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RESEARCHARTICLE

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In silico Analyses and Characterization of an Antimicrobial Peptide 'Sapecin B' Reveals its Molecular Interaction with Bacterial Peptidoglycan

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Abstract

The antibiotics have been used to cure different bacterial diseases for a long time. But the rapidly increasing bacterial resistance against conventional antibiotics has made their use less suitable. Hence, antimicrobial peptides (AMPs) have emerged to be used as an alternative to treat microbial infections. The mechanism of action of antimicrobial peptides includes attraction and binding of AMPs to bacterial cell membrane. The bacterial cell membranes are anionic while the AMPs are cationic in nature. So, there is electrostatic force of attraction that attracts them towards each other, creating pores in the membrane that resulting in destruction of lipid bilayer. The aim of present study was to develop novel protein against antibiotics resistance bacteria. In the present study, an antimicrobial peptide (Sapecin B) was studied. Homology modeling was carried out using SWISS MODEL, Phyre-2 and evaluation of models was done by RAMPAGE server that helped to identify the best model. The structure of peptidoglycan taken from online database PubChem was used as a ligand. Then, the physiochemical properties were checked by online tool Protparam. The study showed that molecular weight of protein was 10040.92 having 88 amino acids, stability index value equal to 40 and the total number of atoms was 1428. The model was then refined to make it ready for docking purpose which was carried out using MOE, Rosetta and Patchdock. According to MOE, the results showed that peptidoglycan had bonding with some of the residues of Sapecin B protein i.e., Lys 87, Val 89 and Cys84. According to Rosetta, the result showed that the protein had shown best docking results with lowest interface_delta score as well as the transform action ratio was also less than 1. According to PatchDock, there exist hydrogen bonding between protein residue and ligand with a length of 2.41. The length higher than 2 was considered best for interaction. So it was considered that there existed strong interaction between AMPs with their ligand.

KEYWORDS
Antimicrobial peptides, Protein-ligand interaction, Sapecin B, *In silico* analysis

1 | INTRODUCTION

The past 50 years have been labeled as the "antibiotic era" in which different life-threatening diseases have been cured using antibacterial chemicals called antibiotics with great success. But that era is going to end as effectiveness of these antibiotics against disease causing bacteria is decreasing (Kleinkauf & Von Döhren, 2008). Globally, antibiotics are considered highly prescribed classes of drug, but their easy availability have led overuse and multidrug resistance, which causes severe threat to human health. According to World Health Organization (WHO), more than 700,000 people die every year due to the infections caused by multi-drug resistance bacteria. It is estimated that by 2050, infections caused

by bacteria would result in 10 million deaths annually (Yang et al., 2024).

Although antibiotic are widely and frequently used for the treatment of different bacterial diseases from centuries. But looking at the past 40 years, only three new classes of antibiotics had entered in medicinal field. All the three classes (lipopeptides, streptogramins, oxazolidinones) were capable of treating infections caused by Gram-positive bacteria. There was lack of new antibiotics that had ability to treat Gram-negative bacterial infections. This resulted into multi-drug resistant issues that demand the development of antimicrobials that could capable of treating these infections (Marr et al., 2006). As large number of antibiotic have been gaining resistance to antibiotics, so alternative therapies for treatment of infections are of great concerned. It is a big challenge for implementation of these alternative therapies in clinical use (Ghosh et al., 2019).

According to Antimicrobial Peptide Database (APD), a total of 3283 AMPs originated from six kingdoms have described so far. Out of these AMPs, 365 came from bacteria, 22 came from fungi, 5 came from archaea, 365 came from plants, 8 came from protists, and 2414 came from animals (Zhang & Yang, 2022). These different peptides were involved in killing both Gram-negative and Gram-positive bacteria and also promoting the elements of innate immunity (Luong et al., 2022). They are small oligopeptides of 12-50 amino acids in length that show antimicrobial activity against wide range of pathogens, including gram positive and gram negative bacteria, fungi, parasite and insects (Rodriguez et al., 2021). These AMPs act as first line of defense against different micro-organisms and help in the removal of invading pathogens (Divyashree et al., 2020). So, these AMPs are short cationic molecules of host defense mechanism that are found in cells and tissues of multicellular organisms. They play role in resisting the invading pathogens as they are strong innate immune effector molecules (Zhu et al., 2022).

These cationic antimicrobial peptides are produced nearly in all species. They are amphipathic molecules that can target the bacteria by two mechanisms. In the first mechanism, these cationic AMPs disrupt the bacterial membrane that resulted into cell lysis and as a result causes cell death. In the second mechanism, the AMPs get into entry into cell without causing any membrane disruption. This will inhibit the important intracellular functions of the cell as they get bind to nucleic acids and intracellular proteins. These AMPs have anticancer properties and they have ability to kill antibiotic-resistant bacteria. They also have ability to destroy wide range of bacteria, viruses, fungi and protozoa (Benfield & Henriques, 2020).

They also show some other advantages like they show less toxicity to eukaryotic cells, lack of resistance, low molecular weight, high solubility as well as strong thermal stability. Thus they have strong applications in the field of medicine due to their strong antimicrobial activities against microorganisms and a lot of AMPs are also in clinical trials (Luo & Song, 2021; Mai et al., 2017). But their use is limited to only pharmaceutical industry because of susceptibility of these peptides to proteases. However, this limitation can be overcome, if antimicrobial peptides are synthesized from D-amino acids that are more stable and have antimicrobial activity for long time period rather than L-amino acids. This will help to make them resistant to proteolytic degradation (Manabe & Kawasaki, 2017).

The AMPs have net positive charge and are divided into different groups based on their amino acid sequences, net charge as well as protein structure and source. These different groups contain different number of amino acids as well different net charges. Most of them range between 10 to 100 amino acids and contained net positive charge ranges between +2 to +9 (Zhang et al. 2021). They show different classification based on the type of micro-organism they target, they may be anti-bacterial, anti-fungal, anti-viral, anti-parasitic, anticancer, anti-inflammatory and anti-fibrotic. The distribution of AMPs is shown in Fig. 1. (Bucataru & Ciobanasu, 2024)

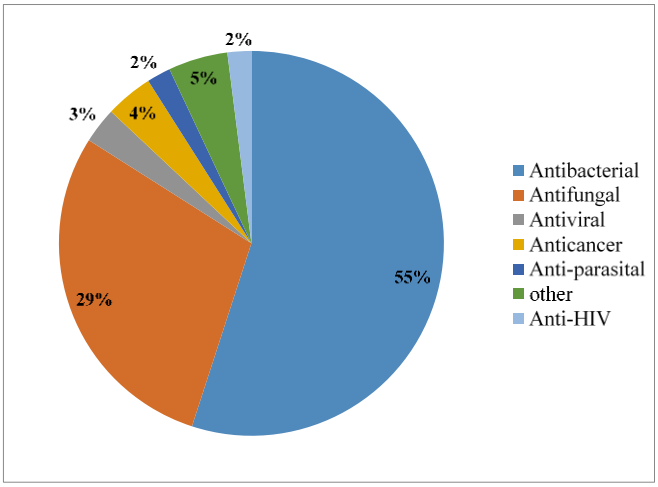

Fig. 1: Distribution of Antimicrobial Peptides

Sapecin B is a bactericidal protein of *Sacrophaga* containing 40 amino acid residues, consisting of six cystein residues with three disulphide bridges. This antimicrobial protein showed structural similarity with defencins, a peptide group that were purified from neutrophils and macrophages (Natori, 2010). This antimicrobial peptide was initially taken from culture medium of embryonic cell line NIH-Sape-4 that was derived from *Sacrophaga peregrine,* and shows strong antimicrobial activity against Gram-positive bacteria (Manabe & Kawasaki, 2017).

The structure of Sapecin B showed four peptides. The peptide contain N-terminal loop, an α helix and a two β-stranded sheets (Takeuchi et al., 2004). Among these four peptides, one of the peptide that was derived from the helical region, handecapeptide had shown more potential to be used as antibacterial agent. Thus this peptide had shown more antimicrobial activity than that of Sapecin B. This peptide gets attached with liposomes containing phospholipids, resulted in the released of trapped glucose. Thus it suggest the site of target of bacterial membrane (Yamada & Natori, 1994).

Now the drug targets can also be identified by using data mining or by bioinformatics. Target identification and validation are the most important steps in drug-discovery. Conventionally target discovery is done by lab experiments which are very time consuming as well as expensive and provide low accuracy. With the use of Bioinformatics, omics, computer-aided drug designing (CADD) and different *in silico* methods, the drug discovery and development cycle reduced. It also reduces the experimental cost. This would also result in lowering the scope of experimental targets (Zhang et al., 2022). The aim of the research is to develop novel protein against antibiotics resistance bacteria using bioinformatics tool.

2 METERIALS AND METHODS

Protein Sequence Analyses

Amino acid sequence of Sapecin B was taken from NCBI with accession number AAB35004.1. The physiochemical properties were determined by Protparam tool that told us about the GRAVY index, stability of protein, no. of atoms etc. then the conserved domains were analysed by NCBI.

Construction and Analysis of 3-D Structure

The 3-D structure of Sapecin B was predicted by online tools Swiss-Model and Phyre-2. Both the models were evaluated. The model with best quality having higher Q-mean value, ramachandran plot value and overall quality factor was selected. Various evaluating tools such as PROCHECK, Q-Mean and ERRAT were accessed by NIH server. Later on superimposition of protein was done by USCF chimera.

The structure of ligand i.e., peptidoglycan was available from PubChem database.The structutre was downloaded in sdf format. So for conversion of this “sdf” file into pdb, an online program smiletranslator was used. The structure downloaded was then used in molecular docking studies.

Refinement and Modification of Model

The refinement of models involved improvement in the qualities of proteins structure during homology modeling and build a structural model that was closer to their native state. This was done using different tools such as ModRefiner and Molecular Operating Environment (MOE). The model energy was then modified.

Molecular Docking Studies

To study the protein-ligand interaction, the protein was subjected to molecular docking analyses using peptidoglycan as ligand by MOE (molecular operating environment), rosetta and PatchDock.

3 RESULTS

Stability Analyses

Here we have described homology modeling, conserved domains, molecular docking of Sapecin B from *Sarcophaga peregrine.* According to our findings, Sapecin B contained 20 residues. From these residues, 12 residues appeared to have positive charge while 8 residues contained negative charge. So the protein has net positive charge.

Then the physiochemical properties of Sapecin B like isoelectric point, no. of amino acids and instability index have determined by Protparam tool. The summary of all properties is given in Table 1.

Table 1: Different parameters of antimicrobial peptides estimated by ProtParam

Sr. #	Parameters	Values
1	No. of amino acids	88
2	Isoelectric point	8.86
3	Molecular weight	10040.92
4	Aliphatic index	110.80
5	Instability index	45.25
6	Total number of atoms	1428
7	Grand average of hydropathicity (GRAVY)	0.106
8	Half-life in mammalian reticulocytes, yeast and E.coli	30hrs , >20hrs, >10hrs

The instability index is equal to 45.25 that show that the protein is unstable. The unstable protein when bind with suitable ligand make it stable. Second parameter that is considered is aliphatic index value. The aliphatic index value is equal to 110.80. High aliphatic index shows thermostablility of protein so it is concluded that the protein under study is thermostable.

Third parameter that is very important is Grand average of hydropathy (GRAVY). The hydrophilic and hydrophobic nature is determined by it. The positive value indicates hydrophobic nature of protein. GRAVY value for Sapecin B was 0.106. So it is concluded that

protein is thermostable as well as hydrophobic in nature.

Domain Analyses

Conserved domain of Sapecin B was determined by CDD tool. It shows that this antimicrobial peptide had only one conserved domain, Defensin-2 having accession no. pfam0109 and had range of residues from 54-87.

Homology Modeling

Homology modeling of Sapecin B was done by Swiss-Model and Phyre-2. After Ramachandran plot assessment it was found that, the model built by Swiss-Model had shown best results with 27 (71.1%) residues fall in favoured region, 9 (23.7%) residues fall in allowed region, 2 (5.3 %) residues fall in outlier region. So Swiss-Model was used for further study and shown in Fig. 2(A). The 3-D structure of ligand i.e. peptidoglycan that was obtained from PubChem database was shown in Fig. 2(B).

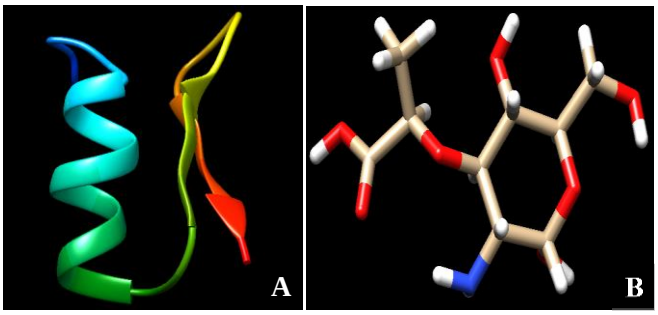

Fig. 2: (A) 3-D structure of Sapecin B (B) 3-D Structure of peptidoglycan

The 3-D structure of Sapecin-B was further evaluated and validated by online programs ERRAT, Q-Mean and PROCHECK,. The summary of ramachandran plot analysis created by PROCHECK was shown in Table 2. The overall evaluation of results was shown in Table 3.

Table 2: Summary of Ramachandran plot analysis created by PROCHECK

Antimicrobial protein	Sapecin B
Most favoured regions	77.4%
Additionally allowed regions	12.9%
Generously allowed region	6.5%
Disallowed regions	3.2%

Table 3: Antimicrobial protein models-overall evaluation and statistics

Antimicrobial protein	Sapecin B
ERRAT (Quality factor)	100
PROCHECK (Overall G-factor)	-0.12
Q-Mean (z-score)	-1.32

After evaluation and validation, the 3-D models were refined by MOE (Molecular Operating Environment). The program helped in energy minimization as well as made improvements in physical quality of local structures. The model generated was refined and thus can be used for docking purpose.

After modification of protein models, docking was done by using different softwares like MOE, Rosetta and PatchDock server. Docking analysis was done to know cellular function. The molecular interactions were very important as they played important role in biological processes. These interactions were responsible in formation of stable protein-ligand and protein-protein complexes that were important in order to perform biological functions. Thus computational docking was considered the best approach for understanding of protein-ligand interactions.

Molecular Docking

PatchDock is a server for protein-ligand docking and for prediction of three dimensional structures of either protein-protein complexes or protein-ligand complexes. The highest top 10 binding protein interactions were provided in the output. These were in PDB format. The top value with highest score was then downloaded.

The evaluation of these amino acids and the output of PatchDock were performed by LigPlus software. This software evaluated the interface residues that were involved in the interaction of antimicrobial proteins with the ligand. These evaluations showed that there is a strong binding affinity between Sapecin B and peptidoglycan.

The interaction of Sapecin B with peptidoglycan showed that there exist hydrogen bonding between protein and ligand. The antimicrobial peptide Sapecin B residue Gln 81 showed hydrogen bonding with peptidoglycan. The length of H-bonding of ligand with Gln 81 was found to be equal to 2.41 which showed that there was strong interaction. Lys82 and Val83 were the non-ligand residues that were involved in hydrophobic contact (Fig. 3).

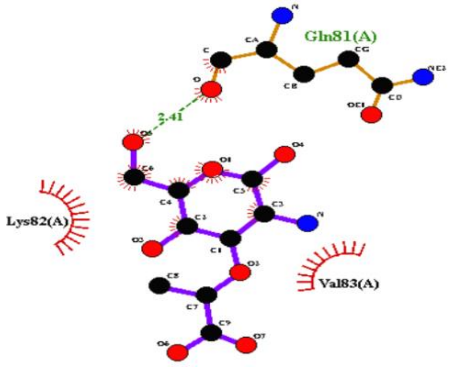

Fig. 3: Protein-ligand interaction of Sapecin B and peptidoglycan visualised by LigPlus

RosettaLigand is a tool for docking of small molecules into proteins and involve in structure prediction as well as designing of proteins. It took “SDF” file of ligand while protein was inputed in “PDB” format. The result of rosetta included 3-D structure of docked proteins, graphs of various scoring parameters as well as it contained tables of scoring and structural evaluation results.

The results of rosetta consisted of 10 protein models. The best model was chosen having lowest interface energy, transform action ratio to be normally between 0.2 and 0.8. For best results it should normally be less than two third of pocket size and should have lowest interface-delta score. So for Sapecin B, the model that showed these properties were downloaded and viewed in Chimera and shown in Fig. 4.

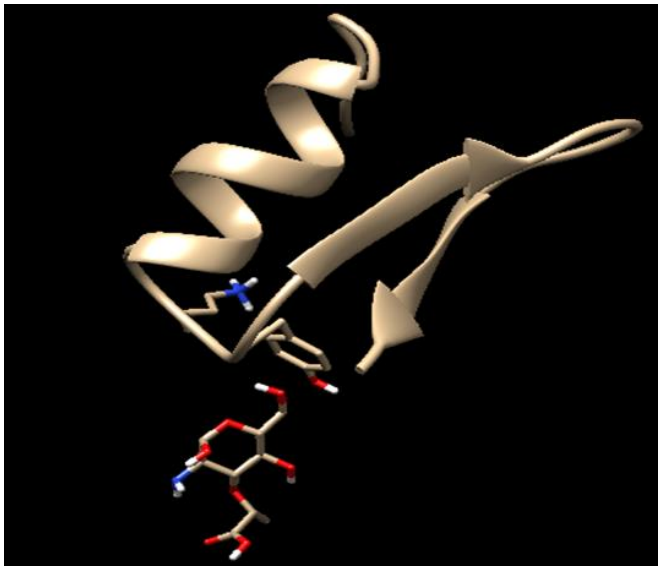

Fig. 4: Visualization of docked protein

Docking with MOE

The proteins were also docked by using MOE. The result showed that peptidoglycan had shown bonding with some of the residues of Sapecin B protein. Lys 87 showed bonding with Oxygen of peptidoglycan while Val 89 had shown bonding with both Oxygen and Hydrogen of peptidoglycan. The Cys 84 had shown bonding with Nitrogen group Fig. 5.

4 | DISCUSSION

Antimicrobial peptides (AMPs) have been gaining focus as a new strategy for the treatment of infections caused by bacteria. They are of global concern because with the emergence of new antibiotics, the resistance against antibiotics increases. So there is a need for the development of antimicrobial agents against conventional antibiotics. These peptides are gaining more and more importance because large number of alarming data has been reporting showing resistance to antibiotics. World health organization (WHO) said the emergence of antibiotic resistant was a major threat to human health. There was an estimation that claimed that by 2050, 10 million people will be killed or died due to infections caused by drug-resistant bacteria per year (Arakal et al., 2023; Pfalzgraff et al., 2018).

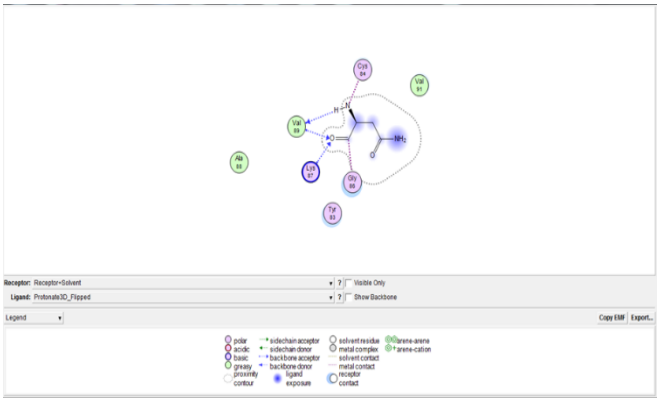

Fig. 5: Docking of Sapecin B by MOE

So AMPs are diverse class of molecules containing 2-50 amino acids and show different antimicrobial activities. More than 3200 peptides have listed in APD database but only few of them are approved by U.S. Food and Drug Administration due to their high toxicity, off-target effects as well as poor performance in different labs (Chen et al., 2022). They are produced by all living organisms for the protection of host from different microbial pathogens. They play an important role in defending the organism from bacterial, fungal and viral infections (Kang et al., 2022). They are gaining more attention because of their unique mechanism as well as their broad-spectrum antimicrobial properties against the conventional antibiotics (Ji et al., 2024).

So to overcome the problems of conventional antibiotics or drug resistance, there is a need for the development of new antimicrobial peptides and by using different therapeutic strategies reduced the effect of resistant pathogens and then control these pathogens (Moghaddam et al., 2015). *In silico* method is considered very important for novel drug development. These methods allow researchers to examine how a drug and targeted protein interact with each other and how mutations in the gene affect functions of the proteins. These methods also used for the screening of medicines to check their effectiveness on different calls and tissues. These methods helped us to understand various features of drug development (Yusuf, 2023).

By following previous work, novel antimicrobial

peptides were designed by using *in silico* methods. The structure of receptor protein i.e., antimicrobial peptide (Sapecin B) weas built by using homology modeling tools. For that purpose, online softwares for construction of three dimensional structures of proteins like SWISS-MODEL and Phyre2 protein fold recognition software were used. The structure of ligand i.e., peptidoglycan was downloaded from PubChem database.

Out of these two models, the best model was predicted on the basis of Ramachandran plot server values. These values were calculated from RAMPAGE tool. The model constructed by SWISS-MODEL was the best because ramachandran values were very good and high. The selected model was more authentic and had good physical appearance as compared to others.

Primary structure analysis was done by ProtParam tool to know about the physiochemical properties of proteins or to know that either the proteins were stable or unstable without the suitable ligand. The structures of proteins were visualized by USCF chimera.

The protein structure was then validated by using different online programs like ERRAT, PROCHECK, QMEAN server. The values of these programs showed that the protein structure of Sapecin B was best and highly reliable. Then the model was modified for docking purpose. For this, modrefiner and MOE were used that involve in energy minimization making the model more refined. Then the protein was prepared for docking. Molecular Operating Environment (MOE), Rosetta and Patchdock were used for docking purpose. The results of Protein-Ligand interaction by Patchock was then viewed by Ligplus.

These docking softwares gave us information about the protein-ligand interaction and showed that there was a strong binding interaction between the ligand i.e., peptidoglycan with our antimicrobial protein Sapecin B.

Conclusion

Due to ever-increasing antibiotic resistance in microbes, the AMPs have emerged as a suitable alternative to antibiotics. We studied an antimicrobial peptide (Sapecin B) using homology modeling and evaluation of models was done by RAMPAGE server that helped to identify the best model. The structure of peptidoglycan was used as a ligand. The results showed that peptidoglycan had bonding with some of the residues of Sapecin B protein i.e., Lys 87, Val 89 and Cys84. According to PatchDock, there exist hydrogen bonding between protein residue and ligand with a length of 2.41. This information provides a step forward in development of antimicrobial peptides effective against antibiotic resistant microbes.

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