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Immune peptides modelling of *Culex pipiens* sp by *in silico* methods

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ABSTRACT

Background: In the past 60 years, antibiotics have been critical in the fight against infectious diseases caused by bacteria and other microbes. Development of resistance to the antibiotics is emerging as a major public health issue which has resulted in the search for new antibiotics in order to maintain a pool of effective drugs at all times. Currently, there is a great interest in cationic peptides as antibiotics. These are reported to destroy the host cell membrane rather interacting with the other cell components, which may not face emergence of resistance. In mosquitoes, peptides like cecropin, defensin and gambicin reported to have inhibitory effect on bacteria, fungi and parasites. These peptides are well-characterized at both the biochemical and molecular level from *Anopheles* and *Culex* species, yet their 3D structures were not reported.

Methods: Defensin, cecropin and gambicin immune peptides of *Culex pipiens* was characterised to have antiparasitic, antibacterial and antifungal activities. Since the crystal structure of defensin, cecropin and gambicin are not yet available their 3D structures were determined using homology modeling and Rosetta fragment insertion methods and were validated.

Results: Stereo chemical evaluation indicated that defensin and gambicin showed that 100% residues of constructed model lie in the most favoured and allowed regions. Cecropin iso-forms A and B showed 100% while C showed 97.6% residues that lie in most favoured and allowed regions, which indicated quality models.

Conclusion: Predicted model provide insight into their structure and aid in the development of novel antibiotic peptides.

Key words Cecropin; *Culex pipiens;* defensin; gambicin; peptide modelling

INTRODUCTION

Mosquitoes upon infection with pathogens elicit innate immune response by producing peptides[1,2]. Such peptides are reported to impede the development and transmission of eukaryotic pathogens. This is a major arm of defence in mosquitoes against microbes, and makes the first line of defence in mosquitoes against invaders. Ample evidence from mosquitoes and other medically important insect vectors suggest that endogenous innate immunity molecules can hinder the development of parasite. These immune peptides have *in vitro* antibacterial activity and reported antiparasitic properties. Insect peptides such as defensin, cecropin, gambicin and transferrin are known to be up-regulated in insect vectors—*Aedes aegypti, Culex quinquefasciatus, Culex pipiens* and *Anopheles gambiae* (Diptera: Culicidae) upon infection and known to own antiparasitic, antimicrobial and anticancer effects[3]. Immune peptides, including defensin, cecropin and gambicin are well-characterised at both the biochemical and molecular levels from *Aedes*, *Anopheles* and *Culex*. Yet there are no crystallographic data available in Protein Data Bank for cecropin and gambicin; except gambicin from *An. gambiae* peptide.

Era of "classical antibiotic" may be over. Yet no truly novel class of antibacterial agent has come in the market in the past 30 years. Currently, there is a great interest in peptide antibiotics, especially the cationic peptides, which help in disruption of cell membrane, therefore, peptide antibiotics may not face the rapid emergence of resistance[4]. Resistance of microorganisms to all approved compounds has rarely been noted and the number of such events will certainly increase with time. The increasing antibiotic resistance of pathogenic bacteria calls for development of alternative antimicrobial strategies. Possible approaches include development of novel broad-spectrum antibiotics as well as specific targeting of personal bacterial virulence factors[5]. Development of novel antibiotics and alternative therapeutic strategies is, therefore, a burning necessity. Among many strategies, studies on natural and artificial amphipathic peptides acting on membranes of microorganisms have yielded promising results. Currently, >800 such peptide antibiotics have been described and some of them have already entered clinical trials[4].

Prediction of three-dimensional structure of protein is one of the fundamental challenges in biology today. Protein sequences are growing rapidly but their structural elucidation is limited by the time and cost. To overcome this limitation, computational predictions of protein structures are more valuable for generating hypotheses[6]. Homology modelling is usually the method of choice when a clear

relationship of homology between sequence of the target protein and at least one known X-ray crystallographic structure is found. To date, the most successful method for structure prediction have been homology–based on comparative modelling and fold recognition[7]. Here, we present computational structure prediction of cecropin, gambicin and defensin peptides. Structural information of cationic peptides may help in designing novel synthetic drugs against super bugs.

MATERIAL & METHODS

Sequence retrieval and sequence analysis

The sequences of cecropin A, B1 & B2, defensin and Gambicin (Accession numbers: AAO38516.1, AAO38517.1, AAO38518.1, AAO38519.1 and AAO38515.1) from *Culex pipiens* were retrieved from NCBI data base (*http://www.ncbi.nlm.nih.gov*). Template search carried out by BLASTp program and Protparam tool[8] was used for physiochemical characterization of cecropin, defensin and gambicin. Disulfide bridges play a major role in stabilization, the folding process and so**,** existence of cysteine residues and disulfide bonding pattern were determined using DISULFIND server[9]. Motif search was performed for the peptides defensin, cecropin, and gambicin using Motif server (*http://www.genome.jp/tools/motif*) to find similar functional domains from other species.

In silico model generation and structure evaluation

Defensin structures were generated by Modeller 9v.9 which implement comparative protein construction by satisfaction of spatial restraints. Cecropin peptides from the species *Cx. Pipiens* have two iso-forms, one designated as Cecropin A; second iso-form has two allelic variants of the same gene designated as Cecropin B1 and Cecropin B2. Robetta server was used to model cecropin and gambicin since templates with enough sequence identity were not available. Stereo-chemical quality of predicted models was improved by subjecting to energy minimization protocol, to correct bond angles and bond lengths. Energy minimization was performed using YASARA Server of Protein Energy minimization[10]. Model quality was assessed through variety of validation tools, such as PROCHECK, VADAR, TM Align, SUPERPOSE and ERRAT[11–13].

RESULTS & DISCUSSION

Defensin

Defensin has 40 amino acids with theoretical iso-electric point 6.86. It has an instability index of 25.67 which shows that it is a stable peptide and the total cationic residues are 5. Template search from BLASTp showed 87.5% sequence identity with sapecin, an antibacterial peptide from *Sarcophaga peregrine* and 1ICA from insect defensin proteins. 3D model was generated using Modeller 9v.9 tool and structures were validated using normalized Dope score, TM align, ERRAT and Ramachandran plot. TM Align score for template 1ICA and query was nearer to one (0.96762) which shows identical structures. Ramachandran plot for defensin model showed normal distribution of phi and psi values, i.e. 75% residues in most favoured region and 25% in additional allowed regions. Overall topology of modeled defensin peptide consists of one α-helix from 13– 24 residues and an antiparallel β-sheet 28–31 & 35–38 respectively. Six cysteine residues in defensin were arranged in such a way that three disulfide bonds could be established. Disulfide bond prediction performed using DISULFIND server showed disulfide bonds among C3 and C30, the second one being formed among C16 and C36 and the third bond formed among C20 and C38. Motif search showed motif in 12–39 and 16–38 shares scorpion toxins like domain and arthropod defensin signature. Defensin has total five cationic residues, four were observed in α-helix, and the other was out of the secondary structures. Most of the cationic residues were observed in the motif regions.

Cecropin

Cecropin peptides from *Cx. pipiens* produce two isoforms (cecropin A and B), among them Cecropin A has four α-helices without any β-sheet, where as Cecropin B has two allelic variants of the same gene designated as Cecropin B1 (three α-helices and one parallel β-sheet) and Cecropin B2 (one α-helix and two parallel β-sheets). Cecropin A, B1 and B2 have theoretical isoelectric points—10.36, 11.24 and 11.24, respectively. The instability index values of these isoforms are 9.22, 25.82 and 34.7 respectively which shows peptides are stable. Cecropin A, B1, B2 iso-forms have 19, 22 and 21 cationic amino acids respectively, and most of them exist other than the secondary structures. Templates with enough sequence identity were not available, so the models were predicted through Rosetta fragment insertion technique. Models were refined and evaluated for their structural quality (Table 1) (Fig. 1), which emphasizes that predicted structures are acceptable. Predicted cecropin A model showed four helices spanning at 2–19, 22–37, 38–48 and 50–59 amino acid residues without β-sheets. Cecropin B1 showed three α-helices spanning at 6–17, 21–46 and 49–58 residues. Cecropin B1 has one parallel β-sheet (9–16

Table 1. Statistical results of protein structure evaluation for defensin, cecropin and gambicin using PROCHECK, ERRAT and TM–align

Structure prediction tool Peptides	Modeller Defensin	Robetta server			
		Cecropin			Gambicin
		A	B1	B2	
Residue in most favoured region (%)	75	95.8	97.9	83.3	97.4
Residues in additional allowed regions (%)	25	4.2	2.1	14.3	1.3
Residues in generously allowed regions (%)	0	0	0	2.4	1.3
Residues in disallowed regions (%)	0	0	0	0	0
ERRAT score	88.88	100	100	96.97	80.519
TM-align	0.9676				
Normalized DOPE score	0.404				

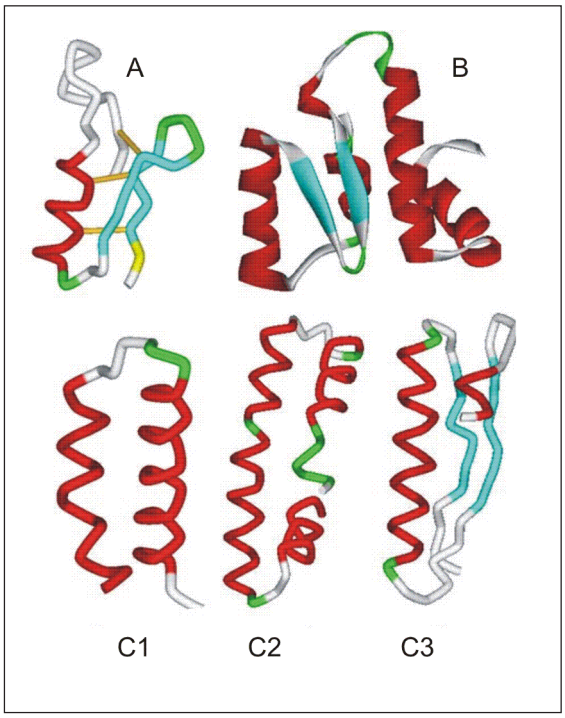

Fig. 1: Model of (A) defensin, (B) gambicin, and (C1, C2 & C3) cecropin iso-forms visualized using ViewerLite5.

and 44–51) and α-helix (26–33). Cecropin B2, has two parallel β-sheets (10–11 & 14–15) and (50–51 & 54–55) and α-helix (27–32). Motif search for cecropin B1 and B2 showed that the peptide shares common domains (28–54 & 22–54 residues) from cecropin family which have an affinity with lipid membrane.

Gambicin

Gambicin peptide has 85 amino acid residues with theoretical isoelectric point of 8.8 and total cationic residues of 11. Theoretical instability index is 48.41 and it classifies the peptide as unstable. Gambicin peptide model has four α-Helices (6–16, 19-34, 58–71 & 79–82 residues) and one anti-parallel β-sheet (45–47 & 53–55 residues). The 11 cationic residues were distributed in α-helices and a β-sheet. DISULFIND server shows that the position of six cysteine peptides of gambicin could not favour disulfide bond formation and shows less stable structure. From the profile search gambicin sequence shows that it has a profile which shares homology with the sequences that are involved in lipid membrane attachment showing the toxicity of peptide due to membrane destruction.

Domain search shows that this peptide's domain has homology with those domains that have an affinity to lipid membrane, a characteristic of endotoxin to Gram-negative bacteria. Maximum cationic residue distribution between secondary structure and domains confer toxicity by cations. Pathogens do not seem to acquire resistance to cationic peptides, which make them attractive drug research. Cationic peptides are present in all organisms and function in the killing of bacteria, viruses, eukaryotic parasites and fungi. So far only few cationic peptides came out of clinical trials. Designing novel peptide with the aid of computers and with new models to predict toxicity could be a possible model for wide range of peptide antibiotics and this may provide a feasible option in combating microbial infections in future.

CONCLUSION

In the present work, three-dimensional structures were predicted for defensin, cecropin and gambicin. Cationic peptides do not interfere with the internal mechanisms, i.e. transcription, translation and immune system of the host, therefore, it could not acquire resistance over time.

Hence, peptides with most cationic residues may have potential to combat bacteria and parasites. Since the effect of cationic peptide is non-specific, wide range of novel cationic antibiotic peptides is possible. Linking cationic peptides to the current class of antibiotics which are facing the problem of resistance could help antibiotic manufacturing industries intern, there are possibilities of lowering medical expenses. Predicted model will give valuable insights towards design of novel antibiotics.

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REFERENCES

1. Paily KP, Agiesh Kumar B, Balaraman K. Transferrin in the mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae), up-regulated upon infection and development of the filarial parasite, *Wuchereria bancrofti* (Cobbold) (Spirurida: Onchocercidae). *Parasitol Res* 2007; *101:* 325–30.
2. Gillespie JP, Kanost MR, Trenczek T. Biological mediators of insect immunity. *Annu Rev Entomol* 1997; *42:* 611–43.
3. Lyric C, Bartholomay, Hoda A, Reda M. Remzy, Bruce M. Christensen. *Culex pipiens pipiens*: characterization of immune peptides and the influence of immune activation on development of *Wuchereria banocrofti*. *Mol Biochem Parasitol* 2003; *130:* 43–50.
4. Hancock REW. Peptided antibiotics. *Lancet* 1997; *349:* 418–22.
5. Adam D, Pawel M, Grzegorz D, Malgorzata R, Justyna SN, Benedy W, *et al*. New generation of peptide antibiotics. *Acta Biochem Polanica* 2005; *52:* 633–8.
6. Eswar N, Marti-Renom MA, Ben W, Madhusudhan MS, Eramian D, Shen M, *et al*. Comparative protein structure modeling with MODELLER. *Curr Protocols Bioinformatics* 2006; *15:* 5.6.1–5.6.30.
7. Richard B, David B. Ab initio protein structure prediction: progress and prospects. *Annu Rev Biophys Biomol Struct* 2001; *30:* 173–89.
8. Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, Bairoch A. Protein identification and analysis tools on the ExPASy Server. In: John M. Walker, editor. *The proteomics protocols handbook*. Humana Press 2005; p. 571–607.
9. Alessio C, Andrea P, Alessandro V, Paolo F. DISULFIND: a disulfide bonding state and cysteine connectivity prediction server. *Nucleic Acids Res* 2006; *34:* W177–81.
10. Krieger E, Joo K, Lee J, Raman S, Thompson J, Tyka M, Baker D. Improving physical realism, stereochemistry, and side-chain accuracy in homology modeling: four approaches that performed well in CASP8. *Karplus Proteins* 2009; *77*(9): 114–22.
11. Laskowski RA, MacArthur MW, Moss D, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structure. *J Appl Cryst* 1993; *26:* 283–91.
12. Zhang Y, Skolnick J. TM-align: a protein structure alignment algorithm based on TM-score. *Nucleic Acids Res* 2005; *33:* 2302–9.
13. Chris C, Todd O. Verification of protein structures: patterns of nonbonded atomic interactions. *Protein Sci* 1993; *2:* 1511–9.

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