Structural Insights into KChIP4a Modulation of Kv4.3 Inactivation *

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Dynamic inactivation in Kv4 A-type K+ current plays a critical role in regulating neuronal excitability by shaping action potential waveform and duration. Multifunctional auxiliary KChIP1–4 subunits, which share a high homology in their C-terminal core regions, exhibit distinctive modulation of inactivation and surface expression of pore-forming Kv4 subunits. However, the structural differences that underlie the functional diversity of Kv channel-interacting proteins (KChIPs) remain undetermined. Here we have described the crystal structure of KChIP4a at 3.0 Å resolution, which shows distinct N-terminal α-helices that differentiate it from other KChIPs. Biochemical experiments showed that competitive binding of the Kv4.3 N-terminal peptide to the hydrophobic groove of the core of KChIP4a causes the release of the KChIP4a N terminus that suppresses the inactivation of Kv4.3 channels. Electrophysiology experiments confirmed that the first N-terminal α-helix peptide (residues 1–34) of KChIP4a, either by itself or fused to N-terminal truncated Kv4.3, can confer slow inactivation. We propose that N-terminal binding of Kv4.3 to the core of KChIP4a mobilizes the KChIP4a N terminus, which serves as the slow inactivation gate.

A-type inactivation, a rapid process of channel closing, leads to the reduction or elimination of potassium currents that can be dynamically regulated by a variety of intracellular factors. These include neurotransmitters, kinases, second messengers, and β-subunits, resulting in diverse mechanisms for the control of potassium channel activity (1–9).

A-type inactivation was first described as a “ball and chain” model in which an intracellular inactivation particle (the α-ball) on a tether physically occludes the pore (10–12). This “ball” was later identified as the first 20 amino acids of the N terminus of the Shaker channel (hence the name “N-type inactivation”), and the contiguous 40 or more residues constituted the chain (10, 11). The inactivation gate possesses two essential chemical characteristics. The first 10 residues are hydrophobic, and the remaining 10 are hydrophilic. This segment of the chain, which is rich in positive charge, pushes the ball toward its receptor site (13, 14). It has been shown that this N-type inactivation occurs through a sequential reaction of a dynamic nature in which the gate initially binds to the T1 domain surface by electrostatic interactions and then enters through the lateral portals to the inner pore and impairs ion movement (13).

Cytosolic Kv channel-interacting proteins KChIP1–4 (216–256 amino acids) co-assemble with Kv4 α-subunits to form a native complex that encodes somatodendritic A-type K+ current, which plays critical roles in shaping the action potential waveforms (15–19). KChIPs 1–4 all have a conserved C-terminal core region that contains four EF-hand-like calcium binding motifs, but KChIPs3 also have a variable N-terminal region, which has been proposed to induce diverse modulation of Kv4 function (15–23). For instance, KChIP1 increases Kv4 surface expression, speeds up steady-state inactivation with a moderate effect on fast inactivation, and accelerates recovery from inactivation. In contrast, KChIP4a functions as a suppressor of inactivation to eliminate the fast inactivation of Kv4 channels but does not promote surface expression and has no effect on the recovery time constant following inactivation (20, 24–26). It is of interest that KChIP4a can be functionally converted to KChIP1 by truncation of a K-channel inactivation suppressor (KIS) domain that resides in the N terminus (residues 1–34) of KChIP4a. However, the underlying mechanisms by which KChIP4a affects inactivation are not understood (20).

Recent evaluation of the co-crystal structure of the KChIP1/Kv4.3 N terminus has revealed that this complex has a clamping mode, in which a single KChIP1 molecule acting as a monomer laterally clamps two neighboring Kv4.3 N termini in a 4:4 manner, and this interaction involves two contact interfaces (27, 28). At the first interface, the proximal N-terminal peptide of Kv4.3 is sequestered and buried deeply within an elongated groove on the surface of KChIP1. The H10 helix of KChIP1 is pushed out of this groove and is replaced by the Kv4.3 N-ter-

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The atomic coordinates and structure factors (code 3D04) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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minal peptide, while this same KChIP1 molecule binds to another adjacent T1 domain, resulting in a stabilized tetrameric Kv4.3-T1-KChIP1 complex. Identification of this clamping mode whereby KChIP1 interacts with Kv4 has provided structural insight into how KChIP1 regulates gating kinetics and increases Kv4 surface expression and possibly trafficking (27–29). However, this clamping mechanism cannot account for the suppression of Kv4 inactivation by KChIP4a that was reported not to promote surface expression, although it shares 79% identity of amino acids in its C-terminal core domain with KChIP1 (20). The question then arises as to whether this distinct modulation of inactivation of Kv4 channels by KChIP4a without increased current density can be attributed to the differences in the N termini of KChIP4a and KChIP1. Therefore, to gain more information about the structure and molecular mechanism by which KChIP4a modulates Kv4 function, we attempted to visualize the structure of KChIP4a. Subsequently, we investigated the features of this structure and how they serve in the modulation of Kv4 function.

Here we describe the crystal structure of KChIP4a, showing that its N-terminal α-helices have a distinctive fork-like conformation that distinguishes it from KChIP1 and that these helices confer the slow gating of Kv4 channels. Taken together with functional and biochemical data, our findings provide a structural mechanism for KChIP4a modulation of Kv4 inactivation.

EXPERIMENTAL PROCEDURES

Molecular Biology and Materials—Constructs of full-length Mouse KChIP4a (229 amino acids, GenBank™ accession number AF453243) and the N terminus of human Kv4.3 containing amino acids 6–145 were cloned by PCR into pET28a and pMAL-c2 vectors, respectively. For electrophysiology, WT or mutant cDNA constructs of either Kv4.3 or KChIP4a were subcloned into pBluescript KSM (8). cRNAs were transcribed in vitro using the T3 mMESSAGE Machine kit (Ambion) following linearization of the cDNA construct with NotI enzyme. All restriction enzymes were purchased from New England Biolabs, Inc. All mutations of human Kv4.3 and KChIP4a cDNAs were carried out using the QuikChange II mutagenesis kit (Stratagene), and insertion of segments in all clones was rigorously verified by sequencing. All materials not specifically identified were purchased from Sigma.

Two-electrode Voltage Clamp Recording in Xenopus Oocytes—For oocyte expression, Xenopus oocytes (stages V and VI) were selected and injected with 46 nl of solution containing 0.5–4.0 ng of the selected cRNA using a microinjector (Drummond Scientific Co.). One to 2 days after injection, oocytes were impaled with two microelectrodes (0.5–1.0 megohms) filled with 3 M KCl in a 40 μl recording chamber, and whole cell currents were recorded using the two-electrode voltage clamp. The chamber was constantly perfused with ND-96 recording solution containing 96 mM NaCl, 2 mM KCl, 1 mM MgCl2, 1.0 mM CaCl2, and 5 mM HEPES, pH 7.6. Currents were recorded in ND-96 solution at room temperature (22 ± 1 °C) using a GeneClamp 500 amplifier (Axon Instruments) or an OC-725C amplifier (Warner Instruments). Data were acquired using Pulse software (HEKA) and digitized at 1.0 kHz using an ITC-16 (Instrutech Corp.). Data were analyzed using PulseFit, Igor, or Microcal Origin 6.1. Inactivation time constants (τ1 and τ2) were obtained by fitting double exponential functions to the decaying phases of Kv4.3 currents. The inactivation time constant, τ1, was defined as the predominant component of current amplitude. The kinetics of recovery from inactivation were analyzed by curve fitting of current to a single exponential function. All data are expressed as mean ± S.E., and statistical significance was assessed by the t test.

Biochemical Analysis by SEC-FPLC—Fast protein liquid chromatography (FPLC) analysis was performed with size exclusion chromatography (SEC) using a Superdex 200 column and monitored at 280 nm absorbance to determine the protein concentration eluted from the column. The purified protein was loaded on a Superdex 200 HR 10/30 column (Amersham Biosciences), and SEC was then performed on an Amersham Biosciences FPLC system using a buffer (containing 25 mM Tris–HCl, pH 8.0, 150 mM NaCl, 3 mM dithiothreitol) with a flow rate of 0.5 ml/min. Fractions of 0.5 ml from the separation were analyzed by SDS-PAGE with Coomassie staining. Size exclusion calibration standards (Amersham Biosciences) were used to generate a calibration curve by calculating the size of proteins eluted from the column.

In Vitro Pulldown Assay—For protein expression and purification, KChIP4aΔ34 (core of KChIP4a), the KIS domain of KChIP4a (N-terminal residues 1–34), and Kv4.3N (residues 6–145) were cloned into bacterial expression vectors pET28a, pGEX-6p1, and pMAL-c2, respectively, and were expressed in Escherichia coli strain BL21(DE3). Expression of fused proteins was induced with 0.5 mM isopropyl 1-thio-β-D-galactopyranoside for 10 h at 22 °C. The cells were subsequently pelleted and resuspended in buffer (25 mM Tris, pH 8.0, 150 mM NaCl) supplemented with protease inhibitors. After lysis by sonication and centrifuging at 15,000 rpm for 50 min, the clear supernatant was decanted and filtered through a 0.45-μm filter, and the lysate was loaded onto a previously equilibrated 1-ml HiTrap Ni2+ chelating column (Amersham Biosciences), GST4B resin column (GE Healthcare), and an amylose resin column (New England Biolabs) at a rate of 1 ml/min. After washing the column, the bound proteins His-core KChIP4a (KChIP4aΔ34), glutathione S-transferase (GST)-KIS domain, and maltose-binding protein (MBP)-Kv4.3N were eluted for further use.

To test the interaction between the KIS domain and Kv4.3N with the core of KChIP4a (KChIP4aΔ34), ~200 μg of His-core KChIP4a was loaded into nickel resin, and the resin was divided into two parts used for flow-through of either ~120 μg of the GST-KIS domain or ~200 μg of MBP-Kv4.3N. After extensive washing with a buffer containing 25 mM Tris, pH 8.0, 150 mM NaCl, 15 mM imidazole, ~10 μl of resin was loaded on SDS-PAGE and visualized by Coomassie staining. The remaining resin was eluted with buffer containing 25 mM Tris–HCl, pH 8.0, 150 mM NaCl, and 250 mM imidazole, and proteins were then concentrated and further fractionated by SEC-FPLC. To test for competition between the KIS domain of KChIP4a and Kv4.3N (residues 6–145) with the core domain of KChIP4a, ~200 μg of His-core KChIP4a was loaded into nickel resin before flow-through of ~240 μg of the GST-KIS domain into the resin. After extensive washing with buffer containing 25 mM...
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Tris, pH 8.0, 100 mM NaCl, and 15 mM imidazole, the nickel resin was evenly divided into three parts and mixed with different concentrations of MBP–Kv4.3N at 4 °C for 2 h before separating the liquid and solid phases by centrifugation at 500 × g for 5 min. After discarding the supernatant and washing the resin with the same buffer, ~10 μl of resin was loaded on SDS-PAGE and detected by Coomassie staining. The remaining resin from part 2 was eluted with buffer containing 25 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 250 mM imidazole, and proteins were then concentrated and further fractionated by SEC-FPLC.

Structural Biology—For protein expression and purification, full-length mouse WT KChIP4a cDNA (GenBankTM accession number AF453243) was subcloned into pET28a prokaryotic expression vectors (Novagen). KChIP4a proteins were expressed in E. coli strain BL21(DE3). Cells expressing KChIP4a were induced with 1 mM isopropyl 1-thio-D-galactopyranoside for 8 h at room temperature, pelleted, then resuspended in buffer A (50 mM Tris, pH 8.0, 100 mM NaCl, supplemented with 1 tablet per 20 ml of buffer A with complete EDTA-free protease inhibitors (Roche Applied Science) using 5 ml of buffer A per gram of cell pellet. The cells were lysed by sonication and centrifuged at 14,000 rpm for 1 h. The recombinant protein was first purified using nickel-nitritolactric acid-agarose (Qiagen) and then further cleaned by anion exchange (Source-15Q, GE Healthcare) as described previously (27, 30).

Crystalization and Data Collection—Crystals of KChIP4a were obtained at room temperature by hanging drop vapor diffusion in which the well solution consisted of 1.6–1.8 M ammonium sulfate, 4% (v/v) isopropanol, and 0.1 M dithiothreitol, and crystals were flash-frozen in mother liquor supplemented with 20% (v/v) glycerol. Data for these KChIP4a crystals were collected using the program DENZO (31). These crystals belong to space group $I4_1$ and contain two KChIP4a molecules per asymmetric unit.

KChIP4a Structure Determination and Refinement—The KChIP4a crystal structure was determined by molecular replacement (AMORE included in CCP4) using the coordinates of bound KChIP1 (PDB code 2NZ0) as the searching model and refined by position refinement with the program CNS (crystallography and NMR system), version 1.1 (32). The N-terminal part (H0–H2) of KChIP4a was manually built into the electron density with the program O (33). All of the residues except 173–183 were built into the density for KChIP4a. The statistics for this data collection and refinement are listed in Table 1. All structural pictures were drawn in PyMOL.

X-ray Coordinates—The PDB ID code obtained for KChIP4a is 3DD4, and the atomic coordinates have been deposited in the Protein Data Bank.

RESULTS

Overall Structure of KChIP4a with Distinctive N-terminal α-Helices—The full-length mouse KChIP4a protein (229 amino acids) expressed in BL21(DE3) cells was purified to homogeneity and crystallized. The structure was determined by molecular replacement using the coordinates of KChIP1 as the initial searching model (27, 30), and the final model was refined to a crystallographic $R_{factor}$ of 23.9% ($R_{free}$ 29.9%) (Table 1). Overall, KChIP4a is composed of an N-terminal region with six α-helices (H0–H5) and a C-terminal region of five α-helices (H6–H10) (Fig. 1). The N-terminal α-helices (H0–H2) of KChIP4a are positioned in such a way that they cause H10 to swing outward ~45° from the hydrophobic pocket (Fig. 1B). After a 90° rotation of the upper panels of Fig. 1B, the lower panels show the relative positioning between H10 and N-terminal α-helices H0–H2 that occupy the hydrophobic pocket and push the H10 out from the pocket as compared with data concerning the bound and free KChIP1 proteins in previous studies (27, 30).

Compared with KChIP1, substantial structural variations are observed in the three N-terminal α-helices (H0–H2) of KChIP4a (Figs. 1B and 2A, left panels). Except for the N-terminal α-helices (H0–H2), which cause H10 to swing up from the hydrophobic pocket, the core structure of KChIP4a shows good alignment with the bound KChIP1 that sequesters the N terminus of Kv4.3 (Fig. 2A, right panel) (27, 28). However, the most striking difference between KChIP4a and KChIP1 is identifiable around the first 30 N-terminal residues of KChIP4a, which form a long α-helix followed by a short rigid coil. These N-terminal residues bind to a well defined hydrophobic pocket formed by the conserved structural components of KChIP4a (Fig. 2B, left panel). Structural comparison indicates that this hydrophobic pocket of KChIP4a is similar to that of KChIP1 (Fig. 2B, right panel). This hydrophobic pocket has recently been shown to form the same type of binding groove that is recognized by the Kv4.3 N terminus in KChIP1 (27, 28).

| Table 1: Data collection and refinement statistics |
|-----------------------------------------------|
| **Data collection**                           |
| Space group                                    | 141                                      |
| Cell dimensions, $a$, $b$, $c$ (Å)             | 96.30, 96.30, 71.10                      |
| Resolution (Å)                                | 99.0-3.0                                 |
| $R_{merge}$ (outer shell)                      | 5.8% (56.7%)                             |
| $I/(O)$                                        | 37.9                                     |
| Completeness (outer shell)                     | 99.6% (100.0%)                           |
| Redundancy                                     | 8.0                                      |
| **Refinement**                                 |
| Resolution (Å)                                | 20.0-3.0                                 |
| No. reflections                                | 6045                                     |
| No. atoms                                      | 1771                                     |
| Protein                                        | 1750                                     |
| Ca$^2+$                                        | 2                                        |
| Water                                          | 19                                       |
| $R_{merge}/R_{free}$                           | 0.239/0.299                              |
| r.m.s.d. bond length (Å)                       | 0.008                                    |
| r.m.s.d. bond angles (degree)                  | 1.210                                    |

$R_{merge} = \frac{\sum_{i} \sum_{h} h \mathbf{i} \mathbf{i}_{h}}{\sum_{i} \sum_{h} \mathbf{i}_{h}}, \text{where } \mathbf{i}_{h} \text{ is the mean intensity of } i \text{ observations of symmetry-related reflections of } h, r = 2(F_{\text{calc}} - F_{\text{obs}})/F_{\text{obs}} \text{ where } F_{\text{obs}} = F_{\text{v}}$ and $F_{\text{calc}}$ is the calculated protein structure factor from the atomic model ($R_{merge}$ was calculated with 5.5% of the reflections). Root mean square deviations (r.m.s.d.) in bond length and angles are the deviations from ideal values.
Release of N-terminal KChIP4a Bound to the Hydrophobic Groove upon Competitive Binding of Kv4.3N—Structural analysis of KChIP4a indicates that the N-terminal H0 (previously called the KIS domain) of KChIP4a binds to the hydrophobic groove shown to sequester the Kv4.3 N terminus in a recent description of the Kv4.3N-KChIP1 structural complex (27, 30). These structural observations suggest that the N termini of both Kv4.3 and KChIP4a can interact competitively with the same KChIP4a hydrophobic pocket (Fig. 2, A, left panel, and B, right panel). We therefore hypothesized that the N-terminal α-helix (H0) of KChIP4a would be released by competitive binding of the Kv4.3 N terminus to the hydrophobic groove. To test this theory, we first purified residues 6–145 of the N-terminal peptide of Kv4.3 (Kv4.3N) and residues 1–34 of the N-terminal peptide of KChIP4a. The interaction between these two peptides with the core of KChIP4a was then evaluated, employing KChIP4a after deletion of N-terminal residues 2–34 (KChIP4aΔ34), which exposes the hydrophobic groove. Fig. 3 demonstrates competition between the Kv4.3 N-terminal peptide (MBP-tagged) and the KIS domain (GST-tagged) with core KChIP4a (His-tagged) proteins by use of an in vitro pulldown assay. For negative controls (Fig. 3A) neither tagged MBP-Kv4.3N nor GST-KIS proteins bound to the nickel resin. Moreover, MBP-Kv4.3N showed no binding to GST-KIS (which was bound to GST-resin), nor did GST-KIS bind to MBP-Kv4.3N (bound to amylose resin) (Fig. 3A).

In contrast, Fig. 3B shows that both Kv4.3N (MBP-tagged) and KIS (GST-tagged) peptides can individually associate with core KChIP4a (His-tagged) and form a complex. To further test competition between Kv4.3N and KIS for binding to the hydrophobic groove of the KChIP4a core, the concentration of Kv4.3N peptide was increased. With increasing concentrations of Kv4.3N, the KIS peptide bound in the complex was released in a concentration-dependent manner (Fig. 3C), indicating that these two peptides competed for the

Contrast, no hydrogen or salt bond is observed between the first α-helix and its binding groove (Fig. 2B, left panel). Another striking difference in KChIP4a as compared with KChIP1 occurs around α-helix 3 (H2). In this locus residues in H2 are rearranged into one long single α-helix that nearly parallels the first α-helix, H0, and this helix is packed against one side of the groove (Fig. 2A, left panel).
**KChIP4a Modulation of Kv4.3**

**N-terminal Peptide of KChIP4a Confers Slow Inactivation Gating—**

The biochemical results of competition between Kv4N and the KIS domain for binding to the core of KChIP4a suggested that the interaction of Kv4.3 may free the N terminus of KChIP4a, which consequently functions as an inactivation suppressor (20). If this hypothesis is correct, an isolated KIS domain would mimic the function of KChIP4a in the modulation of Kv4.3. To investigate this possibility, we synthesized the N-terminal peptide (residues 1–34) of KChIP4a (Fig. 4A). Injection of the N-terminal 34 peptides of KChIP4a into oocytes expressing Kv4.3 resulted in a time-dependent slow inactivation and elimination of fast inactivation within a 30-min interval, similar to the effect observed with co-expression of WT KChIP4a on Kv4.3 inactivation (Fig. 4, B, left panel, and C). In contrast, injection of a scrambled control peptide had no effect on inactivation of Kv4.3 within the same period of time (Fig. 4, B, right panel, and C, right panel).

To further confirm the function of N-terminal KChIP4a in modulating inactivation, we then constructed a KIS-Kv4.3Δ24 chimera in which the N-terminal residues 2–24 of Kv4.3 (Kv4.3Δ24) were replaced with the N-terminal KIS domain of KChIP4a. The latter consisted of residues 2–34 (KIS), as indicated in Fig. 4A. Deletion of Kv4.3 N-terminal residues 2–24 yielded functional currents with a moderately slow inactivation (Fig. 4D, top panel), consistent with previous observations (30, 34). In contrast, the chimera (KIS-Kv4.3Δ24) resulted in Kv4.3 currents with inactivation that was slower as compared with either the N-terminal deletion mutant of Kv4.3Δ24 or co-expression of Kv4.3-WT KChIP4a. This suggested that the N-terminal KChIP4a indeed functions as a separate slow inactivation gate (Fig. 4D, middle and bottom panels). Taken together, these results indicate that the N terminus of KChIP4a directly affects inactivation of the channel and thus may function as an inactivation suppressor that may confer the slow gating of Kv4.3 channels.

**DISCUSSION**

Inactivation of voltage-gated potassium channels is a key process of channel function. Dynamic inactivation of Kv4 A-type K⁺ current is critical in regulating neuronal excitability by shaping action potential waveform and duration. KChIP1–4 are integral components of native A-type Kv4 channel com-

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**FIGURE 2. Structural alignment between KChIP4a and bound KChIP1 proteins.** A, structural alignment between KChIP4a (light orange) and bound KChIP1 (light pink; PDB code 2NZ0) proteins, with the Kv4.3 proximal N terminus (green; residues 6–21) docked in the hydrophobic pocket. The orientation and α-helix labeling of KChIP4a is same as in the upper panel of Fig. 18. The right panel shows alignment between core KChIP4a (without α-helices H0–H2) and bound KChIP1, where H1 and H2 are labeled revealing the hydrophobic cavity, which is conserved in both KChIP4a and KChIP1. B, common hydrophobic binding groove recognized by both the KIS domain and the Kv4.3 N terminus. Surface map of the KChIP4a structure reveals the KIS domain (N-terminal first α-helix), highlighted in deep teal, bound to the hydrophobic groove (left panel) and models the Kv4.3 proximal N terminus (residues 6–21), highlighted in green, binding to the same hydrophobic pocket of core KChIP4a where N-terminal residues 1–34 were removed for clearer visualization of the pocket (right panel). All left and right panels have the same color coding with some secondary structural elements specifically labeled, such as residues of Val-11, Leu-12, Val-14, Ile-15, Phe-18, Val-19, Leu-21, and Phe-25 for the KIS domain (left panel) and Trp-8, Leu-9, Pro-10, Phe-11, Ala-12, Ala-14, Ala-16, Ile-17, and Pro-21 for Kv4.3 N terminus (right panel).
plexes and have distinct modulation effects on the inactivation and current density of Kv4 channels. Moreover, this family of multitasking auxiliary subunits has great influence on somatodendritic excitability. We were particularly intrigued by the fact that KChIP4a causes slow gating of Kv4.3 and lacks the capacity to increase surface expression of the channel (20, 35), yet its C-terminal core region shows a significant degree of homology with other KChIPs 1–3 that speed up steady-state inactivation and increase current density (15, 25, 29, 30, 36–39).

In this study, we attempted to address the molecular mechanism by which auxiliary KChIP4a regulates the slow gating of Kv4.3. We resolved the crystal structure of KChIP4a, for the first time, and found unique N-terminal α-helices that stand out in a fork-like conformation. These helices differentiate it from the known structure of KChIP1. The findings in this study offer two important insights into the structural implications of modulation of Kv4 inactivation by KChIP4a. First, the structural features of KChIP4a reveal how its distinctive N terminus distinguishes it from the known structure of KChIP1, despite the fact that the core structures of both KChIP1 and KChIP4a show a significant overlap because of the high homology of their protein sequences. The N-terminal α-helices (H0–H2) of KChIP4a are positioned in such a way that they bind to the hydrophobic groove and induce an outward swing of H10; this is the same conformational change that is seen in bound KChIP1, which harbors the Kv4.3 N terminus (27, 28). The first α-helix (H0) of KChIP4a, also known as the KIS domain, is an elongate helical structure that is sequestered by a well defined hydrophobic pocket. This pocket was recently shown to dock the proximal N terminus of Kv4.3 (27, 28). This N-terminal
were successfully used in our recent crystallization of the complex KChIP1-Kv4.3N. This attempt was not successful, and the reason is unclear. We speculate that because of its hydrophobic nature and the structural features of the KChIP4a N terminus, KChIP4a may not be stable in solution and may have multiple forms. Nevertheless, based on our present findings, we propose that KChIP4a functions as a monomer.

The second insight into the structural implications of KChIP4a in this study regards the mechanism of slow inactivation induced by KChIP4a. Upon binding to Kv4, the N-terminal of KChIP4a mobilizes and suppresses the inactivation of Kv4 inactivation, leading to a conversion from fast inactivating to otherwise non-inactivating channels. We envision that the slow inactivation induced by KChIP4a is not caused by antagonizing the inactivation ball of the Kv4 channel but, rather, by the N-terminal of KChIP4a acting independently to modulate Kv4 inactivation. However, the exact mechanism of how N-terminal KChIP4a affects Kv4 inactivation, and specifically where the KChIP4a N terminus binds, remain unclear and need further investigation. Moreover, the mechanism by which Kv4 channels inactivate is a complex issue that has not been well understood. This is in part because these channels do not typically operate via classical inactivation mechanisms such as N- and P/C-type inactivation, which has been demonstrated in Shaker or other Kv channels (34, 40–42). It has been reported that deletion of the Kv4 N terminus, which induces fast inactivation presumably by pore occlusion, results in slower inactivation (30, 34). Sequestering the proximal N terminus of Kv4, KChIPs also gives rise to slow inactivation of the channel (27, 42). All of these findings suggest that inactivation of Kv4 channels is also likely to be mediated by other mechanisms such as closed-state inactivation (43). In this study, however, the chimera in which we replaced the proximal N terminus with N-terminal KChIP4a still retained slow modulation, and the resultant phenotype was similar to that found with co-expression of Kv4 and KChIP4a. These findings suggested that the KChIP4a N terminus may act independently to confer
A slow inactivation gate for direct modulation of Kv4 inactivation. Although further investigation is required, it is tempting to hypothesize that the KChIP4a N terminus acts as an inactivation suppressor that can function as a separate slow gate and that may interact with residues around the inner vestibule of the channel pore (13, 20, 40, 44). Based on our present findings, we propose that the N terminus of KChIP4a is released upon binding of the Kv4 N terminus to the hydrophobic groove of KChIP4a. This in turn allows binding of the freed N terminus of KChIP4a to its receptor site(s) close to the inner pore of the Kv4 channel to slow inactivation, as proposed previously (13). Moreover, dynamic association of Kv4 with auxiliary KChIPs 1–4, which modulate inactivation kinetics, could be an important mechanism by which membrane excitability is regulated in native neurons.

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