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A Study on probable binding of cnidarian PLA2 toxins with human TRPV1 receptor through bioinformatics tools

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ABSTRACT

Sea anemones release Phospholipase A2 toxins which block human TRPV1 channel. Here four Phospholipase A2 toxins were retrieved from four Bunodosoma caisarum, Condylatic gigantea, Urticina crassicornis and Adamsia palliata. The present work represents detail in silico study of these four Phospholipase A2 toxins and their probable molecular interaction with human TRPV1 channel. To know the molecular interaction between Phospholipase A2 toxins and TRPV1 channel, Cluspro was used. Homology modeling was performed through Swissmodel. Motif and Domain prediction was performed through GenomeNet and NCBI domain search. Cleft analysis was carried out through PDBsum and energy minimization was done by Deep swiss PDB viewer. Amino acid interaction of docked model was carried out through Ligplot[+]. The focus of this paper is to reveal the probable molecular interaction between the sea anemone Phospholipase A2 toxins and TRPV1 channels find out the amino acid interaction between them. Cleft analysis revealed that large cleft provides increased opportunity for protein to interact with ligand. Lower energy value revealed that docked models are stable and energy minimized. Ligplot+ interaction shows that mainly hydrophobic amino acids are involved and domains are playing important role in docking. Actually PLA2c domain of Phospholipase A2 toxins and EFh domain of TRPV1 channel participate in docking. Thus this study tried to establish probable molecular interaction between Phospholipase A2 toxins with human TRPV1 channel and provides an insight for better understanding of these types of cnidarians toxins.

Key words: Phospholipase A2 toxin, TRPV1 channel, molecular docking, domain, *in silico*

INTRODUCTION

Sea anemones release Phospholipase A2 toxins. Phospholipase A2 toxins (PLA2) are pre-synaptic neurotoxins, blocking nerve terminals by binding to the nerve membrane and hydrolyzing stable membrane lipids. The products of the hydrolysis cannot form bilayer leading to a change in membrane conformation and ultimately blocking the release of neurotransmitters. Phospholipase A2 specifically recognizes the sn-2 acyl bond of phospholipids and catalytically hydrolyzes the bond releasing arachidonic acid and lysophosphatidic acid. Upon downstream modification by cycloxygenase, arachidonic acid is modified into active compounds called Eicosanoids. Eicosanoids include prostaglandins and leukotrienes, which are categorized as anti-inflammatory mediators [1].

In vivo and *in vitro* studies expose that inflammatory mediators like bradykinin, prostaglandin E2 (PGE2), extracellular ATP, glutamate and nerve growth factor can indirectly sensitize TRPV1 channels [2, 3, 4]. Actually Transient Receptor Potential Vanilloid subtype 1 (TRPV1) channel is described as pharmacological target of cnidarians (e.g. sea anemone) venoms and polycyclic ether toxins [5, 6]. The TRPV1 channel, a ligand-gated and

non-selective cation channel that is articulated in peripheral sensory neurons, is one of the important dramatis personae in peripheral pain sensation. TRPV1 is heat and acid sensitive and can be induced by pungent substances like capsaicin. Sensitization of TRPV1 channel via inflammatory mediators is achievable via a variety of mechanisms such as increasing TRPV1 channel expression levels in the membrane [7, 8] inducing TRPV1 channel phosphorylation by protein kinase [9, 10] or releasing channel inhibition by phosphatidylinositol 4,5-biphosphate (PIP2) [11]. In addition, these inflammatory mediators act on G-protein coupled receptors (GPCRs) or, in case of nerve growth factor, on tyrosine kinase pathways. These can release arachidonic acid (AA) and lipoxygenase products of arachidonic acid (e.g. hydroperoxyeicosatetraenoic acid or HPETE), substances which are known as TRPV1 channel agonists [12, 13]. Finally, the endogenous ligand an amide has also been shown to activate TRPV1 channels [14].

This work aimed towards elucidating the probable molecular (amino acid) interaction between the sea anemone PLA2 toxins and human TRPV1 channel and also predicting various scoring functions reflecting binding affinity between PLA2 toxins and TRPVI channel through *in silico* tools.

EXPERIMENTAL SECTION

1. Ligand and receptor retrieval:
The FASTA sequences of four Phospholipase A2 toxins (ligand) of sea anemones were retrieved and coded from UniprotKB [15] and the receptor TRPV1 channel (Transient receptor potential vanilloid receptor1) in human was retrieved from Protein data bank server [16].

2. Homology modeling and validation of predicted 3D model:
Homologies modeling of four sea anemone toxins were carried out with Swissmodel [17].

3. Cleft analysis of both PLA2 toxins and TRPV1 receptor:
Cleft analysis of both ligand and receptor were predicted through PDBsum viewer [18].

4. Domain and motif prediction:
Domains were predicted through NCBI conserved domain search [19] and motifs were predicted by means of GenomeNet server [20].

5. Docking and protein-protein interaction prediction:
Docking of PLA2 toxins with receptor TRPV1 channel was performed with Cluspro [21] and 2D structure of protein- protein interaction was carried out with Ligplot server [22].

6. Energy minimization prediction:
Energy minimization of docked model was predicted through SwissPDB viewer [23].

7. Scoring function prediction:
It was done through seqmol BiochemLab server [24] and Haddock 2.2 Server [25].

RESULTS AND DISCUSSION

Phospholipase A2 type (PLA2), toxins are released from sea anemones and may cause many harmful diseases to human. In Table1, represents four types of PLA2 (as coded) that are released from four different sea anemones, such as: *Bunodosoma caissarum*, *Condylactis gigantea*, *Urticina crassicornis* and *Adamsia palliata*. These four PLA2 toxins catalyze the hydrolysis of 2-acyl ester bonds of 3-sn-phospholipid producing arachidonic acids [26]. This arachidonic acid activates lipoxygenase and cycloxygenase which then through some intermediates such as prostaglandin E2, G-protein coupled receptor, Phospholipase C and hydroperoxyeicosatetraenoic acid converts into phosphatidylinositol bisphosphate that eventually blocks the human TRPV1 channel.

Table 2, represents human TRPV1 channel source, protein sequence and PDB id. It is a non selective cation channel involved in pain sensation and is depicted as pharmacological target of cnidarians venom [27].

Table 1. Brief description about sea anemone toxins

Sl.no	Name of toxins (code)	Sources	Protein sequence	Amino acid length
1.	Phospholipase A2 (PLA2_1)	*Bunodosoma caissarum*	GATIMPGTLWCGKGNSAADYLQLGVWKDTAHCCRDHDGC	39
2.	Phospholipase A2 (PLA2_2)	*Condylactis gigantea*	GVWQFAYMIAKYTGRNPLDYWGYGCWCGLGGKGNPVDAVDRCCYVHDVCYNSITQGPRPTCSRIAPYHKNYYFTGKKCSTGWLTSKCGRAICACDIAAVKCFRRNHFNKKYRLYKKNIC	119
3.	Phospholipase A2 (PLA2_3)	*Urticina crassicornis*	MKNNIILVILLGISVFVDCLPLNDQEEDKSLNAQESEVSAVQKRDILQFSGMIRCATGRSAWKYFNYGNWCGWGGSGTAVDGVDSCCRSHDWCYKRHDSCYPKIIPYIASTSGSHPSCSITCHSANNRCQRDVCNCDKVAAECFARNTYHPNNKH	155
4.	Phospholipase A2 (PLA2_4)	*Adamsia palliata*	MQLYTYFFTFSLVLILALADQENKSLDFTQEGGIAKRGAFQFSYLIKKYTGRNPLDYWGYGCWCGLGGKGTPVDGVDWCCYHHDMCFNSITQGPRPTCSKNAPYHKNYYFSGLKCSTGWLTSKCGRAICACDIAAVKCFMRNHFNNKYQNYKKNIC	156

Table 2. Brief description about TRPV1 receptor

Name of receptor	Sources	PDB id.	Protein sequence
Transient receptor potential vanilloid receptor1 (TRPV1)	*Homo sapiens*	3SUI	MADQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEFVQMMTAK

Identification of clefts on the surface of the protein is important for protein interaction with other molecules (protein/ drug/ lipid). Protein interaction mainly depends on the size of cleft in protein's surface. Protein always interacts with other molecules in carrying out their biological functions. The surface is generally irregular containing many clefts of varying sizes. Clefts in protein surfaces have been studied principally because of their relevance to binding sites. A large cleft provides an increased surface area and hence, increased opportunity for the protein to form interactions with other molecules, particularly small ligands. Cleft volumes in protein relate to their molecular interactions and functions and in single-chain proteins, the ligand is bound in the largest cleft in over 83% of the proteins. Usually the largest cleft is considerably larger than the others, suggesting that size is a functional requirement [28].Table 3, depicts PDBsum cleft analysis of sea anemone four PLA2 toxins i.e. PLA2_1, PLA2_2, PLA2_3 and PLA2_4 and TRPV1 channel receptor. PLA2 toxin of *Bunodosoma caissarum*, cleft (cleft 1) analysis suggest that a region with largest volume (256.92), average depth (7.87), accessible vertices (54.56) and buried vertices (4.73) is the largest and deepest cleft in this toxin which is important for interaction amongst all clefts. Again PLA2 toxin of *Condylactis gigantea*, largest cleft i.e. cleft 1 apart from having largest volume (1393.03) has the highest average depth (10.57), accessible vertices (67.43) and buried vertices (4.72), and act as most important for TRPV1 binding amongst all clefts. Further for PLA2 toxins of *Urticina crassicornis* and *Adamsia palliata* their cleft 1 have largest volume (2001.38 and 2038.53 respectively) highest average depth (13.26 and 14.85 respectively), accessible vertices (66.20 and 72.49 respectively) and buried vertices (14.08 and 11.86 respectively). Cleft 1 of TRPV1 has average depth (12.29), accessible vertices (61.39) and buried vertices (9.57) and is the largest cleft of this receptor and is also important for ligand binding. These results on the whole show that the largest and deepest cleft is located at the top of protein where ligand or receptor binds to it and enter the cell. Amongst four PLA2 toxins, PLA2_4 toxin of *Adamsia palliata* has largest volume (2038.50), largest accessible vertices (72.49) and largest average depth (14.85). Whereas, it is also showed that PLA2_1 toxin of *Bunodosoma caissarum* has smallest depth (256.92), accessible vertices (54.56), buried vertices (7.87) and average depth (7.87) among four PLA2 toxins.

Table 3. Cleft analysis of both TRPV1 receptor and PLA2 toxins

Properties of cleft	PLA2 Toxin				Human TRPV1 Receptor
	Bunodosoma caissarum	***Condylactis gigantea***	***Urticina crassicornis***	***Adamsia palliata***	
Volume	256.92	1393.03	2001.38	2038.50	2249.02
Accessible vertices	54.56	67.43	66.20	72.49	61.39
Buried vertices	4.73	11.72	14.08	11.86	9.57
Average depth	7.87	10.57	13.26	14.85	12.29

Domains are distinct functional units in a protein. Usually they are responsible for a particular function or interaction, contributing to the overall role of a protein. On the other hand, motif is structural unit and is a particular arrangement of amino acids or secondary structure that can be found in protein. Table 4, shows domains and motifs present in four PLA2 toxins and TRPV1 receptor. Here PLA2 toxin of *Bunodosoma caissarum* has PLA2c domain on its 3-39 amino acid position. This PLA2c domain has 4 motifs. PLA2 toxin of *Condylactis gigantea* (1-119 amino acid) and *Adamsia palliata* (38-156 amino acid) have PLA2c domain which both have 7 motifs. PLA2 toxin of *Urticina crassicornis* has PLA2c domain on its 45-155 amino acid position. This PLA2c domain has 10 motifs. So, PLA2c domain is common to four PLA2 toxins of sea anemones and it participates in docking with TRPV1 receptor. TRPV1 channel receptor has two domains; first one is EFh which is present in between 85-147 amino acid and second is PTZ00184 which is present in 1-149 amino acid. These two domains contain 23 motifs each. EFh and PTZOO184 domains are help in ligand binding.

Table 4. Domain and Motif present in PLA2 toxins and TRPV1 channel

Name of toxin / receptor	Name of Domain	Position of Domain	Motif present in each Domain	Position of Motif
PLA2_1	PLA2c	3-39 aa	4	3-39, 4-39, 5-39, 1-39
PLA2_2	PLA2c	1-119 aa	7	1-119, 1-118, 2-119, 21-105, 4-119, 23-50, 53-80
PLA2_3	PLA2c	45-155 aa	10	45-155, 45-154, 46-154, 65-147, 48-146, 65-101, 28-64, 70-93, 6-51, 29-62
PLA2_4	PLA2c	38-156 aa	7	38-156, 40-155, 40-151, 58-142, 41-145, 60-86, 63-150
TRPV1	EFh and PTZ00184	85-147aa, 1-149aa	23	1-149, 4-149, 85-147, 91-146, 97-149, 88-143, 85-114, 5-69, 121-149, 83-146, 85-113, 17-73, 94-146, 86-110, 79-148,83-146, 94-146, 122-147, 3-34, 95-148,101-148, 67-133, 95-147.

Homology modeling of four PLA2 toxins were performed through Swissmodel server and docking of PLA2 toxin of sea anemones (*Bunodosoma caissarum*, *Condylctis gigantea*, *Urticina crassicornis*, *Adamsia palliata*) with TRPV1 channel receptor were performed through Cluspro server. Figure 1, show these four docked models. Total energy of these docked models was analyzed by deep Swiss PDB viewer. Table 5, represented total amount of energy of these docked models that were produced during docking. Total energy of first docked model (PLA2 of *Bunodosoma caissarum*-TRPV1 channel) is -3698.807 KJ/Mol, second docked model (PLA2 toxin of *Condylactis gigantea* – TRPV1 channel) is -7057.977 KJ/Mol, third docked model (PLA2 toxin of *Urticina crassicornis*-TRPV1 channel) is -7846.495 KJ/Mol and fourth model (PLA2 toxin of *Adamsia palliata*-TRPV1 channel) is -6998.157 KJ/Mol. These data showed that total energy production during docking was quite low. Thus these docked model generated through Cluspro are energy minimized and stable.

Table 5. Energy minimization prediction of docked modeled structure

Name of docked model	KJ/mol
PLA2_1 model	-3698.807
PLA2_2 model	-7057.977
PLA2_3 model	-7846.495
PLA2_4 model	-6998.157

Prediction of scoring functions through Biochemlab Seqmol and Haddock 2.2 server of docked models between PLA2 toxins and TRPV1 channel receptor is exhibited in Table 6. Seqmol server predicted various scoring functions such as energy (dGbind) and Kd value and HADDOCK 2.2 predicted burial accessible surface area, desolvation energy, electrostatic energy and vanderwaals energy. Scoring function was used to predict the strength of association or binding affinity between two molecules after they had docked. Lower Kd value increases the binding affinity of docked model. PLA2_1-TRPV1 docked model and PLA2_3-TRPV1 docked model had higher Kd value, i.e. 1.94E-06 and 3.81E-10 respectively. So, these two docked models showed lower binding affinity between ligand and receptor i.e. particular PLA2 toxins bind weakly with TRPV1 receptor. PLA2_4 docked model showed moderate binding affinity (1.89E-14) between ligand and receptor i.e. TRPV1. PLA2_2 -TRPV1 docked model had lowest Kd value (8.03E-17) that means this docked model has highest binding affinity. PLA2 toxin of *Heteractis crispa* partially inhibited TRPV1 channel through docking and Kd value of docked model increased binding affinity [29]. Thus lowest Kd value proved that binding capability of PLA2 toxin of *Condylactis* sp. with TRPV1 channel is the strongest. On the other hand binding capability of PLA2 toxin of *Bunodosoma* sp. with TRPV1 channel is the weakest with highest Kd value. Kd value has positive relationship with dG bind energy. PLA2_1-TRPV1 docked model has highest dG bind energy (-7.83) and PLA2_2-TRPV1 docked model has least energy value (-22.06)

among four docked model. Lower the dG energy value higher the binding affinity, thus PLA2_2-TRPV1 model (*Condylactis sp.*) has highest binding affinity. The energy of desolvation is the amount of energy associated with dissolving a solute in a solvent. If its value is positive, the dissolving process is endothermic; if it is of negative value, it is exothermic. Desolvation energy values of four PLA2-TRPV1 interactions are all negative hence these four PLA2-TRPV1 probable interactions were exothermic in nature. Desolvation energy depends on buried accessible surface area [30]. Chen *et al*. in 2013 revealed direct relationship between buried interfacial surface area and binding affinity, that is, as buried surface area increases, binding affinity also increases [31]. PLA2_2-TRPV1 and PLA2_3-TRPV1 docked model showed highest buried surface area in contrast to PLA2_1-TRPV1 and PLA2_4-TRPV1 docked model. As a result PLA2_2-TRPV1 and PLA2_3-TRPV1 docked models illustrate higher binding affinity than the other two docked model. In 2013, Maleki *et al*. stated that electrostatic energy yields much more efficient production accuracy than desolvation energy [32]. Negative values of electrostatic energy and vanderwaals energy point to the fact that binding affinity would be strong. More negative the value, more efficient is the binding affinity. As predicted vanderwaals energy value of PLA2_2-TRPV1 and PLA2_3-TRPV1 docked models were the lowest thus they have strongest binding affinity. According to predicted electrostatic energy value, exceptionally PLA2_1-TRPV1and PLA2_4-TRPV1 docked models have lowest energy than the other two docked model. But binding affinity of a docked models depend not only on single parameter rather than a combination of various parameters such as desolvation energy, electrostatic energy, vanderwaals energy, Kd value, dG bind energy and buried surface area. So, from the above scoring function prediction, it is clear that PLA2_2 toxin (*Condylactis gigantea*) is the best energy minimized (-7057.977 KJ/Mol) model and has strongest binding affinity (i.e. high quality binding) with human TRPV1 channel.

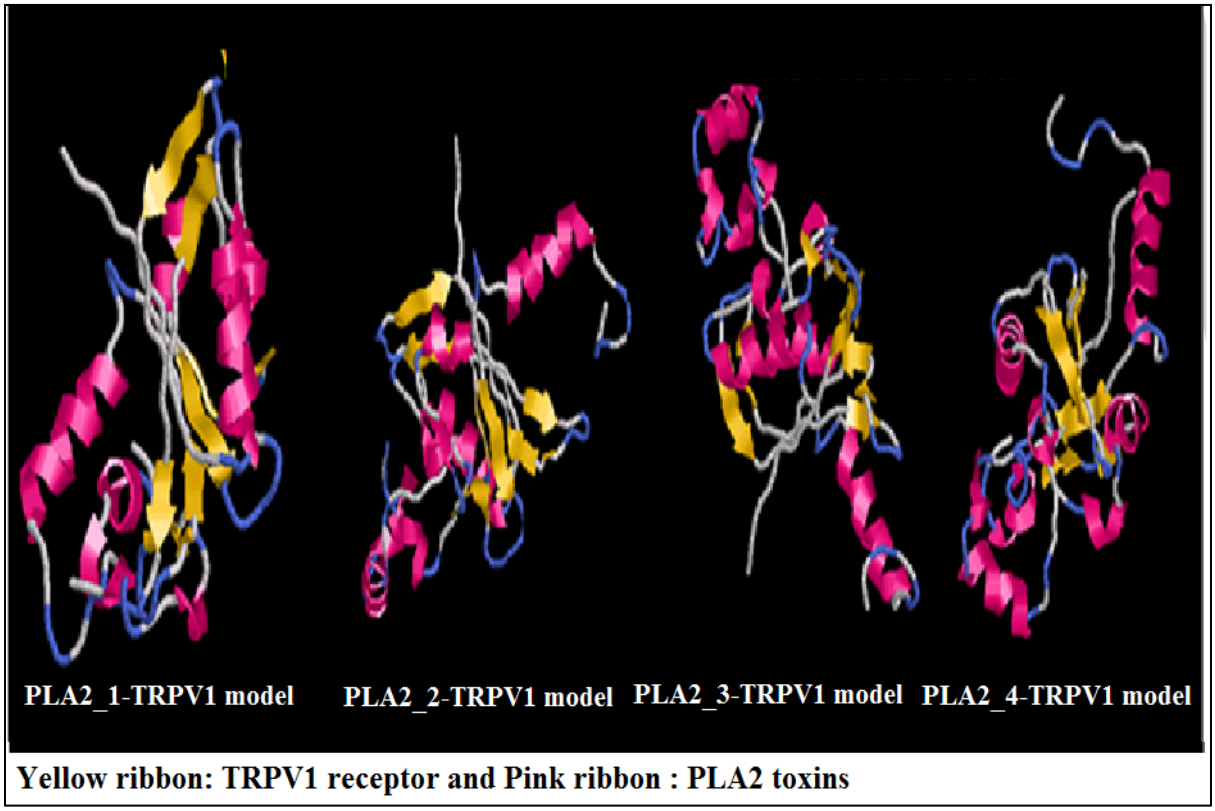

Figure 1. Predicted docked modeled structure between PLA2 toxins and TRPV1 receptor

Table 6. Scoring function prediction of docked modeled structure

Docked model	Scoring function prediction parameters					
	Buried surface area	dG bind (Gibbs free energy)	Kd	Vanderwaals energy	Electrostatic energy	Desolvation energy
PLA2_1 docked model	879.9	-7.83	1.94E-06	-27.7	-138.2	-15.2
PLA2_2 docked model	1131.4	-22.06	8.03E-17	-39.0	-38.3	-66.5
PLA2_3 docked model	1157.5	-12.91	3.81E-10	-46.8	-40.7	-39.3
PLA2_4 docked model	917.5	-18.81	1.89E-14	-28.5	-233.4	-24.8

Figure 2a and Table 7, show protein–protein interaction of docked model PLA2-1. This figure depicts hydrogen bonded and non-bonded contacts between PLA2 toxin (from *Bunodosoma caissarum*) and TRPV1 channel receptor. In non-bonded contacts hydrophobic residues are mainly involved. Polar and charged amino acids are mainly involved in hydrogen bonded contacts. Two aliphatic residues such as glycine and isoleucine are also involved in hydrogen bonded contacts. Here ligand Histidine binds with more than one receptor, such as: $His36^{L}$-$Arg42^{R}$ and $His36^{L}$-$ser35^{R}$. In these cases, oxygen atom of receptor can bind with nitrogen atom of one ligand and carbon atom of another ligand. From the Ligplot^{+} illustration, it is clear that stretch of amino acid of ligand i.e. PLA2 toxin of *Bunodosoma caissarum* having 4-14, 26-37 amino acids stretch were binding with stretch of amino acid stretch i.e. 35-39, 42-53, 90-97, 100-101, 123-124 of TRPV1 receptor . Above mentioned amino acids of both toxin and receptor are located within PLA2c domain and PTZ00184 domain of PLA2 toxin and TRPV1 receptor respectively. Thus the corresponding domains were involved in binding.

Figure 2a. Amino acids involved in PLA2_1 (*Budosoma caissarum*) docked model

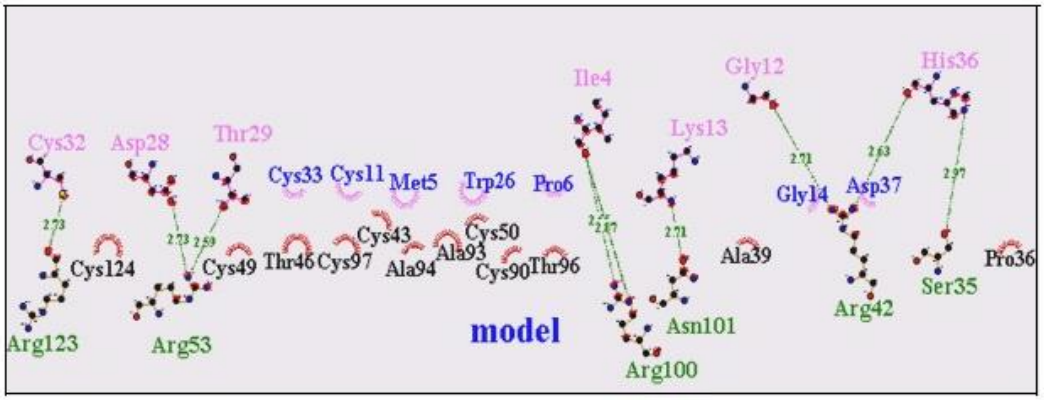

Figure 2b and Table 7, indicate docking model between PLA2 toxin of *Condylactis gigantea* and TRPV1 receptor. Here non-bonded contacts occurred with hydrophobic residues. Polar charged amino acids are involved in hydrogen bonded contacts except Valine. Receptor Lysine [115] binds with two ligands, Threonine and Glutamine. In these condition oxygen atom of Lysine can bind with nitrogen atom of Threonine and carbon atom of Glutamine. From Ligplot^{+} illustration, it is clear that stretch of amino acids of PLA2 toxin of *Condylactis gigantea* i.e. 3-7, 12-21, 54-55, 64-70 amino acid s were binding with amino acids stretch of 1-6, 13-23, 61-64, 111-115 amino acids of TRPV1 receptor . Above mentioned amino acids of both toxin and receptor are located within PLA2c domain and PTZ00184 domain of PLA2 toxin and TRPV1 receptor respectively. Thus the corresponding domains are involved in binding.

Figure 2b. Amino acids involved in PLA2_2 (*Condylactis gigantea*) docked model

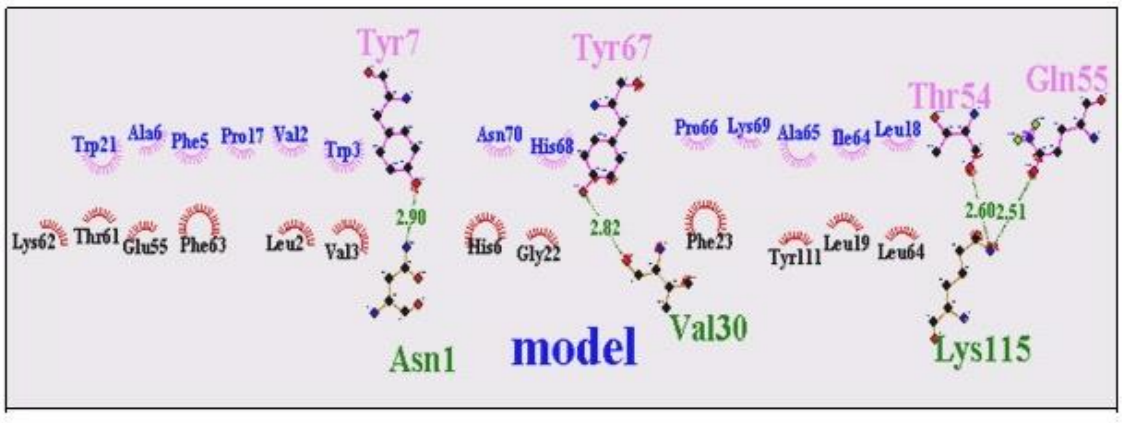

Figure 2c and Table 7, depict amino acids involved I PLA2-3 docked model between PLA2 toxin of *Urticina crassicornis* and TRPV1 channel receptor. In this case, non bonded contacts, hydrophobic amino acids are involved. In hydrogen bonded contacts, polar and charged amino acids were involved except Phenylalanine, Alanine. Receptor Lysine binds with more than two ligands, such as: $Phe144^{L}$ -$Lys115^{R}$, $Asn145^{L}$-$Lys115^{R}$, $His143^{L}$-$Lys115^{R}$. Here oxygen atom of receptor binds with carbon atom of Phenylalanine and Histidine and nitrogen atom of Aspergine. From Ligplot^{+} illustration, it is clear that stretch of amino acid of PLA2 toxin of *Urticina gigantea* i.e. 70-79, 81-89, 137-145 amino acids were binding with stretch of amino acids of i.e. 2-7, 18-29, 62-62, 111-130 of TRPV1 receptor. Above mentioned amino acids of both toxin and receptor are located within PLA2c domain and PTZ00184 domain of PLA2 toxin and TRPV1 receptor respectively. Thus the corresponding domains are involved in binding.

Figure 2c. Amino acids involved in PLA2_3 (*Urticina crassicornis*) docked model

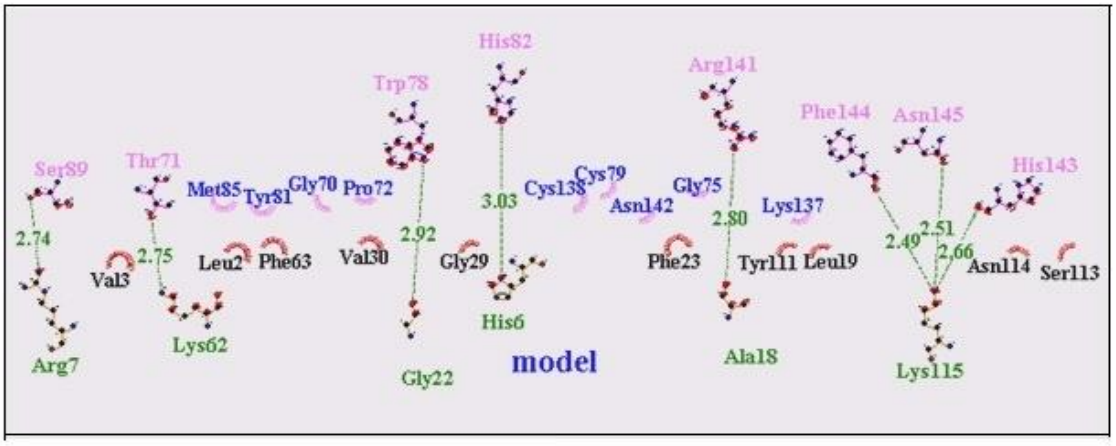

Figure 2d and Table 7, show the protein-protein interaction i.e. amino acid involved in docking between PLA2_4 toxin (*Adamsia palliata*)- human TRPV1 receptor. In non-bonded contacts, hydrophobic residues are mainly involved. In hydrogen bonded contacts, polar and charged amino acids are involved. In hydrogen bonded contacts ligand is rich in hydrophobic residues such as cysteine, valine, proline, and isoleucine. Some ligands are polar in nature such as serine, glutamine and Histidine. Receptor is rich in Arginine i.e. charged amino acid. Sometimes One receptor can bind with more than one ligand, for example, $Arg100^{R}$-$Pro72^{L}$, $Arg100^{R}$-$Val73^{L}$ and $Lys56^{R}$-$Ile90^{L}$, $Lys56^{R}$-$Ser89^{L}$, $Lys56^{R}$-$Gln92^{L}$. In these cases, oxygen atom of receptor can bind with nitrogen atom of one ligand and carbon atom of another ligand. From Ligpilot^{+} illustration, it is clear that stretch of amino acids of PLA2 toxin of *Adamsia palliata* i.e. 70-82, 85-94, 129-143 amino acids were binding with stretch of amino acid i.e. 35-36, 43-49, 53-57, 92-124 amino acid of TRPV1 receptor. Above mentioned amino acids of both toxin and receptor are located within PLA2c domain and PTZ00184 domain of PLA2 toxin and TRPV1 receptor respectively. Thus the corresponding domains are involved in binding.

Figure 2d. Amino acids involved in PLA2_4 (*Adamsia palliata*) docked model

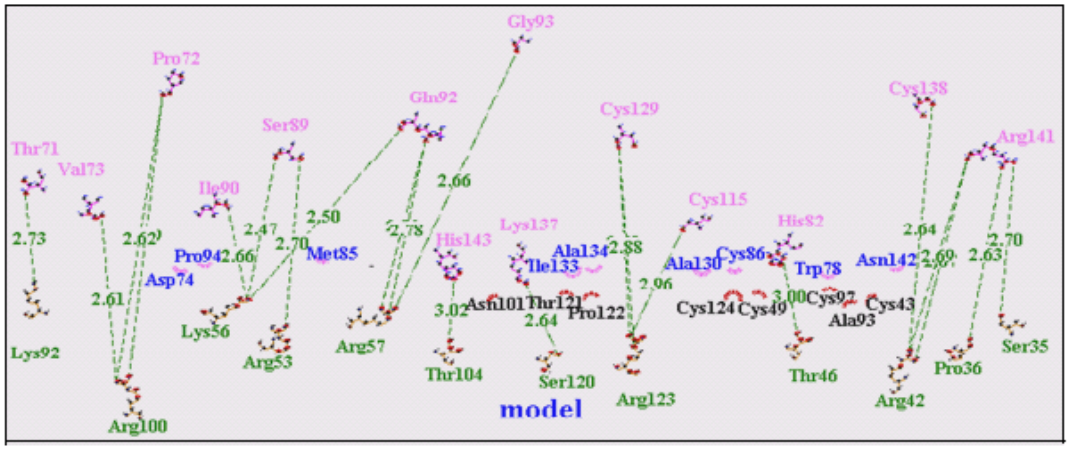

Table 7. Amino acids involved in bond formation during docking of PLA2 toxins and TRPV1 receptor

Model name	H-Bonds	Non-Bonded contacts
PLA2-1 docked model	$Cys32^{L}$-$Arg123^{R}$, $Asp28^{L}$-$Arg53^{R}$, $Thr29^{L}$-$Arg53^{R}$, $Ile4^{L}$-$Arg100^{R}$, $Lys13^{L}$-$Asn101^{R}$, $Gly12^{L}$-$Arg42^{R}$, $His36^{L}$-$Arg42^{R}$, $His36^{L}$-$Ser35^{R}$	$Cys154^{L}$-$Asp28^{R}$, $Cys33^{L}$-$Cys49^{R}$, $Cys33^{L}$-$Thr46^{R}$, $Cys11^{L}$-$Cys97^{R}$, $Met5^{L}$-$Cys43^{R}$, $Met5^{L}$-$Ala94^{R}$, $Trp26^{L}$-$Cys50^{R}$, $Trp26^{L}$-$Cys90^{R}$, $Pro6^{L}$-$Thr96^{R}$
PLA2-2 docked model	$Tyr7^{L}$-$Asn1^{R}$, $Tyr67^{L}$-$Val30^{R}$, $Thr54^{L}$-$Lys115^{R}$, $Gln55^{L}$-$Lys115^{R}$	$Trp21^{L}$-$Lys62^{R}$, $Trp21^{L}$-$Thr61^{R}$, $Ala6^{L}$-$Glu55^{R}$, $Phe5^{L}$-$Phe63^{R}$, $Pro17^{L}$-$Phe63^{R}$, $Val12^{L}$-$Leu2^{R}$, $Trp3^{L}$-$Val13^{R}$, $Asn70^{L}$-$His6^{R}$, $His68^{L}$-$Gly22^{R}$, $Pro66^{L}$-$Phe23^{R}$, $Lys69^{L}$-$Phe23^{R}$, $Ala65^{L}$-$Tyr111^{R}$, $Ile64^{L}$-$Leu19^{R}$, $Leu18^{L}$-$Leu64^{R}$
PLA2-3 docked model	$Ser69^{L}$-$Arg7^{R}$, $Thr71^{L}$-$Lys62^{R}$, $Trp78^{L}$-$Gly22^{R}$, $Arg141^{L}$-$Ala18^{R}$,$Phe144^{L}$-$Lys115^{R}$,$Asn145^{L}$-$Lys115^{R}$, $His143^{L}$-$Lys115^{R}$	$Thr71^{L}$-$Val13^{R}$, $Met85^{L}$-$Leu2^{R}$, $Tyr81^{L}$-$Phe63^{R}$, $Gly70^{L}$-$Phe63^{R}$, $Pro72^{L}$-$Val30^{R}$, $Cys138^{L}$-$Phe23^{R}$, $Cys78^{L}$-$Phe23^{R}$, $Asn142^{L}$-$Phe23^{R}$, $Gly75^{L}$-$Phe23^{R}$, $Lys137^{L}$-$Tyr111^{R}$, $Lys137^{L}$-$Leu19^{R}$, $His143^{L}$-$Asn114^{R}$, $His143^{L}$-$Ser113^{R}$
PLA2-4 docked model	$Thr71^{L}$-$Lys92^{R}$, $Val73^{L}$-$Arg100^{R}$, $Pro72^{L}$-$Arg100^{R}$, $Ile90^{L}$-$Lys56^{R}$, $Ser89^{L}$-$Lys56^{R}$, $Ser89^{L}$-$Arg53^{R}$, $Gln92^{L}$-$Lys56^{R}$, $Gln92^{L}$-$Arg57^{R}$, $His14^{L}$-$Thr104^{R}$, $Lys137^{L}$-$Ser120^{R}$, $Cys129^{L}$-$Arg123^{R}$, $Cys115^{L}$-$Arg123^{R}$, $His82^{L}$-$Thr46^{R}$, $Cys138^{L}$-$Arg42^{R}$, $Arg141^{L}$-$Pro36^{R}$, $Arg141^{L}$-$Ser35^{R}$	$Ile133^{L}$-$Thr121^{R}$, $Ala134^{L}$-$Pro122^{R}$, $Ala30^{L}$-$Cys124^{R}$, $Cys86^{L}$-$Cys49^{R}$, $Trp78^{L}$-$Cys97^{R}$, $Asn142^{L}$-$Cys43^{R}$

Different types of amino acids that were present in all four PLA2 toxins and TRPV1 channel (involved in binding or docking) are represented in Table 8. In case of PLA2 toxins, 2.56% charged amino acid, 4.48% aliphatic amino acid, 1.60% aromatic amino acid, 8.33% polar amino acid and 9.61% hydrophobic amino acid were present that helped in binding or docking. Further, in PLA2 toxins, Histidine is present in highest percentage (2.56%) and Asparagine and Leucine were present in least percentage (0.32%) that assists in binding or docking. In case of TRPV1 channel, 8.97% charged amino acid, 3.20% aliphatic amino acid, 1.60% aromatic amino acid, 8.33% polar amino acid and 9.61% hydrophobic amino acid were present that helped in binding or docking. Again, in TRPV1 channel, Arginine is present in highest percentage (4.80%) and Asparagines, Glutamine, Glutamic acid and Glycine were present in least percentage (0.32%) that aided in binding or docking. Thus most amino acids involved in binding either in PLA2 toxins or TRPVI channel are of positively charged amino acid.

Table 8. Amino acids involved in docking of PLA2 toxins and TRPV1 channel

Docked proteins	% (mean) Amino acid with highest representation in docking	% (mean) Amino acid with lowest representation in docking	Charged amino acid	Aliphatic amino acid	Aromatic amino acid	Polar amino acid	Hydrophobic amino acid
PLA2 toxins	Histidine (2.56%)	Asparagine, Leucine (0.32%)	2.56%	4.48%	1.60%	8.33%	9.61%
TRPVI receptor	Arginine (4.80%)	Asparagines, Glutamine, Glycine, Glutamic acid (0.32%)	8.97%	3.20%	3.20%	6.08%	8.65%

The above results eventually proved that PLA2c domain is common for four PLA2 sea anemone toxins. Results also demonstrated that PLA2c domain of four PLA2 toxins were involved in docking and amino acids that were present in PLA2c domain also participated in bonding. TRPV1 channel took part in docking with its EFh domain. Amino acids that were involved in docking were present in EFh domain. This fact ultimately proved that domains were important part in protein-protein interaction. Actually domains are the subunits of a protein and moderate protein-protein interaction by identifying short peptide sequences. In 1994, J. Schlessinger, proved that SH-2 and SH-3 domains are small protein modules that mediate protein- protein interactions [33]. In 2012, Li *et.al*. proved that PLA2c domain participated in protein-protein interaction [34], on other side Moegenstern and Valencia (2012) proved that EFh is novel domain and it also participated in protein binding [35].

CONCLUSION

Cnidarians' Phospholipase A2 toxins cause harm to human by blocking their TRPV1 channel. This toxicity is due to probable binding of PLA2 toxins with human TRPV1 channel. TRPV1 receptor binds with four PLA2 toxins in their largest cleft. Hydrophobic amino acids are mainly involved in this probable and potential interaction between PLA2 toxins and TRPV1 molecule. PLA2c domain of PLA2 toxins and EFh domain of TRPV1 channel participate in their docking. Among four cnidarians, PLA2 toxin of *Condylactis gigantea* shows highest binding affinity with Human TRPV1 channel as established through various scoring function predictions.

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