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In-silico characterization of mosquito immune peptides (cecropin, defensin and gambicin)

K.Vanlalhruaia[1]*, G.Gurusubramanian[1], N.Senthil Kumar[2]

[1]Departments of Zoology, Mizoram University, Aizawl- 796 004, Mizoram, (INDIA)

[2]Department of Biotechnology, Mizoram University, Aizawl- 796 004, Mizoram, (INDIA)

E-mail : vhruaia2@gmail.com

ABSTRACT

In this paper, nineteen different immune peptides (cecropin, degensin and gambicin) of mosquito retrieved from National Centre for Biotechnology Information (NCBI) database were analysed and characterized using *In silico* tools. Primary structure analysis shows that most of the immune peptides are hydrophobic in nature due to the high content of non-polar residues. The presence of Cysteines residues was found only on defensin immune peptides of *Anopheles gambiae, Culex quinquefasciatus, Aedes aegypti, Aedes albopictus, Culex pipiens* and *Anopheles stephensi* infer that these proteins may form disulphide (SS) bonds, which are regarded as a positive factor for stability. The aliphatic index computed by Ex-Pasy's ProtParam infers that immune peptides may be stable for a wide range of temperature. Secondary structure analysis shows that most of the immune peptides mixed secondary structure. The presence of disulphide (SS) bonds in the Q7PY14.4, ABB00933, EDS293341, AAC36346, AAO38519 and ABM92299 were predicted by CYS_REC tool and also identified from the three-dimensional structure using Rasmol tool. The disulphide bonds identified from the three-dimensional structure using the Rasmol tool might be correct as the evaluation parameters are within the acceptable limits for the modeled 3D structures. © 2015 Trade Science Inc. - INDIA

KEYWORDS

Immune peptides;
Computational analysis;
Disulphide bridges;
Homology modeling;
Proteomics tools.

INTRODUCTION

Computational packages and online servers are the current tools used in the protein sequence analysis and characterization[6].The physicochemical and the structural properties of the proteins are well understood with the use of computational tools. Today, number of computational tools has been developed for making predictions regarding the identification and structure prediction of proteins. The statistics about a protein sequence such as number of amino acid, sequence length, and the physico-chemical properties of a proteins such as molecular weight, atomic composition, extinction coefficient, GRAVY, aliphatic index, instability index, etc. can be computed by computational tools for the prediction and characterization of protein structure. The amino acid sequence provides most of the information required for determining and characterizing the

molecule's function, physical and chemical properties. Sequence analysis and physicochemical characterization of proteins using bio-computational tools have been done by many researchers and reported[4-6].

Mosquito-borne diseases are among the major concerns of public health. Malaria is a particularly threatening disease that is responsible for over one million deaths per year. Dengue fever affects hundreds of millions of people. Other viral and filarial diseases transmitted by mosquitoes are prevalent in many areas of the world[1,2]. Therefore, there is an urgent need to explore every avenue for developing unique control strategies against mosquito-borne diseases. Studies in *Drosophila melanogaster* have provided the basis of our knowledge about the insect innate immune response[16]. Antimicrobial peptides (AMPs) are an important part of the humoral immunity. In *D. melanogaster*, there are seven distinct AMP families, which differ widely in their specificities against microorganisms[16]. Mosquito AMPs significantly differ from *Drosophila* and are mainly represented by defensins and cecropins[17]. Mosquito defensins are primarily active against Gram-positive bacteria, although a Gram-negative bacterium, *Enterobacter cloacae*, is susceptible to *Aedes* Defensin A (DefA)[18]. Mosquito cecropins have a broad spectrum of antimicrobial activity[18,19].

Most of the Cecropins were the first animal inducible AMPs to be isolated and fully characterized. The first insect cecropin was isolated from the blood of experimentally infected diapausing pupae of the moth *Hyalophora cecropia* (Lepidoptera)[16]. Since this first report, expression of cecropin-like peptides have been documented in several other insect species, which all belong to phylogenetically higher insect orders of Diptera and Lepidoptera. Interestingly, it has been noted that the *Anopheles* cecropin without the tryptophan is more efficient against yeast and Gram-positive bacteria than *Drosophila* cecropin A, which has a tryptophan residue in position 2[17]. The combination of increased number of positive charges and no tryptophans may be the reason for the differences in the observed antimicrobial activity.

Defensins, were first reported from cell cultures of the flesh fly *Sarcophaga peregrina*[20] and from experimentally injured larvae of the black blowfly (*Proto)phormia terranovae*[21]. Mosquito defensins are produced and secreted by fat body and midgut tissues in *Ae. aegypti* and *Anopheles gambiae* as precursor molecules, with a signal sequence and propeptide preceding the approximately 40 amino acid mature peptide. Six conserved cysteine residues in the peptide engage in disulfide bridges that stabilize its 3-dimensional structure, which is composed of an N-terminal loop, α-helix and two antiparallel β-sheets[10]. *An. gambiae* gene, gambicin encoding a mature 61-residue cysteine-rich immune inducible peptide. Mature gambicin peptide is active against Gram-positive and Gram-negative bacteria, a filamentous fungus and the ookinete stage of the malaria parasite.

Obviously, if a bacterial pathogen is able to develop or acquire resistance to an antibiotic, this given drug becomes useless in the future treatment of infections caused by the pathogen and alternative treatments are required. Indeed, several new bacterial diseases have been discovered in the past decades. In this context, there is an urgent need for a new generation of antibiotics to complement the panel of drugs that are available to the clinicians and to provide new tools for multitherapy treatment. For decades, one major area of interest for the discovery and study of new antibiotics was the investigation of AMPs derived from insect immune defense reactions. However, physico-chemical characterization of mosquito immune peptides has not been done so far. In this paper, we report the *In-silico* analysis and characterization studies on19-immune peptides sequences of various mosquito.

MATERIALS AND METHODS

Mosquito immune peptides (cecropin, defensin and gambicin) protein sequences of mosquito were retrieved from National Centre for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/). NCBI is scanned for the key word immune peptides (cecropin, defensin and gambicin) of mosquito. From the search result yielded, 19 protein sequences of immune peptides (cecropin, defensin and gambicin) of mosquito were selected (*i.e.* for each immune peptides a protein sequence was chosen for each types of mosquito) by longest amino acids composition and have organized a non-redundant data set. The protein sequences of mosquito immune peptides were retrieved

TABLE 1: Immune peptides (cecropin,gambicin and defensin) sequences of mosquito retrieved from NCBI

Accession number	Sequence description	Organism	Type
XP_565481.2	antimicrobial peptide cecropin	*Anopheles gambiae*	
AAD37702.1	cecropin	*Aedes albopictus*	
CAO83219.1	CEC2 protein	*Anopheles arabiensis*	
ABG29420.1	cecropin	*Culex pipiens*	cecropin
AAF59831.1	cecropin A	*Aedes aegypti*	
EDS36062.1	cecropin	*Culex quinquefasciatus*	
ABU48600.1	salivary cecropin	*Anopheles stephensi*	
ACN38089.1	gambicin	*Anopheles gambiae*	
ACA05576.1	gambicin	*Anopheles arabiensis*	
ACN38078.1	gambicin	*Anopheles quadriannulatus*	Gambicin
AAR18451.1	salivary gambicin immunity-related peptide	*Culex quinquefasciatus*	
AAO38515.1	gambicin	*Culex pipiens*	
Q7PYI4.4	Phagocyte signaling-impaired protein	*Anopheles gambiae*	
ABB82553.1	p38b MAP kinase	*Aedes aegypti*	
ABB00933.1	defensin	*Anopheles arabiensis*	
EDS29334.1	defensin-A	*Culex quinquefasciatus*	Defensin
AAC36346.1	defensin D	*Aedes albopictus*	
AAO38519.1	defensin precursor	*Culex pipiens*	
ABM92299.1	salivary defensin	*Anopheles stephensi*	

TABLE 2 : Amino acid composition (in %) of mosquito immune peptides computed using Expasy's ProtParam tool

Amino acids	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
XP_565481.2	6	3	4.5	3	0	6	4.5	11.9	3	3	14.9	14.9	1.5	6	1.5	1.5	1.5	0	0	13.4
AAD37702.1	8.3	1.7	3.3	0	0	1.7	5	15	0	3.3	20	16.7	1.7	5	1.7	1.7	5	0	1.7	8.3
CAO83219.1	15.3	6.8	3.4	0	0	3.4	3.4	13.6	0	1.7	15.3	11.9	3.4	8.5	1.7	0	1.7	0	0	10.2
ABG29420.1	16.7	3.3	5	0	0	1.7	5	11.7	1.7	3.3	15	11.7	1.7	8.3	3.3	0	3.3	0	0	8.3
AAF59831.1	16.9	3.4	3.4	0	0	1.7	5.1	11.9	0	1.7	18.6	15.3	1.7	5.1	1.7	1.7	3.4	0	0	8.5
EDS36062.1	16.7	3.3	5	0	0	1.7	5	11.7	1.7	3.3	15	11.7	1.7	8.3	3.3	0	3.3	0	0	8.3
ABU48600.1	15	10	2.5	2.5	0	2.5	2.5	12.5	0	0	10	15	0	5	7.5	0	0	2.5	2.5	10
ACN38089.1	11.1	6.2	2.5	4.9	12.3	2.5	2.5	8.6	0	4.9	7.4	6.2	2.5	3.7	1.2	4.9	6.2	0	4.9	7.4
ACA05576.1	11.1	6.2	2.5	4.9	12.3	2.5	2.5	8.6	0	4.9	7.4	6.2	2.5	4.9	1.2	4.9	6.2	0	4.9	6.2
ACN38078.1	11.1	6.2	2.5	4.9	12.3	2.5	2.5	8.6	0	4.9	7.4	6.2	2.5	3.7	1.2	4.9	6.2	0	4.9	7.4
AAR18451.1	9.4	8.2	2.4	5.9	10.6	3.5	1.2	8.2	0	3.5	8.2	4.7	1.2	3.5	0	8.2	7.1	1.2	5.9	7.1
AAO38515.1	8.2	8.2	2.4	5.9	10.6	3.5	1.2	8.2	0	3.5	9.4	4.7	1.2	3.5	0	8.2	7.1	0	5.9	8.2
Q7PYI4.4	9.9	5.6	3.2	5.3	1.6	5.6	7.3	3.7	2.6	4	15.1	5.9	2.7	2.9	3.5	5.9	4.7	1.3	4.8	4.5
ABB82553.1	6.4	4.7	4.7	5.9	0.8	5.3	7	5	4.5	6.1	10.3	5.3	3.6	3.6	4.7	4.7	5.6	1.4	3.9	6.1
ABB00933.1	18.6	5.9	5.9	2	7.8	2	5.9	8.8	2.9	2.9	10.8	2.9	1	1	2	4.9	4.9	0	2.9	6.9
EDS29334.1	13.1	5.1	8.1	4	8.1	5.1	4	8.1	1	2	10.1	3	1	5.1	2	7.1	4	0	3	6.1
AAC36346.1	13.5	4.2	4.2	4.2	8.3	3.1	6.2	7.3	1	4.2	9.4	3.1	1	3.1	5.2	6.2	3.1	0	3.1	9.4
AAO38519.1	10	7.5	10	5	15	0	0	15	2.5	2.5	7.5	5	0	2.5	0	5	2.5	0	2.5	7.5
ABM92299.1	14.6	6.2	7.3	2.1	8.3	5.2	6.2	5.2	2.1	2.1	10.4	2.1	2.1	1	2.1	5.2	4.2	0	3.1	10.4

in FASTA format and used for analysis.

The amino acid composition of immune peptides sequences were computed using Expasy's ProtParam (http://us.expasy.org/tools/protparam.html) prediction

FULL PAPER

TABLE 3 : Parameters of mosquito immune peptides computed using Expasy's ProtParam tool. M. wt., Molecular weight; pI, Isoelectric point; –R, Number of negative residues; +R, Number of positive residues; EC, Extinction coefficient at 280 nm; II, Instability index; AI, Aliphatic index; GRAVY, Grand Average Hydropathy

Accession number	Sequence length	M. wt	pI	– R	+ R	EC	II	AI	GRAVY
XP_565481.2	67	7354.8	10.17	5	12	Nil	10.61	114.78	0.036
AAD37702.1	60	6371.7	10.24	3	11	1490	13.54	123.50	0.350
CAO83219.1	59	6301.6	11.61	2	11	Nil	19.66	110.85	0.442
ABG29420.1	60	6336.6	10.38	3	9	Nil	18.49	112.33	0.445
AAF59831.1	59	6150.5	10.53	3	11	Nil	22.45	120.85	0.405
EDS36062.1	60	6336.6	10.38	3	9	Nil	18.49	112.33	0.445
ABU48600.1	40	4394.2	11.13	2	10	6990	45.25	83.00	-0.400
ACN38089.1	81	8747.3	8.57	6	10	6585	40.99	80.74	0.322
ACA05576.1	81	8795.3	8.57	6	10	6585	40.99	77.16	0.305
ACN38078.1	81	8747.3	8.57	6	10	6585	40.99	80.74	0.322
AAR18451.1	85	9354.8	8.80	6	11	13450	49.07	75.76	0.081
AAO38515.1	85	9309.8	8.80	6	11	7950	48.41	82.59	0.165
Q7PYI4.4	951	108766.2	6.25	119	109	135415	45.35	97.63	-0.236
ABB82553.1	358	41137.9	5.88	46	36	48485	45.21	88.52	-0.367
ABB00933.1	102	10567.1	7.56	8	9	4970	30.88	92.06	0.207
EDS29334.1	99	10573.9	6.52	8	8	4970	36.51	77.98	0.019
AAC36346.1	96	10125.6	4.91	10	7	4970	45.07	93.54	0.309
AAO38519.1	40	4147.7	8.68	2	5	1865	25.67	70.75	0.050
ABM92299.1	96	10317.8	6.69	8	8	4970	36.89	93.54	0.143

TABLE 4 : Transmembrane regions identified by SOSUI server

Accession number	Transmembrane region	Type	Length
XP_565481.2	NVSKLFVIVLLATLLLFGGQAEA	Primary	23
AAD37702.1	NFNKLFALVLLIGLVLLTGQTEA	Primary	23
CAO83219.1	MNFKLIFLVALVLMAAFLGQTEG	Primary	23
ABG29420.1	FNKLFAIVLLAALAFLGQTEAGG	Primary	23
AAF59831.1	NFTKLFLLIAVAVLLLTGQSEAG	Primary	23
EDS36062.1	FNKLFAIVLLAALAFLGQTEAGG	Primary	23
ABU48600.1	Soluable Protein		
ACN38089.1	CILLAVLLCTAAVADAMVFAYAP	Primary	23
ACA05576.1	CILLAVLLCTAAVADAMVFAYAP	Primary	23
ACN38078.1	CILLAVLLCTAAVADAMVFAYAP	Primary	23
AAR18451.1	QTVFVLLALLLVSASCADAWVYV	Primary	23
AAO38515.1	TVFVLLALLLVSASCVDALVYVY	Primary	23
Q7PYI4.4	ELVNGCIEMISLMVFLLAVCYDK	Primary	23
ABB82553.1	Soluable Protein		
ABB00933.1	ATIVCAIAVVLAATLLNGSVQA	Primary	23
EDS29334.1	Soluable Protein		
AAC36346.1	VPTVICFLAMCLVAITGAYPQEP	Primary	23
AAO38519.1	Soluable Protein		
ABM92299.1	KCVTLICAVVVVLAALLNNSVQA	Primary	23

server (TABLE 2). Percentages of hydrophobic and hydrophilic residues were calculated from the primary

TABLE 5 : Percentage of residues forming alpha, beta, and coil structures of immune peptides computed by SSCP server

Accession number	Alpha	Beta	Coil	Class
XP_565481.2	23.7	23.7	52.7	mixed
AAD37702.1	17.8	26.7	55.5	mixed
CAO83219.1	15.4	29.1	55.5	mixed
ABG29420.1	2.5	33.9	63.6	beta
AAF59831.1	20.1	14.9	65.0	mixed
EDS36062.1	34.8	24.5	40.7	mixed
ABU48600.1	65.5	14.7	19.7	mixed
ACN38089.1	2.3	41.1	56.7	beta
ACA05576.1	29.1	36.7	34.2	mixed
ACN38078.1	59.4	1.5	39.1	alpha
AAR18451.1	19.2	41.1	39.7	mixed
AAO38515.1	11.6	51.6	36.9	beta
Q7PYI4.4	47.9	17.4	34.7	mixed
ABB82553.1	47.3	18.7	34.1	mixed
ABB00933.1	33.2	30.8	35.9	mixed
EDS29334.1	78.5	0.0	21.5	alpha
AAC36346.1	66.0	3.4	30.6	alpha
AAO38519.1	81.3	0.0	18.7	alpha
ABM92299.1	25.3	28.4	46.3	mixed

TABLE 6 : Hydrophilic and hydrophobic residues content of immune peptides computed using Protprop software

Accession number	Percentage of Hydrophobic residues	Percentage of Hydrophilic residues	Net hydrophobic Residues content
XP_565481.2	46.27	41.79	High
AAD37702.1	50	35	High
CAO83219.1	55.93	30.51	Very high
ABG29420.1	56.67	31.67	Very high
AAF59831.1	54.24	33.9	Very high
EDS36062.1	56.67	31.67	Very high
ABU48600.1	52.5	35	High
ACN38089.1	55.56	35.8	High
ACA05576.1	55	36.25	High
ACN38078.1	55.56	35.8	High
AAR18451.1	50.59	41.18	High
AAO38515.1	50.59	41.18	High
Q7PYI4.4	50.37	45.95	High
ABB82553.1	47.21	47.77	Low
ABB00933.1	53.92	37.25	High
EDS29334.1	50.51	41.41	High
AAC36346.1	57.29	35.42	Very High
AAO38519.1	47.5	37.5	High
ABM92299.1	54.17	40.63	High

structure analysis results using Protprop software (http://www.mzu.edu.in/schools/biotechnology.html) and tabulated in TABLE 6. The physico-chemical parameters, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, half-life, instability index, aliphatic index and grand average hydrophathy (GRAVY) were computed using the Expasy's ProtParam prediction server and tabulated in TABLE 3. The secondary structure and percentage of residues (5) forming alpha, beta, and coil structures were predicted by a tool - Secondary Structural Content Prediction (SSCP) server (http://coot.embl.de/SSCP//sscp_seq.html). Percentages of hydrophobic and hydrophilic residues were calculated from the primary structure analysis results using Protprop software (http://www.mzu.edu.in/schools/biotechnology.html) developed by Department of Biotechnology, Mizoram University. The SOSUI server performed the identification of transmembrane regions (TABLE 4).

The presence of disulphide bridges (SS bonds) in immune peptides of Q7PY14.4, ABB00933, EDS29334, AAC36346, AAO38519 and ABM92299 were predicted by two methods. The first method involves the prediction of SS bonds using the primary structure (protein sequence data) by the tool CYS_REC (http://sun1.softberry.com/berry.phtml?topic=cys_rec&group=help &subgroup=propt.). CYS_ REC identified the positions and total number of cysteines present and predicted the most probable SS bond pattern of pairs (based on the matrix of pair scores) in the submitted FASTA format protein sequence. The second method involves the visualization and identification of SS bonds using the three-dimensional structure of protein (3D co-ordinates data). The protein sequences were submitted in EsyPred3D Web server 1.0 (http://www.unamur.be/sciences/biologie/urbm/bioinfo/esypred/). The 3D structure of the submitted protein sequences were builded by the server and provided a protein data bank (PDB) file. The tool Rasmol (http://openrasmol.org/) was used to visualize the modelled 3D structures and to identify the SS bonds. The modelled 3D structures were evaluated using the Protein Quality predictor (ProQ)online server (http://www.sbc.su.se/~bjornw/ProQ/ProQ.html).

FULL PAPER

TABLE 7 : Disulphide (SS) bond pattern of pairs predicted by CYS_REC (using primary structure) and identified by Rasmol (using 3D structure modelled)

Accession number	CYS_REC	Rasmol
Q7PY14.4	43-397 349-710 529-917	295-290 349-359
ABB00933	65-92 78-98 82-100	65-92 78-98 82-100
EDS29334	75-95 89-97	62-89 75-95 79-97
AAC36346	59-72 76-92 86-94	59-86 72-92 76-94
AAO38519	20-36 30-38	3-30 16-36 20-38
ABM92299	72-94 86-92	59-86 72-92 76-94

TABLE 8 : Criteria for a good (model) 3D structure based on ProQ score

Pro Q score		Quality of the model
LG score	Maxsub	
>1·5	>0·1	Fairly good model
>2·5	>0·5	Very good model
>4	>0·8	Extremely good model

TABLE 9 : Validation parameters computed for the build 3D structures of targets (immune peptides) of mosquitoes using ProQ

Accession number	ProQ	
	LG Score	Maxsub
Q7PY14.4	1.457	0.135
ABB82553	5.619	0.553
ABB00933	0.484	0.025
EDS29334	0.396	0.068
AAC36346	0.360	0.075
AAO38519	0.602	0.102
ABM92299	0.524	0.030

RESULTS AND DISCUSSION

The results of primary structure analysis suggest that most of the mosquito immune peptides (cecropin, defensin and gambicin) are hydrophobic in nature due to the presence of high non-polar residues content and Aliphatic (Ala) nature was highest as compared to other class of amino acid (TABLE 2). The average molecular weight of GSTe4 calculated is 14938.77 Da. Isoelectric point (pI) is the pH at which the surface of protein is covered with charge but net charge of the protein is zero. At pI proteins are stable and compact.The computed Iso-electric point (pI) indicates that mosquito immune peptides are mostly basic in nature with a high basicity of cecropin peptides. Moreover, Q7PYI4.4, ABB82553.1, EDS29334.1, AAC36346.1 and ABM92299.1 were (pI>7) acidic in character. The computed isolelectric point (pI) will be useful for developing buffer systems for purification by isoelectric focusing method. Extinction coefficient of GSTe4 at 280 nm is ranging from 1490-48485 M^{-1} cm^{-1} with respect to the concentration of Cys, Trp and Tyr. This indicates that these immune peptides can be analyzed using UV spectral methods except in cecropin immune peptides as it does not calculate extinction coefficient value (TABLE 3).

The high extinction coefficient of AAR18451.1, Q7PYI4.4 and ABB82553.1 indicates the presence of high concentration of Cys, Trp and Tyr. The computed protein concentration and extinction coefficients help in the quantitative study of protein- protein and protein-ligand interactions in solution. On the basis of instability index Expasy's ProtParam classifies the gambicin im-

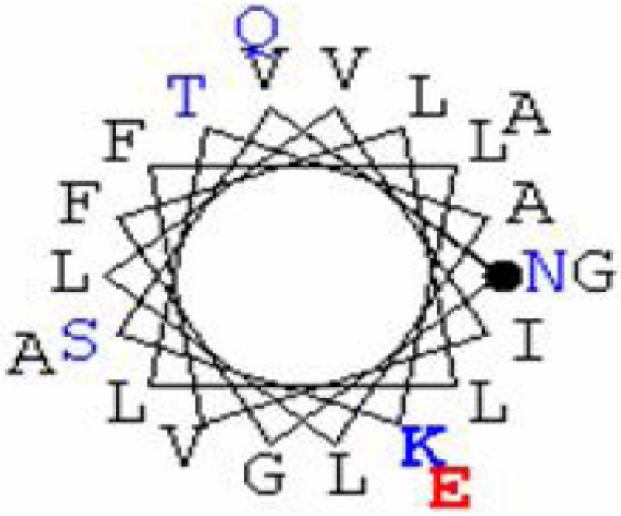

Figure 1 : Helical wheel representation of predicted helix of XP_565481.2 (*Anopheles gambiae*) immune peptides. Hydrophobic residues (V, L, A, I, F, G) are represented as blue, Polar residue (N, Q, T, S) as blue and charged residue as bold blue (K) and bold red (E)

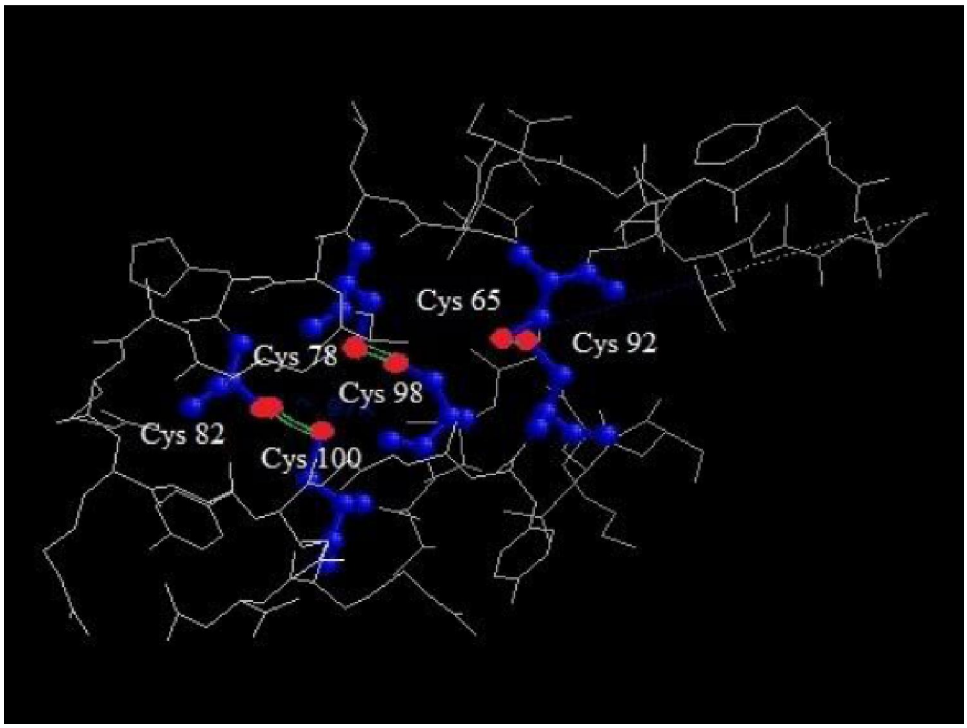

Figure 2 : RasMol representation (wireframe) of the homology modelled 3D structure of ABB00933.1 (*Anopheles arabiensis*). The sulphur atoms present in cysteines and the disulphide bonds (green dotted lines) are shown in red colour

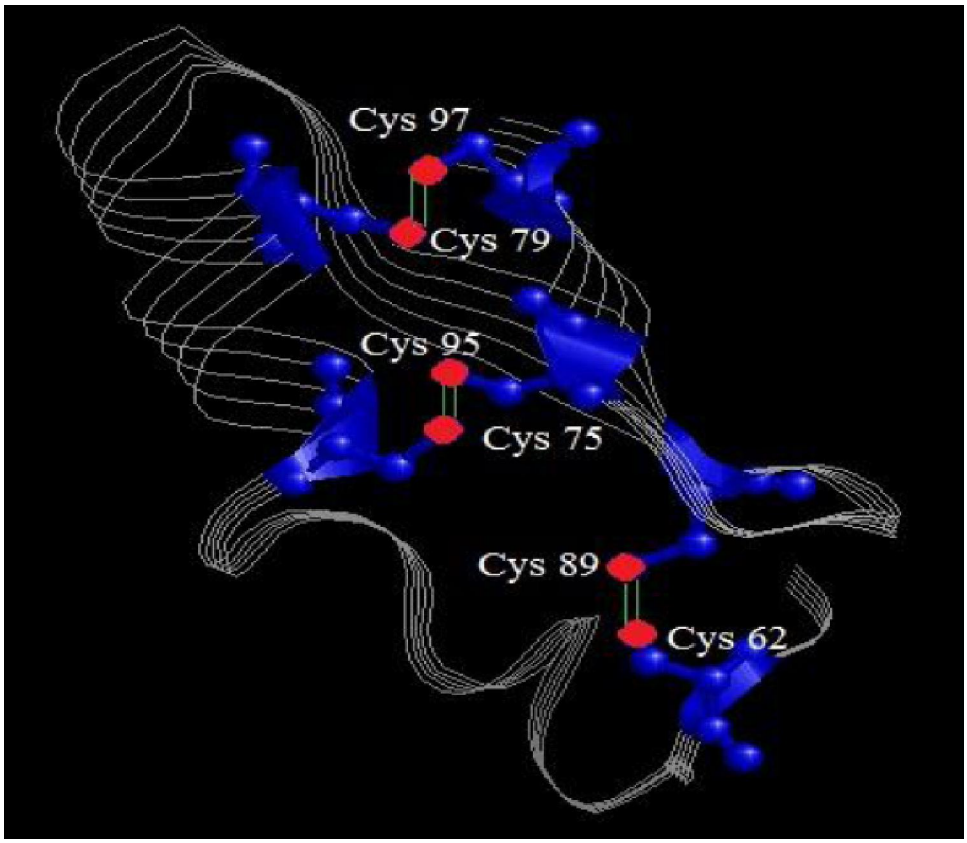

Figure 3 : RasMol representation (strands) of the homology modelled 3D structure of EDS29334.1 (*Culex quinquefasciatus*). The sulphur atoms present in cysteines and the disulphide bonds (green dotted lines) are shown in red colour

mune peptides as stable (Instability index > 40) while other immune peptides (cecropin and defensin) as un-

FULL PAPER

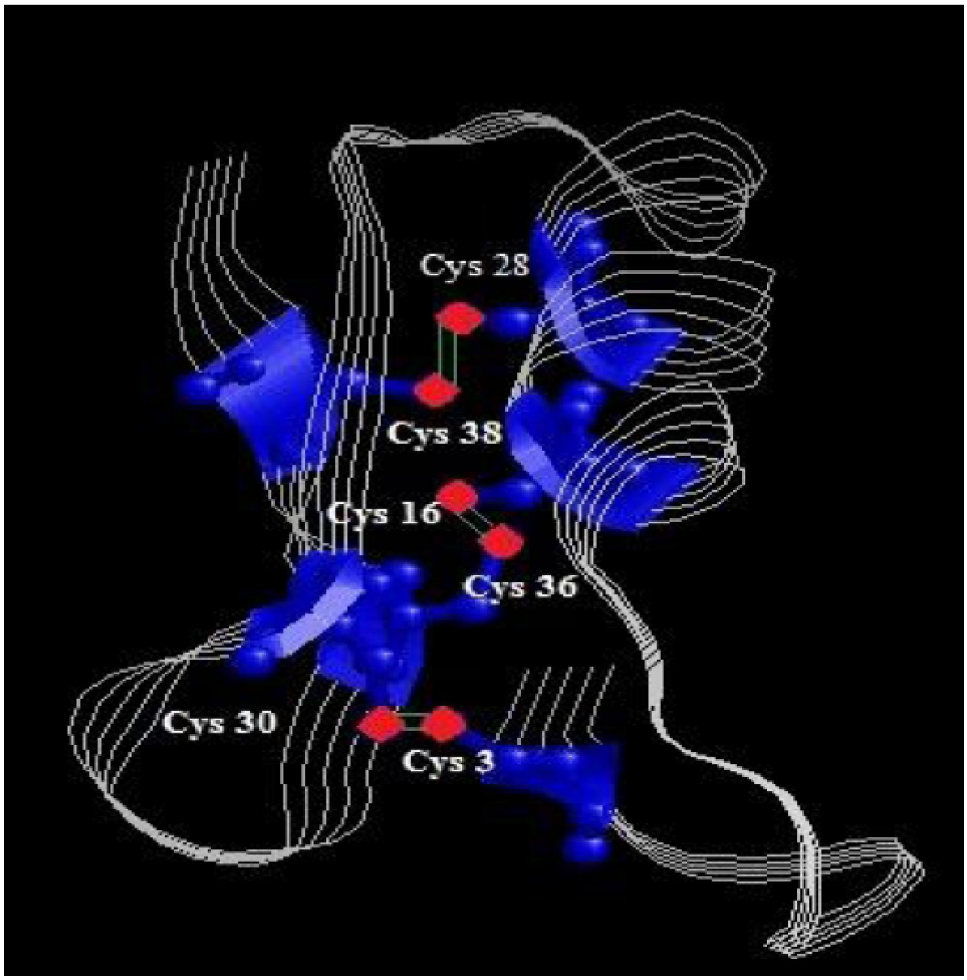

Figure 4 : RasMol representation (strands) of the homology modelled 3D structure of AAO38519.1 (*Culex pipiens*). The sulphur atoms present in cysteines and the disulphide bonds (green dotted lines) are shown in red colour

stable (Instability index < 40) (TABLE 3). The aliphatic index (AI) which is defined as the relative volume of a protein occupied by aliphatic side chains (A, V, I and L) is regarded as a positive factor for the increase of thermal stability of globular proteins. The very high aliphatic index (AI<70) was found on all immune peptides infers that these immune peptides may be stable for a wide range of temperature. Grand Average hydropathy (GRAVY) Index of immune peptides are ranging from –0·2 to 0·4. The very low GRAVY index was computed on all of immune peptides infers that these GST could result in a better interaction with water.

The secondary structure predicted with the help of programs SSCP (Secondary Structural Content Prediction) infers that most of the immune peptides have mixed secondary structure, *i.e.* α-helice, β-strands and coils. ACN38078.1 (59.4%), EDS29334.1 (78.5%), AAC36346.1 (66.0%) and AAO38519.1 (81.3%) have rich alanine content and mostly α-helices (TABLE 5). Protprop software analysis of hydrophobic percentages indicated that immune peptides were hydrophobic and net hydrophobic residues content was high (TABLE 6). Very high hydrophobic residue was found in CAO83219.1 (55.93%), ABG29420.1 (56.67%), AAF59831.1 (54.24%), EDS36062.1 (56.67%) and AAC36346.1 (57.29%). The server SOSUI classifies most of the immune peptides as membrane protein (Figure1) while other immune peptides ABU48600.1, ABB82553.1, EDS29334.1 and AAO38519.1 as soluble proteins (TABLE 4).

The tool CYS_REC recognizes the presences of Cysteines only in defensin immune peptides Q7PY14.4 (*Anopheles gambiae*), ABB00933 (*Aedes aegypti*), EDS293341(*Culex quinquefasciatus*), AAC36346 (*Aedes albopictus*), AAO38519 (*Culex pipiens*) and ABM92299 (*Anopheles stephensi*) while no cysteines

was found in cecropin and gambicin immune peptides (TABLE 7). We speculate that the SS bonds predicted from the primary structure (protein sequence) using CYS_REC tool might not be correct and the SS bonds identified from the three-dimensional structure (3D co-ordinates) using the Rasmol tool might be correct. The SS bonds identified from the three-dimensional structure (3D coordinates) using the Rasmol tool were shown in Figure 2 to 3.

Criteria for a good 3D structure is given in TABLE 8. The modelled 3D structures evaluated using the online servers ProQ (Protein Quality predictor server) concluded that according to Maxsub scores; ABB82553 (Maxsub>0.5) was found as very good model and its LG score (5.619) resulted that it was extremely good model while the rest of the immune peptides were not a good model (TABLE 9).

CONCLUTION

Nineteen different mosquito immune peptides (cecropin, gambicin and defensin) sequences have been chosen mainly to study their physico-chemical properties, primary and secondary structures by using computational tools and servers. Primary structure analysis reveals that most of the immune peptides under study are hydrophobic in nature and six of them contain disulphide linkages. Physico-chemical characterization studies give a good idea about the properties such as pI, EC, AI, GRAVY and Instability Index that are essential and vital in providing data about the proteins and their properties. Secondary structure analysis predicts that most of them contain mixed secondary structure. The presence of Cysteines residues was found only on defensin immune peptides of *Anopheles gambiae*, *Culex quinquefasciatus*, *Aedes aegypti*, *Aedes albopictus*, *Culex pipiens* and *Anopheles stephensi* indicates the presence of disulphide bonds which is also confirmed using CYS_REC and Rasmol tools.

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