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SCREENING AND IN SILICO VALIDATION OF ANTIMICROBIAL PEPTIDE DERIVED FROM ELASTASE AND MYELOPEROXIDASE

Running title: In silico validation of peptides from elastase and myeloperoxidase

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Abstract
Background: Antimicrobial peptides are a group of small molecules intended to serve the purpose as antimicrobial agents. The emergence and resurgence of drug resistant pathogens underscores the need for identifying novel antimicrobial agents. Elastase and myeloperoxidase are enzymes with documented antimicrobial activity. Hence, peptide derivatives of these proteins could be used as antimicrobial agents.
Aim: The study aims to identify and determine the antimicrobial activity of peptides derived from elastase and myeloperoxidase employing *in silico* tools.
Methods: Several *in silico* tools were used to identify the antimicrobial peptides found in the selected proteins. AMPA was used for the identification of peptide units from the antimicrobial proteins. The dPABB and Anti Fp tools were used to deduce the antibacterial and antifungal property of the peptides. Further, toxicity and cell penetrating ability of the peptides were predicted.
Results: Peptides derived from elastase were shown to exhibit antibiofilm property, whereas a peptide derived from myeloperoxidase was found to be anti-fungal in nature. All peptides were found to be non-cell penetrating and non-toxic.
Discussion: The peptide derivatives of elastase and myeloperoxidase were found to exhibit antimicrobial properties. Further experimental validation is necessary to elucidate the molecular mechanisms associated with the mode of action of these peptide molecules.
Conclusion: Identification of peptide molecules from antimicrobial protein would open novel avenues towards the development of peptide therapy.

Key words: Antimicrobial peptide, dental pathogens, elastase, novel peptides, myeloperoxidase, *in silico*

Introduction:

Antimicrobial drug resistance has created a menace globally. Novel strategies have been developed to identify bioactive molecules which can be used as an effective element against microbial pathogens, with a special emphasis on the drug resistant groups. Reports have suggested the emergence of drug resistant pathogens in dental settings (1); (2). Numerous phytocompounds and non-antibiotic drugs were repurposed for use as antimicrobial agents (Priyadharsini *et al.*, 2018); (Nandhini *et al.*, 2020); (Nivethitha, Smiline Girija and Vijayashree Priyadharsini, 2020).

Antimicrobial peptides have opened a new era of peptide therapeutics. These are oligopeptides with varying numbers of amino acid residues. They have been shown to have a broad spectrum of activity which ranges from viruses, bacteria and parasites. These peptides fall into different categories as: cationic peptides, anionic peptides, cationic amphipathic peptides, host defense peptides, alpha helical peptides etc., (6). In line with these facts two antimicrobial proteins were selected for the present study viz., elastase and myeloperoxidase. Elastase is a serine protease, a degradative enzyme playing a vital role in the elimination of pathogens and digest tissues at inflammation sites. It has also been recognized as molecular targets of anti-inflammatory drugs (7); (8). The other protein is myeloperoxidase (MPO), a heme containing peroxidase produced by the neutrophils. MPO catalyzes the formation of reactive oxygen species, which inturn plays a vital role in the destruction of microbes (9). (Vijayakumar *et al.*, 2010; Kavitha *et al.*, 2014; Lekha *et al.*, 2014; Sahu, Kannan and Vijayaraghavan, 2014; Neelakantan *et al.*, 2015)

The present study aims to identify the peptide molecules in the proteins and to predict their anti-biofilm or anti-fungal nature.

Methodology:

The antimicrobial proteins neutrophil elastase (6F5M) and myeloperoxidase (AAA59863.1) were selected for the study based on an extensive text mining process. The protein sequences of the proteins were retrieved from the NCBI (National Centre for Biotechnology Information) database in the FASTA format (https://www.ncbi.nlm.nih.gov/protein/).

Antimicrobial peptide identification

Antimicrobial peptide analysis (AMPA) is a web based application employed for identifying and assessing the antimicrobial domains in a protein. The source is used to design and develop peptide based drugs against microbial pathogens (Torrent *et al.*, 2012); (Torrent, Nogués and Boix, 2009).

Anti-biofilm property

dPABB (design Peptides Against Bacterial Biofilms) algorithm is based on the SVM and Weka models used to identify anti-biofilm peptides based on their amino acid composition, selected residue and position of the residues. The scores generated for each of the peptide molecules are then used to ascertain the anti-biofilm property (Sharma *et al.*, 2016).

Antifungal property:

The tool used in silico prediction of antimicrobial peptides for its antifungal property is Antifp. The module allows users to predict single or multiple sequences for its antifungal properties. The tool can be used for designing peptides and scanning protein sequences to identify peptides and their mutant analogs followed by the screening for antifungal property (Agrawal *et al.*, 2018).

Cell penetrating property:

Identification of newer peptide molecules with the ability to penetrate cells using high throughput methods is known to consume time as well as labour. The in silico screening procedures coupled with experimental validation is considered to be more feasible and cost-effective. The results could be replicated in in vitro conditions with much ease and confidence. CellPPD is one such standalone application developed to predict and design cell penetrating peptide molecules (Gautam *et al.*, 2013); (Gautam *et al.*, 2015).

Toxicity prediction:

Prediction of toxicity of peptides is a vital step in designing antimicrobial peptides. The ToxinPred tool has been used in the present study. The algorithm identifies certain amino acid residues such as Cys, His, Asn and Pro and their placements at various positions which makes them toxic. ToxinPred can be used to predict whether the designed peptide is toxic or non-toxic, consequences of mutations on toxicity and identification of toxic regions in a protein (Gupta *et al.*, 2013).

Results and Discussion:

The antimicrobial proteins - neutrophil elastase and myeloproxidase were tested for anti-biofilm property, anti-fungal property, toxicity and cell penetrating property (Table 1). The neutrophil elastase antimicrobial protein has two antimicrobial peptides NVNVRAVRVVLGA and VVTSLCRRSNVCTLVRGRQAGV. The NVNVRAVRVVLGA peptide has the antibiofilm property with SVM score of 0.09 and also it is non toxic. The VVTSLCRRSNVCTLVRGRQAGV peptide has the antibiofilm property with SVM score of 0.65 and also it is non toxic. The neutrophils possess a heme containing enzymes called the MPO. This protein in combination with hydrogen peroxide and chloride, forms the antimicrobial system (17). The myeloperoxidase antimicrobial protein has three peptides KLRSLWRRPFNVT, RTITGMCNNRRSPT and VLRNLKLARKLM.

The KLRSLWRRPFNVT has the antibiofilm property with SVM score of 0.25 and also it is non-toxic. The RTITGMCNNRRSPT has anti-fungal property with the SVM score of 0.54 and also it is non toxic. The VLRNLKLARKLM has the antibiofilm property with SVM score of 0.40 and also it is non toxic. The hydrophobicity, hydropathicity, hydrophillicity and molecular weight of the peptides were also determined. Animal studies have indicated that MPO and NE deficient mice were more susceptible to bacterial infections *in vivo*. They observed that when MPO and NE deficient mice were challenged with *K. pneumoniae*, it resulted in greater mortality of the mice (18). Thus proving that MPO and NE are key players in exerting antimicrobial effect. In line with the above facts, two proteins, the neutrophil elastase (NE) and myeloperoxidase (MPO) were chosen to identify potent peptide units capable of eliciting antimicrobial activity. Neutrophil elastase (NE) is a potent serine protease with antimicrobial activity. It has been documented that soon after infection with bacterial pathogens, there is recruitment and induction of phagocytes and neutrophils. This process eventually results in the extracellular release of active NE.

A study conducted by Benabid *et al.*, 2012, provided substantial evidence on the effect of NE on infection caused by *Pseudomonas aeruginosa* in a mouse model. The NE-deficient mice when challenged with *P. aeruginosa* resulted in higher mortality due to pneumonia. This observation on the absence of NE was accompanied by the decreased production of protein

and transcript levels of proinflammatory cytokine molecules viz., TNF, MIP-2 and IL-6. The present study identified two peptides of neutrophil elastase that were found to exhibit active anti-biofilm properties (19). Several *in silico* studies have been conducted to elucidate the drug resistant properties of emerging dental pathogens (20); (21). Several research papers have been published related to diseases including, cancer, infectious diseases, autoimmune disorders etc (20 - 36).

Limitations and future scope:

As with any other study, the *in silico* pattern of study design also suffers certain limitations such as (a) the functions of the peptides described here are predictive of the antimicrobial protein, which may or may not exhibit their role in an *in vitro* or *in vivo* set-up, (b) the interactions and pathway network involved in the antimicrobial property of peptides has to be further elucidated. With all the limitations addressed the scope of the study lies in processing extensive data within a short period of time. The computational tool aids in choosing peptide molecules among a vast array of peptide units intended for use in the research and preclinical set-up.

Conclusion:

Thus, from the study it is noted that the elastase has antibiofilm property. The myeloperoxidase has the anti biofilm, antifungal property. Further research has to be performed on this topic to find other properties of the elastase and myeloperoxidase proteins.

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Conflict of Interest:

No potential conflict of interest relevant to this article was reported.

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Table 1: The list of antimicrobial peptides predicted from elastase and myeloperoxidase, their anti-biofilm and anti-fungal properties

Antimicrobial protein	Antimicrobial peptide	Anti-biofilm property	SVM score	Antifungal property	SVM Score	Cell penetrating property	Toxicity
Neutrophil elastase	NVNVRAVRVVLGA	Active	0.09	Non-antifungal	-0.61	Non-CPP	Non-toxic
	VVTSLCRRSNVCTLVRGRQAGV	Active	0.65	Non-antifungal	-0.14	Non-CPP	Non-toxic
Myeloperoxidase	KLRSLWRRPFNVT	Active	0.25	Non-antifungal	-0.33	Non-CPP	Non-toxic
	RTITGMCNNRRSPT	In-active	-1.04	Anti-fungal	0.54	Non-CPP	Non-toxic
	VLRNLKLARKLM	Active	0.40	Non-antifungal	-1.00	Non-CPP	Non-toxic

Table 2: Physicochemical properties of the antimicrobial peptides

Antimicrobial peptide	Hydrophobicity	Hydropathicity	Hydrophilicity	Molecular weight
NVNVRAVRVVLGA	-0.07	0.92	-0.30	1366.82
VVTSLCRRSNVCTLVRGRQAGV	-0.22	0.30	-0.06	2375.13
KLRSLWRRPFNVT	-0.38	-0.79	0.08	1673.18
RTITGMCNNRRSPT	-0.45	-1.18	0.31	1607.03
VLRNLKLARKLM	-0.27	0.23	0.14	1455.07