CHARACTERIZING THE ROLE OF T352 IN THE INHIBITION OF THE LARGE
CONDUCTANCE Ca\textsuperscript{2+}-ACTIVATED K\textsuperscript{+} (BK) CHANNELS BY 1-[1-HEXYL-6-(METHYLOXY)-
1\textit{H}-INDAZOL-3-YL]-2-METHYL-1-PROPANONE (HMIMP)

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Abstract

Large conductance Ca\(^{2+}\)-activated K\(^{+}\) (BK) channels are known to be regulated by both intracellular Ca\(^{2+}\) and voltage. Though BK channel modulators have been identified there is a paucity of information regarding the molecular entities of this channel that govern interaction with blockers and activators. Using both whole-cell and single channel electrophysiological studies we have characterized the possible role that a threonine residue in the pore region of the channel has on function and interaction with BK channel modulators. A threonine to serine substitution at position 352 (T352S) resulted in a 59 mV leftward shift in the voltage-dependent activation curve. Single channel conductance was 236 pS for the wild-type channel and 100 pS for the T352S mutant, measured over the range -80 mV to +80 mV. Additionally, there was an almost 10-fold reduction in the potency of the BK channel inhibitor HMIMP, the IC\(_{50}\) values being 4.3±0.3 nM and 38.2±3.3 nM for wild-type and mutant channel respectively. There was no significant difference between wild type and the mutant channel in response to inhibition by iberiotoxin. The IC\(_{50}\) was 8.1± 0.3 nM for the wild-type and 7.7±0.3 nM for the mutant channel. Here, we have identified a residue in the pore region of the BK channel that alters voltage sensitivity and reduces the potency of the blocker HMIMP.
Introduction

Large conductance Ca\(^{2+}\)-activated K\(^+\) (BK) channels are known to be regulated by both intracellular Ca\(^{2+}\) and voltage (Toro, et al., 1998). These channels are ubiquitously expressed, and have important functions in the physiology of various organs and tissue groups. These include, but are not limited to the brain (Piwonska, et al., 2008), kidney (Grunnet, et al., 2005), urinary bladder (Imaizumi, et al., 1998; Ohi, et al., 2001), and neurons (Vergara, et al., 1998). The diverse role BK channels play in physiology (Ghatta, et al., 2006), as well as the possible link to some pathophysiological states make them tractable targets for therapeutic intervention.

Functional BK channels are formed from the co-assembly of four identical \(\alpha\) subunits, and it is also known that the channel complex has \(\beta\) subunits that are tissue specific in their distribution (Brenner, et al., 2000). BK channels are believed to play a key role in hyperpolarization in excitable cells and changes in the expression pattern of BK (Bloch, et al., 2007), as well as mutations in the channel are associated with some disease states. In particular a missense mutation D434G, resulting in a gain-of-function, is associated with epilepsy and paroxysmal dyskinesia in a large family cohort (Diez-Sampedro, et al., 2006; Du, et al., 2005). Likewise, a mutation in the \(\beta_1\)-subunit resulting in loss-of-function has been identified in some African-American asthmatics (Seibold, et al., 2008), and carriers are believed to be at a greater risk of asthma morbidity. In the present study we limit our analysis to channels that are composed of only the \(\alpha\) subunit.

Amino acid residues that play critical roles in Ca\(^{2+}\) (Xia, et al., 2002) and voltage sensing (Ma, et al., 2006) have been identified, but a lot less is known about residues that interact and form binding pockets for new chemical entities. Understanding how specific residues might influence channel modulation by small molecules will therefore provide a starting point for rational drug design if this channel is to be targeted. BK channel is a member of the class of Ca\(^{2+}\)-activated K\(^+\) channels, which are classified based on their conductance as large, intermediate or small. It was previously reported that a threonine to serine substitution mutation at position 250 in human intermediate conductance Ca\(^{2+}\)-
activated K⁺ channel (hIK) resulted in the elimination of arachidonic acid block (Hamilton, et al., 2003), sequence alignment analysis identified Threonine 352 as the corresponding residue in human BK channel (Fig. 1A).

In the current study we investigated the effect of the T352S substitution mutation on the BK channel function. We also investigated how the substitution mutation at position 352 influenced the ability of the investigational BK channel blocker HMIMP (Zeng, et al., 2008) to modify channel activity. Using homology modeling we identified additional residues that might contribute to the binding and hence the activity of the compound. Using site-directed mutagenesis and whole-cell electrophysiology we were able to test the relative contribution of the identified residues to a putative binding region for HMIMP.
Methods

*Mutagenesis*- The T352S, F380A, A381S and V384I BKα constructs were made by doing site directed mutagenesis using the QuickChange Mutagenesis kit (Stratagene, La Jolla, CA). T352S BKα was subcloned into pFBMNA (BioCat ID: 107399, GlaxoSmithKline), this was then sequenced to confirm the presence of the desired mutation and to ensure that no extra unwanted mutations were present. The plasmid was then transformed into DH10bac cells and the BacMam virus generated using the Bac-to-Bac Baculovirus Expression System (Invitrogen, Carlsbad, CA).

*Cell Culture*- Chinese hamster ovary (CHO) cells were transduced with the BacMam virus to express the human BKα subunit. Cells were either transduced with WT (BioCat ID: 117774) or the T352S mutant BacMam (BioCat ID: 120408). In experiments in which function of the channel mutants for F380A, A381S or V384I were assessed, CHO cells were transfected with the construct of interest using the Fugene 6 Transfection Reagent (Roche, Germany). For uniformity and to account for any changes in compound potency that might have occurred because of the time between experiments, the construct for WT BKα was also transfected using Fugene 6 Transfection Reagent. This was necessary since compound degradation might be an issue so we wanted a time matched control for the effect of HMIMP on WT BKα. All reagents were generated internally at GlaxoSmithKline. Cells were maintained at 37°C with 5% CO₂ in T-75 flasks or 6-well culture dishes in Dulbecco's modified Eagle's medium F-12 supplemented with 10% fetal bovine serum, 0.5 mg/mL geneticin (Invitrogen, Carlsbad, CA).

*Electrophysiology*- All currents were recorded at room temperature (~23°C) using an Axopatch 200B amplifier and Digidata 1322A digitizer (Molecular Devices, Union City, CA). Currents were recorded using the whole-cell or inside-out patch configuration. Cells were placed in a small chamber (volume=0.7 mL) and continuously perfused with an external solution (3-4 mL/min). Electrodes were pulled from thin wall glass (WPI, Sarasota, FL) using a P-97 horizontal puller (Shutter, Novato, CA) and fire polished with MF-830 microforge (Narishige, Long Island, NY). Electrode resistance was 2-3 MΩ for whole-cell and 8-15 MΩ for inside-out patch recordings. Currents were elicited by different voltage
protocols (described in text and figure legends) and acquired with pCLAMP 8 software (Molecular Devices). Single channel currents were filtered at 2 kHz and digitized at 25 kHz.

For experiments to determine G-V relationships in Fig. 2, pipette solution contained (in mM) 140 KCl, 5 EGTA, 1 MgCl₂, 2 CaCl₂, 5 MgATP, 5 HEPES, pH = 7.2 (0.7 µM free Ca²⁺) and external solution consisted of (in mM) 140 KCl, 2 CaCl₂, 10 Glucose, 10 HEPES, pH = 7.4. For inside-out patch recording, bath solution contained (in mM) 140 KCl, 5 EGTA, 1 MgCl₂, 2 CaCl₂, 5 HEPES, pH = 7.2 (100 nM free Ca²⁺), and pipette solution contained (in mM) 140 KCl, 1 MgCl₂, 1 CaCl₂, 5 HEPES, pH 7.2. For the experiments illustrated in figures 4, 5 and 7, bath solution contained (in mM) 140 NaCl, 4 KCl, 1 MgCl₂, 2 CaCl₂, 10 Glucose, and 10 HEPES, pH = 7.4. Pipette solution contained (in mM) 140 KCl, 5 EGTA, 1 MgCl₂, 0.2 CaCl₂, 5 MgATP, 5 HEPES, pH = 7.2 (6.5 nM free Ca²⁺). The free Ca²⁺ concentrations of solutions were calculated using Webmaxc (http://www.stanford.edu/~cpatton/webmaxcS.htm).

Whole-cell currents were analyzed using pCLAMP 8 (Molecular Devices, Union City, CA). Single channel currents were analyzed with Fetchan and PStat bundles in the pClamp 8 software. Events list were created in Fetchan and no minimum-duration level was imposed when detecting transitions between closed and open states. The single channel amplitudes were obtained by forming amplitude histograms of selected regions of recordings that had clear single open and closed current levels. Gaussian distributions were fitted to the amplitude histograms to determine the unitary current. Data were expressed as mean ± SEM.

Drug-1-[1-hexyl-6-(methyloxy)-1H-indazol-3-yl]-2-methyl-1-propanone (HMIMP) was synthesized at GlaxoSmithKline (King of Prussia, PA). HMIMP was dissolved in dimethyl sulfoxide to make 1 mM stock solution and stored at -20°C. The drug stock solution was diluted in external solutions to desired concentrations and used within 3 hours. The effects of HMIMP were determined when drug response reached steady-state after 3-5 min of perfusion with the compound. The highest DMSO concentration in drug solution was 0.1% which has no effect on BK channel function. Iberiotoxin (Sigma, St. Louis, MO) was dissolved into distilled water to make 10 mM stock solutions.
Homology Modeling-The full length sequence of human BK channel subunit alpha-1 (BK alpha) was retrieved from UniProt, accession number Q12791. A BLAST search of the Brookhaven Protein database (PDB) was performed to identify suitable structural templates. In accordance with previously published models (Jiang, et al., 2001; Jiang, et al., 2002; Yusifov, et al., 2008) the highest sequence homology was found in the pore domains of KvAP-33H1 (2a01.pdb) and MTHK (1lnq.pdb). Models were initially constructed and inspected using the protein modeling tools in the Molecular Operating Environment (MOE, Chemical Computing Group, Montreal, Canada), employing the latter crystal structure (MTHK) as a template. The initial automated alignment resulted in the disruption of two helical domains (red lines Fig. 6A); manual adjustment was performed to maintain the secondary structure. The homology model was constructed using 10 initial intermediate structures with the final averaged model being subjected to the Protonate3D protocol for hydrogen placement and side-chain ionization state determination and subsequent optimization with the MMFFx force field. The HMIMP ligand was built in MOE and optimized using the same force field conditions. The ligand was manually docked into the pore domain so that the polar and hydrophobic domains of the protein and small molecule were in correspondence. The whole ligand-receptor complex was then re-optimized to give the final structure.
Results

Threonine352 and BK channel function. The sequence alignment for the putative S5 transmembrane domain and pore region of human BK and IK channels are shown in Fig. 1A. To determine if T352 had any effect on the function or the pharmacology of BK channel, site-directed mutagenesis was used to make a Serine substitution, the position of the mutation is shown in Fig. 1B. To first examine the effect of the point mutation on channel function, CHO cells expressing WT BK channel and the T352S construct were examined in the whole-cell patch clamp configuration. Briefly, cells were stepped from a holding potential of -60 mV to voltages in the range -120 mV to +200 mV, tails currents were then recorded at -60 mV (Fig. 2A&B). The rate of channel deactivation was slower for mutant channels compared to their wild-type counterparts. To quantify the deactivation time constant, the decay of the tail current at -60 mV following a +140 mV depolarization step was fitted with a single exponential function. The $\tau$ of deactivation in the WT channel was 1.1±0.1 ms, compared to 7.7±1.7 ms for the T352S channel mutant. The significantly slower rate of deactivation of the T352S mutant implies that T352 might play an important role in modulating the gating kinetics and specifically the deactivation of BK channels. Conductance-voltage (G-V) curves were obtained by measuring the peak tail current observed at -60 mV for each step to voltages in the range of -120 mV to +200 mV, then normalizing to the value obtained at +200 mV. The voltage at which conductance was 50% of the maximum ($V_{1/2}$) was +86 mV for WT channels and +27 mV for the T352S mutant (Fig. 2C), this significant leftward shift (59 mV) is suggestive of a key role for T352 in channel activation.

Threonine to Serine substitution at position 352 also alters single channel conductance. We then assessed the functional implication of the Threonine to Serine substitution at position 352 by measuring single channel activity in the inside-out patch configuration. In Fig. 3A, we show example single channel recordings from WT (left panel) and T352S (right panel) BK$\alpha$ channels heterologously expressed in CHO cells at +80, +20, -20 and -80 mV. Single channel current-voltage relationship in the range of -80 to +80 mV is summarized in Fig. 3B. Consistent with the observation in whole-cell recordings, the mutant
channel was activated at more negative voltages than WT. Single channel current amplitudes were measured at negative membrane potentials (-80, -60, -40 and -20 mV) for the T352S mutant but not WT channels due to a lack of channel opening at these voltages. The single channel conductance determined from the slope of the fits in Fig. 3B was 236 pS and 100 pS for the WT and the T352S mutant channels, respectively.

**Threonine to Serine substitution at position 352 impairs inhibition by HMIMP.** We previously reported that HMIMP was a potent and selective inhibitor of BK channels (Zeng, et al., 2008), eliciting block by increasing the close dwell time of the channels. However, we did not identify a putative binding region. The contribution of T352S to the activity of HMIMP was therefore investigated using whole-cell recording. Currents elicited in WT BKα channels were completely blocked after cells were perfused with 100 nM HMIMP (Fig. 4A.). In comparison, mutant channels were inhibited approximately 80% at this concentration and complete channel block was observed in the presence of 1 μM HMIMP (Fig. 4B). It should be noted that different voltage protocols (insets of Fig. 4A and 4B) were used because the mutant channels were activated in a voltage range that was more negative than that in the WT channels. The concentration-response curve (Fig. 4C) revealed that there was an almost 10-fold rightward shift in the IC$_{50}$ for the mutant channels (38.2±3.3 nM, n=3) when compared to WT channels (4.3±0.3 nM, n=3). This observation implies that the Threonine residue at position 352 may form part of the binding pocket for HMIMP, but it is also possible that the effects may be due to allostERIC modification of the BKα channel. Further, we can exclude the possibility that this effect is voltage-dependent since we had previously reported that the inhibition of BK by HMIMP was not affected by voltage (Zeng, et al., 2008).

**Threonine to Serine substitution at position 352 has no effect on iberiotoxin activity.** Next we investigated the effect that the residue at position 352 might have on the activity of the known BK channel blocker iberiotoxin (IbTx) (Galvez, et al., 1990; Giangiacomo, et al., 1992). Assessing channel activity in the whole-cell patch clamp configuration we found that there was no significant difference in the inhibition of the WT and mutant channels, there was greater than 90% inhibition after bath perfusion
with 300 nM IbTx in both cases (Fig. 5A&B). As for the experiments with HMIMP, inhibition of WT channel activity was assessed at +140 mV and for the T352S mutant channel inhibition was assessed at +40 mV. The inability of IbTx to differentiate between the WT and T352S channels is probable evidence that the site of action for IbTx is not localized to the selectivity region of the pore where the threonine residue is thought to reside. The IC$_{50}$ values for blockade by IbTx were found to be 8.1±0.3 nM (n=3) and 7.7±0.3 nM (n=3) for the WT and mutant channels, respectively (Fig. 5C).

**Homology modeling of HMIMP docking.** A homology model of the pore domain of the human BK$_{\alpha}$ channel was created using the crystal structure of MTHK as the template. The resulting model was utilized to investigate possible binding modes of the small molecule inhibitor, HMIMP. The modeled pore domain binding pocket is formed by the four threonine 352 (T352) residues from each of the constituent helical bundles creating a polar bottom surface. The remainder of the pore pocket is lined with hydrophobic residues, comprised by F380, A381 and V384. The ligand is characterized by having a more polar lower portion, formed primarily by the carbonyl and methoxy oxygen atoms that can function as hydrogen-bond acceptors and a hydrophobic upper portion formed by the pendant alkyl chain. This is shown by the Connolly surface color-coded according to hydrogen-bonding (magenta), polar (blue) and hydrophobic (green) domains (Fig. 6B). Consequently, the ligand was oriented in the pocket of the pore domain model with the polar oxygen-rich domain directed towards the threonine residues that could potentially act as hydrogen-bond donors. The protein-ligand complex was subsequently optimized with the MOE implementation of the MMFFx force field, where the anticipated interactions were indeed observed (Fig. 6C). The ligand forms hydrogen bonds with two of the T352 residues in the pocket, one via the carbonyl oxygen and the other by means of the methoxy oxygen. The interactions are shown in Fig. 6D (left panel), where the alkyl moieties are oriented towards the hydrophobic protein residues and the solvent front. This orientation of the ligand is an excellent match to the desired complementary interactions sought by the protein, as calculated by the MOE contact preference algorithm. Not surprisingly, the protein seeks a binding partner that is polar in nature around the threonine residues.
Role of F380, A381 and V384 on the activity of HMIMP. Based on homology modeling, F380, A381 and V384 were the amino acid residues that were shown to form part of the binding pocket for HMIMP. To determine the role played by these residues, three individual point mutants were designed and tested for channel activity and to ascertain the effect of HMIMP. Site-directed mutagenesis was used to generate F380A, A381S and V384S, respectively. Mutation of two of these residues A381S and V384I resulted in functional channels, a third mutant F380A was functionally silent. A381S mutant channels had similar voltage dependent activation compared with T352S mutant and the current was recorded in the range of -60 to +40 mV, whereas V384I mutant channels resembled WT in voltage dependent activation and the current was recorded in the range of +40 to +140 mV. For both A381S and V384I channel mutant, the HMIMP potency was affected only slightly compared with the WT BKα channels (Fig. 7). The IC_{50} was 19.3±2.9 nM (n=5) for A381S and 8.9±1.7 nM (n=5) for V384I vs. 13.1±1.9 nM (n=3) for WT.
Discussion

Substitution of a threonine residue at position 352 in BKα channels reveal that this position in the selectivity region of the channel may play an important role in voltage sensitivity and the blocking activity of the compound HMIMP (Zeng, et al., 2008). The corresponding threonine residue in IK channels was previously identified to be a molecular determinant of blockade of this channel by arachidonic acid (Hamilton, et al., 2003). We therefore wanted to determine if this threonine residue might play any role in the function and pharmacology of BK channels. Our findings indicated that this was indeed the case. The possible involvement of T352 in the gating mechanism of the channel is highlighted by the fact that a substitution mutation of threonine to serine revealed a channel that had slower deactivation kinetics. In fact the deactivation time constant was 7-fold greater for the mutant when compared to the wild-type channel.

The Threonine to Serine mutation also seemingly altered the voltage sensitivity of the channel when compared to the WT. We observed a leftward shift of 59 mV for the half maximal activation voltage when WT and mutant channels were compared under the same conditions. This shift in voltage sensitivity was confirmed at the single channel levels where mutant channel openings were recorded at a voltage as low as -80 mV. In addition, the single channel conductance of the mutant was approximately half that of the WT, 100 pS and 236 pS respectively, giving rise to a highly voltage sensitive and low conductance channel.

In assessing the ability of HMIMP, a potent blocker of BK channels whose mechanism of action we previously characterized (Zeng, et al., 2008), to inhibit the mutant channel we found that there was an almost ten-fold shift in the IC50 indicative of the role played by this region of the channel on compound activity. Threonine and serine are related amino acids and since such a marked change was observed for the IC50 values it can be implied that the binding of HMIMP is highly specific. In fact, differences in the overall structural topology between the wild type and Serine 352 (23) mutant homology models are minimal and do not provide a rationale for the observed change in binding affinity of HMIMP. However, there are differences in the rotamer preferences between the threonine and serine side-chains that may
provide a plausible explanation (http:www.chemcomp.com/journal/rotexpl.htm). In the binding model, HMIMP interacts with two threonine residues, forming hydrogen bonds through the hydroxyl proton to either the carbonyl or methoxy oxygen atoms. The T352 residue interacting with the carbonyl oxygen is the low energy rotamer, whereas the methoxy interacting threonine rotamer is 1.5 kcal/mol higher than the low energy conformation. The S352 mutant model requires a much higher energy cost for the side-chain hydroxyls to adopt the binding interaction conformations. The S352 interacting with the carbonyl and methoxy oxygen atoms are 3.94 kcal/mol and 6.92 kcal/mol higher than their low energy rotamers, respectively. Thus, the difference in binding affinity of HMIMP between wild type and S352 mutant may be a function of the energy cost of the amino acid side-chains adopting the required ligand interaction conformation. This specificity is further highlighted by the fact that IbTx inhibition of the channel was not affected by this mutation, the binding region for IbTx was previously identified and is apparently distinct from that of HMIMP (Giangiaco, et al., 1992).

At this point we would like to point out that of the other residues identified to be part of the binding pocket for HMIMP, A381 and V384 appeared to have only minor effect on the inhibitory actions of the compound compared with T352. We were unable to characterize the possible role of F380 since the generated mutant did not form a functional channel.

Here we have reported the characterization of the possible role of a threonine residue at position 352 in the pore region of the BK channel. Our findings reveal that this residue might play an important role in the activation and the deactivation mechanism of the channel as well as its pharmacology. These findings could be the basis for further studies that are geared towards a clear understanding of the structure activity interactions that might be useful for rationale drug design if the BK channel is to be targeted.
References


Legends For Figures

Figure 1. Sequence alignment for the S5 transmembrane domain and the pore region of hBK and hIK α subunits. A: Sequence alignment of the corresponding regions of hBK and hIK using Clustalw (http://www.ebi.ac.uk/Tools/clustalw2). The putative S5 transmembrane domain is shaded in blue and the putative pore region is shaded in red. The conserved threonine residue shown in green was mutated to serine. B: Topology diagram of a single α subunit of the BK channel. The red circle shows the location of the T352S mutation.

Figure 2. Threonine at position 352 modulates the function of BK channels. Exemplar traces of whole-cell currents elicited from the WT (A) and the T352S mutant (B) channels. Currents were elicited by pulses from -120 mV to +200 mV in 20 mV increments from a holding voltage of -60 mV. Tail currents were recorded at -60 mV after each voltage pulse. The voltage-clamp protocol was shown in the inset and the time between each sweep was 2 s. Both internal and external solution contained 140 mM KCl. The current traces above 0 mV were not displayed for clarity. C: Normalized conductance-voltage (G-V) plots for WT (filled square) and T352S (filled triangle) (n= 4).

Figure 3. Threonine to Serine substitution at position 352 alters voltage sensitivity and single channel conductance. A: Single channel current traces from WT (left panel) and T352S (right panel) recorded in symmetrical potassium solutions. Acquisitions done at +80, +20, -20 and -80 mV are shown, arrows indicate the closed state. B: The mean current-voltage (I-V) plot for single channel currents from WT (filled squares) and T352S (open squares). (n=4-6 patches).

Figure 4. Threonine to Serine substitution at position 352 impairs inhibition by HMIMP. A: Exemplar traces of currents elicited from WT channels, using the protocol shown (inset), for control (left panel), after bath perfusion with 10 nM (middle panel) and 100 nM HMIMP (right panel). B: Exemplar traces of currents elicited from mutant (T352S) channels, using the protocol shown (inset), for control (left panel),
after bath perfusion with 100 nM (middle panel) and 1 μM HMIMP (right panel). Currents from cells expressing the WT channels were elicited by pulses from +40 mV to +140 mV in 20 mV increments from a holding potential of -80 mV. In cells expressing the T352S mutant currents were elicited by pulses from -60 mV to +40 mV in 20 mV increments from a holding potential of -80 mV. The time between each sweep was 2 s. C: Concentration-response curves for WT (filled square) and T352S (open square) BK\(\alpha\) channels expressed as current inhibition at +140 mV for WT and +40 mV for the mutant in the presence of increasing concentrations of HMIMP (n=3).

Figure 5. Threonine to Serine substitution at position 352 has no effect on IbTx activity. A: Exemplar traces of currents elicited from WT channels, using the protocol shown (inset), for control (left panel) and after bath perfusion with 300 nM IbTx (right panel). B: Exemplar traces of currents elicited from mutant (T352S) channels, using the protocol shown (inset), for control (left panel) and after bath perfusion with 300 nM IbTx (right panel). Currents from cells expressing the WT channels were elicited by pulses from +40 mV to +140 mV in 20 mV increments from a holding potential of -80 mV. In cells expressing the T352S mutant currents were elicited by pulses from -60 mV to +40 mV in 20 mV increments from a holding potential of -80 mV. The time between each sweep was 2 s. C: Concentration response curves for WT (filled square) and T352S (open square), expressed as current inhibition at +140 mV for WT and +40 mV for the mutant in the presence of increasing concentrations of IbTx (n=3).

Figure 6. Homology modeling of HMIMP docking in the BK\(\alpha\) channel pore. A: Automated (upper panel) and manual (lower panel) alignment of the BK\(\alpha\) and MTHK pore-domain sequence. The actual secondary structure of the MTHK crystal structure is represented by red (alpha helix) domains. B: Connolly surface around HMIMP, color coded according to hydrogen-bonding (magenta), polar (blue) and hydrophobic (green) domains. C: HMIMP (space-filling, colored by element) docked to a homology model of human BK\(\alpha\) pore (left panel) and a close up of the binding pocket showing hydrogen bonds.
between HMIMP and two T352 residues (right panel). D: Ligand-protein interactions in the pore binding pocket (left panel) and protein complementary surface with polar (magenta) and hydrophobic (green) contours (right panel).

Figure 7. A381 and V384 only have minor effect on HMIMP potency. Concentration-response curves for WT (n=3, solid square), A381S (n=5, open square) and V384I (n=5, solid circle). BKα channels expressed as current inhibition at +140 mV for WT and V384I, and +40 mV for A381S in the presence of increasing concentrations of HMIMP.
Figure 1

A

S5 Domain

Pore region

hBK 286 LQFLNILKTSNIKLVNLSSIFISWLTAAGFIHLENSGDPWENFQNNQALTWECVYLLMVSTTVGYGDVYAKTTLG 365
hIK 194 FVAKLYMNTHPGRLGLGLT---LGLWLTTAWVLVSAER--------QAVNATGHLSDTLWLIPITFTIIGYGDVPGTMWG 263

B

Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6 continued
Figure 7

![Graph showing % Inhibition vs. [HMIMP] (nM) with data points for WT, A381S, and V384I.](image-url)