cAMP-dependent Protein Kinase Phosphorylation Produces Interdomain Movement in SUR2B Leading to Activation of the Vascular K\textsubscript{ATP} Channel\textsuperscript{*}\textsuperscript{§}

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Vascular ATP-sensitive K\textsuperscript{+} channels are activated by multiple vasodilating hormones and neurotransmitters via PKA. A critical PKA phosphorylation site (Ser-1387) is found in the second nucleotide-binding domain (NBD\textsubscript{2}) of the SUR2B subunit. To understand how phosphorylation at Ser-1387 leads to changes in channel activity, we modeled the SUR2B using a newly crystallized ABC protein SAV1866. The model showed that Ser-1387 was located on the interface of NBD2 with TMD1 and physically interacted with Tyr-506 in TMD1. A positively charged residue (Arg-1462) in NBD2 was revealed in the close vicinity of Ser-1387. Mutation of either of these three residues abolished PKA-dependent channel activation. Molecular dynamics simulations suggested that Ser-1387, Tyr-506, and Arg-1462 formed a compact triad upon Ser-1387 phosphorylation, leading to reshaping of the NBD2 interface and movements of NBD2 and TMD1. Restriction of the interdomain movements by engineering a disulfide bond between TMD1 and NBD2 prevented the channel activation in a redox-dependent manner. Thus, a channel-gating mechanism is suggested through enhancing the NBD-TMD coupling efficiency following Ser-1387 phosphorylation, which is shared by multiple vasodilators.

ATP-sensitive K\textsuperscript{+} (K\textsubscript{ATP}) channels play an important role in vascular tone regulation (1–3). The K\textsubscript{ATP} channels are expressed in vascular smooth muscles (VSMs) and activated by several vasodilating hormones and neurotransmitters through the G\textsubscript{i}-cAMP-PKA signaling system (1, 4, 5). The channel activation leads to hyperpolarization of VSM cells, decrease in voltage-dependent Ca\textsuperscript{2+} channel activity, and relaxation of resistant arteries (6).

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\textsuperscript{4}The abbreviations used are: K\textsubscript{ATP}, ATP-sensitive K\textsuperscript{+} channel; PKA, cAMP-dependent kinase; MD, molecular dynamics; wt, wild type; NBD, nucleotide-binding domain; TMD, transmembrane domain; ICL, intracellular linkers.

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\textsuperscript{7}K\textsubscript{ATP} channels consist of 4 pore-forming Kir6x and 4 regulatory SURx subunits (7). The Kir6.1/SUR2B channel is the major isoform of K\textsubscript{ATP} channels in VSMs (8–11). The channel does not open spontaneously at rest. Several groups of channel openers activate the channel, including pharmacological K\textsubscript{ATP} channel openers (KCOs, e.g. pinacidil and nicorandil) (12), metabolites (MgADP, acodisic) (13, 14), and hormonal vasodilators and neurotransmitters (calcitonin gene-related peptide, epoxyeicosatrienoic acids, \textbeta-adrenergic receptor agonists, and vasoactive intestinal polypeptide) (5, 15–17). KCOs and Mg\textsuperscript{2+} nucleotides activate the K\textsubscript{ATP} channels via binding to the SUR subunits (12, 18). The hormonal vasodilators activate the vascular K\textsubscript{ATP} channel through direct phosphorylation of the channel protein by PKA (4, 5). Our recent study has shown that Ser-1387 is a key phosphorylation site (5).

It is unclear how phosphorylation at the Ser-1387 residue in SUR2B leads to channel activation. SURs belong to the ABC transporter protein family (19). All ABC proteins have an essential domain assembly, i.e. two transmembrane domains (TMD1 and TMD2) and two intracellular nucleotide-binding domains (NBD1 and NBD2). In addition, SURs have another transmembrane domain containing 5 helical segments termed TMD0 (Fig. 1A). The cytosolic NBDs of several ABC proteins have been crystallized (20–23). They all show similar sandwich-like structures with nucleotides clamped between two NBDs. Several NBD motifs, such as Walker A (13, 24), Walker B (25–27), and signature sequences (28) are known to be important for nucleotide binding. How the channel is activated with nucleotide binding, however, is still not fully understood. Experimental evidence suggests that TMDs play a major role in interacting with Kir6 subunits (29). Such interaction has been observed in recombinant Kir6.2/SUR1 channels using electron microscopy (30). Moreover, the TMD0 and ICL0 are involved in K\textsubscript{ATP} gating by channel modulators and nucleotides (31, 32). Therefore, signals of NBDs must be coupled to TMDs to fulfill channel gating.

Several ABC proteins have been crystallized in full-length with TMDs (33–36). These include the recently crystallized SAV1866 that is a homologue of mammalian ABC proteins (36) and shows the highest sequence similarity to SUR2B. Using the SAV1866 crystal structure, we modeled the core of SUR2B (SUR2B_core) containing TMD1, TMD2, NBD1, and NBD2 with particular attention to conformational changes after Ser-1387 phosphorylation. Our combined studies of modeling, molecular dynamics simulations, functional...
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**FIGURE 1.** SUR2B\_core model. A, skeletal representation of the K\textsubscript{ATP} channel. Kir6.1 (left) has two transmembrane helices (TMHs) with both N and C termini located in the cytosol. SUR2B has 17 TMHs, forming three transmembrane domains (TMD0, TMD1, and TMD2) in a 5-6-6 pattern. The N terminus is located extracellularly and the C terminus intracellularly. The NBD1 is located between TMD1 and TMD2, and NBD2 lies in the C terminus. The ICLs between TMHs are indicated. The SUR2B\_core includes sequences from TMD1 to the C terminus. The region between residues 286 (TM0-NBD1) and 976 (TM-NBD2) were aligned to SAV1866 and used to generate the SUR2B\_core model. A few residues between a2 and a3 were excluded in the SUR2B\_core model as they do not have homology with SAV1866. B, overall structure of SUR2B\_core. TMD1 (residues 286–617, green) was linked with NBD1 (residues 666–913, blue) with 38 residues (loop in gray). TMD2 (residues 976–1289, red) was linked with NBD2 (residues 1310–1546, purple) with 20 residues. The linker regions between TMHs are indicated. The SUR2B\_core includes sequences from TMD1 to the C terminus. The region between residues 286 (arrow a1) and 914 (a2) was designated TM-NBD1. The residues between 976 (a3) and 1546 (C terminus) were designated TM-NBD2. TMD1 and TM-NBD2 were aligned to SAV1866 and used to generate the SUR2B\_core model. A few residues between a2 and a3 were excluded in the SUR2B\_core model as they do not have homology with SAV1866. Overall structure of SUR2B\_core. TMD1 (residues 286–617, green) was linked with NBD1 (residues 666–913, blue) with 38 residues (loop in gray). TMD2 (residues 976–1289, red) was linked with NBD2 (residues 1310–1546, purple) with 20 residues. The linker regions between TMHs are indicated. The SUR2B\_core includes sequences from TMD1 to the C terminus. The region between residues 286 (arrow a1) and 914 (a2) was designated TM-NBD1. The residues between 976 (a3) and 1546 (C terminus) were designated TM-NBD2. TMD1 and TM-NBD2 were aligned to SAV1866 and used to generate the SUR2B\_core model. A few residues between a2 and a3 were excluded in the SUR2B\_core model as they do not have homology with SAV1866. C, overall structure of SUR2B\_core. ATP was inserted into a groove formed by the SUR2B\_core model. ADP was inserted into the nucleotide-binding regions, and the C-loop inserted into NBD1. D, details of the interaction of NBD2 and NBD2. NBD2 formed a big groove to host ICL2, in which β5 formed the bottom, β6, a loop between a3 and a4 (named the C-loop because a cysteine residue, Cys-1408 was located in this segment) formed the walls. The phosphorylation site Ser-1387 was located in the β5-sheet and is shown in cyan.

The model also indicates that the NBD-TMD interaction in SURs can be well represented by the SAV1866 structure.

**MATERIALS AND METHODS**

Modeling of the SUR2B\_core was based on the crystal structure of SAV1866 (PDB: 2HYD). The sequences of SAV1866 were aligned with both halves of the SUR2B\_core (TM-NBD1 and TM-NBD2) using ClustalW. SAV1866 was 21.5% identical to TM-NBD1 of SUR2B (46.5% similarities) and 21.3% to TM-NBD2 (47.8% similarities). The NBDs were highly conserved: 28.7% identical in NBD1 and 33.5% identical in NBD2. SAV1866\_TMD were 16.0 and 12.8% identical to SUR2B\_TMD1 and TMD2, respectively (~35% when amino acids with similar side chains were considered). Although the homology in amino acid sequences of TMDs is low, they can be aligned using the conserved topology within each TMD, i.e. six transmembrane helices (determined by hydrophobicity of the residues) in each domain with similar lengths. The sizes and positions of extracellular and intracellular linker regions were also comparable. There were only a few short gaps in the alignment (4 in TMD1 and 2 in TMD2, supplemental Fig. S1). The atomic coordinates of amino acids, nucleotides, and water molecules in the template (SAV1866) were transferred to the SUR2B\_core model. The ADP molecule in the first nucleotide-binding pocket was replaced with ATP. The coordinates of ATP-binding Mg\textsuperscript{2+} and ADP-binding Mg\textsuperscript{2+} were obtained using the crystal structures of the ABC transporters HlyB (1XEF) and TAP1 (1JF7), respectively. The linker regions between TMD1 and NBD1 (residues 618–665), and between TMD2 and NBD2 (residues 1290–1309) were modeled as surface loops in the structure. The linker between NBD1 and TMD2 (residues 914–975) was omitted from the model because of the lack of a template. Energy minimization was performed using 1000 steps of conjugate gradient optimization with the latest AMMP potential set (version tuna) (37). The model was viewed with PyMOL.
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Molecular dynamics (MD) simulations were carried out to study the conformational change in the NBD-ICL2 interface. The SUR2B_core model contained many hydrophobic transmembrane segments that would require a very large molecular dynamics simulation including the lipid bilayer. Therefore, we constructed two models containing NBDs and an extended segment of ICL2 (residues 500–512) with and without a phosphate group linked to atom $O_5$ of Ser-1387. 7500 H$_2$O were added to the model molecules to ensure an aqueous environment. Additional ions were added to neutralize the total charge of the protein. No screening dielectric term or bulk solvent correction was included. A constant dielectric of one was used. The atom $O_5$ in the Ser-1387 side chain was 3.6 Å from atom $O_3$ of Tyr-506, and −8 Å from the positive charges of Arg-1462 (the middle of the two Nh atoms). B, forskolin (FSK, 10 μM) activated whole cell currents of Kir6.1/SUR2B, which were further activated by pinacidil (Pin, 10 μM) and inhibited by glibenclamide (Glib, 10 μM) (see supplemental Fig. S2 for details). C, channel with SUR2B_R1462A mutation failed to be activated by forskolin. D, SUR2B_Y506A mutant greatly diminished the channel activation by forskolin. E, summary of forskolin effects on wt and mutant Kir6.1/SUR2B channels. Note the Y506F mutant was not activated, and the R1462K mutant was activated by forskolin.

FIGURE 2. Ser-1387 and surrounding sites in PKA phosphorylation. A, Ser-1387 was close to Tyr-506 and Arg-1462. The atom $O_5$ in the Ser-1387 side chain was 3.6 Å from atom $O_3$ of Tyr-506, and −8 Å from the positive charges of Arg-1462 (the middle of the two Nh atoms). B, forskolin (FSK, 10 μM) activated whole cell currents of Kir6.1/SUR2B, which were further activated by pinacidil (Pin, 10 μM) and inhibited by glibenclamide (Glib, 10 μM) (supplemental Fig. S2 for details). C, channel with SUR2B_R1462A mutation failed to be activated by forskolin. D, SUR2B_Y506A mutant greatly diminished the channel activation by forskolin. E, summary of forskolin effects on wt and mutant Kir6.1/SUR2B channels. Note the Y506F mutant was not activated, and the R1462K mutant was activated by forskolin.

Data are presented as means ± S.E. Differences in means were tested with the analysis of variance or Student’s $t$ test and accepted as significant if $p \leq 0.05$.

RESULTS

Modeling of the SUR2B_core—The dimerized SAV1866 was crystallized in the ADP-binding form (PDB: 2hyd). Based on this structure, we built a model of the SUR2B_core in which sequence identities to SAV1866 are 21.5% for the TM-NBD1 of SUR2B (46.5% similarities), and 21.3% for TM-NBD2 (47.8% similarities) (supplemental Fig. S1). NBD1 and NBD2 formed a tight heterodimer (Fig. 1C). There were two nucleotide-binding pockets on the interface between NBD1 and NBD2. A MgATP molecule was incorporated in the first nucleotide-binding pocket and a MgADP in the second pocket. Different nucleotides were used, because the first pocket was likely to be a high affinity ATP-binding site, and the second was likely to bind to MgADP (18, 40). In the SUR2B_core model, TMDs interact with NBDs via four short helices of intracellular linkers (ICLs, Fig. 1C). ICL1 and ICL3 interacted with the NBD surface across the nucleotide-binding regions. ICL2 from TMD1 inserted deeply into NBD2, while ICL4 from TMD2 inserted into NBD1 (Fig. 1C). NBD2 formed a spindle-like groove where a β-sheet (β5) formed the bottom while the Q-loop, C-loop, and several α-helices (α2, α3, and α6) lined the wall. Ser-1387 was located on β5 within the reach of ICL2 (<4 Å, Fig. 1D). The side-chain of an aromatic residue (Tyr-506) in ICL2 was inserted deeply into the NBD2 groove and was close to Ser-1387 in ~4 Å (Fig. 2A). Around Ser-1387 there was a positively charged residue (Arg-1462) in NBD2 at a distance that allows electrostatic interaction between them when Ser-1387 is phosphorylated (Fig. 2A).

Activation of the Kir6.1/SUR2B Channel by Protein Kinase A—In our previous studies (5, 17), we found that β-adrenergic receptor agonists and vasoactive intestinal polypeptide activated vascular $K_{ATP}$ channels in the rat mesenteric artery, a response that could be reproduced in recombinant Kir6.1/SUR2B channels expressed in HEK293 cells. We thereby chose to use the recombinant Kir6.1/SUR2B in our current study because the expression system allowed us to manipulate the channel protein. Also because PKA is a common signal molecule by which multiple hormonal vasodilators activate the channel, we used forskolin (10 μM) to investigate the PKA effects. Kir6.1 and SUR2B were expressed in HEK293 cells. Forskolin was applied to the cell when the baseline currents were stabilized in 4–6 min. The exposure to forskolin activated K$^+$ currents that were sensitive to both pinacidil and glibenclamide (Fig. 2B; supplemental Fig. S2). After currents were normalized...
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between maximum channel inhibition by 10 $\mu$M glibenclamide and maximum activation by 10 $\mu$M pinacidil, the baseline currents averaged 6.0 $\pm$ 1.9% ($n$ = 10) of the maximum channel activity. Forskolin (10 $\mu$M) increased the currents to 38.5 $\pm$ 3.0% ($n$ = 10, Fig. 2E).

Elimination of PKA Activation by Mutation at Arg-1462 or Tyr-506—In the SUR2B_core model, a positively charged residue Arg-1462 was located in the close vicinity of Ser-1387. The distance of charge of Arg-1462 (measured at the center of two N$_h$ atoms) to the Ser-1387 side chain (O$_g$) was $\sim$8 Å (Fig. 2A). Such a distance may allow an electronic attraction when Ser-1387 is phosphorylated. The mutation of Arg-1462 to alanine indeed eliminated the forskolin-induced channel activation (Fig. 2C). Based on our model prediction, a positive charge at this site is important. This prediction was verified by mutating the Arg-1462 to lysine, and the R1462K mutant remained strongly activated by forskolin (68.5 $\pm$ 5.8%, $n$ = 5). The Arg-1462 is located in a consensus PKA site (RKSS), although neither Ser-1464 nor Ser-1465 is a functional PKA phosphorylation site (5). Thus, a positively charged residue critical for the PKA-dependent channel activation is identified in the SUR2B_core model, although it is far from Ser-1387 in the primary sequence.

An aromatic residue, Tyr-506, was found on the ICL2 segment, the side chain of which dipped deeply into NBD2 and physically interacted with Ser-1387 (Fig. 2A). When the Tyr-506 was mutated to alanine, the forskolin-induced channel activation was almost completely abolished (Fig. 2D). A phenylalanine residue is found at the corresponding site in SAV1866 and ICL4 of SUR2B (supplemental Fig. S1). Phenylalanine has a similar side chain as tyrosine but lacks a hydroxyl group. Interestingly, when Tyr-506 was mutated to phenylalanine, the channel failed to be activated by forskolin (Fig. 2E), indicating that the hydroxyl group of Tyr-506 residue is required for channel activation.

Formation of the Ser-1387/Arg-1462/Tyr-506 Triad Following PKA Phosphorylation—Our modeling suggests that PKA phosphorylation at Ser-1387 may induce local conformational changes. To understand these changes, we used MD simulations to reveal changes in residues and the peptide backbone. Our SUR2B_core contained many hydrophobic transmembrane segments that would necessitate time-consuming simulations in the lipid bilayer environment. We therefore constructed two additional models consisting of NBDs and the ICL2 helix with and without Ser-1387 phosphorylation. MD simulations were undertaken at 310 K and 1 atm. After 1000 runs, ICL2 still stayed in the NBD2 groove, suggesting a strong interaction between ICL2 and NBD2. The side chain distances among the three critical residues were monitored (Fig. 3, A and B). Without phosphorylation, the average distance between Tyr-506 and Ser-1387 was 3.8 Å, a distance that allows hydrogen bond formation between these two residues. The Tyr-506/Arg-1462 and Ser-1387/Arg-1462 distances were around 6–7 Å (Fig. 3, A and C), suggesting that there is no direct contact between Arg-1462 and the other two residues before Ser-1387 phosphorylation. When the Ser-387 was phosphorylated, distances of all three were less than 4 Å (Fig. 3C), indicating formation of a compact triad (Fig. 3, D and E). The hydroxyl group of Tyr-506 appeared to be critical for stabilization of the triad, which was able to form hydrogen bonds with oxygen atoms of the phosphate group of pSer-1387 and N$_h$ atoms in Arg-1462, consistent with our observation that forskolin failed to activate the Y506F mutant (Fig. 2E).

The Movement of ICL2—Following phosphorylation, the Tyr-506 C$_\alpha$ atoms moved by 9.2 Å in the mode structures. The movement led to a horizontal shift of ICL2 from a position close to the center of NBD2 toward the edge of NBD2 along the NBD2 groove. Because the MD data were acquired after removing most TMDs, the restriction of ICL2 by TMDs was not evaluated. Therefore, this $\sim$10 Å movement might be overestimated. However, the direction appeared reasonable. The Arg-1462 was located at the end of the $\alpha$6-helix, close to the edge of NBD2, while Ser-1387 was located on the bottom of the NBD2 groove. With the triad formation, ICL2 was pulled toward NBD2 with lateral sliding along the groove. Meanwhile, the $\alpha$2-helix in NBD2 made a lateral move and left space for ICL2 (Fig. 4C).

Reshaping of the NBD2 Groove by Phosphorylation—Studying the conformations of NBD2 of pre- versus post-phosphoryl-
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C-terminal-half (tail) of $\alpha_6$ (residues 1455–1462) but not the N-terminal-half (head) (residues 1448–1454). The average movement of $C_{\alpha}s$ in the tail was $4.2 \pm 0.3$ Å, much greater than the head ($2.2 \pm 0.3$ Å). Consequently, the $\alpha_6$-helix was slightly bent around Phe-1454 and turned toward ICL2 with an angle $-10^\circ$. The movement of the $\alpha_6$-helix appeared to cause the $\alpha_3$-helix to move toward ICL2 about $4.2 \pm 0.7$ Å. Similar to the head of the $\alpha_6$-helix, the Q-loop was quite stable ($2.4 \pm 0.3$ Å). The $C_{\alpha}s$ in the $\beta_5$-sheet moved toward ICL2 with a distance of $3.0 \pm 0.4$ Å. The $\beta_5$ movement was greater than the overall movements of $\beta$-sheets in NBD2 ($2.3 \pm 0.2$ Å, $p = 0.11$) and the corresponding segment in NBD1 ($2.4 \pm 0.2$ Å, $p = 0.07$). The movement of these segments toward ICL2 suggested a stronger interaction between ICL2 and NBD2 following phosphorylation. In the model, we also observed a large movement ($6.9 \pm 0.3$ Å) of the $\alpha_2$-helix. The $\alpha_2$-helix appeared flexible in the SUR2B_core model; it moved toward the edge of NBD2 and yielded space for ICL2.

To make sure that these movements were not random events, the MD simulations were repeated twice for a total of three times with different starting velocities. We observed the same triad formation and protein domain movements in every run. Therefore, even though there may be technical limitations in the MD simulations, we were able to conclude that ICL2 interacted with NBD2 rather loosely before phosphorylation, and the interaction of the two domains was strengthened with the Tyr-506/pSer-1387/Arg-1462 triad formation after phosphorylation.

Restriction of the Interdomain Movement by an Introduced Disulfide Bond—Mutations were engineered in the channel to test the hypothesis that interdomain motion plays a critical role in PKA-dependent channel activation. Forming a disulfide bond between the two domains can restrict the motion thus limiting the channel activation if the motion is critical. Examining all residues in the NBD2 groove and ICL2, we found that a cysteine (Cys-1408) on the wall of the NBD2 groove was close to Ala-507 in ICL2 (Fig. 6A). The distance between the $C_{\alpha}s$ of the two residues was $-8$ Å. When the Ala-507 was mutated to cysteine, the channel activation by forskolin was completely eliminated (Fig. 6B). Because the Ala-507 is located next to Tyr-506, the mutation itself instead of the disulfide bond formation could have affected the channel activation. To address this possibility, we included 3 mM reduced glutathione in the pipette solution and found that the Kir6.1/SUR2B_A507C channel activation by forskolin was nicely restored (29.7 ± 3.0%), $n = 5$, Fig. 6C). To show whether the introduced Cys-507 indeed formed a disulfide bond with Cys-1408, but not with another unidentified cysteine residue, we mutated Cys-1408 to serine, a residue close to cysteine in side chain properties but unable to form disulfide bonds. The mutant channel (Kir6.1/SUR2B_A507C_C1408S) was activated by forskolin to the same extent as the wild-type channel (Fig. 6, D and E). Thus, the formation of an artificial disulfide bond between residues 507 and 1408 locked the channel in the pre-phosphorylation state and disrupted the channel activation by forskolin, supporting the hypothesis that a relative movement between ICL2 and NBD2 is necessary for channel activation by PKA. We also tried

FIGURE 4. The movement of ICL2 following phosphorylation. All panels represented the mode structures of the MD simulations. A–C, top views of ICL2 and NBD2 in pre-phosphorylation, post-phosphorylation, and overlay, respectively. The pre-phosphorylation structure was shown in the same set of colors as Fig. 1. In the post-phosphorylation structure, NBD2 was colored red and ICL2 was colored cyan. Compared with the mode structure of the pre-phosphorylated form, ICL2 helix moved horizontally away from the center of NBDs by $8.4 \pm 0.4$ Å (measured at $C_{\alpha}s$ of residues 500–506) after phosphorylation. Accordingly, the $\alpha_2$-helix in NBD2 moved to yield space for ICL2. The arrows in C indicate the direction of movements of ICL2 and the $\alpha_2$-helix.
DISCUSSION

Modeling SUR2B_Core with SAV1866—The regulatory sub-units SURs (ABCC8 and ABCC9) of K\textsubscript{ATP} channels belong to the ABC transporter superfamily. Four ABC proteins have been crystallized in full-length with TMDs and NBDs. The NBDs are similar to each other in their overall structures and assemblies. The TMDs however, are quite different. There are 10 TMHs in each TMD in HI1470/1 (35) and BtuCD (34), while there are 6 in each TMD in SAV1866 (36) and ModBC (33). The TMD in ModBC is much shorter than that in SUR2B, and no evident sequence homology can be found between them. In contrast, the TMD topology of SAV1866 is the same as that of SUR2B, suggesting the validity of modeling the SUR2B_core based on the SAV1866 structural template.

In our SUR2B_core model, NBD1 and NBD2 dimerize to form two nucleotide-binding pockets on their interface, consistent with the models of SUR1-NBDs in previous studies (41, 42). A significant development of our model is to include TMDs and to show the interaction between TMDs and NBDs. SUR2B_TMD1 interacts with NBDs via two short segments ICL1 and ICL2. ICL1 interacts with both NBDs at their borders around the first nucleotide-binding region, while ICL2 is inserted deeply into NBD2. Therefore, the TMD1 mainly interacts with the NBD2. Similar cross-interaction is found in TMD2-NBD1. Our disulfide bond experi-

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Phosphorylation of Ser-1387 leads to a change in the NBD2 conformation and strengthens the interaction of NBD2 with ICL2 through the formation of an interacting triad of residues in different protein domains. Every residue of the triad is critical, as shown in our mutational analysis. The interaction of pSer-1387 and Arg-1462 is apparently caused by the electrostatic attraction. The interactions of Tyr-506 with the other two seem to rely on hydrogen bonds. Our simulation study indicates that the triad is stable and may act as a primary force for the changing conformations of NBD2 and IC2 on their interface. ICL2 appears to move toward NBD2 with phosphorylation, while NBD2 is reshaped and moves toward ICL2, especially the α6-tail and the α3-helix and probably the β5-sheet as well. Thus the two domains interact with each other more tightly post-phosphorylation, allowing NBDs to couple with TMDs more effectively. Such a strong NBD-TMD coupling is likely to ensure the transfer of mechanical forces and movements necessary for channel gating between these protein domains. Because such a coupling is weakened without Ser-1387 phosphorylation, the mechanical forces and movements produced in NBDs may not be sufficient for channel gating. By enhancing the NBD-TMD interaction, vasodilators seem to be able to use the existing forces to augment the channel activity without producing additional mechanical work. It is noteworthy that the NBD-TMD force transfer depends on elaborate interactions of the triad, as restriction of these domains with a disulfide bond does not lead to channel activation.

Possible Cooperation of PKA and Nucleotide Activators—The SAV1866 is crystallized in ADP-binding forms (PDB: 2hyd), which is represented as a dimer in NBDs. The NBD dimerization has been seen in almost all the ABC proteins including NBDs of SUR1 (41, 42). In our SUR2B<sub>core</sub> model, the two NBDs form a heterodimer with two nucleotide-binding pockets on the interface. Such dimerization of NBD1 and NBD2 in SURs suggests a channel conformation in the active state. Nevertheless, the channel activity is rather low (less than 10% of maximal activity by pinacidil according to our observation in previous and current studies), probably because the interaction between TMDs and NBDs is quite loose at the baseline. PKA phosphorylation enhances the interaction of TMD1 (via ICL2) with NBD2 and thus drastically activates the channel. It is possible that the NBD dimerization by nucleotide binding is necessary for PKA activation. A study (45) on the Kir6.2/SUR1 channel indeed indicates that ADP concentrations determine whether the channel is activated or inhibited by PKA. Although
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we have not observed inhibition on Kir6.1/SUR2B, we did see a reduction in the PKA effects with decreasing ADP concentrations.$^5$ It is of interest to know whether PKA acts on the channel without nucleotides.

In conclusion, the SAV1866-based modeling provides novel information of SUR2B structures, and suggests a mechanism for the Kir6.1/SUR2B channel activation by PKA phosphorylation, information that is necessary for the understanding of how vasodilators activate K$_{ATP}$ channels and relax vascular tension. The discovery of the Ser-1387/Tyr-506/Arg-1462 triad and its critical location for the interaction between TMDs and NBDs contribute significantly to our understanding of SUR-mediated K$_{ATP}$ channel gating. Furthermore, our combined studies of SUR2B_core model with mutational analysis support the accuracy of the SAV1866 crystal structure on the NBD-TMD interface and indicate that the SUR2B can be well represented by the SAV1866 structure.

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