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In silico Molecular Interaction Analysis of LTNF Peptide-LT10 with Snake Venom Enzymes

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Abstract: Lethal Toxin Neutralizing Factor (LTNF) isolated from Opossum (*Didephis virginiana*) has been shown to exhibit anti-venom and anti-allergic property. The small synthetic peptide- LT10 derived from N-terminal of LTNF also showed this property *in vivo*. We applied molecular modeling, docking and molecular dynamic (MD) simulation techniques to compute the interaction of LT10 peptide with few snake venom enzymes, namely PLA2 from *Naja naja* and Atrolysin -C from *Crotalus atrox*. Our *in silico* interaction analyses provides molecular basis that might account for anti-venom activity of LT10 peptide towards these two snake venom toxins. The understanding of such key molecular interactions could be useful for design and development of better anti-snake venom therapies.

Keywords: Atrolysin C, LT10, LTNF, PLA2, snake envenomation, toxin neutralization.

INTRODUCTION

Snake Venoms are complex mixture of enzymes, peptides and metalloproteins [1, 2]. These combinations grant a formidable range of activities, where peptide and polypeptide components of the venom are responsible for a variety of toxic properties. Clinical manifestation of snake venom depends on the toxicity and amount of venom introduced or injected. It affects particularly the central nervous system, cardiovascular system, muscular and vascular system [2].

Snake envenomation has been a major cause of death and disability in the developing countries [1]. Several substances have been evaluated for their effects against snake venoms such as compounds from plant extracts, snake serum plasma, mammals and marine animals, and many synthetic molecules [3, 4].

However most common treatment against snake envenomation is the use of Anti-snake venom (ASV) also referred to as antivenin (venom neutralizing antibodies prepared in horse) [5, 6]. Even though multiple processes purify the antivenins, it may contain other (horse) serum proteins and some individuals may develop an extreme hypersensitive reaction to such proteins upon administration [6]. Thus, an alternative envenomation treatment devoid of side effects and with broad spectrum of action is desirable.

An anti-lethal factor named Lethal Toxin Neutralizing Factor (LTNF) isolated from Opossum (*Didephis virginiana*) serum is known to neutralize the lethality of venoms from all major snake families. The neutralizing effect of LTNF on venom of cobra, rattlesnake, russell's viper, sea snake etc.

along with scorpion venom, honey bee venom, ricin toxin and botulinum toxin have been experimentally shown by Lipps, B.V [7]. Thus, LTNF has potential as a universal therapy for envenomation [7-10]. Synthetic peptides with 10 amino acids designated as LT-10 (LKAMDPTPPL) derived from the N-terminal fraction of LTNF, could evoke similar lethal toxin neutralizing property as that of LTNF for envenomation [8-10]. LTNF and LT-10 inhibited lethality of animal, plant, and bacteria toxins when tested on mice without reacting to the nontoxic substances. Therefore, the use of LT10 peptide as an anti-allergic as well as broad-spectrum therapy for snake envenomation has been suggested [10-12].

This *in silico* work predicts two putative targets of LT10 peptide from snake venom and focuses on their detailed molecular interactions. In the present study, we have modeled the 3D structure of LT10 synthetic peptides, followed by screening of its potential targets. Amongst, the number of binding partners obtained from screening, snake venom enzymes were selected for docking with LT10 peptide and then refined by MD (Molecular Dynamic) Simulation. The interaction analysis of these protein-peptide complexes could help understand the possible molecular mechanism that might be responsible for neutralizing the lethality of these snake venom enzymes by LT10 peptide. Further, understanding of such key interactions would certainly help aid the design and development of better therapeutics against snake envenomation.

MATERIAL AND METHODS

Peptide Modeling

Molecular modeling of LT10 peptide was carried out using PEP-FOLD server (<http://bioserv.rpbs.univ-paris-diderot.fr/PEP-FOLD/>). It's an online resource for de novo modeling of 3D conformations for peptides between 9 and

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25 amino acids. It uses a hidden Markov model-derived structural alphabet of 27 motifs composed of 4 residues. It first determines structural alphabet (SA) letters of the sequence and then builds model by assembling the fragments using a greedy algorithm driven by a coarse-grained force field OPEP (Optimized Potential for Efficient structure Prediction). Starting from an amino acid sequence, PEP-FOLD performs series of 200 simulations and returns the most representative conformations identified in terms of energy and population [13, 14]. It generates clusters of models ranked on the basis of their OPEP (Optimized Potential for Efficient structure Prediction) energy. Top ranked model with least sOPEP score, considered to be the best 3D model generated for LT10 synthetic peptide was selected for further studies.

Target Screening

LT10 peptide model was subjected to ReverseScreen3D server (<http://www.modelling.leeds.ac.uk/ReverseScreen3D/index.html>) for prediction of its probable binding partners. ReverseScreen3D is a ligand-based reverse virtual screening tool that searches against a biologically-relevant and automatically-updated subset of ligands extracted from the RCSB Protein Data Bank [15] in order to identify potential target proteins that are likely to bind a given compound [16]. From the potential targets obtained, snake venom enzymes namely PLA2 and Atrolysin-C were selected for studying their interaction with LT10 peptide.

Molecular Docking

Selected LT-10 peptide model was prepared in Protein Preparation Wizard 2.2 (Epik Version 2.3, Schrödinger, LLC, New York, 2012). After preparation, this LT10 peptide model was subjected to the conformational search of MacroModel (version 9.9, Schrödinger, LLC, New York, NY, 2012) for generating all possible energetically minimum conformers which were then docked on to the snake venom proteins - PLA2 and Atrolysin C, respectively using Glide dock (Schrödinger, LLC, New York, NY, 2012) [17, 18]. The Crystal structure of PLA2 [PDB_ID: 1OWS] and Atrolysin - C [PDB_ID: 1DTH] obtained from Protein Data Bank (<http://www.pdb.org/>) [15] were pre-processed and minimized using Protein Preparation Wizard 2.2 (Epik Version 2.3, Schrödinger, LLC, New York, 2012) after addition of H-atoms. Molecular docking was initiated by generating grid file using receptor grid generation panel of Glide. Grid file contains receptor (protein structure) and binding site information that serves as an input for molecular docking. Sampling of peptide during docking was kept flexible. All three precision methods of Glide viz. high throughput virtual screening (HTVS), standard precision (SP) and extra precision (XP) [19] were used for docking LT10 peptide with PLA2 and Atrolysin-C enzymes, respectively. These series of precision methods generate a subset of best possible peptides by filtering out energetically less preferred peptide conformation.

Computation of Binding Free Energy

The best docked pose obtained from docking was further subjected to Prime MM-GBSA (Molecular Mechanics with

Generalized Born Surface Area) calculations as implemented in Prime 2.0 (Prime, version 3.1, Schrödinger, LLC, New York, NY, 2012), in order to predict the Binding free energy between the receptor and peptide. MM-GBSA is a method that combines OPLS molecular mechanics energies (E_{MM}), surface generalized Born solvation model for polar solvation (G_{SGB}), and a nonpolar solvation term (G_{NP}). G_{NP} includes the nonpolar solvent accessible surface area and van der Waals interactions. The total binding free energy is estimated as:

$$G = E_{MM} + G_{SGB} + G_{NP}$$

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$

Molecular Dynamic Simulation

Docking results were analyzed for selection of best docking pose of PLA2-LT10 and Atrolysin C-LT10 complexes, based on their Glide XP score. Further, these complexes were subjected to MD simulation performed using Desmond 2012 (Maestro-Desmond Interoperability Tools, version 3.1, Schrödinger, New York, NY, 2012) [20]. Optimized Potentials for Liquid Simulations (OPLS) [21, 22] all-atom force field was applied to evaluate the stability. The protein structures were solvated with Monte Carlo simulated TIP3P [23] water model in an orthorhombic box with buffer space of 10 Å from the edges of protein. The pressure was controlled using Martina-Tobias-Klein method [24] and the constant simulation temperature was maintained using Nose-Hoover thermostats [25]. Multistep RESPA integrator [26] was used to integrate the equations of motion with an inner time step of 2.0 fs for bonded interactions and non-bonded interactions within the short-range cut-off. For non-bonded interactions beyond the cutoff, an outer time step of 6.0 fs was used. These periodic boundary conditions were applied throughout the system. The default Desmond protocol which includes a series of restrained minimizations and MD Simulations was applied to equilibrate these prepared systems. Two rounds of steepest descent minimization were carried out with a maximum of 2000 steps and a harmonic restraint of 50 kcal/mol/Å² on all solute atoms followed by a series of four MD Simulations. The first simulation was 12 ps run, at a temperature of 10 K in the NVT (constant number of particles, volume, and temperature) ensemble with solute heavy atoms restrained with force constant of 50 kcal/mol/Å². Similarly, the second simulation was also a 12 ps run except it was run in the NPT (constant number of particles, pressure, and temperature) ensemble. This was followed by a 24 ps simulation with an increased temperature of 300 K in the NPT ensemble and with the force constant retained. The last one was a 24 ps simulation at 300 K in the NPT ensemble without restraints. This default equilibration was followed by a 5000 ps NPT simulation to equilibrate the system. The equilibrated system was simulated for the period of 20ns for both complexes, using NPT ensemble with trajectory saved in every 5 ps of time intervals.

MD Simulation was analyzed using the analytical tools in the Desmond package. In MD simulation quality analysis, potential energy of the protein as well as total energy of the entire system was calculated. Stability of the docked complexes across the trajectory was evaluated from their RMSD

plots. The minimum potential energy conformations for both the docked complexes were then used for interaction analysis. These interactions were plotted using LIGPLOT [27]. Snap shot of the protein–peptide complexes were generated using PyMOL v 1.3 [28].

RESULTS

LT10 Peptide Model and Identification of its Potential

Targets

LT10 synthetic peptide (LKAMDPTPPL) derived from N-terminal of LTNF was modeled (Fig. 1) using PEPFOLD server that predicts the *de novo* peptide structure from given amino acid sequence [14]. It returns an archive of all the generated models, the detail of the clusters and the best conformation from 5 best clusters. These clusters are ranked based on the sOPEP (Optimized Potential for Efficient structure Prediction) energies of their representative models. The model with lowest sOPEP score is considered to be the best model generated. For, LT10 synthetic peptide, 48 clusters of models was generated and model from the 1st cluster with lowest sOPEP score of -5.77 was selected for screening. This peptide model was further optimized and minimized during preparation for Glide docking [22, 23].

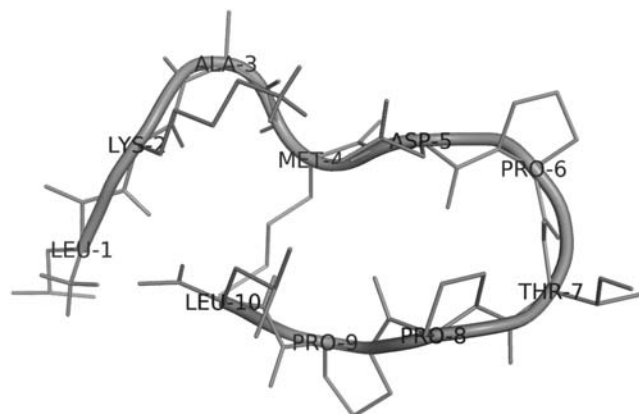


Figure 1. 3D structural Model of LT10 synthetic peptide

The LT10 peptide model was screened for identification of its potential targets using ReverseScreen3D server [16]. From the number of targets obtained with most of them being hydrolyases, we selected snake venom enzymes namely PLA2 [PDB ID: 1OWS] and Atrolysin C [PDB ID: 1DTH], owing to the anti- snake venom activity of LT10 peptide.

Molecular Docking and MD Simulation Analysis

Of the three Glide docking precisions used, Extra Precision (XP) method is highly accurate and generated poses during docking that were ranked based on the XP Glide score. Docked pose with the lowest XP Glide score is considered to be the best pose. Therefore, for both PLA2–LT10 and Atrolysin C–LT10 docking, their best-docked poses were selected for further refinement using MD Simulation. Over the period of simulation, their respective lowest energy conformations were obtained in order to study the detailed interactions. LT10 peptide was successfully docked with

PLA2 (Fig. 2A) and Atrolysin –C (Fig. 2C) proteins with a Glide score of -7.795 Kcal/mol and -11.749 Kcal/mol, respectively for their best-docked poses. Further, the free energy for binding of peptide from these best docked pose was calculated using Prime MM-GBSA method. The MM-GBSA score obtained for PLA2-LT10 and Atrolysin C-LT10 complex was -61.954 Kcal/mol and -79.379 Kcal/mol, respectively. The molecular interactions of these docked complexes were plotted as shown in Fig. (2B, 2D) with detailed description given in Table 1. The molecular interaction of PLA2–LT10 docked complex showed LT10 peptide involved in intermolecular hydrogen bonding with Asp24, Arg53, Lys64 residues and hydrophobic interactions with Gly30, Cys32, Tyr52, Phe65 residues. Similarly, the Atrolysin C-LT10 docked complex showed LT10 peptide involved in intermolecular hydrogen bonding with Glu102, Glu106, His142, His152 residues and hydrophobic interactions with Glu105, Thr107, Leu108, Leu110, His146, Pro168 residues.

PLA2–LT10 and Atrolysin C–LT10 docked complexes were further refined by MD simulation of 20ns. The stability of each of these complex systems during their simulation period was monitored via potential energy plot and RMSD plot. The potential energy plots for both PLA2-LT10 (Fig. 3A) and Atrolysin C –LT10 (Fig. 3C) complexes showed relatively stable complex systems throughout the simulations. RMSD plot (root mean square deviation) of both PLA2-LT10 (Fig. 3B) and Atrolysin C –LT10 (Fig. 3D) complexes during simulation was calculated with respect to their initial docked structure. It provided quantitative output of deviations with respect to time. The RMSD plot for both these protein-peptide complex showed small rearrangement of initial conformations followed by a relative stable region with deviation of less than 1 Å.

From MD simulation trajectories, lowest energy conformation for both the protein complexes were obtained and their interactions were plotted (Fig. 4A, Fig. 4B) and summarized in Table 2. The molecular interaction of PLA2–LT10 complex showed LT10 peptide involved in intermolecular hydrogen bonding with Trp19, His48, Lys64 residues and hydrophobic interaction with Asn1, Thr2, Leu115, Tyr52, Arg53, Phe65 residues. The LT10 residues forming hydrogen bond with PLA2 are Ala3-Trp19, Asp5- Lys 64 and Asp5- His48 as shown in Fig. (4A). Similarly, the Atrolysin C–LT10 complex showed LT10 peptide involved in intermolecular hydrogen bonding with Glu102, Glu106, Leu108, His142, His152, Gly169, Leu170, Zn901 residues and hydrophobic interactions with Leu110, His129, His146 residues. The LT10 residues forming hydrogen bond with Atrolysin-C are Leu1- Glu 106, Leu1- Glu102, Ala3- Glu106, Ala3- Leu170, Ala3-Gly 169, Met 4- Leu108, Asp 5- His152, Asp 5- His142, Asp 5-Zn901 and Thr7 – Zn 901 as shown in Fig. (4B).

Comparative Interaction Analyses

For both the protein-peptide complexes, interactions from their docked and minimum energy structure (from MD Simulation) were compared in Table 3. In case of PLA2–LT10 complex, interacting residues Tyr52, Arg53, Lys64 and Phe65 were retained. Similarly for Atrolysin C –LT10

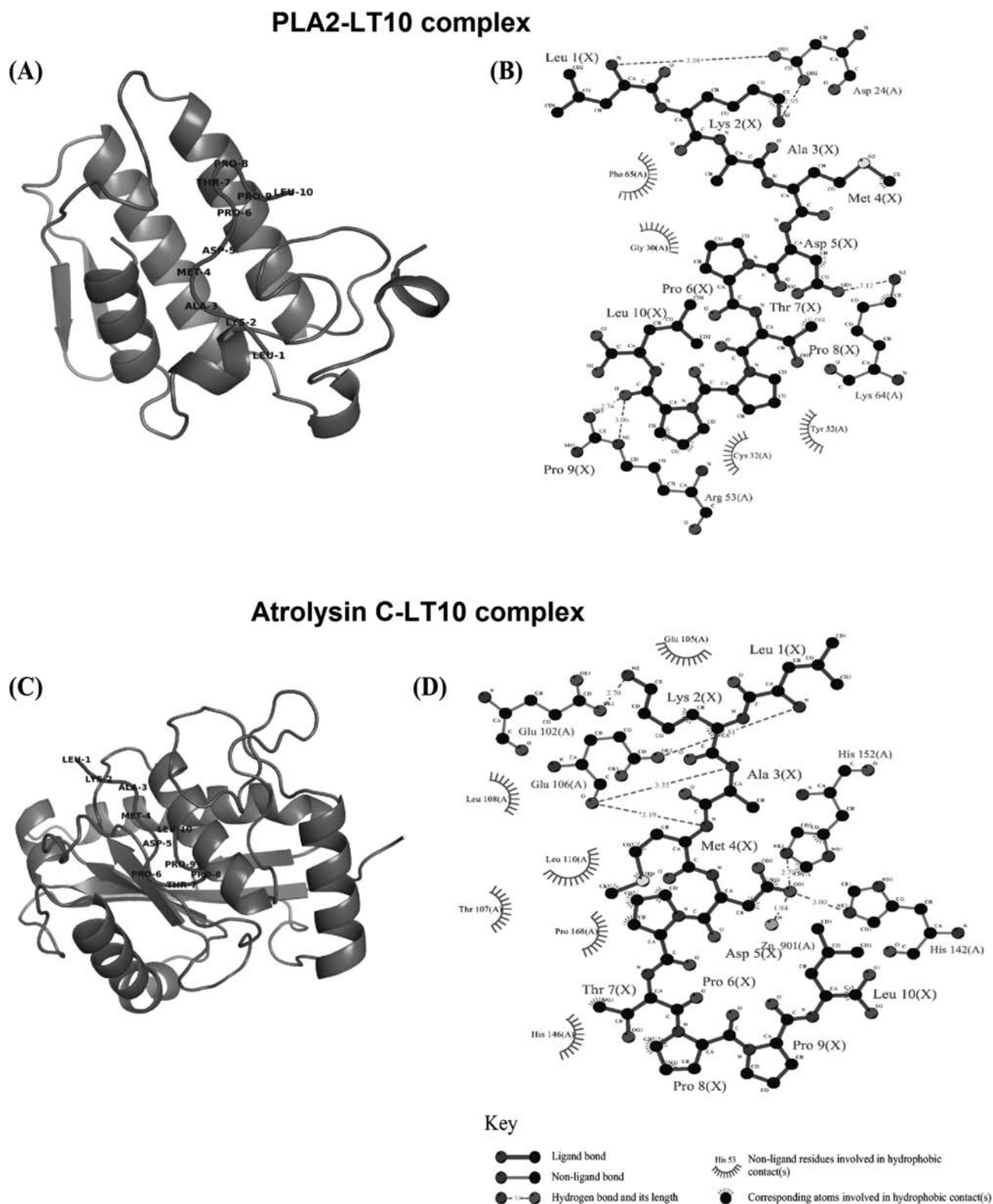


Figure 2. Protein-peptide docked complex with their respective interacting residues.

(A) PLA2-LT10 docked complex. (C) Atrolysin C –LT10 docked complex. The cartoon representation of complex shows protein with labeled N and C-termini and LT10 peptide with labeled residues. (B) Interacting residues of PLA2-LT10 docked complex in ligplot view. (D) Interacting residues of Atrolysin C –LT10 docked complex in ligplot view. The ligplot view represents two-dimensional schematic representation of Hydrophobic (radial lines) and hydrogen bond interactions (dotted lines with distances in Å) of docked complex where residue of peptide are labeled (X). (Please refer to ‘key’ for details).

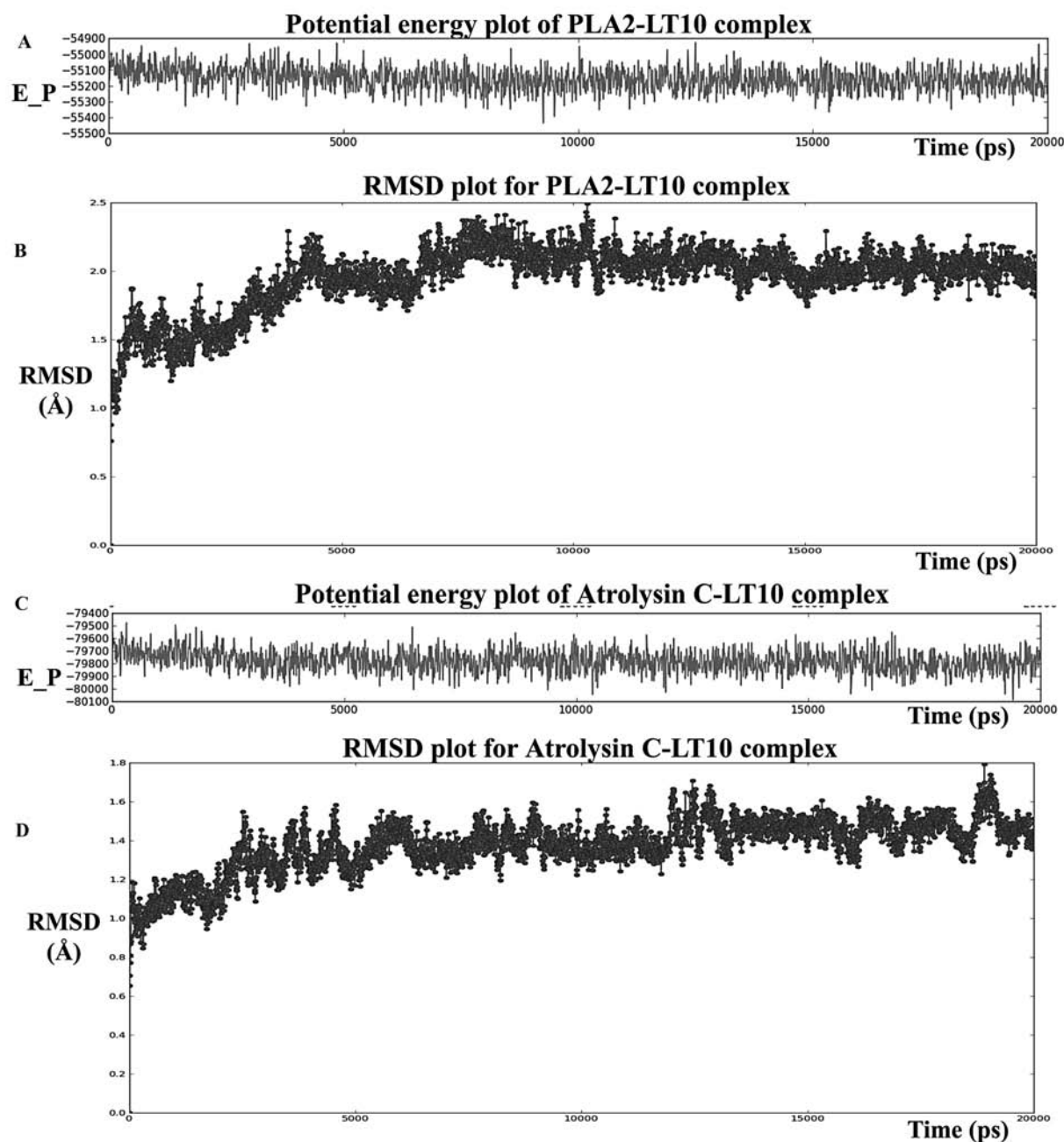


Figure 3. MD simulation (20 ns) trajectory analysis of protein-peptide complexes.

(A) Potential energy plot of PLA2-LT10 complex. (C) Potential energy plot of Atrolysin C –LT10 complex. (B) RMSD plot of PLA2-LT10 complex. (D) RMSD plot of Atrolysin C-LT10 complex.

Table 1. Protein -Peptide docking and identification of interacting residues

Protein-peptide complex	Interacting residues		Glide score Kcal.mol ⁻¹	MM-GBSA Kcal.mol ⁻¹
	H Bond Interactions	Vdw Interactions		
PLA2-LT10	Asp24, Arg53, Lys64	Gly30, Cys32, Tyr52, Phe65	-7.795	-61.954
Atrolysin C- LT10	Glu102, Glu106, His142, His 152	Glu105, Thr107, Leu108, Leu110, His146,Pro168	-11.749	-79.379

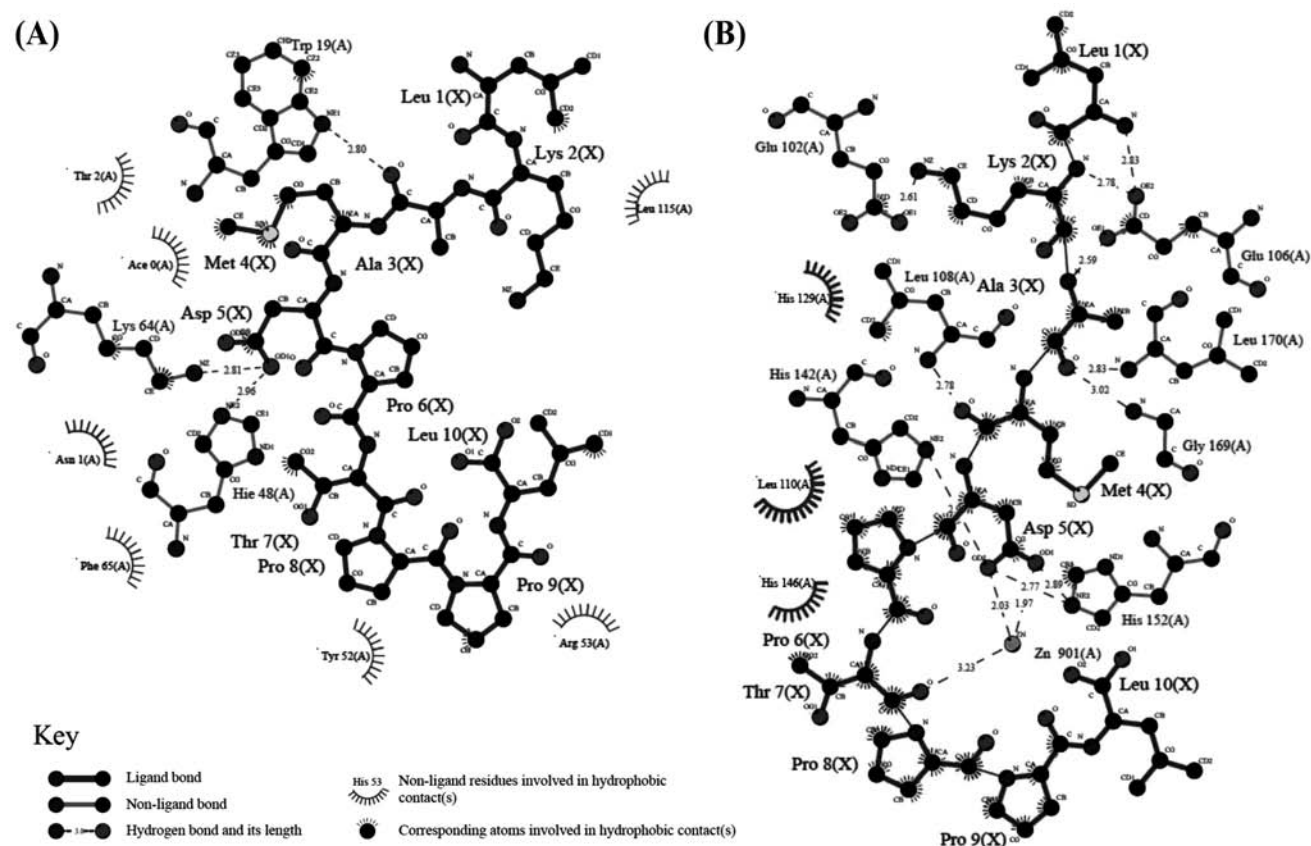


Figure 4. Interactions from minimum energy conformation of Protein-peptide complex obtained over 20ns of MD simulation. **(A)** Interacting residues of PLA2-LT10 complex in a ligplot view. **(B)** Interacting residues of Atrolysin C –LT10 complex in a ligplot view. The ligplot view represents two-dimensional schematic representation of Hydrophobic (radial lines) and hydrogen bond interactions (dotted lines with distances in Å) of protein-peptide complex where residue of peptide are labeled (X). (Please refer to ‘key’ for details).

Table 2. Interacting residues from minimum energy structure of Protein-peptide complex.

Protein-peptide complex	Interacting residues	
	H Bond Interactions	Vdw Interactions
PLA2-LT10	Trp19, Hie48, Lys64	Asn1, Thr2, Leu115, Tyr52, Arg53, Phe65
Atrolysin C- LT10	Glu102, Glu106, Leu108, His142, His152, Gly169, Leu170, Zn 901	Leu110, His129, His146

Table 3. Comparative analysis of interacting residues before and after MD simulation.

Protein-peptide complex	Interacting residues
PLA2-LT10	
Before MDS	Asp24, Gly30, Cys32, Tyr52*, Arg53*, Lys64*, Phe65*
After MDS	Asn1, Thr2, Leu115, Trp19, His48, Tyr52*, Arg53*, Lys64*, Phe65*
Atrolysin C –LT10	
Before MDS	Glu102*, Glu105, Glu106*, Thr107, Leu108*, Leu110*, His142*, His146*, His152*, Pro168, Zn 901*
After MDS	Glu102*, Glu106*, Leu108*, Leu110*, His129, His142*, His146*, His152*, Gly169, Leu170, Zn901*

NOTE: The retained residues are marked with asterisk (*).

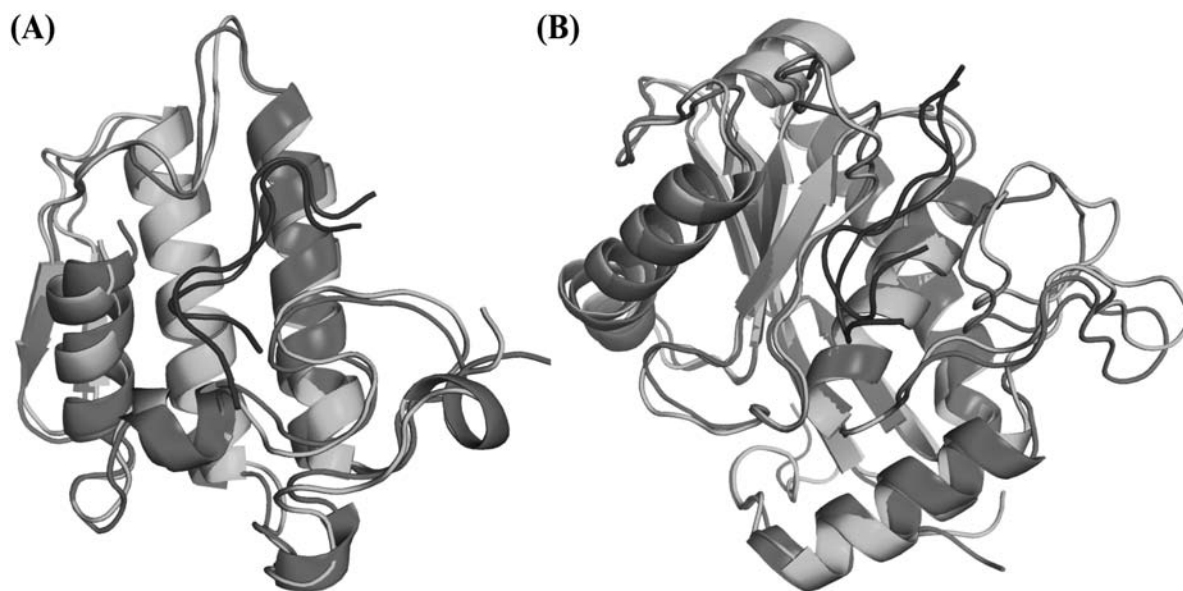


Figure 5. Superimposed protein-peptide complex structures, before and after simulation.

1. Superimposed PLA2-LT10 complex. (B) Superimposed Atrolysin C-LT10 complex.

complex, interacting residues of Glu102, Glu106, Leu108, Leu110, His142, His146, His152 and Zn901 were retained. Also the complex structures before and after simulation were superimposed as shown in Fig. (5A), Fig. (5B). It showed RMSD of 1.50 Å for PLA2-LT10 complex and 1.02 Å for Atrolysin C-LT10 complex.

The interaction of both PLA2-LT10 and Atrolysin C -LT10 complex were computed at regular time interval of 5ns, 10ns, 15ns and 20ns across the simulation trajectory. The trajectory of PLA2-LT10 complex at different point time (Fig. 6) revealed PLA2 residues Asp24, His48, Arg53 and Leu64 to be consistently involved in interactions with LT10 peptide. Similarly, residues Glu102, Glu106, Leu108, His 142, His146, His152, Zn 901 of Atrolysin-C were found to interact frequently with LT10 at regular intervals of trajectory for Atrolysin C-LT10 complex (Fig. 7).

Further, the behavior of LT10 peptide across the simulation trajectory at these regular intervals (5ns, 10ns, 15ns, 20ns) was analyzed by studying its molecular interactions and structural deviation. For PLA2-LT10 complex, LT10 residues Leu1, Asp5 and Thr7 were consistently involved in interactions (Fig. 6), whereas for Atrolysin C-LT10 complex, the residues Leu1, Lys2, Met4 and Asp5 of LT10 showed frequent interactions across the simulation trajectory (Fig. 7). RMSD of LT10 peptide with respect to its conformation in respective docked structures was calculated (using PyMOL) at 5ns, 10ns, 15ns and 20ns. It showed RMSD of 2.5 Å, 2.7 Å, 0.9 Å and 1.3 Å at 5ns, 10ns, 15ns and 20ns respectively for PLA2-LT10 complex. Similarly, it showed RMSD of 1.2 Å, 2.2 Å, 1.7 Å and 2.3 Å at 5ns, 10ns, 15ns and 20ns respectively for Atrolysin C-LT10 complex. However, the deviation of generated LT10 peptide model was found to be significant with respect to its conformation in the docked complex. It showed RMSD of 4.058 Å in PLA2-LT10 docked complex and 3.456 Å in Atrolysin C-LT10 complex.

DISCUSSION

In this work, we have predicted two putative target of LT10 peptide from snake venom, namely Atrolysin-C and PLA2 and further reported the molecular characterization of their interactions. Although, the anti-venom activity of LTNF and LT10 peptide has already been experimentally studied [7-10], the present work provides the possible molecular bases of stable and significant inhibitory interactions underlying the lethal toxin neutralizing property of LT10 peptide.

The docking studies suggested relatively good binding of LT10 peptide to PLA2 and Atrolysin-C with a docking (XP GScore) score of greater than -7 kcal/mol. Moreover a high docking score of -11.749 Kcal/mol for Atrolysin-C marked its comparatively stable interaction with LT10 peptide. Further the binding free energy (ΔG) calculation for Atrolysin C-LT10 gave a better score (-79.379 Kcal/mol) as comparison to that of PLA2-LT10 complex (-61.954 Kcal/mol), suggesting greater binding affinity of LT10 peptide for Atrolysin C as compared to that with PLA2. These docked complexes were further refined by 20 ns MD Simulation, trajectory of which was evaluated via potential energy and RMSD plots. The potential energy plots for PLA2-LT10 (Fig. 3A) and Atrolysin C -LT10 (Fig. 3C) represented well equilibrated and relatively stable complex systems throughout the simulations. The RMSD plots (Fig. 3B, Fig. 3D) of these complex systems showed deviation of less than 1 Å suggesting relatively stable interaction of LT10. So also the post MD simulation interaction analysis (Table 3) showed retention of most of the interacting residues. Furthermore, the structural comparison of these complexes before and after simulation (Fig. 5, Fig. 6, Fig. 7) indicated significant deviations despite retention of most of their interactions. All of these implied reasonably stable interaction of LT10 peptide with PLA2 and Atrolysin-C.

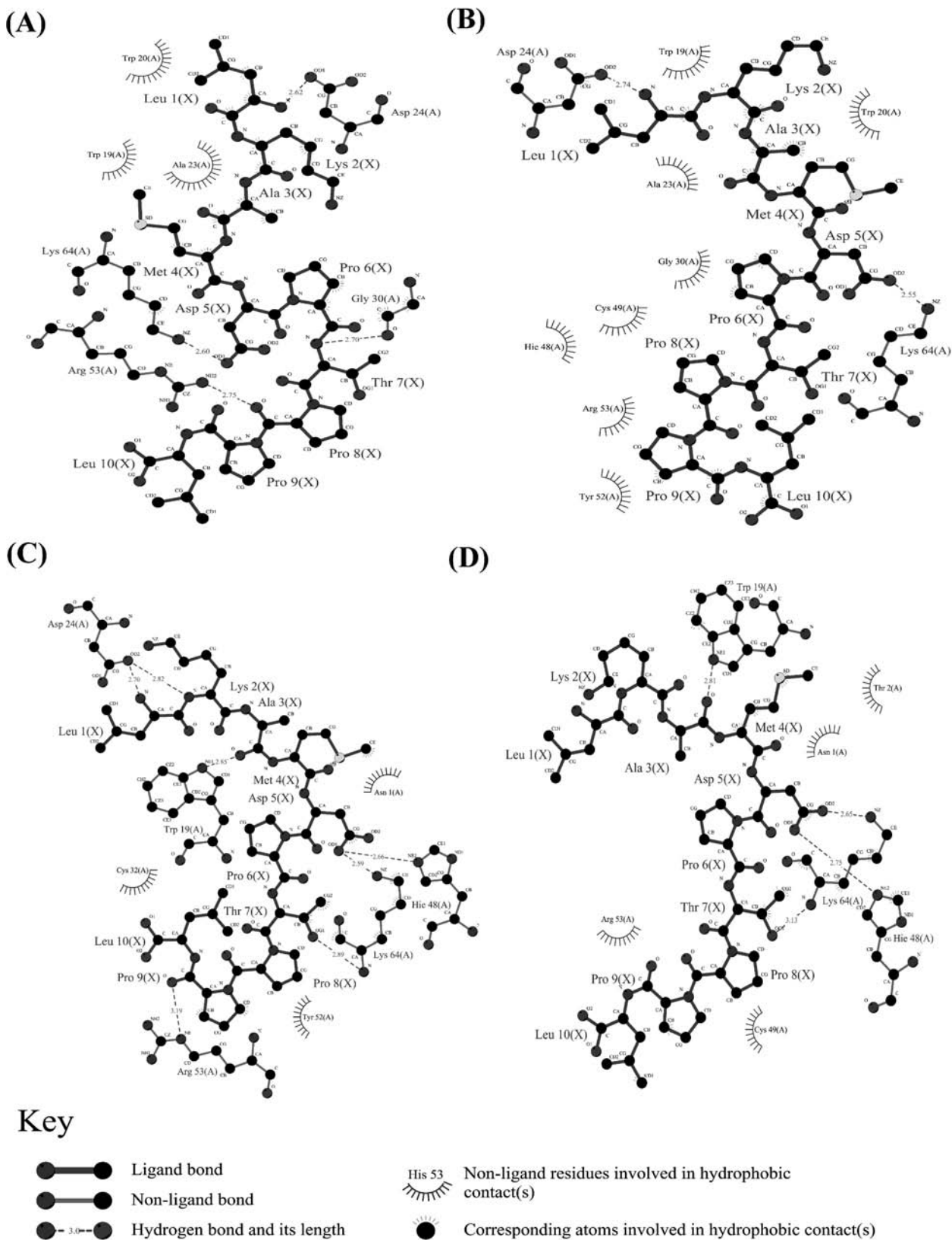


Figure 6. Interaction analyses of PLA2-LT10 complex at different time point along the simulation trajectory. (A) 5ns, (B) 10ns, (C) 15ns and (D) 20ns. This ligplot view represents two-dimensional schematic representation of Hydrophobic (radial lines) and hydrogen bond interactions (dotted lines with distances in Å) of docked complex where residue of peptide are labeled (X). (Please refer to 'key' for details).

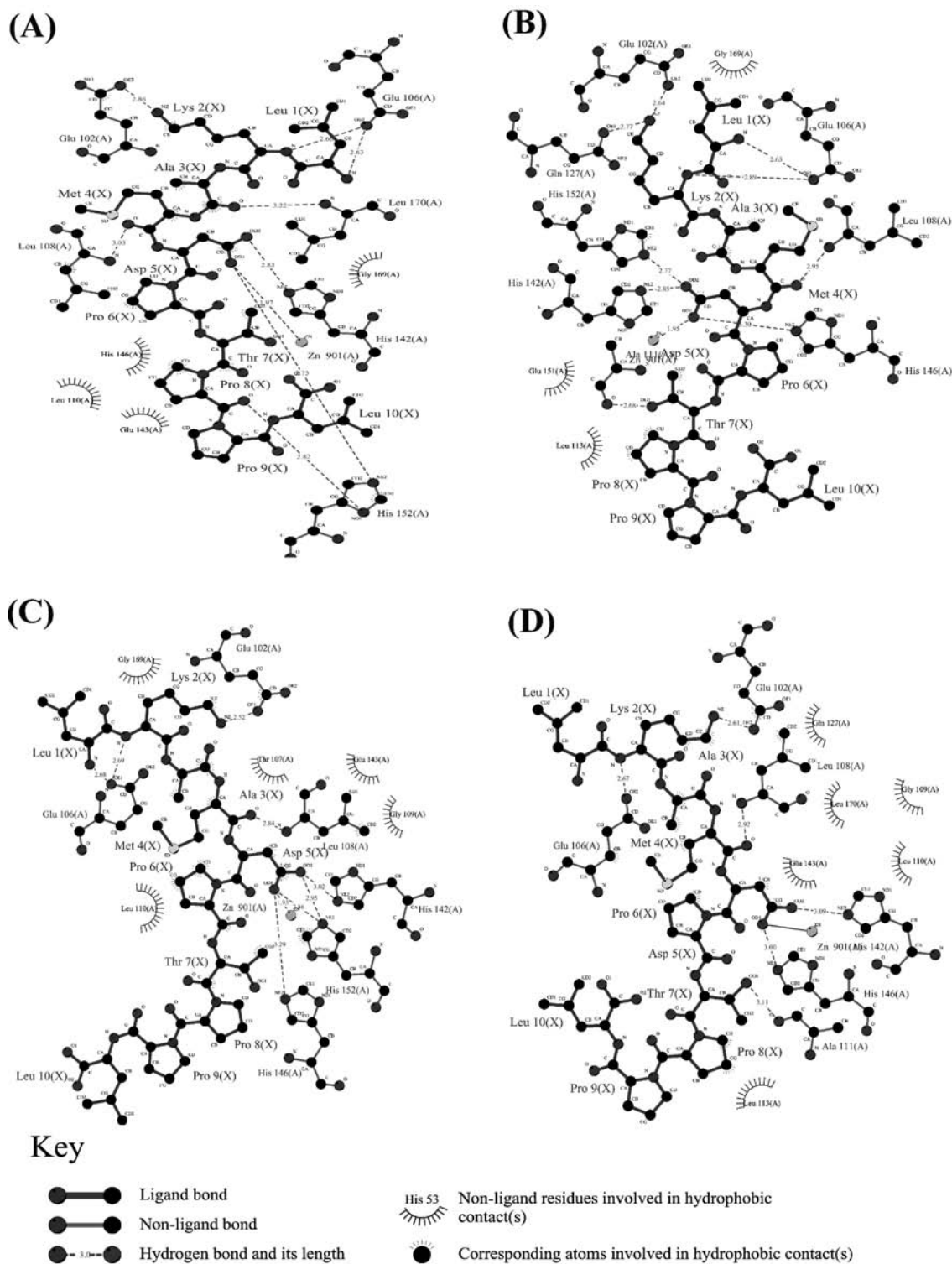


Figure 7. Interaction analyses of Atrolysin C-LT10 complex at different time point along the simulation trajectory. (A) 5ns, (B) 10ns, (C) 15ns and (D) 20ns. This ligplot view represents two-dimensional schematic representation of Hydrophobic (radial lines) and hydrogen bond interactions (dotted lines with distances in Å°) of docked complex where residue of peptide are labeled (X). (Please refer to 'key' for details).

Secreted Phospholipase A2 (PLA2), a major protein constituent of the Indian cobra venom is a Myotoxin and belongs to Group IA of PLA2 family. It is the most abundant muscle-damaging components of these venoms and brings

about vacuolation, lysis and necrosis of skeletal muscle cells in the victim, apart from other pathological effects [29-32]. This enzyme is characterized by presence of histidine in the catalytic site, Ca²⁺ bound in active site as well as 6-

conserved disulfide bond [31]. It catalyzes phospholipid hydrolysis reaction by cleavage of the 2-acyl ester bond of phospholipids *via* activating water molecule in presence of a histidine/aspartic acid dyad in a Ca^{2+} dependent manner [33–35]. The enzymatic activity of PLA2 is considered to be its key step in the induction of myonecrosis and inflammation [29, 36]. Apart from this, lysine 6 and lysine 65 residues of PLA2 are also found to be important for its biological activities [37]. The comparative interaction analysis of PLA2–LT10 complex (Fig. 4A, Fig. 6) showed interaction of LT10 residue ‘Asp5’ with active site histidine (His48) as well as with functionally important residue lysine 65, which thus provides molecular explanation for inhibition of PLA2 and its possible neutralization by LT10 peptide.

On the other hand, the significant interaction of other target *i.e.* Atrolysin-C with LT10 peptide provides a molecular basis for its possible neutralization. Atrolysin-C, a snake venom metalloproteinase (SVMP) from western diamond-back rattlesnake is a hemorrhagic toxin also called HT-d (hemorrhagic toxin d). It belongs to P-I class of SVMP owing to the presence of single metalloproteinase domain and Reprolysin (M12B) family of Zinc metalloprotease. It is a primary factor responsible for hemorrhage in victim and is known to induce the leakage of blood components by the proteolytic destruction of basement membrane and extracellular matrix surrounding capillaries and small vessels [38–40]. The active site region of Atrolysin-C has the catalytic zinc ion, which is coordinated by His146, His142, His152 and a water molecule bound to Glu143, thus forming a tetrahedral co-ordination sphere [41]. These three histidine residues are found in the conserved sequence (HEXXHX XGXXH) which forms a common locus for the active site Zn atom and an exceptionally large and deep S1' primary specificity binding pocket [42, 43]. The Hemorrhagic activity of this enzyme has been shown to be associated with its enzymatic proteolytic activity, since chelation of the zinc ion abolishes both proteolytic and hemorrhagic effects [39, 44]. Also, It has been inferred that the requisite for inhibition of these Matrix Metalloproteinases (MMPs) include (i) chelation of the active-site zinc ion (ii) at least one functional group that provides a hydrogen bond interaction with the enzyme backbone, and (iii) one or more side chains, which undergo effective van der Waals interactions with the enzyme sub-sites [45, 46]. The comparative interaction analysis carried out showed favorable hydrogen bond and van der Waals interactions of LT10 peptide with Atrolysin-C (Fig. 4B, Fig. 7). LT10 peptide residue ‘Asp5’ showed interactions with catalytic zinc ion as well as with the three histidine residues-His142, His146 and His152 from the conserved sequence (HEXXHX XGXXH) that run across the catalytic domain. This interaction analysis thus effectively elucidated the possible inhibition of enzymatic activity of Atrolysin-C by LT10 peptide thereby rendering deactivation of its toxicity. Such an inhibition of this SVMP will hold significant therapeutic value in anti-venom therapy owing to its lethal hemorrhagic activity.

It is interesting to note that despite of significant deviation in conformation of LT10 peptide from its the initial generated model, it showed vital interactions, most of which were retained across the simulation. Especially, both the protein-peptide complexes showed the amino acid ‘Asp5’ of

LT10 peptide to interact consistently (Fig. 6, Fig. 7) with the active site residues, thereby inhibiting these venom enzymes. Negatively charged aspartic acid pairs with positively charged amino acid (such as Histidine, lysine) as well as positively charged zinc ion from the active site forming stabilizing hydrogen bonds [47] and provide binding stability of LT10 peptide. Aspartic acid (D5) of LT10 peptide therefore appears to play a key role in binding as well as deactivating these snake venom toxins.

In summary, our *in silico* interaction study predicted two putative targets (from snake venom) of LT10 peptide, namely PLA2 (from *Naja naja*) and Atrolysin -C (from *Crotalus atrox*). Their detailed interaction analysis revealed ‘Asp5’ of LT10 peptide as a key residue for its significant contribution in interaction with snake venom enzymes. Thereby inhibiting their enzymatic and biological activity resulting in loss of their toxicity. Such interaction analyses thus gave an insight into the molecular details that might account for possible neutralization of these snake venom toxins by LT10 peptide. Further, the knowledge of these key interactions can be exploited for their potential use in drug discovery and development of better anti-venom treatments against snake envenomation.

ABBREVIATIONS

LTNF	=	Lethal Toxin Neutralizing Factor
SVMP	=	snake venom metalloproteinase
ASV	=	Anti-snake venom
MD	=	Molecular Dynamic
MMPs	=	Matrix Metalloproteinases
sOPEP	=	Optimized Potential for Efficient structure Prediction
RMSD	=	Root mean square deviation

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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