Review

Journal of Food Safety and Hygiene

Journal homepage: http://jfsh.tums.ac.ir

Characterization, application and action mechanism of bioactive peptides (BAPs) derived from microbes, plants and recombinant proteins against food borne pathogens; a review

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ARTICLE INFO

Article history:
Received 19.04.2024
Received in revised form 21.06.2024
Accepted 24.06.2024

Keywords:
Bioactive peptides;
Antimicrobial effects;
Beneficial effect;
Food pathogens

ABSTRACT

The preventive properties of bioactive peptides (BAPs) derived from microbes and plants have stimulated extensive research and discovery. The antimicrobial properties of BAPs against foodborne pathogens have made them effective and potential alternatives to existing antibiotics. Antitumor, antihypertensive, antioxidant, anti-obesity, and antidiabetic activities are just a few of the other beneficial properties of BAPs. An increasing number of harmful microorganisms, including foodborne pathogens, are developing resistance to various antibiotics. The goal of scientific research is to discover new, innovative and safe antimicrobial agents to fight these infections. Foodborne pathogens are among the many pathogenic microorganisms against which plant and microbial BAPs have shown significant antimicrobial activity. These compounds (BAPs) have the ability to eliminate pathogenic microorganisms by disrupting membrane integrity, inhibiting DNA and RNA synthesis, preventing protein synthesis, blocking protein activity or interacting with specific intracellular targets. In addition, the beneficial effect of BAP consumption includes the regulation of intestinal microbiota and modulation of the balance of reactive oxygen species in the digestive tract. This review article deals with different sources of antipathogen peptides, the food application of peptides from distinct sources, and their Mechanism of action.

Citation: Nabgan M, Shariatifar N. **Characterization, application and action mechanism of bioactive peptides (BAPs) derived from microbes, plants and recombinant proteins against food borne pathogens; a review.** J Food Safe & Hyg 2024; 10 (2): 91-108 https://doi.org/10.18502/jfsh.v10i2.17683

1. Introduction

The health benefits of bioactive peptides and their potential application as functional foods and nutraceuticals have been extensively researched (1).

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Emerging antibiotic resistance presents significant challenges to the economy, animal populations, and human health. Therefore, it is vital to find a suitable alternative to antibiotics that can be less harmful and more effective in addressing various foodborne and other diseases (2).

Therefore, these compounds can be incorporated into food as safe food supplements, as they exhibit less bacterial resistance compared to antibiotics (3).

Microbes, plants, and animal tissues can produce BAPs, which may also arise from microbial enzymes and food processing. Key sources include flaxseed, peas, cauliflower, eggs, soy products, cheese, yogurt, sausage, and milk (4). Currently, chemical or recombinant versions of several classes of BAPs are sold (5).

Many plant-derived BAPs, such as glycine-rich peptides and thionins, occur in stems, roots, seeds, flowers, and leaves. These BAPs are active against harmful bacteria and phyto-pathogens (6). Along with a wide range of other physiologically active peptides, microbes also produce gramicidin, endolysins, apicidin, nisin, propionicin, and enterocin. Bacteria, fungi, and viruses are the sources of these peptides (7). Peptides regulate many vital processes, particularly hormones. They promote health by reducing cardiovascular risks, modulating immunity, lowering blood pressure, binding minerals, chelating metals, and exhibiting antimicrobial, anticoagulant, and antioxidant properties. They also impact food's nutritional value and inhibit disease-promoting enzymes (8). Certain BAPs directly extend the shelf life of prepared foods, which makes them highly sought-after in this industry. Additionally, their antimicrobial activity shields mammals from a variety of foodborne pathogens (9). BAP functions as an antimicrobial peptide by acting on specific intracellular targets, disrupt membrane integrity, impede DNA and RNA synthesis, and kill pathogens through a range of mechanisms (10). Furthermore, the regulation of gut microbiota is one of the benefits of consuming BAP (11). Foodborne pathogens represent a serious threat to human health and food safety. They are responsible for a number of serious illnesses that affect people. Furthermore, a lot of these foodborne bacteria are constantly becoming resistant to antibiotics (12). To safeguard health and food safety, It is essential to develop safe and effective antimicrobial agents against foodborne pathogens. Bioactive peptides can target various pathogens, including hepatitis. This review focuses on bioactive peptides from microbes and plants, as well as recombinant peptides and their future applications.

2. An overview of various BAPs

2.1. Plant-based BAPs

Bioactive peptides can be obtained from protein sources found in plants, such as beans, seeds, grains, legumes, and leftover olive oil, in addition to milk and meat. Because plant proteins have a lower carbon footprint than animal proteins, they are essential (13). The usage of plant-based proteins in food products is limited due to their low protein content and absence of essential amino acids. However, several methods of modification—chemical, physical, and enzymatic—enhance their efficacy, turning them into multipurpose ingredients in food systems (14).Peptides are more readily absorbed by the small intestine epithelium than intact proteins, which means that while both can have bioactivity, peptides have been shown to have a higher potential for in-vivo bioactivity (15). In nutritional and

therapeutic applications, plant proteins are increasingly being used in place of animal proteins as an alternative to animal proteins (16).

2.2. Animal peptide

Animal proteins have physiological effects and act as substrates for enzymatic hydrolysis when food is processed and consumed (17). Animal proteins contain peptide sequences that link short chains of two to twenty amino acids to form animal bioactive peptides, which have a variety of physiological effects on the body (18,19). 32 of the most effective milk proteins were created from low-molecular-weight antimicrobial peptides (20). Bioactive peptides can be produced during food processing by digestive and microorganism-derived enzymes (17). Studies have demonstrated the efficacy of peptides derived from edible insects (21), Proteins found in cow's milk (22), and fermented probiotic drinks made with whey (23) in the fight against pathogens like *C. albicans, S. typhi, B. cereus, E. coli, S. aureus,* and *Shigella dysenteriae*. Meat, fish, milk, and dairy products are among the foods that contain animal peptides, which have antithrombotic, antihypertensive, antioxidant, antimicrobial, and antiproliferative qualities (24). Examples include calcitonin gene-related peptide and parathyroid hormone, which both considerably contribute to bone health. They play critical roles in preserving cell integrity, tissue regeneration, and bone healing (19). The importance of animal bioactive peptides in promoting general health and well-being is being highlighted by the growing amount of research being done on their possible therapeutic benefits in treating conditions like inflammation, hypertension, and microbial infections (25). Therefore, antimicrobial activity against a wide range of foodborne pathogens by animal-derived bioactive peptides is promising.

2.3. *BAPs derived from Microbes*

Bacteria, fungi, and viruses produce microbe-derived BAPs, which are classified based on their sources and properties. Viral BAPs include phage proteins like holins, lysins, and peptidoglycan hydrolases, with two main types: phage-encoded lytic factors and phage-tail complexes (26). Two kinds of fungal BAPs are defensins and peptaibol. The term 'peptaibol' refers to compounds consisting of amino alcohols, peptides, and α-amino isobutyrate (27). Defensins consist of amino acids that are connected by disulfide bonds, they are actually the immune response of fungi to survive and compete with other microorganisms (28).

2.4. Gram-negative and gram-positive bacteria: Both bacteria can produce bacterial BAPs bacterial BAPs. Bacteriocins can be categorized into ribosomally produced BAPs and nonribosomally or enzymatically produced BAPs (29). Lantibiotics and non lantibiotics are the two categories of bacteriocins; lantibiotics include the artificial amino acid lanthionine (68). Colicins, microcins, colicin-like bacteriocins, and phage-tail-like bacteriocins are the different categories of gram-negative bacterial bacteriocins (30).

2.5. Fermentation-derived BAPs: Gram-positive lactic acid bacteria (LAB), especially *Lactiplantibacillus plantarum*, produce bioactive compounds like short-chain peptides and fatty acids. They are vital in the food industry, providing bio protective effects against pathogens (31). Studies indicate that *L. plantarum* can inhibit foodborne pathogens like *S. aureus* and *E. coli*, enhanced by bacteriocin activity from its supernatants. (32). *L. Furthermore*, when fermented, *L. plantarum* in

camel milk demonstrated antimicrobial effects against various pathogens (33). *Lactobacillus casei ATCC 334* also showed potent antibacterial activity against multiple microbes when producing BAPs (34). Additionally, *Bifidobacterium lactis BB-12* and *Lactobacillus acidophilus LA-5* exhibit antibacterial properties in milk formulations against gram-positive and gram-negative bacteria (35). *Saccharomyces cerevisiae* plays a key role in producing bioactive biomasses for fermented foods, with its protein enolase II contributing significantly to antimicrobial BAPs that combat microbial resistance, making it a valuable source for next-generation compounds in the global market. (36).

2.6. Synthetic BAPs

After identification, peptide sequences can be produced chemically or via recombinant DNA technologies. Enzymatic hydrolysis is simple but requires complex purification and is time-consuming. Natural proteins yield low BAP content. While chemical synthesis is advanced, it lacks specificity and uses hazardous reagents. In contrast, recombinant DNA technology is environmentally friendly and achieves high yield and purity with fewer chemicals (37). Recombinant production of BAPs can be divided into two classes based on the expression of the bioactive peptide gene in a specific expression system, either in vitro or in vivo, as per several studies (38). To facilitate purification and enable mass production of the required peptide, the targeted peptide gene is connected to another well-known carrier protein gene in the in vivo expression method. For example, yeast expresses peptides called ecallantide and desirudin (39). While the in vitro expression method is not cost-effective, the in vitro expression method, a cell-independent system, has the advantage of producing the desired result quickly (40). On the other hand, peptide flexibility and effectiveness have led to a recent focus on the engineering of BAPs. In type II diabetes, for instance, engineered insulin is more important and has a longer effect than natural insulin (41).

3. Bacterial antimicrobial peptides against foodborne pathogen

Microorganisms, especially lactic acid bacteria (LAB), produce a wide range of chemical substances with antimicrobial properties (42). LAB naturally exist in various foods such as dairy products, meat products and vegetables or are used as pure kills or starters in the production of dairy products such as milk, yogurt, cottage cheese, and both hard and soft cheeses (43). Acetic acid bacteria can produce health-beneficial peptides by synthesizing short amino acid chains. Cyclic dipeptides and protein compounds are natural antimicrobials, especially antifungals, which are produced by acetic acid bacteria and increase the shelf life and health of food (44).

3.1. Cyclic dipeptides

Cyclic dipeptides, or 2,5-dioxopiperazines, are common in nature. Lactobacillus plantarum VTTE-78076 produces leucyl-L-glycyl, which inhibits the growth of Gram-negative bacteria Pantoea agglomerans and Fusarium avenaceum (45).

3.2. Bacteriocin

Bacteriocins, discovered in E. coli in 1925, are antibiotic alternatives. Their use in the food industry meets modern consumers' demand for safe, minimally processed food (46). Bacteriocins are complex synthesized proteins with low molecular weight (below 10 kilodaltons) that can be produced by ribosomes in gram-positive and gram-negative bacteria and show their bactericidal and inhibitory effects against strains

close to or distant from bacteriocin-producing strains (46). Bacteriocins can be classified into the following 4 categories according to different characteristics, including: genetic, biochemical, thermal and enzymatic stability, post-translational modifications, and antimicrobial activity:

Lantibiotics, which are small and heat-resistant peptides with post-translational modifications, which contains unusual amino acids lanthiene and methyl lanthiene in their structure (47). Lantibiotics are divided into two groups based on their structure and antimicrobial function: Subgroup A includes linear and cationic peptides with a maximum of 34 amino acids, the mechanism of antimicrobial action of this group involves the destruction of the target cell membrane. Subtype B are cyclic peptides with a maximum of 19 amino acids, which stop the vital actions of the enzymes of the target cell. The antimicrobial activity of nisin is studied as the most famous lantibiotic (46). (2) Small heat-resistant peptides (II Class) with an amphiphilic helical structure (47). (3) Larger bacteriocins with complex activity and flexible protein structure (Class III) (47). (4) Complex bacteriocins that contain fat and carbohydrate parts (47). The antimicrobial effect of bacteriocin on gram positive bacteria is much higher and it is insignificant against gram negative bacteria, the main reason of which is the presence of external lipopolysaccharide layer in the cell wall of gram negative bacteria (46).

4. Antimicrobial properties of plant-derived peptides against foodborne pathogen

A wide variety of structural and functional methods have been employed to identify and characterize plant derived BAPs with antimicrobial properties. PaDef was identified and isolated from a library of cDNA obtained from avocado fruits grown in Mexico. The peptide bears similarities to defensin. Because PaDef exhibits antibacterial qualities against both *E. Coli* and *S. aureus* (60). Moreover, the peptides with cysteine demonstrated powerful action against intestinal pathogenic bacteria like *E. coli* and *S. aureus* and Salmonella, and did not cause harm to human cells. AMPs also belong to the 2S albumin protein family which have been discovered to exist in plants. Filamentous fungi isolated from passion fruit, such as *Fusarium oxysporum*, *Aspergillus fumigatus*, *Trichoderma harzianum*, and *Colletotrichum gloeosporioides*, were found to be effectively suppressed by Pa-AFP-1 (61). A thionin-like peptide from chili that has been isolated and described is called CaTi. There are reports that CaTi works effectively against several harmful bacteria, such as *Candida albicans*, *F. Solani*, the species *S. cerevisiae* and *C. tropicalis* (62).

The most common protein in the world, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), is the primary enzyme for photorespiration and photosynthesis in plants and other living organisms. RuBisCO is a long-term and desired resource for BAPs (63). The original hydrolysate, parts produced by hydrolyzing RuBisCO with pepsin, and the RuBisCO 407 large subunit-derived peptides ELAAAC (f454-459) and MDN (472-474) all demonstrated antimicrobial activity against gram positive (*Bacillus subtilis, Micrococcus luteus, and L. innocua*) and gram-negative (like *E. coli*) microbes (64). Undoubtedly, using microorganisms to ferment protein from various sources is a novel way to produce BAPs with a range of beneficial health effects. It is more cost-effective to purify BAPs directly from microbial fermentation than to use enzymes for hydrolysis (65).

Table 1. Characteristics and applications of some bioactive peptides that have been recently used to eliminate food pathogens

Peptide sequencing	Weight	Amino acids number	Mechanism of action	action model	Applications in food categories	Limitations	References
	2846.46 Da	26	Interacting with lipid membranes and compromising cellular integrity. Creation of pores or disturbances in the membrane configuration	barrel-stave	meat products, included in food coatings	hemolytic and cytotoxic toxicity, at higher concentrations	(81)
His-Phe-Asp-Asn-Leu/Ile-Asn-Lys-Leu/Ile-Gln-Aib-Asn-Leu/Ile	1422.76 Da	11	Disruption of histidine metabolism, Stimulation of Reactive Oxygen Species (ROS) Generation, Reduction of Genes Associated with Na+/H+ Antiporter and Cell Wall	barrel-stave	Meat and poultry products Dairy products Seafood Processed foods Beverages Bakery products Ready-to-eat meals Fermented foods	Solubility and Stability, Specificity, Resistance Development, Optimal Concentration, regulatory approval and safety assessments	(82)
	14 kDa	129	disturbing the integrity of bacterial cell walls, breaking the glycosidic bonds within the peptidoglycan	barrel stave	Meat products Dairy products Seafood Beverages Baked goods Processed foods	lower lytic activity than native lysozyme, low Stability, Heat sensitivity, Resistance of S. typhi and A. hydrophila	(83)
		137-337	The addition of Cecropin P1 may enhance LysC02's ability to penetrate Gram-negative bacteria's outer membrane, facilitating the lysin's more efficient delivery of its peptidoglycan target.	synergistic action model	Therapeutic agent for treating infections and as a disinfectant for food contact surfaces to combat Cronobacter sakazakii	specificity, stability, and effectiveness against various strains or species	(84)
			Interaction of positively charged peptides with membrane phospholipids increases the permeability of membranes and causes cell death in negatively charged bacteria.	barrel-stave	dairy products	Further validation of its antimicrobial activity, potential challenges in large-scale production, restrictions related to target pathogens or food matrices	(85)
	3350 Da	34	Disrupting bacterial cell membranes by creating pores that result in cell death	Toroidal-pore model	dairy products, meats, and canned foods	risk of bacterial resistance development over time and Gram-negative bacteria has a different outer membrane structure, which limits its effectiveness against them	(86)
	4.5 kDa for tdAMP-1 and 5.8 kDa for tdAMP-2	37 for tdAMP-1 and 48 for tdAMP-2	Causing disruption of bacterial cell membranes, resulting in bacterial cell death	Toroidal-pore model	various food categories involve the potential use of natural food additives, food preservation, and improved food safety	possible cytotoxicity to mammalian cells, restricted stability in specific situations, and the eventual emergence of resistance in the target pathogens	(87)

Applications in food categories	Limitations	References	peptide's name	importance of peptides	Peptide extraction methods			
					Sources	Recombinant or	Enzymatic hydrolysis	Microbial fermentation
Marine products, and it is appropriate for use in food processing due to its high thermal stability and packaging industries	May have potential toxicity to non-target organisms, a restricted spectrum of activity against particular pathogens, gradual development of resistance in target pathogens and challenges in large-scale production	(88)	Melittin	*E. coli, L. monocytogenes, S. aureus*	honey bee venom	recombinant protein expression and purification		
			Bacipeptin	bactericidal against *Listeria monocytogenes, Bacillus cereus, Enterococcus faecalis*, and *Staphylococcus aureus* that are resistant to methicillin	*Bacillus licheniformis* M1			naturally produced by marine bacterium
applications in functional foods	lack of efficacy of antimicrobials against some pathogens	(89)	lysozyme	*Pseudomonas, S. enteritidis and E. coli*	egg white lysozyme		using pepsin enzyme	
functional foods	Optimizing its efficacy requires further research, as well as assessing its practical applicability in real food systems with the aid of validation studies	(89)	LysC02-Cecropin P1	Effectively eliminates *Cronobacter sakazakii* strains including ATCC 29544, BAA-894, ES15, NCTC 11467, and KCTC 2949, displaying bactericidal properties.	LysC02 from phage ΦC02- Cecropin P1	Cecropin P1 was fused with LysC02		
various food categories	potential issues related to stability, scalability, and cost-effectiveness in large-scale applications	(90)	*MP-4*	*S. aureus*				
in various food categories particularly in the dairy industry	potential destruction of secondary disulfide structure when temperatures rise	(91)	Nisin	Gram-positive bacteria, including L.monocytogenes	Koumiss, a traditional milk-based beverage		peptidomics analysis	
various food applications	Challenges in large-scale production and cost-effectiveness	(92)	both TdAMP-2 and TdAMP-1	Shows bactericidal properties and efficiently eradicates uropathogenic *E. coli* strains, *s. Typhimurium*, and *s. Typhi.*	Lactococcus lactis			it is produced by specific strains of bacteria
					Solanum lycopersicum			

https://doi.org/10.18502/jfsh.v10i2.17683

peptide's name	importance of peptides	Peptide extraction methods				Peptide sequencing	Weight	Amino acids number	Mechanism of action	action model
		Sources	Recombinant or engineered	Enzymatic hydrolysis	Microbial fermentation					
KK12YW	bactericidal against *Pseudomonas aeruginosa, Aeromonas sobria, E. coli,* as well as *Aeromonas salmonicida, S. epidermidis, Aeromonas hydrophila, E. tarda, methicillin-resistant S. aureus, V. parahaemolyticus*	de novo designed	designed and synthesized in the laboratory using a knowledge-based methodology					12	It folds into alpha helices with hydrophobic and hydrophilic residues (net charge +7) and interacts with microbial membranes (negatively charged) through electrostatic and hydrophobic mechanisms	Toroidal-pore model
ABF	*S. Typhimurium*	adzuki bean protein concentrates		using Flavourzyme enzyme			below 3000 Da	9	interaction with the phospholipids in the bacterial cell membrane, causing a rupture.	Toroidal-pore model
MBF	*S. aureus*	mung bean protein concentrates		using Flavourzyme enzyme			below 3000 Da		peptides with proline–proline being effective against S. Typhimurium and lysine being prominent against S. aureus → formation of pores in the bacterial membrane	Toroidal-pore model
KTA / KTR	*S. aureus* strains, including MRSA	chickpea storage protein legumin hydrolysates		were not explicitly mentioned in the provided document		for kta is riktatwrlalrwlkl and for ktr is riktrtwrlalrwlkl		16	. electrostatic forces, insertion into the peptidoglycan layer, inhibition of peptidoglycan synthesis, binding to the negatively charged cell surface of Staphylococcus aureus, disruption of the cell wall and membrane, and interaction with amino acid residues in the peptidoglycan synthetase PBP2a.	Barrel-stave model
Pediocin PA-1	anti-listerial	pediococci and lactobacilli species			As a part of its antimicrobial defense strategy, bacteria produce Pediocin PA-1 during fermentation				Within the lipid layer of the plasma membrane, the hydrophilic structure acts as a hinge, Integration of the β-sheet region with the hydrophobic alpha-helix structure. probability of interaction of peptide with the mannose phosphotransferase system	Toroidal-pore model
β-purothionin, Defensin-2, Purothionin-α2	*Puccinia triticina*	various plant species	use of heterologous expression systems						Protein synthesis is inhibited, cell membrane disruption occurs, and interference with cell wall synthesis occurs.	toroidal-pore model

Cc-AFP1, which was isolated from Carum carvi, exhibits antifungal activity against Aspergillus species (66). The bagasse peptides NLWSNEINQDMAEF and VSNCL inhibit food pathogens, namely *Bacillus subtilis*, *Burkholderia cepacia*, and *Pseudomonas aeruginosa*, according to shotgun proteomics investigation (67).

5. Synthetic antimicrobial peptides against foodborne pathogen

Food-borne pathogens are effectively combated by the antimicrobial peptide HX-12C. Both Gram-positive and Gram-negative bacteria exhibit susceptibility to its potent antimicrobial activity HX-12C (68). The peptides W2R3V10R11 and K2W3V10R11 have demonstrated promise against food-pathogens and as antibiotic substitutes (69).

The structure–function correlation of antimicrobial peptides P4 and P5 was examined. Strong antibacterial and antifungal activity was demonstrated by peptides P4 and P5. Specifically, P4 and P5 exhibited robust activity against Pseudomonas aeruginosa (70). Using Solanum granuloso leprosum, the antimicrobial dipeptide IQ, which is composed of N-α-[Carbobenzyloxy]-Ile-Gln (Z-IQ), is synthesized enzymatically. In tests against different bacterial strains, IQ dipeptide revealed MIC values (71).

Membrane penetration assays, electron microscopy, and flow cytometry all support the molecular mechanism of RI12[K3W], an antibacterial dodecapeptide from pig containing Trp, binding to LPS components, increasing membrane permeability, and inflicting membrane damage (72). One study involving the construction of peptides with thermostability, pH stability, and antibacterial activity against multiple bacterial foodborne pathogens found that the two distinct functions attributed to different peptide segments—membrane destabilization and intracellular trypsin inhibition—were responsible for the antibacterial effects of peptide (73). Peptide derivatives (A5, A6, A9, and A11) were synthesized from acidocin J1132β by truncating and substituting amino acids. A11 exhibited the best safety profile and the highest antimicrobial activity among them, particularly against *S. Typhimurium*. In environments mimicking negative charges, A11 tended to form an α-helix structure. The inhibitory effects of A11 were sustained at 100°C. The monophasic variant strains of *S. Typhimurium* and drug-resistant strains were inhibited by it (74). Through a series of enzymatic hydrolysis processes, bioactive peptides were engineered from mackerel byproducts and demonstrated antibacterial activity against gram-negative pathogens such as *E. coli, P. aeruginosa*, and *K. pneumoniae*. Additionally, they demonstrated antibacterial activity against *S. aureus* and other gram-positive bacteria (75).

6. Mechanism

Interference with the synthesis of proteins and nucleic acids, disruption of bacterial metabolism, interaction with particular intracellular targets, disruption of the formation of cellular building blocks (e.g. cellular wall), and suppression of enzyme function are some of the methods used to suppress foodborne pathogens (76). Peptide interactions with bacterial cells begin with the attraction to the cell membrane. Positively charged amino acids in the peptide create electrostatic interactions with the negatively charged components of the bacterial membrane, such as phosphate groups in

lipopolysaccharides and lipoteichoic acids. Specific receptors are not necessary for these interactions to occur (76). Peptides enter gram-negative bacteria via hydrophobic interactions with the outer membrane, adopting conformations that form peptide-membrane complexes. This disrupts the membrane, facilitating further peptide entry. Transport occurs through "self-promoted capitation," allowing peptides to reach the membrane's interfacial region, driven by hydrophobic interactions and electrostatic forces. The abundance of negatively charged lipids in bacterial cytoplasmic membranes is crucial for the selectivity of antimicrobial peptides, unlike in eukaryotic cells, which mainly have uncharged lipids (76). Nevertheless, different models have been put forth and there is still disagreement regarding the biomolecular events that take place at the membrane surface (e.g. "carpet," "toroidal pore," and "barrel-strip") (77). These models indicate an intermediary event leading to actions like membrane translocation, micellization, or channel formation. Thus, peptides can penetrate bacterial membranes without causing damage. Antibacterial peptides work via two mechanisms: at intracellular sites or directly interacting with the membrane. Research shows some peptides disrupt crucial intracellular targets by crossing the membrane intact (76). Most research and theories remain untested in vivo. For example, using physicochemical models may overestimate membrane-permeating activity by ignoring important physiological factors like pH or salt. Thus, various test parameters must be considered to fully understand peptide and bacterial membrane interactions (76, 78). The amphipathic nature of antimicrobial peptides is key in membrane permeation. Peptides often adopt a linear shape upon interacting with membranes due to hydrophilic and hydrophobic group interactions. Thus, research should be conducted under conditions that accurately reflect in vivo systems (78). Peptide molecules with hydrophobic segments interact with bacterial membranes, while hydrophilic parts face the pore lumen or phospholipid heads. The pore formation by α-helical antimicrobial peptides is well understood, but the mechanisms of β-sheet peptides in pore formation remain unclear (76).

The structure of lipid bilayers is disrupted by the interaction of antimicrobial peptides with bacterial membranes, which in turn causes cell lysis. This is in contrast to the fairly slow enzyme inhibition process that underlies the traditional antibiotics' modes of action (79). Antimicrobial peptides are thought to function by combining hydrophobic and electrostatic interactions, according to various models (80). Nevertheless, the exact mechanisms through which peptides penetrate bacterial membranes and exert their antimicrobial effects remain unclear, leading to the proposal of several models to explain how peptides interact with lipid bilayers (78). For α-helical peptides, two basic models have been proposed: "carpet-like" and barrel-stave (81).

The Shai-Matsuzaki-Huang (SMH) model explains how cholesterol molecules at the phospholipid membranes influence the biological activity of peptides and is another model that characterizes the method of interaction for most antimicrobial peptides (82). In recent times, there has been evidence to suggest that the "detergent" or "detergent-like" model of action is more comprehensive, encompassing a multitude of antimicrobial peptides (78). Few peptides utilize the barrel-forming mechanism. Helical peptides bind to the

lipid bilayer, with cationic domains facing the phosphate groups. At a certain concentration, peptide monomers aggregate and insert perpendicularly into the lipid bilayer, creating linear pores. The hydrophobic regions attach to nonpolar membrane areas, while hydrophilic regions expose channels for ions and small molecules to pass through (81). The quantity of monomeric peptide at the membrane can have a significant impact on pore size (83). The "carpet-like" model explains how peptide molecules align parallel to the lipid bilayer, forming a covering. Hydrophilic regions attach to phospholipid phosphate groups, while hydrophobic side chains enter the bilayer. This rearrangement of lipids alters membrane curvature. Increased peptide amounts correlate with deformation, leading to membrane breakdown (80). The barrel-stave model suggests smaller peptides can't form pores, while the SMH model applies to most antimicrobial peptides.

Pore formation and surface interactions together explain the SMH model, as peptides displace phospholipids similarly to the "carpet-like" model, leading to membrane disorder (84). Peptide molecules may be able to enter the target cell in certain instances due to the lipid bilayer structure is partially disrupted by this disorder. As a result, the SMH model suggests various ways that antimicrobial peptides work, i.e. interference with intracellular targets or disruption of the lipid bilayer (85). The detergent model, based on peptide intercalation in lipid bilayers, better explains amphipathic molecule behavior. Detergent interactions with lipid membranes create a complex micellar state above the critical micelle concentration (CMC). Antimicrobial peptides can also form oligomeric structures, interacting with lipid membranes differently than their monomers (78). These peptide oligomers can still form micelle aggregations on lipid bilayers after interacting with the membrane and adsorbing phospholipids. Antimicrobial peptides can destabilize cell membranes, while detergents do not affect membrane structures at low concentrations, below their CMC (86). The molecule's aggregation is key to the detergent model, and each peptide must be assessed individually with regard to factors like charge density and hydrophobicity. For example, different peptides show dimeric or tetrameric aggregation states (87), and δ-lisin exhibits larger states of aggregation (88). Aggregation states depend on specific concentration and pH levels. Peptide oligomers form in lipid bilayer domains via the detergent mechanism, differing from aggregates that form on the membrane surface. Recent studies show that aggregate formation within the phospholipid bilayer is thermodynamically favorable compared to in solution, enhancing understanding of detergent mechanisms (89). According to Yang et al. (90), Antimicrobial peptides contain cationic amino acids with a net positive charge (+2 to +9) and abundant hydrophobic amino acids such as phenylalanine and leucine. This positive charge helps them accumulate on negatively charged phospholipids in pathogen membranes, leading to the formation of holes or channels (91). It is therefore possible to hypothesize that raising antimicrobial peptides' net positive charge will improve the peptide-microbiome electrostatic interaction, which will raise the antimicrobial activity (92). As the strongest bidentate hydrogen bonding amino acid, Arginine (Arg), one of the three cationic amino acids, helps

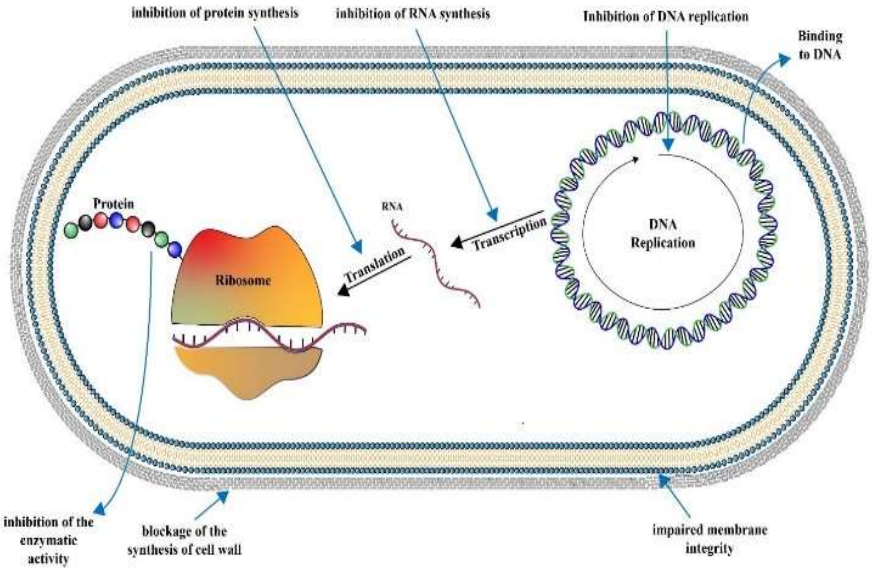

Figure 1. antipathogen activity mechanism of BAPs

antibacterial peptides enter microbial membranes, and enhances their ability to disrupt membranes. Nonetheless it has a slightly lower isoelectric point than Arg, lysine (Lys) is more biocompatible (93). by substituting arginine (Arg) for isoleucine (Ile) and valine (Val), Michailidis et al. (94) showed that β-defensin-8 peptides were more effective against E. coli, but replacing Val and Ile with negatively charged aspartic acid reduced activity. The antimicrobial properties of peptides relate to their hydrophobicity, defined by the content of hydrophobic amino acids. Tan et al. analyzed numerous antimicrobial peptides, and Peng Tan found that 40 to 60 percent of all amino acids are hydrophobic. (93).

4. Conclusion

It is crucial to comprehend the mechanisms underlying BAPs activity in order to facilitate successful industry applications. With a deeper understanding of both macro- and micro-level aspects, researchers can develop more effective methods to investigate and assess the potential of BAPs. While still in its early stages, the data presented here may help in selecting molecules based on their intended applications, designing more effective peptides from scratch, or even overcoming target microorganisms' resistance mechanisms. Numerous studies on synthetic BAPs are currently underway, and as the mechanisms are better understood, these engineered peptides will soon be optimized. Although the binding, action, and resistance mechanisms of BAPs remain largely unproven and unclear, ongoing research and new methodologies will

soon enable the precise identification of BAPs' mechanisms of action. Given that many of these molecules interact with one another and that the antimicrobial effect is influenced by a number of other complementary factors, it is crucial to remember that knowledge of the invitro mechanisms serves as the foundation for the application of the isolated BAPs. Within BAPs studies, the phenomenon of synergistic effects between different peptides is peptides has gained increasing attention in BAP research and holds considerable promise. In summary, BAPs' molecular mechanisms of action are frequently disregarded despite their importance. It is necessary to conduct fundamental research on the mechanisms that underlie the action of BAPs.

Funding

The authors declare that no financial support was received for the conduct of the research and/or preparation of the article.

Authorship contribution

MN: Original writing, Main text preparation, Supervising. NSh: Conceptualization, Finalizing

Declaration of competing interest

The authors do not have any competing interests to report.

Data availability

Data are available in the manuscript and will be available on demand.

Acknowledgements

The authors gratefully acknowledge the support of the Tehran University of Medical Sciences in publishing this review.

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