BTK-2, a new inhibitor of the Kv1.1 potassium channel purified from Indian scorpion *Buthus tamulus*

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Abstract **A novel inhibitor of voltage-gated potassium channel was isolated and purified to homogeneity from the venom of the red scorpion *Buthus tamulus*. The primary sequence of this toxin, named BTK-2, as determined by peptide sequencing shows that it has 32 amino acid residues with six conserved cysteines. The molecular weight of the toxin was found to be 3452 Da. It was found to block the human potassium channel hKv1.1 (IC_{50} = 4.6 μM). BTK-2 shows 40–70% sequence similarity to the family of the short-chain toxins that specifically block potassium channels. Multiple sequence alignment helps to categorize the toxin in the ninth subfamily of the K^+ channel blockers. The modeled structure of BTK-2 shows an α/β scaffold similar to those of the other short scorpion toxins. Comparative analysis of the structure with those of the other toxins helps to identify the possible structure–function relationship that leads to the difference in the specificity of BTK-2 from that of the other scorpion toxins. The toxin can also be used to study the assembly of the hKv1.1 channel.**

Key words: Scorpion; Toxin; Homology; Voltage-gated potassium channel; Patch clamp; *Buthus tamulus*

1. Introduction

Scorpion venom consists of a large number of toxins, which act as blockers of ion channels, showing neurotoxic effects. This tool has long been used by the biologists to unravel the structure of ion channels such as K^+ and Na^+. The determination of the tetrameric structure of the K^+ channels [1] and the identification of their pore region [2] were made possible by the use of scorpion toxins. These small peptide inhibitors thus help in the characterization of ion channels structurally as well as pharmacologically. The binding studies of these inhibitors with ion channels further provide an insight into the specificity of these toxins towards different ion channels. In addition, these peptides have been valuable tools for understanding the physiological role of specific channel proteins [3].

The scorpion toxins are divided into two classes. The long-chain scorpion toxins consist of 60–70 amino acid residues compacted by four disulfide bonds. Their targets are the voltage-sensitive Na^+ channels of excitable nerve and muscle cells and they can be classified into α-toxins, which slow down Na^+ channel inactivation [4] and β-toxins, which affect the channel activation process [5]. Short-chain scorpion toxins contain up to 40 amino acid residues with three or four disulfide bridges, and have affinity towards the K^+ and Cl^- channels [6]. These two classes of toxins share a common structural framework consisting of a double-stranded antiparallel β-sheet linked to a short α-helix. Insect defensins produced by the insects in response to body injury or bacterial infection [7] and the plant γ-thionines [8] also possess a similar kind of structural configuration.

The short toxins affecting potassium channels have been divided into three structurally distinct groups, designated as α-KTx [9], β-KTx [6] and γ-KTx [10]. The peptides belonging to the α-KTx are the best studied. They block the potassium channel by physically occluding the pore of the channel [3].

Toxins specific to potassium channels show a common structural motif with one α-helix and two to three antiparallel β-strands forming a β-sheet structure, compacted with three to four disulfide bonds [11]. In spite of the similar α/β scaffold there are variations among these toxins in the size of the β-sheet, the type of β-turns and the type of the helix. These differences in structure lead to variations in the specificity of the toxins towards different ion channels [12]. Binding studies on mutants of charybdotoxin indicated the significance of 'crucial' residues that participate in the toxin–ion channel interactions. The most crucial residue Lys27 has been found to occlude the pore of the potassium channel [13].

We have purified a novel peptide from the venom of the red Indian scorpion *Buthus tamulus*. An attempt has been made to characterize this peptide structurally as well as functionally by the determination of the primary structure, mass spectrometry and electrophysiological studies. BTK-2 isolated from *B. tamulus* was found to show highest similarity to the ninth subfamily of the α-KTx group of potassium channel inhibitors [9]. This toxin blocks the human voltage-gated K^+ channel, hKv1.1.

2. Materials and methods

2.1. Material

Venom of the scorpion *B. tamulus* was obtained in a lyophilized state from the Haffkine Institute, Parel, Mumbai, India. All solvents used for separation were of high-performance liquid chromatography (HPLC) grade obtained from Spectrochem (India). The chemicals used for sequencing were purchased from Wako (Japan). Bovine serum albumin (BSA) used as a standard for protein assay was obtained from SRL (India). All chemicals used for preparation of buffers were

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of analytical grade. Buffers were filtered through 0.45 μm filters (Sartorius, USA). The crude venom was filtered using 0.2 μm filters (Pall Gelman Labs, USA). Amino acid sequencing was done on a gas phase sequencer (PPSQ-10) from Shimadzu, consisting of a HPLC system with a reverse-phase C18 column (4.6×250 mm) and a SPD-10 UV detector. Absorbance was measured on an UV-265 spectrophotometer from Shimadzu. Centrifugation was done in Beckman's J2-21M/E using J-18 rotor. A strong cation exchange column, Resource S (1 ml) (6.4×30 mm) from Pharmacia was used for ion exchange chromatography and reverse-phase HPLC was done on C4 and C18 semi-preparative columns (22×250 mm) from Vydac. Purified fractions were lyophilized in the speed vac concentrator from Savant.

2.2. Purification

115 mg of crude venom of *B. tamulus* obtained in the lyophilized form was weighed and dissolved in 20 ml of water and stirred at 4°C for 4 h. The solution was centrifuged at 13 300×*g* at 4°C for 20 min to separate the mucous material of the venom. The clear supernatant was separated and the pellet was resuspended in 20 ml water and stirred for 4 h and again centrifuged. The supernatants were pooled and filtered through a 0.2 μm filter and then lyophilized in a speed vac concentrator. The filtered water extract of the venom was used for further purification. The crude venom after processing was applied on a Resource S (Pharmacia 6.4×30 mm) cation exchange HPLC column, equilibrated with 20 mM sodium acetate buffer, pH 5. Separation was performed using a linear gradient of 0–100% of 0.5 M NaCl added to 20 mM sodium acetate buffer, pH 5, at a flow rate of 0.5 ml/min. The fractions were collected manually according to the absorbance. The fraction eluting at retention time 20–23 min was collected, lyophilized in the speed vac concentrator, and applied on a C4 (22×250 mm, Vydac) semipreparative reverse-phase HPLC column, equilibrated with 0.1% of trifluoroacetic acid (TFA) in water. Separation was done using a linear gradient of 0.1% TFA in acetonitrile starting from 15% for the first 5 min, then increasing from 15 to 60% in the next 45 min with a flow rate of 2.5 ml/min. The absorbance was measured at 215 nm as well as 280 nm. The manually collected fractions were again lyophilized and applied on a C18 (22×250 mm, Vydac) semipreparative reverse-phase HPLC column. Separation was performed using a gradient starting from 15% of 0.1% TFA in acetonitrile for the first 5 min and then going from 15% to 31% in the next 20 min, with a flow rate of 2.5 ml/min. The final fraction obtained was denoted as BTK-2, which was lyophilized and the amount of protein estimated by the method of Lowry et al. [14].

2.3. Mass spectroscopy

The purity and molecular weight of the components isolated and fractionated from the crude venom were assessed by electrospray ionization mass spectrometry (ESI-MS). The ESI-MS spectra were recorded on a Hewlett-Packard (model HP-1100) electrospray mass spectrometer. The mass spectra corresponding to each peak present in the total ion current (TIC) chromatograms were averaged to determine the molecular masses accurately.

2.4. Amino acid sequencing

The disulfide bonds of BTK-2 were simultaneously reduced and alkylated by adding tri-*n*-butyl phosphine and 4-vinylpyridine. The position of the cysteines was identified by immobilizing the toxin on Immobilon-PSQ polyvinylidene difluoride membrane (Millipore) and 5 μl of the mixture of 0.6% tri-*n*-butylphosphine and 1.2 μl of 4-vinylpyridine in 80% isopropanol was added for the simultaneous reduction and alkylation of the toxin, followed by Edman degradation reaction. The reaction was performed at 60°C for 50 min. The N-terminal amino acid sequence analysis of the native and the reduced and pyridylethylated toxin was performed on PPSQ-10 sequencer.

2.5. Electrophysiological experiments

Stage V and VI oocytes were isolated by mini-laparotomy from adult female *Xenopus laevis*. The frogs were anesthetized by immersion in 0.04% benzocaine for 15–20 min, and then placed on a wet platform during dissection and removal of ovarian lobes. The incision was suture-closed and the frog was allowed to recover for about 2 months before removal of another batch of oocytes. Clusters of oocytes were treated with 1 mg/ml Type 1A collagenase (Sigma) in OR-Mg solution that contained (mM): 82 NaCl, 2 KCl, 20 $MgCl_2$, 5 HEPES (pH 7.7). The isolated oocytes were then incubated for microinjection at 18°C in ND96-HS solution containing (mM): 96 NaCl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, 2.5 Na-pyruvate (pH 7.7) supplemented with 100 U/ml of Pen-Strep and 5% heat-inactivated horse serum.

Capped polyadenylated RNA of hKv1.1 was generated using T7 RNA Polymerase (mMessage mMachine transcription kit, Ambion, USA). 46 nl of transcribed RNA (150–300 ng/μl) was injected per oocyte. Electrophysiological recordings were carried out 2–5 days after microinjection. Methods for cRNA injection and two-electrode voltage clamp of *Xenopus* oocytes have been described in [15]. In short, we used an OC-725 oocyte clamp amplifier (Warner Instruments) to maintain the holding potentials and record membrane currents. The external recording solution was modified ND96, containing (mM): 96 sodium gluconate, 2 potassium gluconate, 1.8 calcium gluconate, 1 magnesium gluconate, 5 HEPES (pH 7.7). Solution exchange was achieved by gravity flow. Analog data from the amplifier were sampled at 15 kHz and filtered at 5 kHz by low-pass filter (LPF-100, Warner Instruments), digitized and stored on a 486PC hard disk for further analysis. The pCLAMP 6.0 (Axon Instruments) software package was used to generate voltage-clamp commands, acquire membrane currents and analyze digitized data. All experimental data were first analyzed with Clampfit 6.0 and exported to Sigma Plot 5.0 for subsequent analysis, curve fitting and display. 1.0 μg/μl (290 μM) stock solution of BTK-2 was made in 0.1 mg/ml BSA solution with 10 mM HEPES buffer (pH 7.6) and was added to the 'bath' in cumulative fashion. All electrophysiological experiments were performed at room temperature.

2.6. Homology modeling

The primary structure of the toxin BTK-2 as obtained from the N-terminal sequencing was aligned using the multiple sequence alignment program CLUSTAL W. The alignment was done on the basis of the conserved cysteine residues. The algorithm used was BLASTP and the scoring was done using the BLOSUM62 matrices. We used Swiss PDB Modeler to generate the three-dimensional model of the toxin. It was developed by substituting its residues in the known three-dimensional structure of the toxin BmP02 with PDB code 1DU9. This model was then subjected to checking, using WHAT IF [16] and the PROCHECK [17] programs. Four C-terminal amino acids GKKD were added to the model structure obtained from Swiss PDB modeler using Insight II (MSI) and the structure was further energy-minimized using Discover and Builder module, until the root mean square derivatives became less than 10^{-3} kcal/mol.

3. Results

3.1. Purification

The fractionation of the crude venom was done on a cation exchange Resource S column, which divided the mixture into nine fractions (S1–S9) as shown in Fig. 1A. The fractions were separated on the basis of their basicity and the less basic fraction S3 was further purified on a C4 HPLC reverse-phase column as shown in Fig. 1B. The fraction eluting at 9.8 min was collected and was finally purified by passing it through C18 HPLC column. The peptide eluted at 11.5 min was named BTK-2 as in Fig. 1C. The molecular weight of the toxin was found to be 3452 Da as shown in Fig. 2. The primary amino acid sequence of BTK-2 was determined by Edman degradation and the positions of the amino acids were unequivocally assigned. The cysteine positions were confirmed by sequencing the reduced and alkylated toxin. The primary structure of the toxin shows 32 amino acid residues with six conserved cysteine residues as shown in Fig. 3. The sequence of BTK-2 is

BTK-2 VGCAECPMHCKGKMAKPTCENEVCKC-NIGKKD

with a calculated molecular weight of 3450 Da.

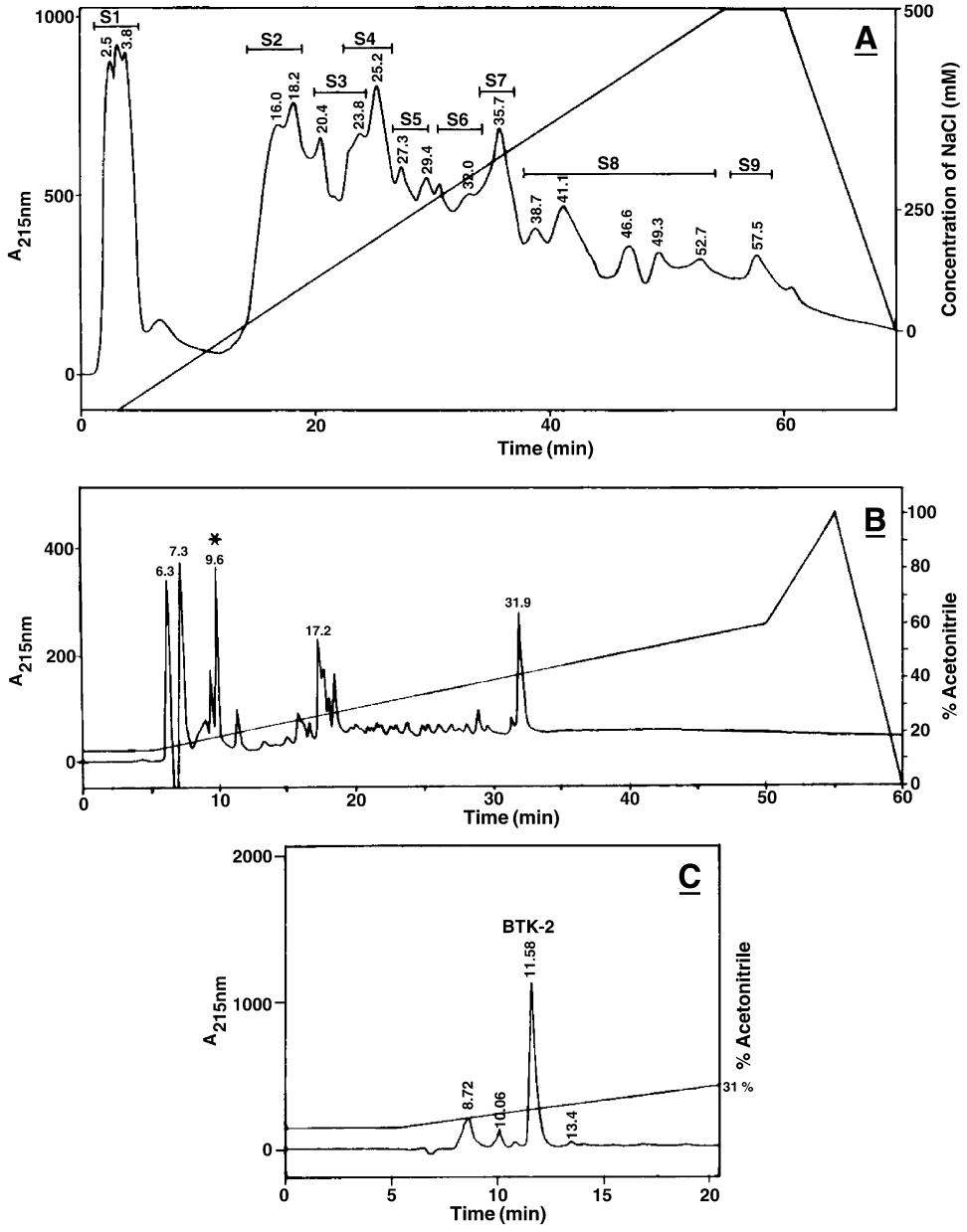

Fig. 1. Purification of the crude venom. A: The crude venom was applied on an ion exchange Resource S (1 ml) column. The fractionation was done using a linear gradient of 0–0.5 M NaCl in 20 mM sodium acetate buffer, pH 5, with a flow rate of 0.5 ml/min. The mixture was divided into nine fractions (S1–S9). B: The S3 fraction was further purified by passing on a reverse-phase C4 column. The elution was done using a linear gradient of 0.1% TFA in acetonitrile starting from 15% for the first 5 min, 15–60% in the next 45 min, 60–100% in the next 5 min and 100–0% in the next 5 min. The flow rate was 2.5 ml/min and the absorbance was measured at 215 and 280 nm. The fractions were collected manually as per the absorbance and retention time. C: The labeled fraction obtained from the C4 column was finally purified by passing it on a C18 reverse-phase column. The final fraction, named BTK-2, was isolated using a linear gradient starting from 15% of 0.1% TFA in acetonitrile for the first 5 min and then going from 15 to 31% in the next 20 min, with a flow rate of 2.5 ml/min.

3.2. Structural analysis

Fig. 3 shows the multiple sequence alignment based on the conserved cysteines and other conserved N and C terminal residues using CLUSTAL W. The three conserved disulfide bonds between Cys3–Cys19, Cys6–Cys24 and Cys10–Cys26 were determined with the help of homology. The toxin BTK-2 shows homology to the α-KTX family of toxins, which are known to block the potassium channel. It shows highest similarity to leiuropeptides LpI, LpII and LpIII (78.6%, 42.3% and 42.3%, respectively) from *Leiurus quinquestriatus hebraeus* [18], BmP01, BmP02 and BmP03 (42.3%, 75.0% and 71.4%, respectively) from *Buthus martensii* [19], Bs5 (71.4%) from *Buthus eupeus* [20] and P01 (75.0%) from *Androctonus mauretanicus mauretanicus* [21].

The three-dimensional structure of several scorpion K^+ channel blockers has been determined by NMR (nuclear magnetic resonance) spectroscopy, such as KTx [22], IbTx [23], ChTx [24], MgTx [25], NTx [11]. The data reveal that all these toxins are made up of one β-sheet linked with an α-helix by disulfide bridges. The helical part of these toxins includes a Cys-X-X-X-Cys pattern with both the cysteines forming disulfide bonds with the Cys-X-Cys sequence in the β-sheet. This

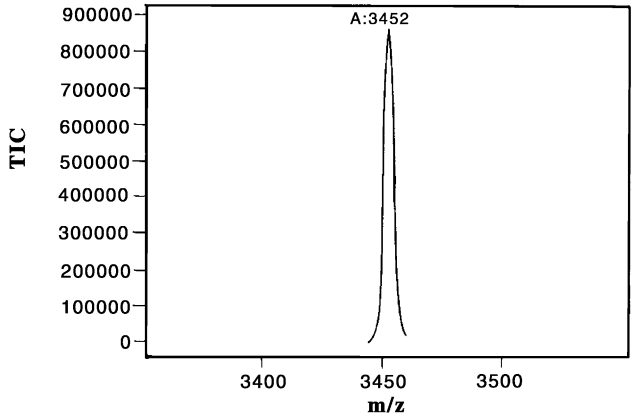

Fig. 2. Mass spectra of the toxin BTK-2. The molecular mass was determined by ESI-MS. The *x* and *y* axes show mass-to-charge ratio (*m*/*z*) and TIC, respectively.

arrangement helps in bringing the specific residues in juxtaposition to each other and is involved in binding the toxin to the channel protein [26]. The presence of a similar motif and high sequence similarity of the toxin BTK-2 with the above-described K^+ channel blockers increases the probability of this toxin adapting a similar fold. Therefore this toxin was modeled using the three-dimensional structure of BmP02 (PDB: 1DU9). This model provides an insight into understanding the structure–function relationship of BTK-2 peptide based on its preliminary structure. Comparison of the structure of BmP02 with the model structure of BTK-2 shows a similar backbone structure as depicted in Fig. 4, with Cys6-His9 forming the α-helix and the two β-strands formed by residues Pro17 to Glu20 and Val23 to Cys26, connected by a β-turn.

3.3. hKv1.1 blockage studies

Potassium channels are among the molecular targets of scorpion toxins. Sequentially BTK-2 belongs to the ninth subfamily of scorpion toxins (Fig. 3) that affects potassium channels. This suggests a similar pharmacological identity for BTK-2. The effect of pharmacological screening of BTK-2

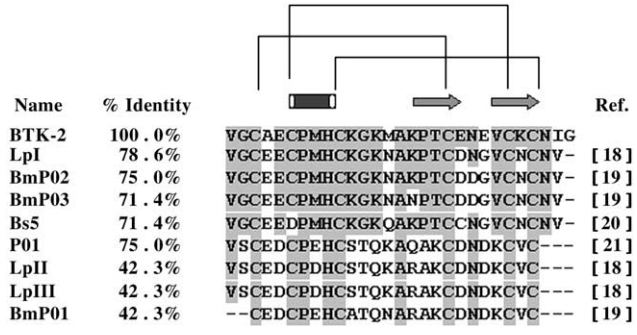

Fig. 3. Multiple sequence alignment of known scorpion toxins with BTK-2. The primary sequence of the toxin BTK-2 was aligned with other known scorpion toxins using CLUSTAL W. The alignment is based on the conserved cysteine residues and a few other amino acid residues. The sequences showing homology are as follows: LpI, LpII and LpIII leiuropeptide from *L. quinquestriatus hebraeus* [18], BmP01, BmP02 and BmP03 from *B. martensii* [19], Bs5 from *B. eupeus* [20], P01 from *A. mauretanicus mauretanicus* [21]. The disulfide bonds are shown by the straight lines connecting two cysteine residues. The region showing α-helix and two β-strands is shown by the symbols.

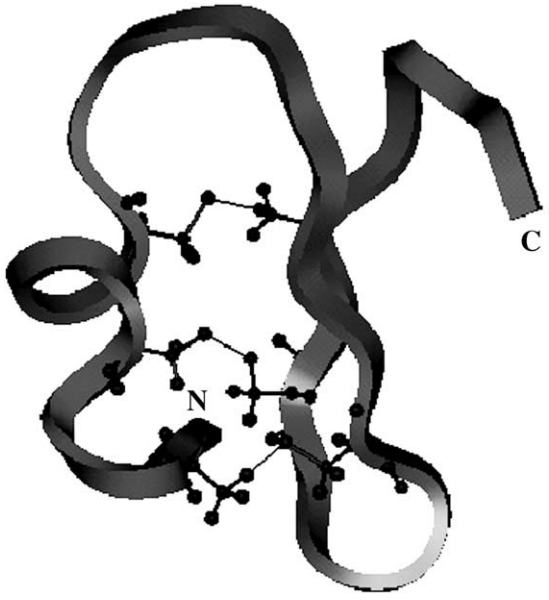

Fig. 4. Molecular modeling for BTK-2 toxin. The PDB file 1DU9 for the known structure of the toxin BmP02 was downloaded from the structural database. The three-dimensional structure of BTK-2 was modeled with the help of the Swiss PDB modeler. The structure was visualized using InsightII. The structure shows the presence of one α-helix, two β-strands and three conserved disulfide bonds.

peptide on human K^+ channels was performed using the *Xenopus* oocyte system for heterologous channel expression, and standard two-electrode voltage-clamp setup for K^+ current recording (see Section 2). Oocytes from *X. laevis* expressing either human Kv1.1 or hKv1.4 were voltage-clamped at −70 mV and stepped to a range of test potentials. Outward currents were seen on depolarizing to potentials less negative than −40 mV shown in Fig. 5A. Application of BTK-2 to these oocytes resulted in reduction of hKv1.1 currents as in Fig. 5B, but had no effect on hKv1.4 currents (data not shown). Fig. 5C shows the concentration dependence of block by BTK-2 on currents monitored at −20 mV. These data have been analyzed in Fig. 5E to yield a half-maximal block at 4.6 μM. Washing out the toxin over a period of 15 min resulted in negligible recovery of the current (Fig. 5A, trace labeled wash). This is in keeping with block of K^+ channels by other toxins such as IbTx and ScyTx [27], which is usually irreversible, and in contrast to block by agents such as 4-amino pyridine (4-AP) or tetraethylammonium (TEA), which is essentially completely reversible.

hKv1.1 is a voltage-dependent channel and the currents observed are non-Ohmic due to channel opening on depolarization. The blocker, however, is effective at all potentials tested shown in Fig. 5A,D, and has no significant effect on the kinetics of activation (data not shown). Further, the selectivity of the channel, as estimated from the reversal of tail currents, is unperturbed (data not shown). BTK-2 thus appears to have all the characteristics of a simple pore blocker as reported for other scorpion toxins.

4. Discussion

In this study we have identified and characterized a new potassium channel inhibitor from *B. tamulus*. The purified toxin BTK-2 was found to be a single polypeptide chain of

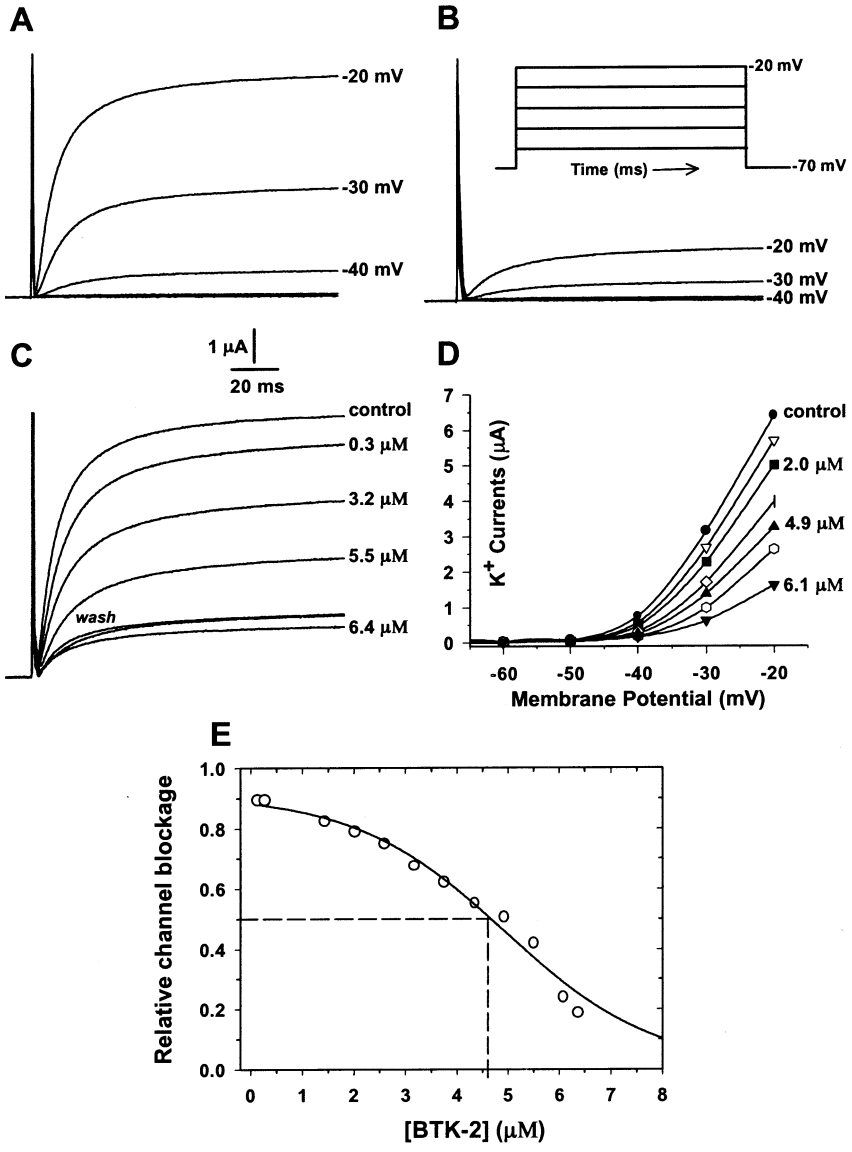

Fig. 5. hKv1.1 blockage by BTK-2 toxin. A: Currents elicited in a *Xenopus* oocyte expressing the hKv1.1 channel. Oocyte was held at −70 mV and stepped to the depolarizing potentials indicated against the current traces, using a pulse protocol shown in the inset to panel B. B: The effect of 6 μM BTK-2 on the Kv1.1 currents. C: An aqueous stock solution of BTK-2 (290 μM) was applied in a cumulative fashion to the oocyte and the currents were recorded at different concentrations of BTK-2 in the bath as indicated against the traces. In order to achieve a binding equilibrium, the K^+ currents were recorded 15 min after the addition of BTK-2 to the bath. The 'wash'-labeled trace indicates the currents 15 min after the manual washing of the clamped oocyte with ~5 ml of the recording solution. D: Current–voltage relationship (*I–V* curves) for hKv1.1 in the presence of various concentrations of BTK-2, unblocked control (●); 0.15 μM (∇); 2.0 μM (■); 3.8 μM (◇); 4.9 μM (▲); 5.5 μM (○); and 6.1 μM (▼). Membrane was held at −70 mV before stepping to various depolarizing potentials as described in panel A. E: The concentration dependence of BTK-2 block of hKv1.1 current at a test potential of −20 mV. The normalized currents at this potential were plotted as a function of BTK-2 concentration in the bath. The data points were fitted to a sigmoidal function (solid line). The half-maximal effect of BTK-2 was observed at 4.6 μM (dotted lines).

32 amino acid residues, with a molecular weight of 3452 Da. The toxin shows 40–70% similarity to other short scorpion toxins belonging to the α-KTx family of toxins that block the potassium ion channel. It shows highest similarity to the toxins belonging to the ninth subfamily of the potassium channel blockers. The amino acid mutation is seen mainly in the C-terminus of the toxins between 20 and 32 amino acid residues. Although the overall fold of the scorpion toxins is similar, there are subtle variations among them in terms of their amino acid sequence, the size of the β-sheet, the type of β-turn and/or the type of helix (i.e. α-helix vs. 3_{10} helix). These variations result in the sub-type specificities of scorpion toxins towards different K^+ channels [28], i.e. a given toxin usually blocks only one type of K^+ channel such as Kv1.1 without affecting very closely related channels such as Kv1.2 or Kv1.4. The utility of this specificity for the scorpion may lie in it having to use less venom for the job, for the researcher in using these bioactive peptides for neuro-pharmacological dissection of physiological processes and for the drug designer in providing templates that may lead to specific blockers. There is variability in the ion channel in terms of the key residues, which are present in the α-subunit that forms the tetrameric K^+ channels [29]. The toxin BTK-2 is a blocker of the Kv1.1 channel. The classification of the toxin in the ninth subfamily

of the α-KTx family is controversial as the members of this subfamily, BmP02, BmP03 from *B. martensii* and LpI from *L. quinquestriatus hebraeus* are known to be either non-toxic or weakly toxic to the apamin-sensitive calcium-activated potassium channel. The toxin also shows similarity to the fifth-family toxins, which are potent blockers of the apamin-sensitive potassium channel and include toxins like Leiurotoxin 1 from *L. quinquestriatus*, PO5 from *A. mauretanicus mauretanicus*. PO5, a 31 residue polypeptide cross-linked by three disulfide bridges [30], also possesses binding and physiological properties similar to those of the bee venom toxin apamin (18 residue, two disulfides). The similarity between the two toxins lies only in the Arg-Arg-Cys-Gln motif, which is located on the α-helix. The conformation of PO5 includes two and a half turns of α-helix (residues 5–14) connected by a turn to the β-sheet, which has two antiparallel strands (sequences 17–22 and 25–29). The three functionally important arginine residues, Arg6, Arg7, and Arg13 as judged by K^+-channel blockage studies, are all located on the solvent exposed part of the helix and form a positively charged surface, which includes Gln9. The presence of His31 together with the presence of the C-terminal carboxyl amidation is necessary to obtain the irreversibility of the PO5–NH2 binding [31]. This residue is remote from the positively charged surface, thus suggesting a multipoint interaction of PO5–NH2 with the receptor. The C-terminal analysis of the toxin BTK-2 shows that it does not have the C-terminus amidated as the rest of the fifth-family members which shows affinity towards the apamin-sensitive calcium-activated potassium channel. PO5 replacement of the two Arg6 and Arg7 by Lys induces a 10-fold decrease in the activity and is reduced to almost nil when replaced by Leu [32]. Arg13 is also found to be involved in the binding activity. The electrostatic potential is highly asymmetric and the greatest positive potential is centered on Gln9. This subfamily is different from that of charybdotoxin in the presence of the functionally important residues which are present in the helix, unlike the ChTx where they are present in the β-sheet.

BmPO2 is a 28 amino acid residue peptide and is a weak blocker of apamin-sensitive calcium-dependent K^+ channel with its α/β fold largely distorted. The α-helix is shortened to one turn and the loop connecting the helix to the first β-strand exhibits conformational heterogeneity. The instability of BmPO2 could be attributed to the presence of a proline in the 17th position, which is usually a glycine [33]. Since the residue at this position makes intense contact with the α-helix, it is supposed that the bulky side chain of proline pushes the helix away from the β-sheet. This has a significant influence on the structure and function of BmPO2, as the α-helix gets rotated by about 40° to avoid Pro17 while forming the two disulfides with the second β-strand. The rotation further causes both ends of the helix to unwind due to covalent restrictions. According to its structure, BmPO2 was supposed to interact with its target via the side chain of Lys11 and Lys 13. The two arginines located in the beginning of the helix in PO5, which are believed to be involved in binding to K^+ channels, are replaced by two acidic amino acids in both PO1 and BmPO2. This is the major cause of the decrease in the affinity of these toxins towards the apamin-sensitive calcium-activated K^+ channel. The relatively weak toxicity of BmPO2 must also be associated with the instability of the binding region centered at Lys11 and Lys13.

The sequence of the isolated toxin BTK-2 shows conserved cysteine residues such as that found in the potassium channel blockers. The other conserved residues are the same as those present in the ninth subfamily of potassium channel-inhibiting toxins. This peptide also shows the presence of two prolines at the same positions as above (7 and 17), which again might lead to an unstable α-helix conformation. The toxicity for this toxin is different from that possessed by the other members of its family. It has Lys11 and Lys13, but the presence of Glu in the fourth position reduces the binding affinity of the toxin to the ion channels. The binding affinity towards hKv1.1 is accounted for by the presence of Lys25, which might be exposed to the solvent surface and thus binds to the potassium channel. Kaliotoxin, however, binds to both the calcium-activated channel as well as the voltage-gated potassium channels just like charybdotoxin but with opposite affinities. It shows very high affinity for the voltage-gated potassium channel. The toxins having proline in the helical region are found to exhibit a high blockage of the voltage-dependent potassium channel [22]. The toxin BTK-2 possesses a high positive charge (+3), which gives an indication of this peptide to be toxic. The exposure of Lys25 might be more in BTK-2 according to this hypothesis, as it also has a proline in its helical region and hence we can predict this to be the main reason for the blockage of the hKv1.1 channel.

The toxin blocks hKv1.1 with half-maximal efficiency at 4.6 μM (Fig. 5). The currents observed in the presence of the toxin have the same properties as unperturbed hKv1.1 in terms of activation kinetics and ionic selectivity. The toxin thus appears to have the hallmarks of a pore blocker. The affinity of BTK-2 for hKv1.1 is in the micromolar range, as opposed to the nanomolar affinities of other scorpion toxins such as ChTx, IbTx and MgTx for their cognate channels. However, this may be contrasted with the millimolar affinities of blockers such as 4AP and TEA. Moreover, the off-rates for the binding reaction are slow for BTK-2 hKv1.1, as demonstrated by the negligible extent of washout in 15 min. Both 4AP and TEA can be completely washed out in this time frame. Finally, the lack of interaction with hKv1.4 is indicative of specific interactions with amino acid residues at the mouth of the hKv1.1 pore.

In contrast to BTK-2, its structural relatives block slow-conductance calcium-gated K^+ (SK) channels and not Kv channels [26,29]. Further mutational and structural studies on BTK-2 are required to find out the residues involved in the specificity of the toxin towards the hKv1.1 channel and therefore, the toxin could be used to analyze the architectural differences between the outer mouth of SK and Kv channel pores. The toxin could also be used as a molecular caliper to explore the topology of the pore region of voltage-gated potassium channels as reported in the case of Kv1.3 [34].

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