Molecular characterization of a novel secretory phospholipase A2 as coding gene from venom glands of Iranian *Scorpio maurus* (Arachnida: Scorpionidae)

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Abstract

Background

Scorpions as a venomous arthropod can defend and capture the prey by secretion of venom into their venom sacs. The venom is a mixture of a variety of compounds with specific biological activities, such as phospholipase A2 (PLA2) enzyme to be present in scorpion venom. This study is the first investigation of structural and molecular characterization of PLA2 from Iranian scorpion, *S. maurus* that can be added to the literature when targeting molecular characterization of PLA2 coding gene from venom glands of Iranian *S. maurus*.

Results

S. maurus PLA2 with 432 base pair (bp) nucleotide length encoded a protein of 144 amino acid residues and 16.34 kilodaltons (kDa). According to DiANNA 1.1 web server prediction, this protein had 5 disulfide bridges. The deposited PLA2 in GenBank (MW241004) was very similar to *S. maurus* (MF347455.1) with 95.14% identity. The phylogenetic analysis indicated that our PLA2 had a close relationship with insect PLA2. Our PLA2 had 38.83% identity with bee venom PLA2 according to SWISS-MODEL prediction and 100% confidence and 39% identity with insect phospholipase A2 family, which was predicted by Phyre2. Our PLA2 was similar to chain A of bee venom PLA2. Conserved domains on target PLA2 contained 17–118 amino acid in length and 45–52 amino acids in the active site. There was no signal peptide.

Conclusions

In the light of our results, the coding sequence of characterized PLA2 was a member of the PLA2 superfamily. According to the three-dimensional structure prediction, the target PLA2 with five disulfide bonds has a very high similarity to the structure of phospholipase A2 belonged to the group III subfamily. Interestingly, the active site residue of target PLA2 also has the His-Asp acid pair and the effective amino acid in catalytic domain of it was His⁴⁹.

Background

The order of scorpions is the oldest one of venomous arthropods that have been living over 400 million years ago [1, 2]. So far, over 2,360 scorpion species have been identified and classified as 19 families [3]. The scorpion species have venom glands used to secrete venom into venom sacs to defend and capture the prey [4]. The secreted compounds from the scorpion venom gland are a complex mixture of proteins, non-proteins, and some unknown compounds with unknown function including inorganic salts, mucoproteins, carbohydrates, peptides, lipids, free amino acids, nucleotides, water, and enzymes such as phospholipase A2 (PLA2) [5–7].

PLA2 is an enzyme that hydrolyzes glycerophospholipids at position of sn-2 of phospholipids and releases free fatty acids [oleic acid (OA) and arachidonic acid (AA)] and lysophospholipids [8, 9]. This superfamily is classified into 15 groups and many subgroups [10]. PLA2 superfamily has six principal types including cytosolic PLA2 (cPLA2), platelet-activating factor cetyl hydrolases (PAF-AH), Ca^{2+} -independent PLA2s (iPLA2s), lysosomal PLA2s, adipose specific PLA₂ (AdPLA₂) and secreted phospholipase A2 (sPLA2) [10–12].

sPLA2 as calcium-depend enzyme, is a typical enzyme in the venom of snake, lizard, bee, and scorpion that has been identified till now [8, 13–15]. This low-molecular-weight enzyme is 13–15 kilodaltons (kDa) [16]. So far, 17 isoforms of sPLA2 have been identified and divided in groups I-III, V, IX-XIV [17]. sPLA2 from the venom glands of scorpions is classified as group III subfamily [18]. sPLA2 has been identified from different species of scorpions till now, including *Pandinus imperator* [19, 20], *Hemiscorpius lepturus* [21, 22], *Anuroctonus phaiodactylus* [18], *Heterometrus fulvipes* [23], *Heterometrus laoticus* [8] and *Scorpio maurus* [24].

Iran as a country located in the Middle East, due to its climate, has a good potential for scorpions living [25]. Fars province which is located in the southwest of Iran has hot and humid weather and scorpions are important public health problem in this region [26]. *Scorpio maurus* as a species of *Scorpionidae* family is a considerable species in this province [27]. The aim of this study was to identify PLA2 from the venom glands of Iranian scorpion, *S. maurus* based on a molecular characterization.

Results

Characterization of the PLA2 coding gene

To decide the PLA2 coding sequence, polymerase chain reaction (PCR) reaction, which was performed by specific primers on the synthesized complementary DNA (cDNA), appeared as a fragment close to the awaited size of 432 base pair (bp) (Figure1). The PLA2 sequence was deposited in GenBank under accession number (MW241004).

Analysis of cDNA and protein sequence of *S. maurus* PLA2

S. maurus PLA2 protein contained a 432 bp open reading frame (ORF) that encoded a protein of 144 amino acid residues with a predicted molecular mass of 16.34 kDa (Figure 2). The amino acid sequence of concluding PLA2 was evaluated by GenRunner software. The BLAST result showed that the protein sequence was very similar to *S. maurus* PLA2 (GeneBank: MF347455.1) with 95.14% identity. Other listed proteins with high identity levels in BLAST search were 74.07% to Hemilipin from *H. lepturus* (GeneBanke: KX924472.1), 78.12% to PLA2 from *O. cayaporum* (GeneBanke: FM998793.1), and 74.12% to PLA2 from *A. phaiodactylus* (GeneBanke: EF364043.1). To ensure, *S. maurus* PLA2, protein sequence was assessed using protein BLAST alignment. At first glance, all proteins revealed association with PLA2 enzyme.

Phylogenetic analysis

Initial tree(s) for the heuristic search were received automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with higher-level logarithm likelihood value. Instructive branch lengths were typically drawn to scale and showed the number of substitutions per site (0.2). The significant relationship between insect PLA2 was quite obvious. In addition, the phylogenetic analyses showed that the enzyme detected in human differed significantly from that of insects (Figure 3).

Structural characteristics of *S. maurus* PLA2

The consequences indicated that all selected proteins were associated with insect PLA2. Above all, high similar to *S. maurus* PLA2 (GeneBank: AVD99009.1) with 90.28% identity. Sequence alignment of target *S. maurus* with three other species of scorpions including *S. maurus* (AVD99009.1), *H. fulvipes* (Q3YAU5.1), and *H. lepturus* (A0A1L4BJ46.1) were performed by Clustal Omega and conserved amino acids were highlighted (Figure 2). The conserved amino acids between the two species sequences of target *S. maurus* and *S. maurus* (AVD99009.1) were analyzed. The target *S. maurus* PLA2 did not have similarity with *S. maurus* PLA2 (AVD99009.1) in 13 amino acids. The amino acid compounds of *S. maurus* PLA2 were shown in Table 1. The predicted disulfide bonds in five positions were between (Cys8 and Cys46), (Cys25 and Cys46), (Cys45 and Cys46), (Cys52 and Cys75), and (Cys77 and Cys84) (Figure 4).

Three-Dimensional structure prediction

The three dimensional (3D) structure of *S. maurus* PLA2 by SWISS-MODEL demonstrated that the target PLA2 was similar to chain A of bee venom PLA2, group III subfamily, with 38.83% identity. The predicted 3D structure demonstrated that target PLA2 of *S. maurus* was similar to chain A of bee venom PLA2. The 3D structure of *S. maurus* PLA2 by Phyre2 displayed that the target sequence close to insect PLA2 family with 100% confidence and 39% identity. On the other hand, The *S. maurus* PLA2 was similar to vertebrate PLA2 with 97.3% confidence and 33% identity.

Active site structure

There was no signal peptide in target PLA2. The comparison of our PLA2 sequence with similarly characterized related proteins displayed a conserved catalytic site (active site), which was common in PLA2 superfamily of secretory and cytosolic enzymes. Conserved domains on target PLA2 contained 17-118 amino acids length (Figure 5). According to ExPASy-PROSITE online tool, the active site of target PLA2 contained 45-52 amino acids length which was conserved among different species of scorpions (Figure 2). The catalytic domain included "CCRTHDXC motif," which was the binding domain that supported the active site in PLA2 superfamily.

Prediction of protease cleavage sites

Based on PROSPER, three types of protease families cleavage the target PLA2 sequence were determined and shown in Table 3. According to PeptideCutter web server, the prediction enzymes including Caspase-1

to -10, Enterokinase, Factor Xa, GranzymeB, and Thrombin were demonstrated not to cut the target PLA2.

Discussion And Conclusion

The current study was the first investigation of structural and molecular characterization of PLA2 from venom glands of Iranian *S. maurus* that can be added to the literature when targeting molecular characterization of PLA2 coding gene from venom glands of Iranian *S. maurus*. Phospholipase A2 (PLA2) is functionaries of the inflammatory process which catalyzes the hydrolyze phospholipids at the sn-2 position of the glycerol backbone and releases fatty acid and lysophospholipids [28, 29]. Group III subfamily of PLA2s have been identified from various sources such as reptiles [30, 31], mammals [32], parasites [33], and arthropods including scorpion [18, 21]. The coding sequence of PLA2 was detected from *S. maurus* venom glands for the first time in Iran. The detected PLA2 has encoded a protein of 144 amino acid residue to be 16.34 kDa. Till now, several studies reported the genes encoding phospholipases A2 from different species of scorpions including Hemilipin from *H. lepturus* [21], Imperatoxin I and Phospholipin from *P. imperator* [19, 20], Phaiodactylipin from the *A. phaiodactylus* [18], Heteromtoxin from *Heterometrus laoticus* [8], PhospholipaseA2 from *H. fulvipes* [34], MtPLA2 from *Mesobuthus tumulus* [35], Sm-PLVG from Tunesian *S. maurus* [24], and PhospholipaseA2 from *Hadrurus gertschi* [36].

The results of Blast in NCBI showed that the detected sequence from the Iranian *scorpio maurus* has a very high similarity to the same sequence of Tunesian *S. maurus* (GeneBank: MF347455.1) [37], while this level of similarity is very low compared to other species of scorpions. Comparing the coding sequence of target PLA2 with other characterized PLA2 from different species of scorpions, was shown that our PLA2 was a member of the PLA2 superfamily.

Phylogenetically, there is a distant relationship between group I, II, and III of phospholipase A2, but at the site of calcium-binding and the region of active site, they are quite similar [38, 39]. In the current study, the result of likelihood analysis showed that the characterized PLA2 from Iranian *S. maurus* and other arthropods, scorpions and *Apis mellifera* (FE373554.1), are well clustered, whereas this one from *Homo sapience* (M86400.1) is distantly located. similar to the phylogenetic relationship of phospholipases A2 from *A. phaiodactylus* [39].

The difference in 13 amino acids residue in the gene encoding sequence of phospholipase A2 enzyme between the Iranian and Tunisian *S. maurus* is most likely due to differences in the geographical condition in which they live. The residues Asp², Leu⁹, Phe¹⁷, Glu³⁰, Glu³², Ser⁶⁷, Pro⁶⁹, Met⁸⁶, Asp⁹⁰, Thr⁹⁵, Asn¹⁰², Lys¹⁰⁹, Tyr¹¹⁰ of target *S. maurus* PLA2 were Val, Ser, Leu, Val, Lys, Phe, Phe, Thr, Asn, Asp, Asp, Asp, Asn in Tunisian *S. maurus* (AVD99009.1), respectively. Certainly, the difference in the amino acid residue is greater among different species of scorpions.

Based on the results of the three dimensional structure prediction, the phospholipaseA2 encoding gene isolated from the Iranian *S. maurus* has a very high similarity to the structure of phospholipase A2

belonged to group III subfamily, but similarity to vertebrates' phospholipase A2 was smaller. Phylogenetic analysis also confirms these results. Similar to characterized PLA2 of *A. phaidactylus* [39], Imperatoxin I of *P. imperator* [19], our PLA2 is closely related to genomic structure of *A. mellifera*, the only known representative structure in group III PLA2. Evaluation of the 3D structure of the target gene revealed that the identified protein was very similar to the chain A of characterized PLA2 from bee venom. Four disulfide bonds were predicted for phospholipase A2 of *M. tamulus* at the position of eight cysteines (Cys8–Cys30), (Cys29–Cys68), (Cys35–Cys61) and (Cys59–Cys96) [35]. It is similar to the position of the human group III PLA2 disulfide bonds in ten cysteines (Cys8-Cys30), (Cys29-Cys68), (Cys35-Cys61), (Cys59-Cys91), and (Cys101-Cys113) [40], while different positions were predicted to the five disulfide bonds in target PLA2. The histidine–aspartic (His-Asp) acid pair has been suggested to be necessary for the catalytic mechanism of the phospholipase A2 [41]. In this study, the active site residue of target PLA2 also has the His-Asp acid pair and the effective amino acid in catalytic domain of target PLA2 was His⁴⁹, while His³⁴ played a major role in the active site residue of bee's venom and Tunisian *S. maurus* [37].

Methods

Scorpion collection

The scorpions were collected from Fars Province, Zarrin Dasht County, south-west of Iran, and transferred alive to the laboratory of Medical Entomology in Shiraz University of Medical Sciences, Shiraz, Iran. Samples were identified via a valid key [42]. Before RNA extraction, venom of the collected scorpions was milked manually to release the venom. Three days after venom milking, the telson of the scorpions were separated and stored at -70°C. Other parts of the body were stored at ethanol 96% and were kept in the archives of the Museum of the department of Medical Entomology in the Shiraz University of Medical Sciences.

Primer design

To design the gene-specific primer (GSPs), the mRNA sequences of PLA2 from different species of scorpions such as *S. maurus* (GeneBanke: MF347455), *H. leptorus* (GeneBanke: KX924472), *A. phaidactylus* (GeneBanke: AY571967.1), and *Opisthacanthus cayaporum* (GeneBanke: FM998793.1) were obtained from national center for biotechnology information (NCBI). The normal size bound was 432 bp. The specific primers were 5'-TCCAAAGAAGAAATGGA (forward primer) and 5'-GTCTTTGTAGCTCTTTTCCAGG (reverse primer).

Preparation of cDNA

Total RNA was extracted from the venom glands of *S. maurus* telson by High Pure RNA Isolation Kit, Roche®. RNA sample was treated enzymatically by DNase based on manufacturer's manual. Extracted RNA concentration was measured using a Nanodrop (analytikjena®).

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

According to manufacturer's instruction, 3 µL of total RNA was used as a template for cDNA synthesis by AccuPower® CycleScript RT Premix with (d) (Bioneer Company, Korea). 0.1 to 1 µg of RNA template was filled up to the 20 µL volume with Diethylpyrocarbonate-treated water (DEPC DW) and was dissolved by vortexing. cDNA synthesis reaction was performed in four steps according to manufacturer's manual including 30 sec at 25°C for primer annealing, 4 min at 45°C for cDNA synthesis, 30 sec at 55°C for melting secondary structure and cDNA synthesis, and 5 min at 95°C for heat inactivation. The synthesized cDNA was kept at -70°C for PCR amplification steps as a template.

Polymerase Chain Reaction (PCR)

The desired DNA sequences were amplified in total 20 µL volume containing 10 µL Taq DNA Polymerase Master Mix RED (2X), 1 µL forward primer, 1 µL of reverse and finally 1 µL of synthesized cDNA. It was performed to 35 cycles of 30 s at 94°C as denaturation, 30 sec at 50°C as annealing temperature, 30 sec at 72°C as extension and 10 min at 72°C for a final extension.

DNA gel purification and sequencing

PCR products were run onto 2% tris borate EDTA (TBE) agarose gel with appropriate DNA ladder. Our specific band was observed in the gel documentation instrument. Selected band was purified according to protocol of Gene All kit. Expected size bands were sequenced using GSPs forward and reverse.

Phylogenetic analysis

To evaluate the target PLA2 protein, a phylogenetic tree was created based on different characterized related protein sequences including scorpion species, *Apis mellifera*, and *Homo sapiens* by utilizing the maximum likelihood method. Multiple sequences alignment for evolutionary analyses were conducted in MEGA7 software based on the Clustal W method [43]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches [44].

Structural characteristics of *S. maurus* PLA2

MEGA software (Version 7.0) was used for alignments. All primers were designed by the GeneRunner (Version 4) and Oligo 7 software. The designed primers specificity was determined using Primer-BLAST on NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). To identify the structural features of the protein coded by PLA2 and guarantee that obtained sequence could be a part of phospholipase family proteins, the *S. maurus* PLA2 was translated by GeneRunner software (Version 4.0), and the concluded sequence was evaluated by diverse instruments. Protein BLAST was performed to decide proteins with great closeness to recognize and record their characterization. To compare and classify protein structure, similar proteins were chosen for alignment with Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). Amino acid compounds of *S.maurus* PLA2 were analyzed by utilizing ProtParam online tool

(<https://web.expasy.org/protparam/>). The PLA2 coding gene was translated by GeneRunner software (version 4). A protein BLAST was also carried out for more comparative relation. To disulfide bridge prediction of the target protein, DiANNA 1.1 web server was used [45, 46]. Disulfide bridges formation are essential for biological activity in many proteins [47]. To predict disulfide bonds, DiANNA 1.1 web server (<http://clavius.bc.edu/~clotelab/DiANNA/>) was used.

Three-Dimensional structure prediction

In order to predict the 3D structure, the SWISS-MODEL online tool (<https://swissmodel.expasy.org/>) and Phyre2 (<http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>) were used, which predicted the 3D structure of a query protein through the sequence alignment of template proteins. This model was chosen for superimposition. The predicted 3D structure was evaluated by UCSF Chimera software (Version 1.14).

Active site structure

Several online tools were used to evaluate the signal peptide of the gene in question. PLA2 breaks the sn-2 position of the glycerol backbone of phospholipids, mainly in a metal-dependent reaction, to produce lysophospholipid (LysoPL) and a free fatty acid (FA) [8, 9]. Superimposition of active site was carried out by UCSF Chimera (Version 1.14) and DeepView/Swiss-pdbviewer (Version 4.10) software (Figure 3) (34). The root-mean-square deviation (RMSD) of bee venom PLA2 and target protein active site residue was calculated by Chimera software (Version 1.14) to measure the average distance between corresponding atoms in two protein chains based on carbon alpha atoms (Table 2). Active-site residues were universally conserved within protein families, displaying their key role for substrate catalysis [48]. The active site prediction was undertaken by ExPASy-PROSITE (<https://prosite.expasy.org/>). Several online devices were used for signal peptide analysis such as SignalP 4.1 Server (<http://www.cbs.dtu.dk/services/SignalP/>), Signal Signal-3L 2.0, and BLAST (<http://sigpep.services.came.sbg.ac.at/signalblast.html>).

Prediction of protease cleavage sites

PROSPER (protease specificity prediction Server), (<https://prosper.erc.monash.edu.au/webserver.html>) and PeptideCutter (http://web.expasy.org/peptide_cutter/) servers were used for prediction of the activated form of target protein. ProtParam (<https://web.expasy.org/protparam/>) was used for amino acid composition.

Abbreviations

PLA2: Phospholipase A2; cPLA2: cytosolic PLA2; PAF-AH: platelet-activating factor cetyl hydrolases; iPLA2s independent PLA2s; AdPLA2: adipose specific PLA2; sPLA2: secreted phospholipase A2; OA: Oleic acid; AA: Arachidonic acid; cDNA: complementary DNA; PCR: polymerase chain reaction; bp: Base pair; kDa: Kilodaltons ; ORF: open reading frame; MCL: Maximum Composite Likelihood; 3D: three dimensional structure; NCBI: national center for biotechnology information; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; μ L: Microliter; Met: Methionine; TBE: Tris borate EDTA; GSPs: Gene-Specific Primers;

RMSE: root-mean-square deviation; Thr: Threonine; Asn: Asparagine; Lys: Lysine; Tyr: Tyrosine; Asp: Aspartic acid; Cys: Cysteines; His: Histidine; Val: Valine; Leu: Leucine; Phe: Phenylalanine; Glu: Glutamic acid; Ser: Serine; Pro: Proline.

Declarations

Ethics approval and consent to participate

No human or animal data or tissue was used in this study. But all experiments were performed in accordance with the ethical standards of the Shiraz University of Medical Sciences ethics committee (ethics committee code: IR. SUMS. REC.1399.87). Specimens (Scorpions) were not client-owned animal and we collected them from the natural habitats (Zarrin Dasht county, Fars province, South of Iran) with the permission of the Shiraz University of Medical Sciences and in coordination with the Fars Environmental Protection Organization.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are available upon reasonable request to the corresponding author.

Competing interests

None declared.

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Authors' contributions

PS-A and KA conceived and designed the study and contributed to the discussion of results, HA, AS, QA and AR conceived the study and analyzed data, PS-A, KA and DM drafted the manuscript. All authors have read and approved the final manuscript.

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Tables

Table 1. Amino acid compounds of *S.maurus* PLA2 were analyzed by ProtParam online tool.

Amino acid type	Number	Percentage
Glu (E)	12	8.3
Gly (G)	12	8.3
Lys (K)	12	8.3
Asp (D)	11	7.6
Ser (S)	11	7.6
Cys (C)	11	7.6
Thr (T)	10	6.9
Asn (N)	9	6.2
Ala(A)	8	5.6
Arg (R)	8	5.6
Leu (L)	8	5.6%
Tyr (Y)	8	5.6
Phe (F)	6	4.2
Pro (P)	4	2.8%
Trp (W)	3	2.1
Val (V)	3	2.1
Gln (Q)	2	1.4
His (H)	2	1.4
Ile (I)	2	1.4
Met (M)	2	1.4

Table 2. The RMSD of active site residues in an important domain in *Scorpio maurus* and bee venom PLA2.

Amino acid	Cys	Cys	Arg	Thr	His	Asp	His	Cys
Position in <i>Scorpio maurus</i> PLA2	45	46	47	48	49	50	51	56
Amino acid	Cys	Cys	Arg	Thr	His	Asp	Met	Cys
Position in bee venom PLA2	30	31	32	33	34	35	36	37
RMSD	0.000	0.000	0.000	0.000	0.724	2.035	2.401	1.531

Table 3. Predicted cleavage sites of individual proteases. Restriction site have been shown by (⌘).

Protease family	Proteases type	Position	Segment
Cysteine protease	Cathepsin K	56	DYIG⌘SGET
		86	QCLM⌘EAYD
Metalloprotease	Matrix metalloproteinase-9	63	TKYG⌘LTNS
		84	FDQC⌘LMEA
		102	STKN⌘LRNF
		115	RPQC⌘YVVT
Serine protease	Chymotrypsin A (cattle-type)	81	EEAF⌘DQCL
		89	MEAY⌘DKEE
		138	VATW⌘KKS Y
	Elastase-2	18	RFFI⌘WGGT
	Cathepsin G	17	GRFF⌘IWGG
		54	HCDY⌘IGSG
		62	ETKY⌘GLTN
		116	PQCY⌘VVTC
		121	VTCN⌘SNRS
	Glutamyl peptidase I	41	GPLE⌘ADKC
		79	KCEE⌘AFDQ
		132	AGCE⌘NGVA
	Thylakoidal processing peptidase	52	HDHC⌘DYIG

Figures

Figure 1

Amplification of *Scorpio maurus* PLA2 mRNA; Lane 1 is ladder 100 bp DNA marker, Lane 2 and 3 are PLA2 (432bp), and lane 4 is negative control

<i>Heterometrus fulvipes</i>	-----TMW-GTKWCGSGN	12
<i>Hemiscorpius lepturus</i>	ECRMINSMHELSRELMDSP EHTVKRASKEEMDDL VQRCSGSAEGRSWFIWPD TKWCGPGT	120
<u><i>Scorpio maurus</i></u>	-----MDDLARRCLDSSEGR-FFIWGGTKWCGPGN	29
<i>Scorpio maurus</i>	-----MVDLARRCSDSSEGR-FLIWGGTKWCGPGN	29

<i>Heterometrus fulvipes</i>	KAINYTDLG YFSNLDSCCRTHDHCDNIAAGETKYGLTNEGKYTMMNCKCEATFQQCLRDV	72
<i>Hemiscorpius lepturus</i>	DAKNESDLGPLE-ADKCCRTHDHCDYIGAGETKYGLTNKSFFTKLNCKCEAAFDQCLKES	179
<u><i>Scorpio maurus</i></u>	E A ENESDLGPLE-ADKCCRTHDHCDYIGSGETKYGLTN SSP FTKLNCKCEEAFDQCL MEA	88
<i>Scorpio maurus</i>	V A KNESDLGPLE-ADKCCRTHDHCDYIGSGETKYGLTN S FFTKLNCKCEEAFDQCL TEA	88

<i>Heterometrus fulvipes</i>	HGPLEGKA AF---TIRKLYFGLYGNGCFNVQCPS-----	103
<i>Hemiscorpius lepturus</i>	IDRAEGSAKSSMEGLHSFYFNTYSPECYEVKCSRK---RDAECTNGIAIWKDSYKS	232
<u><i>Scorpio maurus</i></u>	YDKEEGTAKTSTKNLRNFYF KY YRPQCYVVT CNSNRSSRDAGCENG VATWKKSYKD	144
<i>Scorpio maurus</i>	YNKEEGDAKTSTKDLRNFYF DN YRPQCYVVT CNSKRSSRDAGCENG VATWKKSYKD	144

Figure 2

Sequence alignment of target *Scorpio maurus* (underlined) with three other species of scorpions including *S. maurus* (AVD99009.1), *Heterometrus fulvipes* (Q3YAU5.1), and *Hemiscorpius lepturus* (A0A1L4BJ46.1). Gaps are displayed by (-). Conserved amino acids among species are shaded in gray. Conserved amino acids between the same species of *S. maurus* are bolded. Conserved catalytic site is assigned by (*).

Figure 3

3D structure of PLA2 was predicted by SWISS-MODEL web server. Predicted 3D structure of homo-dimer PLA2 of *S. maurus* with 38.83% identity to bee venom PLA2.

Figure 4

Superimposition of 3D structure of bee venom and *S. maurus* PLA2. 3D structure of *S. maurus* PLA2 is similar to the chain A of bee venom PLA2. (A) and (B): the 3D structure predicted by DeepView/Swiss-pdbviewer (version 4.10) software. (C): The Superimposition predicted by UCSF Chimera (version 1.14) software. In both pictures, the blue color shows *S. maurus* PLA2 and the red color shows bee venom PLA2. (D): The yellow color shows *S. maurus* active site.

Figure 5

Phylogenetic relationship between different species of scorpion, *Apis mellifera*, and *Homo sapiens* based on PLA2 sequences. The accession number of each sequence has been shown in front of its name. The percentage of replicate trees in which the related taxa clustered together in the bootstrap test (1000 replicates) has been displayed next to the branches. The scale bar corresponds to 0.2 substitutions per nucleotide

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1  M D D L A R R [C] L D S S E G R F F I W G G T K
1  ATGGATGATTTGGCAAGAAGATGCTTAGATTCATCAGAGGGAAGATTTTTTATATGGGGAGGGACCAAGT
71  W [C] G P G N E A E N E S D L G P L E A D K [C] [C] R
71  GGTGTGGGCCAGGCAACGAGGCGGAAAATGAATCTGATCTTGGTCCTCTTGAAGCTGATAAGTGTTGCCG
141  T H D H [C] D Y I G S G E T K Y G L T N S S P F
141  CACTCATGACCACTGCGACTATATAGGGTCTGGAGAAACGAAATATGGACTAACTAACAGCTCTCCTTTC
211  T K L N [C] K [C] E E A F D Q [C] L M E A Y D K E E
211  ACTAAGTTGAACTGCAAATGCGAAGAAGCTTTCGATCAGTGCTTGATGGAAGCTTATGACAAAGAGGAGG
281  G T A K T S T K N L R N F Y F K Y Y R P Q C Y V
281  GCACTGCAAAGACATCGACTAAAAATTTGCGAAACTTTTATTTCAAATATTATAGACCACAGTGCTATGT
351  V T C N S N R S S R D A G C E N G V A T W K K
351  TGTGACATGTAACAGTAATAGGTGCGAGCAGGGATGCAGGATGTGAAAATGGAGTTGCTACCTGGAAAAAG
421  S Y K D
421  AGCTACAAAGAC

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Figure 6

Nucleotides and predicted amino acid sequence of *S. maurus* PLA2. Nucleotide and amino acid numbers appeared on the left. The middle-letter at above the nucleotide sequence is coded the amino acid sequence. Coding sequence and predicted amino acid sequence. Coding sequence and predicted amino acid sequence of PLA2. The disulphide bonds between cysteine residues in the protein structure have been signed by black boxes. The conserved domines of target PLA2 amino acid length with PLA2 superfamily have been shown by black line.