domain of Kir3.2 insensitive to PIP\(_2\). Based on the functional analyses, we propose a model where His-69 on the N terminus directly interacts with Asp-228 on the CD loop. In addition to the electrostatic interaction, a hydroxyl group of Tyr-353 may contact with the side chains of these two residues. B, alignments of the amino acid sequences at the interface between the N and the C termini in mouse Kir channels. The residues involved in the direct association and a conserved arginine in the CD loop, which is thought to be crucial for the interaction with PIP\(_2\), are enclosed by red and blue squares, respectively. Charged residues in the squares are indicated in boldface. Numbers above the alignments correspond to amino acid numbers in the longest isoform of mouse Kir3.2.
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(36). Thus, the mechanism to maintain the channel in the closed state cannot be accounted for by a bond between the aspartate and the arginine on the CD loop.

The structures of whole Kir channels (4–6, 17) indicate that the N terminus in one subunit associates with the C terminus of the adjacent subunit in functional channel assembly. This implies that the ionic bond between the N terminus and the CD loop in Kir3.2 is an intersubunit interaction. The pair of amino acids forming the bond in Kir3.2 is conserved in Kir3.4, Kir6.1, and Kir6.2, and His-69 in Kir3.2 is also observed in Kir3.1 (Fig. 5B). This suggests that the electrostatic interaction between the N terminus and the CD loop may control the conformational changes in the cytoplasmic domain not only in the homomeric KG channels and KATP channels, but also in the heteromeric KG channels assembled with Kir3.1 and other KG channel subunits.

Functional Implications of Residues Involved in Ionic Bond—The Asp-228 in the CD loop of Kir3.2 (27, 28) and the corresponding aspartates in Kir3.4 (16) and Kir5.1 (30) are known to be involved in Na\(^+\)-dependent activation of these Kir channels. Furthermore, the histidines equivalent to His-233 in Kir3.2 in the same CD loops in Kir3.4 and Kir5.1 appear to participate in the coordination of Na\(^+\) (30). The order of ion selectivity for Kir3.2 activation by monovalent cations is Na\(^+\) \(>\) Li\(^+\) \(>\) K\(^+\) = Cs\(^+\) (data not shown) (13), which corresponds to Eisenman’s selectivity sequence X (37). This suggests that the Na\(^+\)-binding site of Kir3.2 consists of an anionic and relatively compact pocket. However, the point mutations at two histidine residues on the N terminus reduced the Na\(^+\)-sensitivity of Kir3.2 (Fig. 4 and supplemental Fig. S1). This may be either an indirect effect upon Na\(^+\)-binding to the site largely contributed to by the CD loop or the conformational changes following the association with Na\(^+\) (38). Alternatively, the N terminus might provide additional Na\(^+\)-binding sites associated with channel activation. Because Na\(^+\) and water molecules have an equal number of electrons, it is difficult to distinguish between them on the electron density map. A high resolution structure of Kir3.2 in the presence of a maximal or supramaximal concentration of Na\(^+\) would help to address the question of how Na\(^+\) interacts with Kir3.2.

On the other hand, the histidine residues in Kir3.1 and Kir3.4 (His-57 and His-64, respectively), which correspond to His-69 in the N terminus of Kir3.2, are involved in channel activation by G\(\beta\)Y (25). Furthermore, the equivalent histidine in Kir3.4 participates in the inhibition of channel activity by intracellular acidification (39). Based on our observations, the effect of lowering pH on the reduction of the macroscopic current of Kir3.4 could be attributed to facilitation of the formation of the ionic bond between His-64 and Asp-223 on its CD loop. Thus, these reports strongly support our idea that electrostatic interaction between N and C termini is a mechanism crucial for the control of Kir3.2 channel activity.

Activator-induced Conformational Changes in Cytoplasmic Domain—The CD loop possesses an arginine that is conserved among the Kir channel family, and it is thought to be essential for PIP\(_2\)-dependent channel activation (Fig. 5B) (16, 22). In addition, some of the Andersen-Tawil syndrome mutations in the CD loop of Kir2.1 cause a reduction in the sensitivity to PIP\(_2\) (22, 23). The direct interaction between the N terminus and the CD loop, therefore, seems crucial for lowering the sensitivity to PIP\(_2\) by constraining the conformation of the CD loop via the control of the pivoting movement of Asp-228 (Fig. 6). However, whereas Kir3.2 mutants that lacked the ionic bond showed increased sensitivity to PIP\(_2\), they were also further activated by Na\(^+\) and/or G\(\beta\)Y (Figs. 3 and 4 and supplemental Fig. S1). Thus, the disruption of this ionic bond is not the only effect of the activators. The coordination of Na\(^+\) (30) and multiple contacts with G\(\beta\)Y (24, 26, 40) would also be required for channel activation. Therefore, in addition to the disruption of the ionic bond, the activators appear to induce other conformational changes to further facilitate the sensitivity to PIP\(_2\) or stabilize the open channel conformation. Taken together, the intersubunit ionic bond between the N terminus and the CD loop seems to be characteristic of KG channel subunits and acts as an inhibitory mechanism for channel activation (Fig. 6). The two different KG channel activators, G\(\beta\)Y and Na\(^+\), would disrupt the bond, increase the sensitivity to PIP\(_2\), and open the gate.

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REFERENCES

1. Hibino, H., Inanobe, A., Furutani, K., Murakami, S., Findlay, I., and Kurauchi, Y. (2010) Physiol. Rev. 90, 291–366
2. Yamada, M., Inanobe, A., and Kurachi, Y. (1998) Pharmacol. Rev. 50, 723–750
3. Lüscher, C., and Slesinger, P. A. (2010) Nat. Rev. Neurosci. 11, 301–315
4. Kuo, A., Gulbis, J. M., Antcliff, J. F., Rahman, T., Lowe, E. D., Zimmer, J., Cuthbertson, J., Ashcroft, F. M., Ezaki, T., and Doyle, D. A. (2003) Science 300, 1922–1926
5. Nishida, M., Cadene, M., Chait, B. T., and MacKinnon, R. (2007) EMBO J. 26, 4005–4015
6. Tao, X., Avalos, J. L., Chen, J., and MacKinnon, R. (2009) Science 326, 1668–1674
7. Hilgemann, D. W., and Ball, R. (1996) Science 273, 956–959
8. Bichet, D., Haass, F. A., and Jan, L. Y. (2003) Nat. Rev. Neurosci. 4, 957–967
9. Logothetis, D. E., Jin, T., Lupyan, D., and Rosenhouse-Dantsker, A. (2007) Pflugers Arch. 455, 83–95
10. Logothetis, D. E., Kurachi, Y., Galper, J., Neer, E. J., and Clapham, D. E. (1987) *Nature* 325, 321–326
11. Ito, H., Tung, R. T., Sugimoto, T., Kobayashi, I., Takahashi, K., Katada, T., Ue, M., and Kurachi, Y. (1992) *J. Biol. Chem.* 267, 961–983
12. Reuveny, E., Slesinger, P. A., Inglese, J., Morales, J. M., Iglesias-Lluhi, J. A., Letkowitz, R. J., Bourne, H. R., Jan, Y. N., and Jan, L. Y. (1994) *Nature* 370, 143–146
13. Lesage, F., Guillermare, E., Fink, M., Duprat, F., Heurteaux, C., Fosset, M., Romey, G., Barhanin, J., and Lazdunski, M. (1995) *J. Biol. Chem.* 270, 28660–28667
14. Sui, J. L., Chan, K. W., and Logothetis, D. E. (1996) *J. Gen. Physiol.* 108, 381–391
15. Huang, C. L., Feng, S., and Hilgemann, D. W. (1998) *Nature* 391, 803–806
16. He, C., Yan, X., Zhang, H., Mirshahi, T., and Logothetis, D. E. (1999) *Nat. Cell Biol.* 1, 183–188
17. Clarke, O. B., Caputo, A. T., Hill, A. P., Vandenberg, J. I., Smith, B. J., and Gulbis, J. M. (2010) *Cell* 141, 1018–1029
18. Nishida, M., and MacKinnon, R. (2002) *Cell* 111, 957–965
19. He, C., Yan, X., Zhang, H., Mirshahi, T., Jin, T., Huang, A., and Logothetis, D. E. (2002) *J. Biol. Chem.* 277, 6088–6096
20. Pastler, N. M., Tawil, R., Tristani-Firouzi, M., Canin, S., Bendahhou, S., Tsonoda, A., Donaldson, M. R., Iannaccone, S. T., Brunet, E., Barohn, R., Clark, J., Deymeer, F., George, A. L., Jr., Fish, F. A., Hahn, A., Nitu, A., Ozdemir, C., Serdaroglu, P., Subramony, S. H., Wolfe, G., Fu, Y. H., and Ptacek, L. J. (2001) *Brain* 105, 511–519
21. Lopes, C. M., Zhang, H., Rohacs, T., Jin, T., Yang, J., and Logothetis, D. E. (2002) *Neuron* 34, 933–944
22. Donaldson, M. R., Jensen, J. L., Tristani-Firouzi, M., Tawil, R., Bendahhou, S., Suarez, W. A., Cobo, A. M., Poza, J. J., Behr, E., Wagstaff, J., Szepetowski, P., Pereira, S., Mozaffar, T., Escolar, D. M., Fu, Y. H., and Ptacek, L. J. (2003) *Neurology* 60, 1811–1816
23. Finley, M., Arrabit, C., Fowler, C., Suen, K. F., and Slesinger, P. A. (2004) *J. Physiol.* 555, 643–657
24. He, C., Yan, X., Zhang, H., Mirshahi, T., Jin, T., Huang, A., and Logothetis, D. E. (2002) *J. Biol. Chem.* 277, 6088–6096
25. He, C., Zhang, H., Mirshahi, T., and Logothetis, D. E. (1999) *J. Biol. Chem.* 274, 12517–12524
26. Ho, I. H., and Murrell-Lagnado, R. D. (1999) *J. Biol. Chem.* 274, 8639–8648
27. Ho, I. H., and Murrell-Lagnado, R. D. (1999) *J. Physiol.* 520, 645–651
28. Ito, H., Tung, R. T., Sugimoto, T., Kobayashi, I., Takahashi, K., Katada, T., Ue, M., and Kurachi, Y. (1992) *J. Gen. Physiol.* 99, 961–983
29. Sui, J. L., Chan, K. W., and Logothetis, D. E. (1996) *J. Gen. Physiol.* 108, 381–391
30. Huang, C. L., Feng, S., and Hilgemann, D. W. (1998) *Nature* 391, 803–806
31. Zhang, H., He, C., Yan, X., Mirshahi, T., and Logothetis, D. E. (1999) *Nat. Cell Biol.* 1, 183–188
32. Clarke, O. B., Caputo, A. T., Hill, A. P., Vandenberg, J. I., Smith, B. J., and Gulbis, J. M. (2010) *Cell* 141, 1018–1029
33. Nishida, M., and MacKinnon, R. (2002) *Cell* 111, 957–965
34. He, C., Yan, X., Zhang, H., Mirshahi, T., Jin, T., Huang, A., and Logothetis, D. E. (2002) *J. Biol. Chem.* 277, 6088–6096
35. He, C., Yan, X., Zhang, H., Mirshahi, T., Jin, T., Huang, A., and Logothetis, D. E. (1999) *J. Biol. Chem.* 274, 12517–12524
36. Ho, I. H., and Murrell-Lagnado, R. D. (1999) *J. Biol. Chem.* 274, 8639–8648
37. Ho, I. H., and Murrell-Lagnado, R. D. (1999) *J. Physiol.* 520, 645–651
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