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Conserved Phe-Arg in *AVBD10* May Mediate Membrane Disruption

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ABSTRACT: The accelerated evolution and growth of multidrug resistant superbugs has motivated the development of novel antimicrobial agents. Cation – π interactions are important in antimicrobial peptide- lipid membrane interaction, for substrate binding, catalysis, as well as ion channel activity. In this study, three dimensional structures of AvBD10 of 15 birds were predicted and dipole moment and cation- π interactions of the selected peptides were determined. The results revealed that the entire dipole vector observed in the selected AVBD10 passes through or near the center of Phe- Arg π-cation system of the peptide and this π-cation system may help AVBD10 in interacting and disrupting the membrane lipids.

KEYWORDS: Antimicrobial peptides; Avian Beta Defensin 10 (AvBD10); π-cation system; Arginine; Phenylalanine; Dipole moment

I. INTRODUCTION

The emergence of the superbugs, that are resistant to nearly all of the existing antibiotics, forms a prime communal health problem [1]. Recent outbreaks of epidemic causing antibiotic resistant strains demand the development of novel anti-infective approaches. Antimicrobial peptides (AMPs) are an ideal substitute of antimicrobial agents against widespread increase of antibiotic resistance. AMPs are rather small molecular weight molecules (mostly <100 amino acid residues) comprising cationic and amphipathic amino acids which permits the binding and disruption of microbial membrane causing cell death [2, 3]. Since non-specific physical interactions exist between AMPs and microbial membranes, the development of resistance to AMPs is difficult [4]. Antimicrobial peptides are primarily synthesized as inactive prepropeptides, including a signal peptide, an 'anionic' propiece and mature peptide upon infection mature peptides are released by the host proteases following the cleavage of the N-terminal [5].

Host defense peptides were first discovered from avian tissues [6]. Birds are generally documented as a pool of zoonotic pathogens, comprising of bacteria and viruses, however they remain asymptomatic [7]. The health of birds would be significantly enhanced by elevating the innate immune system and by confining the colonization and spreading of these pathogenic microbes. The two major categories of AMPs seen in vertebrates are cathelicidins and defensins [2]. Defensins are small cationic AMPs that are present in almost all the organisms, including fungi, plants, invertebrates and vertebrates [8]. This class of peptides can protect the host against a wide range of microbes such as bacteria, some fungi, enveloped viruses and pathogenic protozoa either by disrupting the membrane or by targeting specific intracellular components, and can also regulate and promote innate and adaptive immune response to pathogens [9].

In vertebrates, defensins are subdivided into α-, β- and θ- defensin based on the order of six cysteine residues and disulfide bridge combination [10]. Of the three defensins in vertebrates, only β-defensins have been reported in birds and is termed as avian β –defensins (AvBDs) [11]. At present, more than 40 AvBDs have been acknowledged in birds, comprising chicken, turkey, king penguin, king pigeon, duck and other avian species [12]. AvBDs have three antiparallel β-sheets, with or without an N-terminal helix, and three distinctive intramolecular disulfide bridges formed by six cysteine residues. Among avian AMPs, currently tertiary structures of two β-defensins (chicken AvBD2 and penguin AvBD103a/spheniscin-2), three cathelicidins (CATH1–3), and a chicken ovodefensin (gallin1/2) have been

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resolved using nuclear magnetic resonance in solutions [13].AMPs kill microbes either by forming pores in the cell membrane or by targeting specific intracellular or membrane bound components [14]. Different models that have been proposed to explain the mechanisms involved in membrane pore formations are barrel stave pore, toroidal pore and carpet model [15].

Many AMPs have higher proportion of Arginine (Arg), Lysine (Lys) and Phenylalanine (Phe). Cationicity and hydrogen bonding properties of Arg and extensive π–electron system of the indole side chain of Phe seem to balance each other for the functioning of these peptides. Cation–π interactions between the residues promote the deeper penetration of peptide into the membrane and thereby heightening the peptide-membrane interactions [16]. In the current study, three dimensional structures of AvBD10 of 15 birds were predicted and dipole moment and cation- π interactions of the selected peptides were determined.

II. MATERIALS AND METHODS

Sequence retrieval and analysis:

AvBD10 protein sequences from 15 different birds such as *Corvus brachyrhynchos* **,** *Gallus gallus, Aptenodytes forsteri, Balearica regulorum gibbericeps, Gavia stellata, Nipponia nippon, Columba livia, Opisthocomus hoazin, Podiceps cristatus, Charadrius vociferous, Cariama cristata, Manacus vitellinus, Calypte anna, Cuculus canorus* and *Picoides pubescens* were retrieved from Uniprot (http://www.uniprot.org/). These selected sequences were aligned using Clustal W. The retrieved FASTA sequences of the selected AvBD10 sequences were subjected to Protparam analysis to analyse physiochemical properties. Disulfide bonding patterns, an important characteristic of defensins were determined using DISULFIND server.

Three Dimensional Structure Generation & Validation:

The selected AvBD10 structures were generated using Phyre 2. Signal peptides were cleaved using SignalP 4.1 Server prior to modelling. Structural representations were visualized using Rasmol. Model quality was assessed through Ramachandran Plot.

Calculation of Dipole moment and Cation- π interactions in AVBD10:

Since most proteins are charged at physiological pH 7.4 [17], determining their dipole moments is extremely important for understanding many biological processes, such as protein–lipid recognition, where electrostatic interactions play a dominant role. Dipole moment was calculated for all the AVBD10.

Arg, Lys and Phe residues are found in unusually high proportion in many antimicrobial peptides. Their roles have been examined in detail but it is not entirely clear what specific properties they contribute to antimicrobial peptides, in addition to their positive charge and hydrophobic bulk, respectively. Another important factor is the general π –electron system of the aromatic sidechain that gives rise to a significant quadrupole moment. A quadrupole moment can be envisaged as two dipole moments extending perpendicularly out of either side of the ring plane. That is, their positively charged tails lie close to the plane of the ring, while the negative charges make up the ends of the dumb bell-type shape that is formed.

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III. RESULTS

Sequence analysis:

The alignment of the selected AvBD10 proteins using Clustal W (Fig. 1) showed the presence of six cysteine residues in all the sequences. Conserved residues like Arg and Phe which may take part in the lipid peptide interaction are highlighted in red colour.

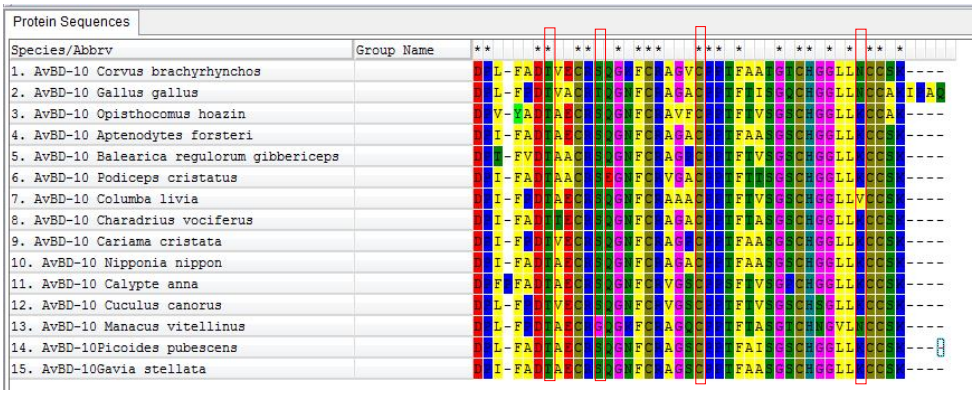

Fig. 1. Multiple sequence alignment of AvBD10 peptide sequences, all the six essential cysteine residues are conserved. Conserved residues which may take part in the lipid peptide interaction is highlighted using line coloured in red

Disulphide bond prediction using DISULPHIND Server showed that bonds were formed connecting C10 and C32, C17 and C39 and C22 and C39. (Fig. 2).

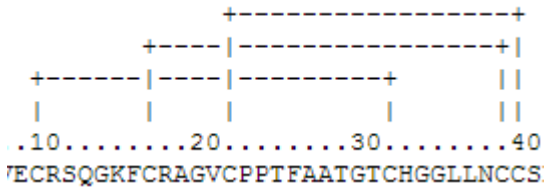

Fig. 2. DISULPHIND result showing 3 disulphide bridges

Cationicity plays an important role in anchoring the peptide to negatively charged lipid head regions of the membrane. Table. 1 shows that the number of overall cationic residues seems to outnumber that of anionic residues in all the selected peptides.

AvBD10 protein Sequences	Theoretical pI	Total cationic residues	Total anionic residues	Molecular weight	Grand Average of hydropathicity (GRAVY)
Ophisthocomus hoazin	8.29	5	3	6830.0	0.616
Gavia stellata	8.3	5	3	6731.8	0.506
Podiceps cristatus	8.29	5	3	6718.9	0.588
Cuculus canorus	8.3	5	3	6902.1	0.509
Charadrius vociferus	8.30	5	3	6761.9	0.464
Calypte anna	8.3	5	3	6897.1	0.480
Cariama cristata	8.30	5	3	6796.0	0.467
Balearica regulorum	8.66	5	2	6743.9	0.527

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gibbericeps					
Aptenodytes forsteri	8.3	5	3	6715.8	0.536
Nipponia nippon	8.29	5	3	6687.8	0.545
Manacus vitellinus	8.29	5	3	6848.0	0.309
Picoides pubescens	8.3	5	3	6775.9	0.492
Corvus brachyrhynhos	8.29	5	3	6792.0	0.586
Columba livia	7.59	4	3	6671.8	0.592
Gallus gallus	8.24	4	2	7I30.4	0.649

Table. 1. Physiochemical properties of AvBD10

Three dimensional structure generation and validation:

Structures of the selected birds AVBD10 were generated using Phyre2 and visualized by Rasmol. The quality of the structures was validated by Ramachandran Plot (Table. 2). Ramachandran Plot for the all the 15 predicted models showed normal distribution of phi and psi values. For most of the predicted models no residues were in the disallowed region, and a very small percentage of residues in the outlier regions for the remaining structures.

AVBD10 peptides	Residues in favored region	Residues in allowed region	Residues in disallowed region
Ophisthocomus hoazin	95.0%	5.0%	0%
Gavia stellata	97.5%	2.5%	0%
Podiceps cristatus	97.5%	2.5%	0%
Cuculus canorus	95.0%	5.0%	0%
Charadrius vociferus	97.5%	2.5%	0%
Calypte anna	92.7%	7.3%	0%
Cariama cristata	87.5%	12.5%	0%
Balearica regulorum gibbericeps	92.5%	7.5%	0%
Aptenodytes forsteri	97.5%	2.5%	0%
Nipponia nippon	95.0%	2.5%	2.5%
Manacus vitellinus	95.0%	2.5%	2.5%
Picoides pubescens	92.5%	5.0%	2.5%
Corvus brachyrhynhos	92.5%	5.0%	2.5%
Columba livia	90.0%	7.5%	2.5%
Gallus gallus	84.1%	9.1%	6.8%

Table. 2. Statistical evaluations using Ramachandran Plot

Calculation of dipole moment and cation – π interactions:

The π –cation electron system of Phe – Arg was observed to be conserved in all the selected AVBD10. Calculated dipole moment of *Corvus brachyrhynchos, Ophisthocomus hoazin* and *Charadrius vociferus* was 162.536, 176.079 and 161.158 respectively. Dipole vector was found to be 92.031 12.056 133.427; 85.46 143.94 54.605 and 147.313 -34.911 55.244 respectively. Fig. 5 shows the interaction of Phe-Arg present in three species namely *Corvus brachyrhynchos, Ophisthocomus hoazin* and *Charadrius vociferus.*

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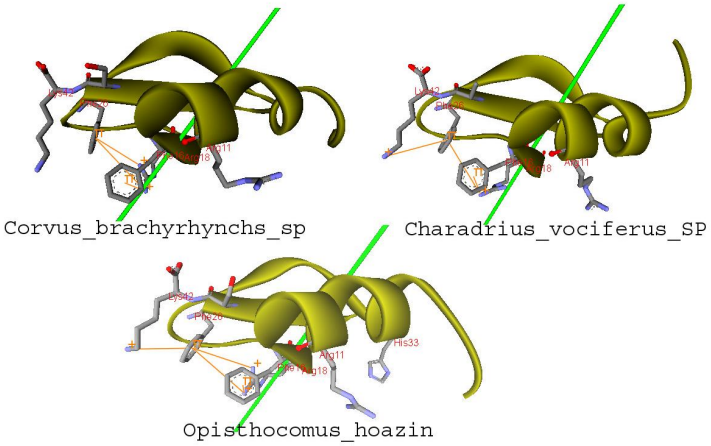

Fig. 3. Cation – π interaction of the conserved Phe-Arg residues and the overall dipole moment of three selected peptides.

IV. CONCLUSION

Cation – π interactions are important in antimicrobial peptide lipid interaction, for substrate binding, catalysis, as well as ion channel activity. Of the 15 selected AVBD10, it is observed that all of the structures carry a Phe- Arg π-cation system which may help AVBD10 in interacting and disrupting the membrane lipids. Even though there exists a well characterized dipole moment the number of overall cationic residues seems to outnumber that of anionic resides which is essential for anchoring the peptide to negatively charged lipid head regions. All the dipole vector observed in the selected AVBD10 passes through or near the center of Phe- Arg π-cation system of the peptide. The Phe- Arg π-cation system lies on the cationic face of the peptides, which may along with other cationic residues help in anchoring and promoting the deeper penetration of peptide into the membrane disrupting the membrane integrity.

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