Potential Mechanism of Action of Antimicrobial Peptide through Computer-Assisted Method

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***Abstract* - This research have been carried out to study the mechanism of actions of antimicrobial peptides using bioinformatics technique. The aim of this research is to determine and visualize the 3-dimensional structure of the secondary structure of the antimicrobial peptide, to classify and compare the antimicrobial peptides based on the secondary structure: α-helical, β-strand, α+β structure and non-α+β structure and to determine the effect of net charge of the antimicrobial peptides on their mechanism of action. The mechanism of action of antimicrobial peptides has studied by bioinformatics techniques, using any related databases such as APD, PDB, UniProt and DBAASP. The results shows that most of antimicrobial peptides are α-helical and α+β secondary structure. The peptides are also cationic which makes them interact with negative-charges of bacterial cell membranes.**

I. INTRODUCTION

Bioinformatics has been defined in many different ways. Basically, bioinformatics has been commonly considered as a combination of both biological and computer sciences, along with other contributing disciplines. The words of 'bio' from bio-informatics is referring to the biology in the terms of molecules (in the sense of physical chemistry). Whereas, the 'informatics' is applying to the "informatics techniques" in order to understand and organize the information associated with these molecules [1]. It is derived from disciplines such as applied maths, computer science and statistics. In short, bioinformatics is a management information system for molecular biology for processing any biologically-derived information, whether DNA sequences or breast X-rays [2].

In this research, bioinformatics approaches is used that involves biological information for anti-microbial peptide. Peptide is a short protein that is approximately contains of 12-50 amino acids long [3]. In other words, antimicrobial peptide is the protein that may be a part of an evolutionarily ancient system for immune defense that produced as a first line of defense by all multicellular organisms that have broad activity to directly kill bacteria, yeasts, fungi, viruses and even cancer cells [4]. These antimicrobial peptides can be found in various different organisms including mammalians, amphibians, birds, insects, as well as plants.

In this new era, the bacterial resistance and emerging infectious diseases has become potential threats to human. This scenario has leads to ribosomally synthesized antimicrobial peptides to become a promising focus area in antibiotic research. This antimicrobial peptides can be classified as either non-ribosomally synthesized peptides or ribosomally synthesized peptides (RAMPs). The non-ribosomally synthesized peptides are commonly found in bacteria and fungi. These antimicrobial peptides are assembled by peptide synthetases as opposed to ribosomal-supported synthesis [5]. Gramicidin, polymyxin B, bacitracin and vancomycin are some of the examples from non-ribosomally synthesized antimicrobial peptides [6].

Among the 500 RAMPs that have been described, they are all derived from a diverse range of species,that comes from all types of organism including prokaryotes to humans. All of these peptides are not only possess antibiotic, in fact may also possess antiviral, antiparasitic as well as antineoplastic activities. In antibiotic research, AMPs has their different amino acid sequences and structural conformation that make them contains unique antibiotic spectrum. In killing the target bacteria, the mechanism of action of antimicrobial peptides firstly involves peptide binding to the bacterial cell surface. It will then causes conformational change to the peptide and leads to the aggregation of multiple peptide monomers. Pore formation will then occurred through the bacterial cell wall. The AMPs bind to lipopolysaccharides in the negatively-charged, Gram-negative bacterial outer cell wall or to the acidic polysaccharides of the Gram-positive bacterial outer cell wall. After the binding step, permeabilization of the bilayer membrane occurs by transient pore creation. The permeabilization leads to a leakage of cell components and cell death [7].

With the increase of such peptides annually, it is realized that a database would be much useful to help manage the basic information of AMPs. Furthermore, new features are added to the database continuously to help the researcher for better use this resource. From the database, each entry is highly integrated with additional peptide information that covering various aspects of AMPs.

II. MATERIALS AND METHODS

A. Lists of Database

All the listed databases were visited to find the targeted information (**Table 1**).

Table 1: List of databases have been used

Name of Database	URL Link
Antimicrobial Peptide Database, DBAASP	https://dbaasp.org/home
Antimicrobial Peptide Database, APD	http://aps.unmc.edu/AP/about.php
Protein Data Bank, PDB	https://www.rcsb.org/pdb/static.do?p=help/explore/sequence_details.html
UniProtKB	http://www.uniprot.org/uniprot/

B. Finding Information from Database

i) From DBAASP

Database of Antimicrobial Activity and Structure of Peptides, DBAASP is opened. The 'Search' tab in the database is clicked. From the list, the peptides are chosen and clicked to see the information provided. Next, the 3D structure by JSMol is clicked to see the secondary structure of peptides. The image is then print screened and saved. The name of organism, amino acid sequence and net charges of the peptides are recorded.

ii) From APD

If the 3D structure is not provided, Antimicrobial Peptide Database, APD is opened. The 'Search Database' tab is clicked. In the form, all the information such as name of organism and amino acid sequence that obtained from DBAASP are filled in the space provided. Next, the PDB or UniProt ID from the database are recorded. Protein Databank, PDB or UniProt is opened. The PDB or UniProt ID is filled in the 'search' tab. Lastly, the secondary structure is opened and print screened.

III. RESULTS AND DISCUSSION

The result found 36 sources of antimicrobial peptides from different groups of organisms. **Table 2** shows different antimicrobial peptides have found from mammals, amphibians, plants, insects and invertebrates together with their types of secondary structure based on databases used.

Table 2: Sources of antimicrobial peptides from different groups of organisms

Mammalians		
No	**Source**	**Name of AMPs**
1	Cattle *(Bos Taurus)*	Bovine tracheal antimicrobial peptide,TAP
2	Chinchilla *(Chinchilla lanigera)*	Beta-defensin 1, BD-1, cBD-1
3	Hamster *(Mesocricetus auratus)*	Neutrophil defensin 1, HANP-1
4	Monkey *(Macaca mulatta)*	Cathelicidin, RL-37
5	Mouse *(Mus musculus)*	Beta-defensin 14, BD-14, mBD-14
6	Wild boar *(Sus scrofa)*	Protegrin 1
Amphibians		
No	**Source**	**Name of AMPs**
1	Australian frog *(Litoria raniformis)*	Aurein 1
2	Chinese red belly toad *(Bombina maxima)*	Maximin 3
3	Frog *(Phyllomedusa distinta)*	Distinctin
4	Sauvage's leaf frog (*Phyllomedusa sauvagei)*	Dermaseptin S4
Plants		
No	**Source**	**Name of AMPs**
1	Argentine mistletoe *(Phoradendron liga)*	Ligatoxin-B
2	Broom-corn *(Sorghum bicolor)*	Defensin-like protein 1, S1 alpha-1
3	Butterfly pea *(Clitoria ternatea)*	Defensin AMP1, CT-AMP1
4	Dahlia flower *(Dahlia merckii)*	Defensin AMP1, DmAMP-1
5	European spindle tree *(Euonymus europaeus L.)*	E. europaeus chitin-binding protein, Ee-CBP
6	Four o'clock flower *(Mirabilis jalapa)*	Antimicrobial peptide 2, MJ-AMP2, AMP2
7	Onion *(Allium cepa)*	Antimicrobial protein Ace-AMP1
8	Pea *(Pisum sativum)*	Defensin-2, PsD2
9	Potato *(Solanum tuberosum)*	Snakin-1
10	Radish *(Raphnus sativus)*	Defensin-like protein 3
11	Spinach *(Spinacia oleracea)*	Defensin-2, So-D2
12	Wheat *(Triticum aestivum)*	Alpha-purothionin
13	Wild grass *(Echinochloa crus-galli)*	Herpin-like peptides, EcAMP1
Insects		
No	**Source**	**Name of AMPs**
1	Bug *(Podigus maculiventris)*	Thanatin
2	Fruit fly *(Drosophila melanogaster)*	Drosomycin
3	Moth *(Galleria mellonella)*	Defensin
4	Wasp *(Vespa magnifica)*	Mastoparan-like peptide 12d
5	Yellow fever mosquito *(Aedes aegypti)*	Defensin-A, AaDef
Invertebrates		
No	**Source**	**Name of AMPs**
1	Mediterranean mussel *(Mytillus galloprovincialis)*	Mussel defensin, MGD-1
2	Scorpion *(Androctonus australis)*	Androctonin

3	Shrimp *(Litopenaeus setiferus)*	Penaeidin-4d
4	Japanese horsehoe crab *(Tachypleus tridentatus)*	Tachystatin-C
5	Sand fly *(Phlebotomus duboscqi)*	Defensin, PduDef

In many recent years, antimicrobial peptides has been studied as the microbial resistance to the traditional antibiotics. With the strong ability of killing target cells α-helical and β-sheet cationic antimicrobial peptides have been proposed as the potent candidates. They are both have a wide spectrum mechanism of their activity which can affect both gram-negative as well gram-positive bacteria. Other than that, they can also interact with pathogens that is resistant to the traditional antibiotics. Basically, antimicrobial peptides are interacting with the membranes which cytoplasmic membrane is the main target of some peptides. The peptides used will accumulated in the cell's membrane which eventually increased the permeability and which cause the cell to lose their barrier function. Recently, there are several important factors that have been studied which can affect the mechanism of these antimicrobial peptides. The factors include the hydrophobicity of the peptides, the charged of the peptides, amphipathic nature that segregates the basic and hydrophobic residues of the peptides, and the secondary structure of the antimicrobial peptides [8].

A. Charges of Peptides

Table 3*:* The Net Charges of AMPs

Mammalians			
No	**Source**	**Net Charge**	**Amino Acid Sequences**
1	Cattle *(Bos Taurus)*	+9	NPVSCVRNKGICV PIRCPGSMKQIGT CVGRAVKCCRKK
2	Chinchilla *(Chinchilla lanigera)*	+11	GIINTIQRYFCRVR GGRCAALTCLPRE TQIGRCSVKGRKC CRTRK
3	Hamster *(Mesocricetus auratus)*	+3	VTCFCKRPVCDSG ETQIGYCRLGNTF YRLCCRQ
4	Monkey *(Macaca mulatta)*	+5	GFCRCLCRRGVC RCICTR
5	Mouse *(Mus musculus)*	+12	FLPKTLRKFFCRIR GGRCAVLNCLGK EEQIGRCSNSGRK CCRKKK
6	Wild boar *(Sus scrofa)*	+7	RGGRLCYCRRRF CVCVGR

Amphibians			
No	**Source**	**Net Charge**	**Amino Acid Sequences**
1	Australian frog *(Litoria raniformis)*	+1	GLFDIIKKIAESF
2	Chinese red belly toad *(Bombina maxima)*	+4	GIGTKILGGVKTA LKGALKELASTY AN
3	Frog *(Phyllomedus a distinta)*	+4	NLVSGLIEARKYL EQLHRKLKNCKV
4	Sauvage's leaf frog (*Phyllomedus a sauvagei)*	+5	ALWKTLLKKVLK A
Plants			
No	**Source**	**Net Charge**	**Amino Acid Sequences**
1	Argentine mistletoe *(Phoradendro n liga)*	+4	KSCCPSTTARNIY NTCRLTGASRSVC ASLSGCKIISGSTC DSGWNH
2	Broom-corn *(Sorghum bicolor)*	+3	RVCMGKSQHHSF PCISDRLCSNECV KEEGGWTAGYCH LRYCRCQKAC
3	Butterfly pea *(Clitoria ternatea)*	+3	NLCERASLTWTG NCGNTGHCDTQC RNWESAKHGACH KRGNWKCFCYFN C
4	Dahlia flower *(Dahlia merckii)*	+1	ELCEKASKTWSG NCGNTGHCDNQC KSWEGAAHGACH VRNGKHMCFCYF NC
5	European spindle tree *(Euonymus europaeus L.)*	+5	QQCGRQAGNRRC ANNLCCSQYGYC GRTNEYCCTSQG CQSQCRRCG
6	Four o'clock flower *(Mirabilis jalapa)*	+3	CIGNGGRCNENV GPPYCCSGFCLRQ PNQGYGVCRNR
7	Onion *(Allium cepa)*	+1	QNICPRVNRIVTP CVAYGLGRAPIAP CCRALNDLRFVN TRNLRRAACRCL VGVVNRNPGLRR NPRFQNIPRDCRN

			TFVRPFWWRPRIQCGRIN
8	Pea *(Pisum sativum)*	+3	KTCENLSGTFKGPCIPDGNCNKHCRNNEHLLSGRCRDDFRCWCTNRC
9	Potato *(Solanum tuberosum)*	+8	GSSFCDSKCKLRCSKAGLADRCLKYCGICCEECKCVPSGTYGNKHECPCYRDKKNSKGKSKCP
10	Radish *(Raphnus sativus)*	+3	KTCENLSGTFKGPCIPDGNCNKHCRNNEHLLSGRCRDDFRCWCTNRC
11	Spinach *(Spinacia oleracea)*	+8	GIFSSRKCKTPSKTFKGICTRDSNCDTSCRYEGYPAGDCKGIRRRCMCSKPC
12	Wheat *(Triticum aestivum)*	+10	KSCCRSTLGRNCYNLCRARGAQKLCAGVCRCKISSGLSCPKGFPK
13	Wild grass *(Echinochloa crus-galli)*	+4	GSGRGSCRSQCMRRHEDEPWRVQECVSQCRRRRGGGD
Insects			
No	**Source**	**Net Charge**	**Amino Acid Sequences**
1	Bug *(Podigus maculiventris)*	+6	GSKKPVPIIYCNRRTGKCQRM
2	Fruit fly *(Drosophila melanogaster)*	+1	VFIDILDKVENAIHNAAQVGIGFAKPFEKLINPK
3	Moth *(Galleria mellonella)*	0	DKLIGSCVWGATNYTSDCNAECKRRGYKGGHCGSFWNVNCWCEE
4	Wasp *(Vespa magnifica)*	+4	INLKAIAAMAKKLL
5	Yellow fever mosquito *(Aedes aegypti)*	+3	ATCDLLSGFGVGDSACAAHCIARGNRGGYCNSKKVCVCRN
Invertebrates			
No	**Source**	**Net Charge**	**Amino Acid Sequences**
1	Mediterranean mussel *(Mytillus galloprovincialis)*	+5	GFGCPNNYQCHRHCKSIPGRCGGYCGGWHRLPCTCYRCG
2	Scorpion *(Androctonus australis)*	+8	RSVCRQIKICRRRGGCYYKCTNRPY
3	Shrimp *(Litopenaeus setiferus)*	+5	HSSGYTRPLRKPSRPIFIRPIGCDVCYGIPSSTARLCCFRYGDCCHL
4	Japanese horsehoe crab *(Tachypleus tridentatus)*	+7	DYDWSLRGPPKCATYGQKCRTWSPPNCCWNLRCKAFRCRPR
5	Sand fly *(Phlebotomus duboscqi)*	+3	ATCDLLSAFGVGHAACAAHCIGHGYRGGYCNSKAVCTCRR

Based in **Table 3**, all of the antimicrobial peptides that found have positive net charges regardless of their groups of organisms.

Basically, antimicrobial peptides works by targeting the lipid bilayer of the membrane of the target cells. The reason is because the peptides are able to increase the rate of leakage of the internal aqueous contents of liposomes. Furthermore, most of the antimicrobial peptides are cationic, which makes it clearer why they interacts with bacterial membranes [9]. Bacterial membranes has anionic phospholipids which allows the cationic antimicrobial peptides to react with their membranes. For Gram-negative bacteria, they contains of anionic molecules that are oriented in both of their outer leaflet of the plasma membrane as well as the outer membrane. However, it is contrast with mammalian membranes because they are zwitterionic amphiphiles in their extracellular monolayer. This makes the antimicrobial peptides to not interact with the membrane layer of mammalians.

Nevertheless, the mechanism of action of these peptides in disturbing the membranes are not directly related to their mechanism of cytotoxic action. They are simply related to the manner of the peptides by which they enter the cell to reach an alternative target [10].

B. Amphipathic Nature and Hydrophobicity of Peptides

Many studies have shown that the mechanism of action of antimicrobial peptides are also related to the amphipathicity of these peptides. By eliminating the helical amphipathicity or helical structure of the peptides is prevented, results in significant reducing the activity of peptides [11]. The results also shows that the activity of the peptides towards the cells are completely abolished when reducing the depth and hydrophobicity of the nonpolar sector of the peptides [12].

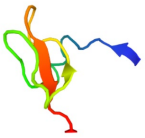
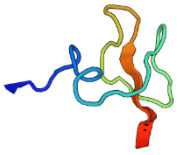
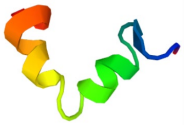
However, it can be explained that this peptides is basically unstructured when in solution and

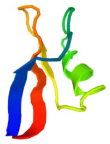
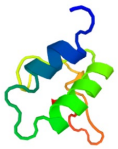
electrostatically attracted to the negatively charged groups that contains in the membrane of the cell. When these peptides accumulated on the membrane of the cells, it will form transient pores or channels, or even a detergent-like disaggregation of the bilayer [13]. This mechanism leads to depolarization, which causing the leakage of essential metabolites and eventually the membrane lose their compositional specificity. It will then cause the cellular inactivation [11].

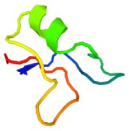
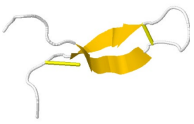
C. Secondary Structure of Peptides

The secondary structure for all the 36 AMPs found are listed in the table below.

Table 4: The Secondary Structure of AMPs

Mammalians		
No	**Source**	**3D Structure**
1	Cattle *(Bos Taurus)*	 β-strand
2	Chinchilla *(Chinchilla lanigera)*	 β-strand
3	Hamster *(Mesocricetus auratus)*	 β-strand
4	Monkey *(Macaca mulatta)*	 β-strand
5	Mouse *(Mus musculus)*	
		β-strand
6	Wild boar *(Sus scrofa)*	 β-strand
Amphibians		
No	**Source**	**3D Structure**
1	Australian frog *(Litoria raniformis)*	 α– helix
2	Chinese red belly toad *(Bombina maxima)*	 α– helix
3	Frog *(Phyllomedusa distinta)*	 α– helix
4	Sauvage's leaf frog (*Phyllomedusa sauvagei)*	 α– helix
Plant		
No	**Source**	**3D Structure**

1	Argentine mistletoe *(Phoradendron liga)*	 α+β structure
2	Broom-corn *(Sorghum bicolor)*	 α+β structure
3	Butterfly pea *(Clitoria ternatea)*	 α+β structure
4	Dahlia flower *(Dahlia merckii)*	 α+β structure
5	European spindle tree *(Euonymus europaeus L.)*	 α-helix
6	Four o'clock flower *(Mirabilis jalapa)*	 β - strand
7	Onion *(Allium cepa)*	
		α– helix
8	Pea *(Pisum sativum)*	 α+β structure
9	Potato *(Solanum tuberosum)*	 α – helix
10	Radish *(Raphnus sativus)*	 α+β structure
11	Spinach *(Spinacia oleracea)*	 α+β structure
12	Wheat *(Triticum aestivum)*	 α+β structure
13	Wild grass *(Echinochloa crus-galli)*	 α– helix
Insects		
	Source	**3D Structure**

1	Bug *(Podigus maculiventris)*	 α+β structure
2	Fruit fly *(Drosophila melanogaster)*	 α+β structure
3	Moth *(Galleria mellonella)*	 α+β structure
4	Wasp *(Vespa magnifica)*	 α - helix
5	Yellow fever mosquito *(Aedes aegypti)*	 α+β structure
Invertebrates		
	Source	**3D Structure**
1	Mediterranean mussel *(Mytillus galloprovincialis)*	 α+β structure
2	Scorpion *(Androctonus australis)*	
		 β – strand
3	Shrimp *(Litopenaeus setiferus)*	 α - helix
4	Japanese horsehoe crab *(Tachypleus tridentatus)*	 α+β structure
5	Sand fly *(Phlebotomus duboscqi)*	 α+β structure

(Source: https://dbaasp.org/home, uniiprot.org/uniprot/, rcsb.org/structure/)

Based on **Table 4**, we found out that most of the antimicrobial peptides are α- helices. This because α-helices is a common structure for protein-protein structure as mentioned earlier [14]. Basically, most of the antimicrobial peptides are present in amphipathic structures which they are either α- helices or β- sheet conformation, especially in the state of membrane-binding. However, there are still antimicrobial peptides that exist with no specific secondary structure, such as random coiled [8]. In this research, α- helices and $\alpha + \beta$ structure have shown in **Figure 1** and **Figure 2**.

There are many short peptides are helical structure at their lipid bilayers. Antimicrobial peptides can also dent with the helices in transmembrane orientation. In the mechanism of action of the antimicrobial peptides, the tilt angles of the helical axis, the angle of rotation around the axis, the degree of helicity and the interaction are include in the important parameters for the activity of the peptides [15].

There are few studies shows that the secondary structure is also related to the cell concentration. When research is carried out in low cell concentration, the outcomes results shows it is likely higher content of

random coil rather than helical peptides. It has explained that this phenomenon is occurred due to the concentration of availability of lipopolysaccharides that the peptides will bind to, which increases the cell concentration [16].

IV. CONCLUSION

In this study, bioinformatics technique has been used to study the mechanism of action of antimicrobial peptides. All the databases that related to this study have been listed out. Based on this study, we found out that most of antimicrobial peptides are α-helical and $\alpha + \beta$ structure in their secondary structure. There are 36 organisms from different groups gives their secondary structure. They are also cationic which explains why they are interacting to the negative-charges membrane of bacteria. The results also shows that amphipathicity of the peptides plays an important roles in their mechanism of action toward targeted cells.

Based on the studies that have been done. There are certain improvements can be done to increase accuracy of the research for the next studies. Firstly, the bioinformatics specialist needed to be increase. There are certain limitations in finding the secondary structure for the antimicrobial peptides. There are still certain antimicrobial peptides that have unknown secondary structure. There should be more bioinformatics specialist in the field to find out and visualize the secondary structure. Furthermore, in finding the peptides using the database, researcher must ensure the amino acid sequences is accurate. This because there are many peptides with similar name but different in their amino acid sequences. Only one different of amino acid presence in the peptides can be a different peptides. In order to obtain accurate results, the amino acid sequences must be precise.

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