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Discoveries of Serine Protease Inhibitors from Scorpions

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Abstract

Protease inhibitors (PIs) are proteins or peptides capable of inhibiting the catalytic activity of proteolytic enzymes. They are widely distributed in nature and can be found in all kingdoms of cellular life and also in viral genomes. It has previously been stated that a typical mammalian genome contains 2% – 4% of genes encoding for proteases or protease inhibitors, indicating the importance of proteolysis in their biological processes. However, many protease inhibitors have been reported to be present in animal venoms, suggesting that venomous animals use these proteins / peptides in their biological process of survival. Similarly, in recent years, some protease inhibitors have been reported to be present in scorpions and most of them have been identified by cDNA library screening or transcriptomic analysis. These protease inhibitors identified from scorpions have been described in two classes, such as 1) Kunitz-type inhibitors, and 2) Ascaris type inhibitors. Therefore, all the protease inhibitors identified to date from scorpions should be compiled based on their functional categories to help further understanding and research. Here, on the basis of published reports, this review describes about functions, primary sequences, structures, molecular mechanisms as well as functional diversity of the proteins/peptides from scorpions which have been reported to act as protease inhibitors. Summarily, here, we provide updated as well as amassed information about the scorpion protease inhibitors.

Keywords: Scorpion; Venom; Protein / peptide; Protease; Protease inhibitor; Kunitz-type; Ascaris-type

Introduction

Protease inhibitors (PIs), comprising a family of proteins, inhibit their target proteases. These proteins inhibit activity of proteases by a conserved pathway using a profound conformational change [1-3]. Among all PIs, serine protease inhibitors are the largest and most widely distributed superfamily of PIs [4-6]. Serine protease inhibitors (SPIs) are ubiquitous in animals, plants as well as microorganisms and they play important roles in physiological processes such as blood coagulation, tissue remodeling, and proteolysis regulation [7-10]. According to previous reports, SPIs have been found in a variety of animal venoms, including snakes, scorpions, spider, cnidarians, cone snails, platypus, and hymenopterans, as well as the salivary secretions of hematophagous insects and leeches [11-13]. Importantly, serine protease inhibitors are a class of proteins involved in the regulation of serine and other types of proteases. In humans, most of the SPIs regulate the functions of proteases involved in the body's response to injury, including roles in coagulation, fibrinolysis, inflammation, wound healing, and tissue repair. In addition, SPIs have been reported to be involved in various animal and human pathologies due to the loss of a functional serpin gene through deletion or mutation, which causes a defect in functional protein. Examples of sestorically called anti-thrombin III are first described [14].

SPIs can be subdivided in many classes based on their conserved functional motifs. Among all classes, the Kunitz-type inhibitors are well characterized of them, possibly due to their abundance in several creatures [13,15,16]. Many studies reported that animal venoms contain Kunitz-type serine protease inhibitors [12,17-19]. Similarly, Kunitz-type as well as Ascaris-type serine protease inhibitors have been reported to be present in scorpions, and most of them have been identified by cDNA cloning and transcriptomic analysis [10,11,20-22].

However, interest in characterizing new PIs and understanding their physiological significance has increased due to their biological relevance for all living processes, such as blood coagulation system, complement cascade, apoptosis, cell cycle and hormone processing pathways [17,23-25]. Furthermore, deficiencies or alterations in the regulation of these enzymes lead several pathological conditions, such as cancer, arthritis, neurodegenerative and cardiovascular diseases [26,27].

Serine proteases, serine protease inhibitors, and protease-activated receptors have been intensively investigated in the periphery and their roles in a wide range of processes, such as coagulation, inflammation, and digestion [3,4,28-30]. Protease inhibitors, trypsin inhibitors, such as ulinastatin and aprotinin, are already being clinically used in anti-inflammatory therapy [31].

Therefore, these medically important protease inhibitors should be given more emphasize for further research. According to published reports, some protease inhibitors have been reported to be present in scorpions, which belong to Kunitz-type and Ascaris-type sub-family. In order to help further research, all the protease inhibitors reported from scorpions should be compiled and described according to their functions. Here, in this review, we report updated information about the protease inhibitors described to date from scorpions.

Protease Inhibitors

It has been reported that scorpion venoms contain a variety of peptides / proteins, which are used as a molecular arsenal for predation and defense [32]. It is interesting how scorpions protect their venom peptides / proteins from degradation. Under the natural selection

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pressure, scorpions produce more efficient toxic peptides / proteins to be evolutionarily successful [32-34]. Suggestively, these peptides / proteins act as protease inhibitors to protect their venom peptides from degradation. Based upon previously and recently published papers, the protease inhibitors identified in scorpions are described in two categories- 1) Kunitz-type inhibitors and 2) Ascaris-type inhibitors.

Kunitz-type protease inhibitors

Kunitz-type inhibitors, a class of serine protease inhibitors, which are characterized by a conserved spacing between their cysteine residues. These inhibitors hold one or more Kunitz domains and possess a typical disulfide bonding pattern [35,36]. These are a paradigm for the canonical inhibition of serine proteases [37]. The Kunitz domain is an earliest as well as prevalent domain possessing a disulfide rich α + β fold, which is stabilized by three highly conserved disulfide bridges with the bonding patterns C1–C6, C2–C4, and C3–C5. Two of three disulfide bonds (C1–C6 and C3–C5) are involved in the conservation of native conformation [36,38], whereas the third bond (C2–C4) stabilizes the two binding domains [35,36].

Kunitz-type protease inhibitors have been reported to be present in scorpions, most of them identified by cDNA library screening and transcriptomic analysis. First, a novel Kunitz-type venom peptide gene precursor, SdPI, was cloned and characterized from a venom gland cDNA library of the scorpion *Lychas mucronatus* [22]. It codes for a signal peptide of 21 amino acid residues and a mature peptide of 59 residues. The recombinant SdPI peptide, being a thermo-stable peptide, showed potent inhibitory activity against trypsin with K_i value of 1.6×10^{-7} M. SdPI is the first functionally characterized Kunitz-type trypsin inhibitor from scorpion. Another Kunitz-type protease inhibitor, named SdPI-2, was identified from venom gland cDNA library of *Lychas mucronatus* [22], but its functional characterization has not yet been reported details. A Kunitz-type protease inhibitor named BmKPI was characterized from the venom gland of the scorpion *Buthus martensi* [10]. Experimentally BmKPI showed potent inhibitory activity against trypsin ($K_i = 1.8 \times 10^{-6}$M), chymotrypsin ($K_i = 3.2 \times 10^{-8}$ M), and elastase ($K_i = 1.6 \times 10^{-7}$ M). Mutagenesis on cysteine indicated that the disulfide bridge between C53–C61 has little effect on its inhibitory activity against elastase. Thus, BmKPI is a new multifunctional serine protease inhibitor, as well as it is the first functionally characterized Kunitz-type elastase inhibitor from scorpion.

The mature peptide, named Hg1, from the Mexican scorpion *Hadrurus gertschi*, was expressed using *Escherichia coli* BL21(DE3) cells, purified and tested on trypsin [11]. Along with Hg1, LmKTT-1a, LmKTT-1b and LmKTT-1c from *Lychas mucronatus* venom gland cDNA libraries, and BmKTT-1, BmKTT-2 and BmKTT-3 from venom gland cDNA libraries of scorpion *Buthus martensii* were also expressed and characterized as trypsin inhibitors. It should be mentioned that LmKTT-1b and SdPI are the same peptide. Experimentally Hg1, LmKTT-1a, LmKTT-1b, LmKTT-1c, BmKTT-1, BmKTT-2 and BmKTT-3 were found to exhibit trypsin inhibitory activity with K_i value of 107, 140, 160, 124, 136, 420 and 760 nm, respectively, with no activity against chymotrypsin or elastase even at high concentrations [11]. Among them, recombinant Hg1 (rHg1) was found to show the lowest dissociation constant against trypsin, while rBmKTT-3, from *Mesobuthus martensii*, had the highest [11].

Primary sequence of Kunitz-type inhibitors: All the Kunitz-type protease inhibitors identified from scorpions, till date, have been compiled here. Primary amino acid sequences of some inhibitor peptides have been found in NCBI protein data bank, whereas some other sequences have been adapted directly from published papers. The names of the inhibitors and their respective protein sequences are listed in the Table 1.

The inhibitor peptide rLmKTT-1b (SdPI), from scorpion *Lychas mucronatus*, was found to share sequence similarity with other Kunitz-type inhibitors. Nevertheless, rLmKTT-1b along with the scorpion peptides rLmKTT-1a, rLmKTT-1c, and rBmKTT-1 [11,39] contain a unique cysteine framework, different from the classical Kunitz-type motif, where the normal C2–C4 disulfide bridge is absent, but two additional cysteine residues are present at the C-terminus of the mature peptide, which might create a new disulfide bond. Consequently, a distinct disulfide bridge may be generated [22,39]. Additionally, among other scorpion protease inhibitors, recombinant BmKTT-2 was found to possess eight cysteine residues connected by four disulfide bridges, which is different architectural property from all known Kunitz-type animal toxins [11,39] (Figure 1).

Functional sites of Kunitz-type protease inhibitors: According to published reports, the circular dichroism (CD) spectrum, mutation in cysteine residues as well as molecular dynamics (MD) simulation have been employed to describe about the functional mechanism or active site of some scorpion Kunitz-type protease inhibitors.

To detect whether SdPI possesses a similar active site to other Kunitz-type venom peptides, four residues (Lys12, Gly13, Lys14, and Ala15) were mutated [13,22,40]. The circular dichroism (CD) spectrum was performed for each of the mutants and it was found that each of the mutants, compared with that of the wild-type peptide, indicated no significant change in secondary structure, suggesting that they all adopted the same structural topology. The inhibitory constants (K_i) of wild-type SdPI and four mutants against trypsin were determined and the results indicated that the Lys14Ala mutant displayed no inhibitory activity to trypsin up to 40 mM. Molecular dynamics (MD) simulation was performed to investigate the stability of SdPI-trypsin complex model and it was confirmed that Lys14 was located in the P1 position [22].

LmKTT-1a was a Kunitz-type toxin that adopted unique disulfide

PIs	Amino acid sequences	Sources
BmKPI	SEEADCHSPSRSGLCLAYFERYFYQPELGKCQKFVYGGCGGNGNNYESEAECCKACGDDRCLKK	[10]
SdPI	KNKCQLPSDVGKGKASFTRYYYNEEGGKCETFIYGGVGGNSNNFLTKEDCCRECAQGSC	NCBI accession: P0DJ45
SdPI-2	KKKCQLPSDVGKGKASFTRYYYNEESGKCETFIYGGVGGNSNNFLTKEDCCRECAQGSC	[22]
LmKTT-1a	KKKCQLPSDVGKGKASFTRYYYNEESGKCETFIYGGVGGNSNNFLTKEDCCRECAQGSC	NCBI accession: P0DJ46
LmKTT-1c	KNKCQLPSDVGKGKASFTRYFYNEESGKCETFIYGGMGGNSNNFLTKEACCRECAQGSC	NCBI accession: P0DJ48
BmKTT-1	QKDCSLPVDTGRGKGWFLRYYYNKNSKTCESFIYGGVGGNKNNFLNIENCCKICKAKNC	NCBI accession: P0DJ49
BmKTT-2	EGVDCTLPSDTGRCKAYFIRYFYNQKAGECQKFVYGGCEGNSNNFLTKSDCCKQCSPGKC	NCBI accession: P0DJ50
BmKTT-3	KHGSINCRLPPERGPCRGNITKYYYHNESRTCRTFSYGGCEGNSNNFRNRHYCMKYCARKRHGWLGTGWI	NCBI accession: P0DJ47
Hg1	GHHNRVNCLLPPKTGPCKGSFARYYFDIETGSCKAFIYGGCEGNSNNFSEKHHCEKRCRGFRKFGGK	NCBI accession: P0C8W3

Table 1: Primary amino acid sequence of Kunitz-type PIs from scorpions.

Figure 1: Sequence alignment of Kunitz-type protease inhibitors from scorpions.

bridges, C51-C59 [41]. To assess the function of this characteristic disulfide bridges in LmKTT-1a, a mutant LmKTT-1a-C51A / C59A was designed, possessing the same form of disulfide bridges as the Kunitz-type toxin ConK-S1. Circular dichroism spectroscopy showed that rLmKTT-1a-C51A / C59A had a secondary structure similar to LmKTT-1a. Enzyme and inhibitor reaction kinetics experiments using recombinant LmKTT-1a-C51A / C59A exhibited inhibition on trypsin with 5-fold lower activity than wild-type LmKTT-1a [41].

BmKPI was also found to be a Kunitz-type toxin with a unique disulfide bridge C53-C61 [10]. Similarly, In order to investigate the function of this characteristic disulfide bridge in BmKPI, a mutant BmKPI-C53A / C61A was designed, possessing the same disulfide bridges as the classical Kunitz-type peptide BPTI. The rBmKPI-C53A / C61A was found to have secondary structure and elastase inhibition, similar to wild-type BmKPI. These results indicated that the new C53-C61 disulfide bridge in BmKPI has only a weak effect on its ability to inhibit elastase [10]. Experiments performed in disulfide bridges of LmKTT-1a and BmKPI indicates the evolutionary diversity and functional conservation of Kunitz-type toxins that have different disulfide bridge patterns.

Ascaris-type inhibitors

Almost all Ascaris-type peptides possess a conserved structural feature with four short β-strands organized in two approximately vertical β-sheets and stabilized by five disulfide bridges: C1–C7, C2–C6, C3–C5, C4–C10, and C8–C9 [42]. The active site loop of these peptides is almost bounded by two disulfide bridges (C2–C6 and C3–C5), which is a part of the long loop connecting strands β1 and β2, as seen in the *Ascaris* sp. trypsin inhibitor (ATI), *Apis mellifera* cathepsin G / chymotrypsin inhibitor-1 (AMCI-1), chymotrypsin / elastase inhibitor-1 (C/E-1), and *Bombina bombina* skin trypsin inhibitor (BSTI) [43-45].

From scorpion, first Ascaris-type protease inhibitor, SjAPI, was identified from the venom of the *Scorpiops jendeki* [20]. SjAPI is a peptide of 64 amino acid residues with a typical Ascaris-type cysteine framework connected by five disulfide bridges. SjAPI is the first functionally characterized animal toxin peptide with an Ascaris-type fold, which inhibits α-chymotrypsin and elastase with a K_i value of 97.1 nM and 3.7 μM, respectively [20].

Along with SjAPI, three other serine protease inhibitors belonging to the Ascaris-type peptides were identified from the cDNA libraries constructed from the venom glands of scorpions: SjAPI-2 (*Scorpiops jendeki* Ascaris-type protease inhibitor 2), CtAPI (*Chaerilus tricostatus* Ascaris-type protease inhibitor), and BmAPI (*Buthus martensii* Ascaris-type protease inhibitor) [20]. Notably, functional characterization of SjAPI-2, CtAPI and BmAPI as protease inhibitors has not yet been revealed in details till date. A new peptide precursor, named Sj7170, containing 62 amino acid residues and stabilized by five disulfide bridges has been identified and characterized from the venomous gland cDNA library of the scorpion *Scorpiops jendeki*. Recombinant Sj7170 inhibits α-chymotrypsin with the inhibitory constant (K_i) of 1.0×10^{-7} M [21].

Recently, by screening scorpion venom gland cDNA libraries, four new serine protease inhibitors with a conserved Ascaris-type structural fold were identified [46]. These are Ascaris-type toxins *Lychas mucronatus* Ascaris-type protease inhibitor (LmAPI), *Pandinus cavimanus* Ascaris-type protease inhibitor (PcAPI), *Pandinus cavimanus* Ascaris-type protease inhibitor 2 (PcAPI-2), and *Hottentotta judaicus* Ascaris-type protease inhibitor (HjAPI). Among four, the detailed characterization of only Ascaris-type toxin LmAPI has been reported. LmAPI contains 60 residues and possesses a classical Ascaris-type cysteine framework reticulated by five disulfide bridges. The study confirmed that recombinant LmAPI inhibits the activity of chymotrypsin potently with a K_i value of 15.5 nM, but exhibits little effect on trypsin and elastase [46].

Primary sequence of Ascaris-type inhibitors: The sequences of all the Ascaris-type protease inhibitors identified from scorpions, up to now, have been compiled here. Primary amino acid sequences of some inhibitor peptides have been found in NCBI protein data bank, whereas some other sequences have been adapted directly from published papers. The names of the inhibitors and their respective protein sequences are listed in the Table 2.

All the Ascaris-type protease inhibitors from scorpions share homology and possess a conserved Ascaris-type structural fold. However, according to the sequences reported by Liu et al. [46] only the peptide HjAPI identified from *Hottentotta judaicus* was shown to contain nine cysteine residues, odd number of cysteine (Figure 2).

Functional sites of Ascaris-type protease inhibitors: Based upon sequence analyses, LmAPI contains a conserved Ascaris-type structural fold. According to structure-function relationship, the binding loop of Ascaris-type peptides might be located in a conserved region between cysteine V and VI [47], and in LmAPI, this region corresponds to the sequence "TQD", indicating that Thr30 might be the P1′site of LmAPI, Gln31 might be the P1 site of LmAPI, and Asp32 might be the P2 site of LmAPI [46]. 6 ns unrestrained molecular dynamics (MD) simulation employed to probe the stability of a constructed LmAPI–chymotrypsin complex model suggested that the hydrophobic interactions mainly contributed to the interaction between LmAPI and chymotrypsin. Thr30 of LmAPI interacts with five amino acid residues of chymotrypsin Cys38, His53, Cys54, Ser189 and Ser208. Asp32 of LmAPI interacts with Met186 residue of chymotrypsin. Gln31, the P1 site of LmAPI, fits into the pocket of chymotrypsin and interacts with eleven amino acid

Inhibitors	Amino acid sequences	Sources
Sj7170	QMTCRISGEVFTWCGTTCPLTCENFRNPPKHCPQGCFVGCMCRRGLVRHRNGRCVRPPRCYY	[21]
LmAPI	QRRGFCGPNEEIKPCGPCDGTCRNPNPICTQDCRPPACGCVRGTVRGPSGLCIPLRFCFR	[46]
PcAPI	YEKCGKNEYYTTCGACDGTCAKPEVPCPRICHPPGCYCVLDSVRGPDGNCIPLGECP	[46]
PcAPI-2	QSDEGKCGENEFFMRCGGCDGDCYQPMVPCTMICHAPGCYCKEGTVRGPDGSCIPEEECH	[46]
HjAPI	MSSSRECKRENEEYQECGTARPITCANYHNPPTGCTEQCVSDCFCKNGYYRASNRACVLLKDCF	[46]
BmAPI	QSYFRCRDNEVFDNCISNCGPPRCSNILNTYPCTNLGPLCTPGCKCKDGRVYDNQGRCVLQTECFQK	NCBI accession: Q86RQ7
CtAPI	QPNLWRCEKDEEFVNCAPRCPQNCRNIRSYQPCLVLTPVCAPGCVCRSGKVKNDRGDCVSITDCFK	NCBI accession: P0DM57
SjAPI	QKCSSKNEEFQQCGSSCPETCANHKNPEPKSCAAVCFVGCVCKPGFIRDDLKGSICVKPEDCSK	NCBI accession: P0DM55
SjAPI-2	QMTCRISGEVFTWCGTTCPLTCENFRNPPKHCPQGCFVGCMCRRGLVRHRNGRCVRPPRCYY	NCBI accession: P0DM56

Table 2: Primary amino acid sequence of Ascaris-type PIs from scorpions.

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Sj7170      ---QMTCRISGEVFTWCGTTC-PLTCENFRNP-PKHCP-QGCFVGCMCRRGLVRH--RNGRCVRPPRCYY- 62
SjAPI-2     ---QMTCRISGEVFTWCGTTC-PLTCENFRNP-PKHCP-QGCFVGCMCRRGLVRH--RNGRCVRPPRCYY- 62
SjAPI       ----QKCSSKNEEFQQCGSSC-PETCANHKNPEPKSCA-AVCFVGCVCKPGFIRDDLKGSICVKPEDCSK- 64
HjAPI       MSSSRECKRENEEYQECGTAR-PITCANYHNP-PTGCT-EQCVSDCFCKNGYYRA--SNRACVLLKDCF-- 64
BmAPI       -QSYFRCR-DNEVFDNCISNCGPPRCSNILNTYPCTNLGPLCTPGCKCKDGRVYD--NQGRCVLQTECFQK 67
CtAPI       QPNLWRCE-KDEEFVNCAPRC-PQNCRNIRSYQPCLVLTPVCAPGCVCRSGKVKN--DRGDCVSITDCFK- 66
PcAPI       ---YEKCG-KNEYYTTCGACD--GTCAKPEVPCPRICH----PPGCYCVLDSVRG--PDGNCIPLGECP-- 57
PcAPI-2     QSDEGKCG-ENEFFMRCGGCD--GDCYQPMVPCTMICH----APGCYCKEGTVRG--PDGSCIPEEECH-- 60
LmAPI       -QRRGFCG-PNEEIKPCGPCD--GTCRNPNPICTQDCR----PPACGCVRGTVRG--PSGLCIPLRFCFR- 60
                  *   .*    *        * :     .           * *  .          *:    *
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Figure 2: Sequence alignment of Ascaris-type protease inhibitors from scorpions.

residues Ser184, Cys185, M186, G187, Ser189, Val207, Trp209, Gly210, Ser211, Ser212 and Cys214 [46].

In case of Ascaris-type peptide SjAPI, the binding loop region corresponds to the sequence "AAV," in which Ala34 is the P1 site, Ala33 is the P2 site, and Val35 is P1' site. MD simulation performed to probe inhibitor-protease interactions confirmed that the inhibitory activity of SjAPI was more potent for chymotrypsin than for elastase [20]. Beside the hydrophobic interactions, the amide group of Ala34 in SjAPI and the hydroxyl group of Ser189 in chymotrypsin formed hydrogen bond pair and contributed to the interaction between SjAPI and chymotrypsin. The carboxyl group of Ala34 in SjAPI formed hydrogen bond pairs with the groups of Ser185 and Gly183 in elastase, and the carboxyl group of Ala33 in SjAPI formed hydrogen bond pair with the group of Gln185 in elastase, and all these bond pairs contributed to the interaction between SjAPI and elastase. According to the report, based on bioinformatics analyses and chimera experiments, SjAPI contains the unique short side chain functional residues "AAV" and might be a useful template to produce new serine protease inhibitors [20].

Structural properties of scorpion protease inhibitors

Most of the scorpion protease inhibitors reported so far have been identified by screening the cDNA libraries constructed from the venom glands of scorpions, whereas native protease inhibitors have hardly been reported by direct purification from the venom of scorpions. However, till date, little is known about the structural information of scorpion Kunitz-type protease inhibitors, whereas structural information about Ascaris-type inhibitors has not yet been revealed in details. The solution structure of Kunitz-type serine protease inhibitor, LmKTT-1a, was determined by NMR in previous report [41]. The solution structure of LmKTT-1a presents a typical Kunitz-type fold, containing an N-terminal helix from Lys2 to Cys4, double-stranded anti-parallel β-sheets from Phe17 to Asn23 and Lys28 to Tyr34. LmKTT-1a presents a C-terminal helix from Asp49 to Ala55 containing a unique disulfide link between Cys51 and Cys59. The structural integrity of LmKTT-1a was maintained by two additional disulfide bridges located at Cys4–Cys54 and Cys29–Cys50. The β-sheet was found to be stabilized by the connection between Cys29 and the C-terminal Cys50, which is the most rigid region of the structure [41]. Different from the typical Kunitz motif, SdPI lacks the normal C2–C4 disulfide bond but obtains another two cysteine residues at the C-terminus. A computer-based molecular dynamics simulation was used to predict the 3D structure of recombinant SdPI to determine the active site [22] where α-helix, β-sheets as well as active sites were determined, suggesting that the change in cysteine positions may lead to the formation of a novel disulfide bridge in the SdPI peptide, but it interacts with trypsin in similar way to other Kunitz-type venom peptides [22]. The 3-D structure of BmKPI was reported to be modeled using LmKTT-1a (PDB Code: 2M01) as a template. 3D structure of scorpion Kunitz-type toxin Hg1 was also modeled [11] by SWISS-MODEL using BPTI as a template (PDB code 6PTI).

Functional diversity of protease inhibitors

Some of Kunitz-type protease inhibitors from scorpions have been reported to block potassium channel $K_V1.3$ [11]. Seven Kunitz-type protease inhibitors (LmKTT-1a, LmKTT-1b, LmKTT-1c, BmKTT-1, BmKTT-2, BmKTT-3 and Hg1) were tested on voltage gated potassium channel subtype 1.3 ($K_V1.3$ channel) and it was found that six of seven scorpion toxins, except rBmKTT-3 having weak activity, inhibited ~ 50% – 80% of Kv1.3 channel currents at a concentration of 1 μM. The IC_{50} values of rBmKTT-1, rBmKTT-2, and rHg1 for Kv1.3 channels were ~ 129.7 nM, 371.3 nM, and 6.2 nM, respectively. In pharmacological experiments, rHg1 was found to be a highly selective Kv1.3 channel inhibitor.

A new function of Ascaris-type peptide SjAPI-2 has been discovered recently [48]. It has been reported that SjAPI-2 of 62 amino acid residues including 10 cysteine residues can selectively inhibit KCNQ1 potassium channel. The report confirmed that SjAPI-2 was a selective KCNQ1 potassium channel inhibitor, also showing weak effects on other potassium channels, such as Kv1.1, Kv1.2, Kv1.3, SKCa2, SKCa3, and IKCa channels. Experiment of concentration-response exhibited

that SjAPI-2 inhibited the KCNQ1 potassium channel with an IC_{50} value of 771.5 nM ± 169.9 nM. However, SjAPI-2 is the first neurotoxin with a unique Ascaris-type fold, which provides novel insights into the divergent evolution of neurotoxins from venomous animals [48]. In addition, Sj7170 was reported as a unique dual-function peptide, i.e., a specific α-chymotrypsin inhibitor and a potent tumorigenesis / metastasis activator [21]. Chymotrypsin inhibitor, Sj7170, was found to promote cell proliferation and colony formation by up-regulating the expression of cyclin D1 *in vitro*. Besides, it was also found to enhance tumor growth in nude mice. Sj7170 was finally reported to accelerate cellular migration and invasion by increasing the expression of the transcription factor Snail and then inducing the epithelial-mesenchymal transition. Furthermore, Sj7170 was found to change cell morphology as well as cytoskeleton of U87 cells by the GTPase pathway [21].

Conclusion

In this review we present the protease inhibitors, Kunitz-type and Ascaris-type serine protease inhibitors, from scorpions, most of which have been identified by screening cDNA library constructed from scorpion venom glands. Here, we have presented all the protease inhibitors reported till date according to their biological sources, together with their main characteristics and activities against different proteases. Then, their dual function including potassium channel blocking activity is discussed, followed by the functional active sites of protease inhibitor compounds.

Because of the increasing diversity of protease inhibitors, primary sequence comparisons, along with studies comprising site-directed mutagenesis and conformational analysis could help better understand about key amino acid residues which are essential for protease inhibition and also for the Kv channel blocking activity. The sequences of these scorpion PIs could be better analyzed, which could lead to the design of more potent protease inhibitors and dual-function polypeptides and also to their strategically minimization in size, fashioning enhanced and low-priced drugs for diverse therapeutic and biotechnological applications. Besides, comparative analysis could lead to a global evolutionary model that comprises all the PIs with important physiological functions in the source organisms.

Notably, serine protease, trypsin, has been reported to be involved in many inflammatory reactions in the human body, such as pancreatitis as well as other cardiovascular and nervous systems diseases [49,50]. Trypsin inhibitors, such as ulinastatin and aprotinin, are already being clinically used in anti-inflammatory therapy [31]. Accordingly these serine protease inhibitors, from scorpions, might also be usefully exploited in drug development to cure protease-related diseases. Moreover, to open the gate of new molecules to treat emphysema, coagulation, inflammation, dermatitis and cancer, serine protease inhibitors that can be exploited by the medical industry.

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