Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(12): 58-66

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Epitope Based Vaccine Strategy against Sea Anemone Toxins through in Silico Route

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ABSTRACT

Sea anemone toxins block voltage gated sodium and potassium channel of human through paralyzing and promote inflammation and pain. Haemolysis and coronary artery diseases are also associated with these toxins. Usually treatment for this issue is taking oral pain killers and tropical antibiotics. Prognosis is generally favourable but some species are highly toxic and cause lethality. As there is no successful drug available, in this study, epitope prediction for vaccine development using computational tools may show the light for treatment. Thus Aller Hunter server was used to predict allergenicity of these toxins. Antibody and MHC molecule prediction was carried out by IEDB analysis resource server and docking simulation was carried out through Haddock 2.2 server. Amino acid interaction was made by Ligplot+ server. Results show that the efficient epitope sequences reveal greatest chance for eliciting cell mediated immunity in human body against sea anemone toxins. Predicted MHC peptides were docked with HLA molecules. Lower energy scores represent better binding between receptor and ligand. Mainly polar and charged amino acids are involved in these docking. So it is therefore concluded that designing of a vaccine by using these epitope may elicit cell mediated immunity against sea anemone toxins.

Keywords: Sea anemone toxins; Docking simulation; Vaccine development; Epitope; MHC molecule

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INTRODUCTION

The venom of sea anemone is a mixture of toxins including neurotoxins (that paralyze the prey) which act on voltage gated sodium and potassium channel and these channels in turn help in excitability of most animals as they are responsible for triggering the initiation, mediation and finally propagation of action potentials. Some sea anemone toxins act as cytolysin causing haemolysis. Phospholipase A2 type sea anemone toxins promote inflammation and pain. Excess level of Phospholipase A2 causes vascular inflammation correlated with coronary events in coronary artery diseases and acute coronary syndrome. Usually treatment for such issues involves removing stings, application of tropical antibiotics and use of oral painkillers. Prognosis is generally favorable with this treatment but some highly toxic species (*Actidodendron*, *Phyllodiscus* and *Stichodactyla*) have caused severe

injuries and are potentially lethal [1] and therefore vaccine therapy becomes essential. Recent developments in the field of computational tools enable us to predict epitope from antigenic proteins in a specific way resulting in specific, secured and optimized peptide-based vaccine design planning. Consequently it is easy to predict the peptide binding to leukocyte antigen (HLA) alleles using structural and modeling methodologies. In this chapter to design vaccine against sea anemone toxins epitope and MHC-I, MHC-II molecule were predicted through IEDB analysis resource server. Aller Hunter server is used to predict allergenicity. *In silico* docking of toxins with HLA alleles are carried out through Haddock 2.2 server. Lower energy score represents good binding capability of receptor and ligand.

EXPERIMENTAL SECTION

Allergenicity of sea anemone toxins was predicted by Aller Hunter method [2]. Epitope database IEDB analysis resource server [3] was used for T-cell epitope prediction. T-cell epitopes determine each subsequence's ability to bind to a specific MHC-I and MHC-II molecule. During MHC-I peptide analysis, only frequently occurring alleles were selected and seven set of alleles were selected in case of MHC-II peptide. Docking simulation was carried out through Haddock2.2 server [4]. Human MHC-I and MHC-II molecule are retrieved from protein data bank [https://www.rcsb.org/]. Amino acid interaction was predicted by Ligplot+ [5].

RESULTS AND DISCUSSION

Antibody epitope prediction epitope database (IEDB) analysis resource server [3] was used which predicted the sites that produce antigenic response against an antigenic protein. Table 1, represents brief discussion of NaV blocker, Kv blocker, Cytolysin, Other type and Phospholipase A2 sea anemone toxins. Here one representative toxin is chosen from each toxin family. Representative has been chosen on the basis of maximum number of amino acids i.e. long chain amino acid toxin.

Table 1. Prediction of antibody epitope of sea anemone toxins

Name of toxins	Length	Protein sequences
Toxin Am-1 (NaV blocker toxin)	233 amino acid	MKRIFIVALLFATCLVNAKPSINDADIKREPEPNVAVPPCGD CYQQVGNTCVRVPSLCPSRKREPEPNVAVPPCGDCYQQVG NTCVRVPSLCPSRKREPEPNVAVPPCGDCYQQVGNTCVRV PSLCPSRKREPEPNVAVPPCGDCYQQVGNTCVRVPSLCPSR KREPEPNVAVPPCGDCYQQVGNTCVRVPSLCPSRKREPEP NVAVPPCGDCYQQVGNTCVRVPSLCPSRKR
Kappa-actitoxin Aer1a(Kv blocker toxin)	83 amino acid	MKGQMIICLVLIALCMSVVVMAQNLRAEELEKANPKDER VRSFERNQKRACKDYLPKSECTQFRCRTSMKYKYTNCKKT CGTC
Toxin PsTx-60A (Cytolysin toxin)	501 amino acid	MSPYFKLSSALIFLAITMEALCSPIENTSTSNKDNDKETEHIE ISAKPSGISRGALGQGFEIHREDLLSKQFEATGEKIFEDLPM DECTVTTTLGTIERDDSFYNSTESLYQSVASSTKISGSLKGA YTLGVSVAAVTNNIASSEEEVQGLSLNLKAYSMSSILKKNC VNTKPLSKDLVSDFEALDSEITKPWKLSSWKKYKVLLEKY GSRIVKESISGSSIYQYVFAKSSQKFNHRSFTVKACVSLAGP TKVGKLSFSGCTGVSQQEIEQSSSQSMIKKLVVRGGKTETR

		ASLIGELDPDQINKFLIEAETDPSPIQYKFEPIWTILKTRYVG TEHFAKAVNLEQFYKGFLHFGCSYLHTTSAENKVAEMQKF DFAKTSDPDAPTYVCKVGPEGCQHHEDCHYRAAFWCECG GPYDLARTCFRHKFKKLKSGLTKKECYPNKESGFAWHGC RLHGLTCWCSAPNRSWEESWSGEDTNNALNDVHQVLME KKRRDNAQQQY
Toxin Avt120 (Other type toxin)	995 amino acid	MLLCIVFLTMLSTSLLNVEGLKASSLSKGLERIGRNIRSFGD PQRMLKAGSAISSVYVGATGIYMRSGVIGSINQLEQGKKED AMETLNVAIASMAVFDLTQSTVSPIASELIHQLVKHKGHFA QTLQGFSSYNNALKTQVLTESIDDAGDIIGRLTAAKKRIAQ YFDNEVKTFFQGTELYDSLVKSLQGAKKWAKALTWADTI SGPLFDAANVAFSSWQLHEAIHDTVSSKEERALNIANSSLG VASGTVGLVSFVVSALAIAGSTLAAVAGPIGAIIGCILGVAA IIIDLVNSVNPHTKIKHHLETIQALKEGSLQYLENHVNLTQA MTSSINRDVGFDTVYQVNQGNLITGVFGEKGKSVRGVDTD LFLDFKSKNFPGQENGYLTMGQNRDFDKSKYANSVWRPS GSVKLGYDFYGKRVNSEGIGTSVFATTPMLTDNFYIRSVHI DTRLDNDQEAPDNVIIGEMTNLELSGNTFYIFTGAGDDLVQ IAGLVCNQWDVPCLNVYLGTGVNTLSFQGMNYDRLEFPR NGRHQPYTLTAMEIDLNFERQTTSVKYILRVPEDPKRRVVY EIGKIENVDVLHGSPFDDEIWLSDIKDQIVKSDNGTNKYVIR IFTKRDFTHTIIDNSDHFGSIYLASKRLGQEKYQSIHESDVV YNSETQTLVIYVRDSNDRHVYTGRIIFKRTKAGDIGQMHRY LASTQNTHRSCEVHGLKKDGLYCSHPTRLSEERNEMGTDQ RMARPVPNIPFRYCTDVAGDFNADPIPGLCGDFMLILSRKH RVIPFETTSKYTLTLPQNSLLIIDDEYISEWSTEEKMRYSPFS AFYYERSHKKLFSESRNTRLGWRHDRLAQVRGTIELSVPQ ANGIVFGTAEATNFYLISNMTTGMALLESEYLALASRPRYA FNFTEDHIDESDKPKQVVLGIPYRSAITKQVTITLGKATTAY GEYAHSIGQKATGDYAENALIVNNILQEYLTREGDTMEIKF RRDKTQENTWKM
Phospholipase A2	119 amino acid	GVWQFAYMIAKYTGRNPLDYWGYGCWCGLGGKGNPVD AVDRCCYVHDVCYNSITQGPRPTCSRIAPYHKNYYFTGKK CSTGWLTSKCGRAICACDIAAVKCFRRNHFNKKYRLYKKN IC

For the prediction of MHC binding peptides of sea anemone toxins, IEDB analysis resource server was used. IEDB provides two options. For MHC-I binding peptide prediction, proteasomal cleavage/transporter of antigenic peptides transporter/ MHC Class-I prediction server [6,7] and for MHC-II binding peptide, MHC-II binding prediction are used. In both the prediction servers, artificial neural network prediction method [8] is used to predict the potential nonamers that may efficiently bind to the binding groove of the MHC molecule. In case of sea anemone toxins, twenty seven types MHC-I molecules (belong to two super types A and B) and seven types MHC-II molecule (belong to six complexes) are predicted.

Table 2. MHC –I and MHC-II of sea anemone toxins

Name of toxins	**Type of MHC-I molecule**	**Type of MHC-II molecule**
Am-1 (NaV blocker toxin)	HLA-A*01:01 HLA-A*02:01 HLA-A*02:03 HLA-A*02:01 HLA-A*03:01 HLA-A*11:01 HLA-A*23:01 HLA-A*24:02 HLA-A*26:01 HLA-A*30:01 HLA-A*30:02 HLA-A*31:01 HLA-A*32:01 HLA-A*33:01 HLA-A*68:01 HLA-A*68:02 HLA-B*07:02 HLA-B*08:01 HLA-B*15:01 HLA-B*35:01 HLA-B*40:01 HLA-B*44:02 HLA-B*44:03 HLA-B*51:01 HLA-B*53:01 HLA-B*57:01 HLA-B*58:01	HLA-DRB1*03:01 HLA-DRB1*07:01 HLA-DRB1*15:01 HLA-DRB3*1:01 HLA-DRB3*2:01 HLA-DRB4*1:01 HLA-DRB5*1:01
Kappa-actitoxin-Aer1a (Kv blocker toxin)		
PsTx-60A (Cytolysin type)		
.		
Avt120 (Other type toxin)		

Phospholipase A2 toxin		

To assay the degrees of allergenicity, Aller Hunter server [2] is used. This server predicts allergens as well as non-allergens with high specificity. The prediction score of all sea anemone toxins is zero.

In silico docking simulation is performed to find out whether or not the predicted epitope will bind to the MHC molecule when these will be applied for further in vivo experiments. For docking simulation Haddock2.2 server [4] is used. Table 3 depict MHC-I and MHC-II binding peptide sequence of five different sea anemone toxins. Antigenic peptide and HLA (1I7T) binding result is exhibited in Table 4 and Table 6 represent docking result of antigenic with HLA (5JLZ).

Table 3. MHC-I and MHC-II peptide sequence of five different sea anemone toxins

Representative of toxin name	Type of MHC-I	Sequences	Type of MHC-II	Sequences
Am-1	HLA-A*02:01	ALLFATCLV	HLA-DRB1*07:01	CGDCYQQVGNTCVRV
Kappa-actitoxin-Aer1a	HLA-A*02:01	VLIALCMS	HLA-DRB1*03:01	CLVLIALCMSVVVMA
PsTx-60A	HLA-A*02:01	KLSSALIFL	HLA-DRB1*07:01	MSPYFKLSSALIFLA
Avt120	HLA-A*01:01	NSDHFGSIY	HLA-DRB3*01:01	PFDDEIWLSDIKDQI
Phospholipase A2	HLA-A*01:01	YTGRNPLDY	HLA-DRB5*01:01	GVWQFAYMIAKYTGR

The predicted epitope “ALLFATCLV” for Am-1 toxin, “VLIALCMS” for Kappa-actitoxin-Aer1a, “KLSSALIFL” for toxin PsTx-60A, “NSDHFGSIY” for Avt-120 toxin and “YTGRNPLDY” for Phospholipase A2 toxin were docked with one MHC-I molecule (HLA-A*02.01,PDB 1I7T). These epitope peptides have chosen among many on the basis of low IC_{50} score. IC_{50} is a method, through which peptides are sorted. Low percentile rank of IC_{50} is a sign of good binder.

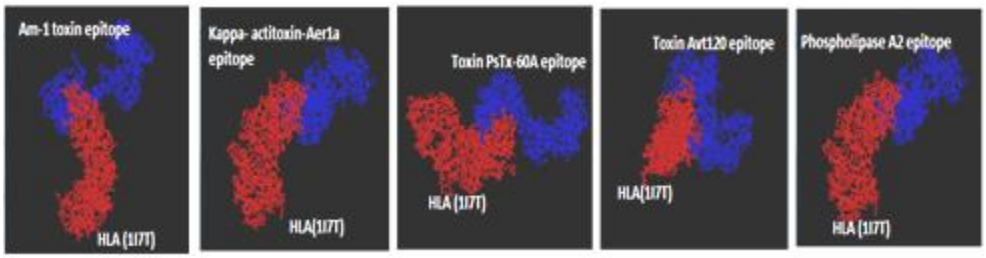

Figure 1. Docking between toxin epitopes of five toxin families with HLA (1I7T)

Table 4. Binding score of toxin epitopes of five toxin families with HLA (PDB 1I7T)

Toxin name	Type of MHC-I	Haddock Score	RMSD Value	Vander Waal energy	Electrostatic energy	Desolvation energy	Buried surface area	Z-score
Am-1	HLA-A*02.01 (PDB 1I7T)	-93.7	1.5	-55.4	-122.5	-42.7	1155.2	-1.2
Kappa-actitoxin-Aer1a	HLA-A*02.01 (PDB 1I7T)	-89.2	0.9	-61.8	-121.7	-39.8	951.7	-0.8
PsTx-60A	HLA-A*02.01 (PDB 1I7T)	-98.1	0.9	-67.1	-119.4	-45.1	816.5	-1.2
Avt120	HLA-A*02.01 (PDB 1I7T)	-95.3	0.9	-56.7	-121.1	-49.1	1157.3	-0.8
Phospholipase A2	HLA-A*02.01 (PDB 1I7T)	-89.5	1.7	-57.7	-138.1	-35.2	979.8	-1.6

Table 5. Amino acid interaction between toxin epitopes of five toxin families with HLA (1I7T)

Name of docked model	H-bonded contacts	Non-bonded contacts
Am-1-HLA	Val269^{Lignad}-Asp53^{Receptor} Leu261^{L}-Ser55^{R}, Lys265^{L}-Tyr63^{R}, Asp256^{L}-Lys58^{R}, Trp232^{L}-Glu36^{R}, Gln231^{L}-Glu36^{R}	Gly268^{L}-Asp55^{R}, Ala264^{L}-Ser55^{R}, Leu260^{L}-Phe56^{R} Ala245^{L}-Phe56^{R}, Leu241^{L}-Leu54^{R}, Thr257^{L}-Ser57^{R}, Arg249^{L}-Trp60^{R}, Ala235^{L}-Asp34^{R}, Leu238^{L}-Ser333^{R}, Leu242^{L}-Phe62^{R}, Tyr63^{L}-GIle35^{R}, Tyr63^{L}-Tyr66^{R}, Tyr63^{L}-His51^{R}
Kappa-actitoxin-Aer1a-HLA	Lys22^{L}-Ser55^{R}, Lys22^{L}-Asp53^{R}, Lys22^{L}-Leu54^{R}, Ser20^{L}-Ser33^{R}, Arg18^{L}-Asp34^{R}, Phe15^{L}-Met0^{R}	u7^{L}-Trp60^{R}, Arg16^{L}-His31^{R}, Tyr23^{L}-Phe62^{R} Tyr63^{L}-Phe56^{R}, Phe15^{L}-Pro32^{R},
PsTX-60A-HLA	Gln53^{L}-Lys58^{R}, His368^{L}-Leu54^{R}, Cys369^{L}-Ser33^{R}, Lys371^{L}-Ile35^{R}, Lys376^{L}-Asp34^{R}, Lys370^{L}-Val37^{R}	Gly351^{L}-Trp60^{R}, Gly348^{L}-His31^{R}, Cys345^{L}-Phe62^{R} His368^{L}-Ser52^{R}, His368^{L}-Asp53^{R},

Avt120-HLA	Ser143^{L}-Arg3^{R}, Asp109^{L}-Lys58^{R}, Asp7^{L}-Leu54^{R}, Lys6^{L}-Asp53^{R}, Lys6^{L}-His51^{R}	Lys113^{L}-Trp60^{R}, Val141^{L}-His31^{R}, Ala146^{L}-Phe62^{R} Lys6^{L}-Ser52^{R}, Gln49^{L}-Asp34^{R},
PLA2-HLA	30^{L}-Met0^{R}, Leu19^{L}-Asp34^{R}, Asn6^{L}-Asp34^{R}	n72^{L}-Trp60^{R}, Leu2^{L}-His31^{R}, Arg3^{L}-Thr70^{R}, Trp3^{L}-Ser33^{R}, Pro18^{L}-Asp34^{R},

The predicted epitope “CGDCYQQVGNTCVRV” for Am-1 toxin, “CLVLIALCMSVVVMA” for Kappa-actitoxin-Aer1a, “MSPYFKLSSALIFLA” for toxin PsTx-60A, “PFDDEIWLSDIKDQI” for Avt-120 toxin and “GVWQFAYMIAKYTGR” for Phospholipase A2 toxin were docked with one MHC-II molecule (PDB 5JLZ). These epitope peptides have sorted among many on the basis of low percentile rank. Low percentile rank is a sign of good binder.

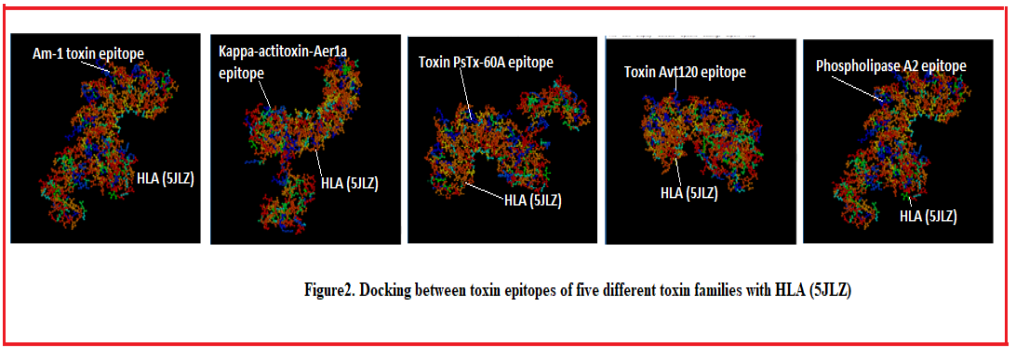

Figure 2. Docking between toxin epitopes belonging to different toxin families with HLA (PDB 5JLZ)

Table 6. Binding score of toxin epitopes of five different toxin families with HLA (PDB 5JLZ)

Toxin name	MHC - II	Haddock Score	RMSD Value	Vander Waal energy	Electrostatic energy	Desolvation energy	Buried surface area	Z-score
Am-1	HLAA* DRB1 (PDB 5JLZ)	-81.2	0.9	-41.3	-167.7	-35.7	875.7	-0.8
Kappa-actitoxin-Aer1a	HLAA* DRB1 (PDB 5JLZ)	-116.7	0.9	-31.8	-154.2	-46.2	899.0	-0.8
PsTx-60A	HLAA* DRB1 (PDB 5JLZ)	-100.3	1.2	-45.2	-134.5	-41.2	912.0	-0.9
Avt120	HLAA* DRB1 (PDB	-98.1	1.6	-40.5	-116.8	-46.6	946.2	-0.9

	5JLZ**)**							
Phospholipase A2	HLAA* DRB1 (PDB 5JLZ**)**	-95.4	1.2	-54.9	-123.1	-44.9	970.1	-0.9

Table 7. Amino acid interaction between toxin epitopes of five different toxin families with HLA (5JLZ)

Name of docked model	H-bonded contacts	Non-bonded contacts
Am-1-HLA	$Arg266^{Lignad}$-$Glu3^{Receptor}$ $Thr270^{L}$-$Glu4^{R}$, $Gly268^{L}$-$His5^{R}$, $Ala264^{L}$-$Ile7^{R}$, $Gln583^{L}$-$Arg76^{R}$, $Ag584^{L}$-$Arg76^{R}$	$Asp301^{L}$-$Lys2^{R}$, $Leu242^{L}$-$Ser53^{R}$, $Leu260^{L}$-$Phe56^{R}$ $Ala558^{L}$-$Ser77^{R}$, $Gly234^{L}$-$Phe26^{R}$, $Trp232^{L}$-$Asp29^{R}$
Kappa-actitoxin-aer1a-HLA	$s9^{L}$-$Glu4^{R}$, $Arg18^{L}$-$Asp27^{R}$, $Arg18^{L}$-$Asp29^{R}$, $Arg18^{L}$-$Phe26^{R}$, $Lys22^{L}$-$Glu55^{R}$	$n14^{L}$-$His5^{R}$, $Arg24^{L}$-$Ile31^{R}$, $Leu25^{L}$-$Phe54^{R}$,$Met21^{L}$-$Phe32^{R}$
PsTX-60A-HLA	$Ser409^{L}$-$Glu4^{R}$, $Arg50^{L}$-$Asp192^{R}$, $Gln353^{L}$-$Asp192^{R}$, $His368^{L}$-$Asn62^{R}$, $Lys370^{L}$-$Glu11^{R}$, $Arg414^{L}$-$Asp29^{R}$	$Gly351^{L}$-$Phe51^{R}$, $GIle352^{L}$-$Ala52^{R}$, $Gly348^{L}$-$Phe32^{R}$ $Cys369^{L}$-$Asp66^{R}$, $Ile411^{L}$-$His5^{R}$,
Avt120-HLA	$Lys33^{L}$-$Glu55^{R}$, $Leu30^{L}$-$Glu55^{R}$	$Ile200^{L}$-$Ser53^{R}$, $Gly31^{L}$-$Phe54^{R}$, $Glu28^{L}$-$Phe24^{R}$,$His29^{L}$-$Phe32^{R}$, $Ala1699^{L}$-$Ile7^{R}$
PLA2-HLA	$Asn72^{L}$-$Ser53^{R}$, $Asn71^{L}$-$Phe51^{R}$, $Lys57^{L}$-$Glu4^{R}$	$)3^{L}$-$Glu55^{R}$, $val165^{L}$-$ala52^{R}$, $Asp66^{L}$-$Phe48^{R}$, $Thr703^{L}$-$Phe32^{R}$, $Met56^{L}$-$His5^{R}$,

DISCUSSION

Prediction of epitope on the protein surface is a critical step for epitope–based vaccine design and a number of approaches had been attempted in earlier studies but in this study we tried to predict the epitope more accurately relying on very basic step – like detection of the allergenicity of sea anemone toxins and ending by docking of epitope to their respective receptors. The Aller Hunter score value is probably that a particular sequence is cross reactive allergen. The threshold value for prediction of cross reactive allergen is adjusted in such a manner that a sequence is predicted as a cross reactive allergen that its probability is > = 0.06. Aller Hunter has optimum prediction result of that particular threshold. The FAO and WHO provide guidelines for sequence based allergenicity prediction and the protocol clearly states that a sequence can be potentially allergic if it either has an approximated identity of at least 6 contiguous amino acids or >35% sequence identity over a window of 80 amino acid chains when compared to known allergens. All the sea anemone toxins were predicted as potential non-allergen with a prediction score 0.0. Thus if a vaccine is designed developed by using venom peptides, it will not create allergic reaction.

The binding between antigenic peptides and the MHC molecule of human (HLA) is main step in cellular immune response. For the prediction of MHC binding molecule in both cases (MHC-I & MHC-II) IEDB analysis resource was used. Five nonamers were predicted (Table 4) and they exhibited sufficiently high results in the prediction methods which were used in this paper. The predicted nonamers are "ALLFATCLV" for NaV blocker utilized protein family, "VLIALCMS" for Kv blocker family, "KLSSALIFL" for Cytolysin family, "NSDHFGSIY" for Other toxin family and "YTGRNPLDY" for Phospholipase A2 family. These peptides showed interaction with multiple MHC Class I and MHC Class II alleles. Interaction among different alleles with these peptides is summarized in Table 3. The predicted epitopes of five different sea anemone toxins are interacted with two HLA (1I7T and 5JLZ).

In this paper, minimization of predicted epitope and pinpointing the efficient epitope sequence is done to reveal greatest chance for eliciting cell-mediated immunity in human body against sea anemone toxins. Predicted antigenic peptides were docked with HLA molecules to find out whether or not the vaccine designed by using the predicted epitope will elicit sufficient immunological response in vivo. Lower energy scores represent better binding between receptor and ligand (Table 4 and Table 6) [9]. Figure 1 displays docking between antigenic peptides of five toxins of five different families with HLA (1I7T) and Figure 2 shows docking between antigenic peptides of five toxins of five different families with HLA (5JLZ). Table 5 and Table 7 displays hydrogen bonded contacts and non-bonded contacts of both docked models as predicted through Ligplot+. In non-bonded contacts mainly hydrophobic amino acids are involved. Polar and charged amino acids are mainly involved in hydrogen bonded contacts.

So, it is therefore concluded that designing of a vaccine by using these epitope may elicit cell mediated immunity against sea anemone toxins.

ACKNOWLEDGEMENT

Author Sriparna Ray is indebted to the Dr. Sudip Hajra, Asansol, West Bengal regarding software application.

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