Computer Simulation Characterisation of the Coral Antimicrobial Peptide AmAMP1 PT07

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Abstract. To investigate the biological functions of the antimicrobial peptide AmAMP1 PT07 isolated from coral, bioinformatics tools were employed to predict and analyse its physicochemical properties, structural features, amphiphilicity, modification sites and biosafety. The results indicate that the coral antimicrobial peptide AmAMP1 PT07 is a cationic antimicrobial peptide comprising 43 amino acids. Its secondary structure consists of α-helices (53.49%), unfolded regions (41.86%) and β-sheets (4.65%). It has a theoretical isoelectric point of 11.09, lacks transmembrane domains and a signal peptide, and is classified as an intracellular protein. It contains one phosphorylation site, is susceptible to cleavage by 18 proteases, exhibits no protein toxicity, and demonstrates high stability, hydrophilicity and biosafety. This indicates that AmAMP1 PT07 possesses good stability and biosafety, making it suitable for development as a novel biological preservative with potential application value.

1. Introduction

As consumer attitudes evolve, natural and organic foods are becoming increasingly popular, whilst the use of traditional chemical preservatives is facing growing restrictions due to potential health risks and concerns regarding antibiotic resistance [1]. Antimicrobial peptides offer advantages such as broad-spectrum antimicrobial activity, rapid bactericidal action and a low likelihood of resistance development, and are therefore expected to emerge as a new type of green preservative. However, natural antimicrobial peptides suffer from various drawbacks, including poor stability, complex extraction and purification processes, and cytotoxicity [2]. Consequently, the use of bioinformatics tools to predict and analyse the properties of antimicrobial peptides provides a theoretical foundation for optimising and modifying the sequences of natural antimicrobial peptides and for heterologous expression, thereby helping to improve the safety and expression yield of these peptides.

As key members of the phylum Cnidaria, corals occupy a central position in marine ecosystems. Their unique living environment and immune mechanisms facilitate the production of bioactive substances—antimicrobial peptides—within their bodies, thereby enabling them to defend against pathogen attacks [3]. The coral antimicrobial peptide AmAMP1 PT07 is a cationic antimicrobial peptide comprising 43 amino acids and exhibiting broad-spectrum antimicrobial activity; however, its key characteristics—including structure, physicochemical properties, stability and biosafety—remain unclear, and these data form the core basis for assessing its stability and safety as a food preservative.

This study employs a combination of bioinformatics tools to conduct predictive analyses of the physicochemical properties, structural information, stability and biosafety of AmAMP1 PT07. It systematically evaluates the potential of this peptide as a novel biological preservative, providing a theoretical basis for its structural optimization, heterologous expression and large-scale production.

2 Materials

The amino acid sequence of coral antimicrobial peptide AmAMP1 PT07 is:

GTCIDLGSRLYCKLIRRRGMCRSRSHRARIAM MRCERSCGRCH (43), from http://aps.unmc.edu/AP/.

3 Method

3.1 Bioinformatics analysis of coral antimicrobial peptide AmAMP1 PT07

Bioinformatics software was used to analyse the physicochemical properties, structure and intracellular localisation signal peptide of the coral antimicrobial peptide AmAMP1 PT07.

(1) Prediction of physicochemical properties: The physicochemical properties of AmAMP1 PT07 were predicted using ProParam (https://web.expasy.org/protparam/), including: theoretical molecular weight, isoelectric point (PI), instability index, lipophilicity index, hydrophilicity index, and half-life in different organisms.

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(2) Structure prediction: Using SOPMA (https://npsa.lyon.inserm.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) and Swiss Model (https://webs.iiitd.edu.in/raghava/eippred), to predict the secondary and tertiary structures of AmAMP1 PT07, further illustrating the composition of local secondary structural elements and the overall three-dimensional conformation of the protein.

(3) Prediction of subcellular localisation: Preliminary predictions of the transmembrane domains of AmAMP1 PT07 were performed using DeepTMHMM (DeepTMHMM 1.0 - DTU Health Tech - Bioinformatic Services), whilst the intracellular localisation of AmAMP1 PT07 was preliminarily predicted using the Molecular Bioinformatics Centre (NetWheels: Peptides Helical Wheel and Net projections maker) laying a theoretical foundation for investigating its mechanism of action.

(4) Prediction of signal peptides and restriction sites: The signal peptide and restriction sites of AmAMP1 PT07 were predicted using SignalP 6.0 (https://services.healthtech.dtu.dk/services/SignalP-6.0/). This allowed for a preliminary assessment of whether the protein possesses a secretory signal and facilitated the selection of suitable restriction enzymes, thereby providing a theoretical basis for the subsequent construction of recombinant plasmids.

(5) Glycosylation and phosphorylation site prediction: Firstly, the O- and N-glycosylation sites of AmAMP1 PT07 were predicted using NetOGlyc (https://services.healthtech.dtu.dk/services/NetOGlyc-4.0/), and NetPhos (NetPhos 3.1 - DTU Health Tech - Bioinformatic Services), to predict the O- and N-glycosylation sites of AmAMP1 PT07, respectively, to further analyse the post-translational modification sites of the protein.

(6) Amphiphilicity prediction: The amphiphilicity of AmAMP1 PT07 was preliminarily assessed using ProtScale (Expasy - ProtScale), whilst NetWheels (NetWheels: Peptides Helical Wheel and Net projections maker) was employed to predict the arrangement and specific locations of amino acid residues within AmAMP1 PT07.

(7) Biosafety prediction and analysis: Predict the cytotoxicity of AmAMP1 PT07 using ToxinPred (ToxinPred) to conduct a preliminary assessment of its biosafety.

4 Results and discussion

4.1 Analysis of amino acid composition and physical and chemical properties

Antimicrobial peptides generally consist of 10–60 amino acids, with hydrophilic amino acids distributed at the N-terminus and hydrophobic amino acids at the C-terminus [4]. The composition of amino acids not only influences the spatial structure of the protein but also has a certain impact on the biological activity of antimicrobial peptides [5]. Analysis of AmAMP1 PT07 using ProParam software yielded the results shown in Tables 1 and 2. The molecular formula of this antimicrobial peptide is $C_{20}H_{353}N_{81}O_{54}S_9$; it comprises 43 amino acids from 14 different types, with a molecular weight of approximately 5057.06 Da. Generally, proteins with lower molecular weights exhibit greater protease stability [6]. The theoretical isoelectric point of AmAMP1 PT07 is 11.09, indicating that it carries a net positive charge, which is closely related to the abundance of arginine (Arg) and lysine (Lys) in its amino acid composition. Within a certain range, the greater the number of positive charges carried by an antimicrobial peptide, the stronger its electrostatic interactions, and its antimicrobial activity generally increases accordingly; these positive charges enable the antimicrobial peptide to selectively bind to the negatively charged surface of bacterial cell membranes, disrupting the integrity of the cell membrane structure and preventing adverse effects on mammalian cells whilst exerting its action [7]. The instability coefficient of AmAMP1 PT07 is 82.84, which is greater than the threshold of 40, indicating that it is relatively unstable in its free state. However, AmAMP1 PT07 is rich in cysteine, which facilitates the formation of disulphide bonds; under identical conditions, this allows it to maintain a more stable spatial conformation, enhancing the structural stability of the antimicrobial peptide and enabling it to retain good antimicrobial activity in various environments [8]. AmAMP1 PT07 has a lipophilicity index of 59.07, indicating that it possesses strong thermal stability and is unlikely to undergo structural changes due to high temperatures that could impair its antimicrobial activity [9]. The half-life of AmAMP1 PT07 in mammalian cells is approximately 30 hours, in yeast cells it exceeds 20 hours, and in E. coli it exceeds 10 hours, indicating strong protease stability across different biological environments and a capacity to resist proteolytic degradation [10]. Therefore, although the free form of AmAMP1 PT07 has a high instability index, its lipophilicity index and half-life predictions suggest that the peptide exhibits good stability in practical applications, particularly once disulphide bonds have formed. The average hydrophilicity coefficient of AmAMP1 PT07 is –0.567, indicating that it is a hydrophilic peptide. In summary, AmAMP1 PT07 exhibits strong cationic properties, stability and hydrophilicity, suggesting that AmAMP1 PT07 may interact with negatively charged cell membranes.

Table 1. Amino acid composition of coral antimicrobial peptide AmAMP1 PT07.

Amino acid	Count	Percentage (%)
Alanine(Ala,A)	2	4.7
Arginine(Arg,R)	11	25.6
Aspartic acid(Asp,D)	1	2.3
Cysteine(Cys,C)	6	14.0
Glutamic acid(Glu,E)	1	2.3
Glycine(Gly,G)	4	9.3
Histidine (His,H)	2	4.7
Isoleucine (Ile, I)	3	7.0
Leucine (Leu,L)	3	7.0
Methionine(Met,M)	3	7.0
Serine(Ser,S)	4	9.3

Threonine(Thr,T)	1	2.3
Tyrosine(Tyr,Y)	1	2.3
Lysine(Lys,K)	1	2.3

Table 2. Prediction results of physicochemical properties of coral antimicrobial peptide AmAMP1 PT07.

Item	Result
Number of amino acids	43
Molecular weight (Da)	5057.06
Isoelectric point (pI)	11.09
Coding sequence (CDS) (bp)	129
Number of positive charges (Arg + Lys)	12
Number of negative charges (Asp + Glu)	2
Half-life in mammalian cells (h)	30
Half-life in yeast (h)	>20
Half-life in E. coli (h)	>10
Instability index	82.84
Aliphatic index	59.07
GRAVY (hydropathicity)	-0.567

4.2 Structural prediction

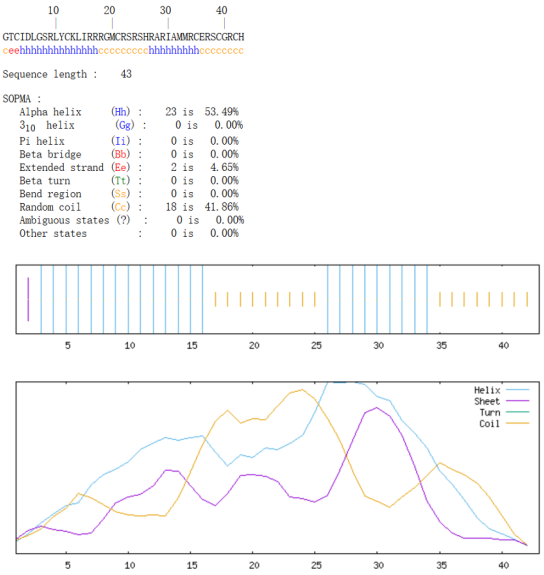

Figure 1. Prediction results of secondary structure of Antimicrobial Peptide AmAMP1 PT07. H, blue line. α-helix structure; C, yellow line. irregular curling; S, purple line. β - Folding structure.

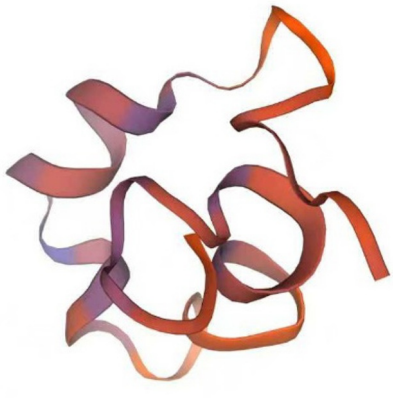

Figure 2. Prediction results of tertiary structure of Antimicrobial Peptide AmAMP1 PT07.

Antimicrobial peptides exhibit a wide variety of structural types, which are closely correlated with their antimicrobial activity. According to predictions made using the SOPMA software, the secondary structure of AmAMP1 PT07 consists primarily of α-helices (53.49%) and random coils (41.86%), with a small proportion of β-sheets (4.65%), as shown in Figure 1. Tertiary structure prediction provides a more accurate understanding of the intuitive characteristics of the antimicrobial peptide's structure. The tertiary structure of AmAMP1 PT07 was predicted using homology modelling techniques in the Swiss-Mold software, with the results shown in Figure 2. Consequently, the tertiary structure of this antimicrobial peptide is primarily centred on α-helices and random coils, with a small amount of β-sheets, consistent with the secondary structure prediction results. AmAMP1 PT07, with its predominant α-helical structure, is capable of forming an amphiphilic conformation, enabling it to readily insert into the interior of the phospholipid bilayer and exert its antimicrobial activity via a membrane-mediated mechanism [11]; this also helps to enhance the selectivity of AmAMP1 PT07, enabling it to preferentially inhibit bacterial growth whilst having minimal impact on the integrity of mammalian cell membranes, thereby demonstrating good biosafety [12]; the α-helix and β-sheet structures confer high thermal stability upon AmAMP1 PT07, making it resistant to proteolytic degradation under physiological conditions and exhibiting strong tolerance to high temperatures and various proteases, consistent with the predicted physicochemical properties [13].

4.3 Amphiphilia prediction

The amphiphilicity of a protein is determined by its hydrophilic and hydrophobic properties, manifested specifically by a relative equilibrium between hydrophilic and hydrophobic residues in the protein structure [14]. Amphiphilicity is one of the key characteristics underlying the interaction between antimicrobial peptides and the lipid bilayer of target cell membranes [15]. When the amphiphilicity of an antimicrobial peptide is significantly enhanced, its antimicrobial activity and its ability to damage mammalian cells increase simultaneously, leading to enhanced haemolytic activity and a substantial reduction in biosafety [16]. Hydrophobic amino acids facilitate insertion into the phospholipid bilayer of the bacterial cell membrane, whilst hydrophilic amino acids bind to the negatively charged phospholipids on the cell membrane. Through electrostatic interactions, they efficiently disrupt the integrity of the bacterial membrane, leading to the leakage of cellular contents and bacterial death [17]. Firstly, as shown in Figure 3, the ProtScale prediction results indicate that the coral antimicrobial peptide AmAMP1 PT07 possesses a typical amphiphilic structure, yet exhibits an overall hydrophilic nature. Among the amino acids, arginine has the highest hydrophilicity score, whilst isoleucine has the highest hydrophobicity score; however, as arginine occurs most frequently, AmAMP1 PT07 exhibits an

overall hydrophilic nature, consistent with the physicochemical property predictions. Secondly, the HelixWheel prediction can analyse the arrangement of hydrophobic and hydrophilic amino acids in the peptide, thereby clearly illustrating the distribution of amino acids across the polar and non-polar regions of the sequence. As shown in Figure 4, AmAMP1 PT07 exhibits typical amphiphilic structural characteristics, with a high proportion of hydrophilic amino acids distributed on both sides of the hydrophobic amino acids. This is consistent with previous predictions of its amphiphilic nature and is crucial for maintaining the structural stability of AmAMP1 PT07 and the overall function of the protein.

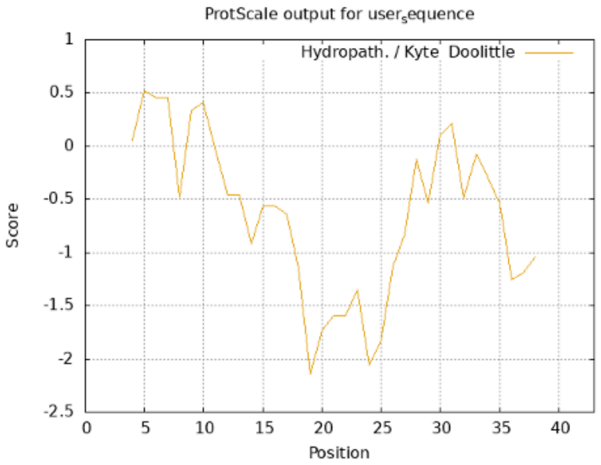

Figure 3. Prediction results of amphiphilism of Antimicrobial Peptide AmAMP1 PT07.

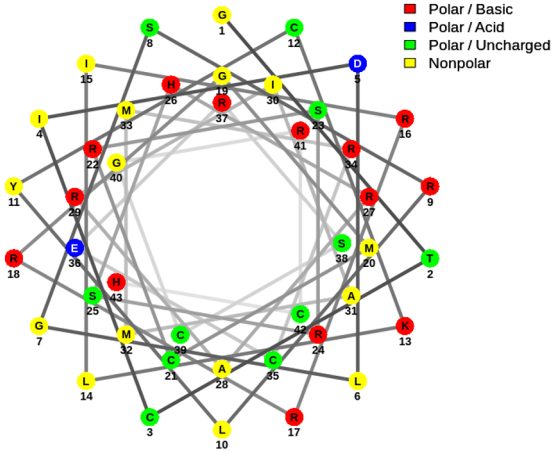

Figure 4. Prediction results of Antimicrobial Peptide AmAMP1 PT07.

4.4 Prediction of enzyme cleavage sites

The prediction of enzyme cleavage sites helps to elucidate the processing mechanisms of proteins and provides precise cleavage site sequences for the recombinant expression of proteins, thereby yielding mature and highly active peptides. As shown in Table 3, there are 18 proteases capable of cleaving the coral antimicrobial peptide AmAMP1 PT07, with each protease possessing at least one cleavage site

Table 3. Digestion site analysis results of coral antimicrobial peptide AmAMP1 PT07.

Name of enzyme	No. of cleavages	Positions of cleavage sites
Arg-C proteinase	11	9 16 17 18 22 24 27 29 34 37 41
Asp-N endopeptidase	1	4
Asp-N endopeptidase + N-terminal Glu	2	4 35
CNBr	3	20 32 33
Chymotrypsin-high specificity (C-term to [FYW], not before P)	1	11
Chymotrypsin-low specificity (C-term to [FYWML], not before P)	9	6 10 11 14 20 26 32 33 43
Clostripain	11	9 16 17 18 22 24 27 29 34 37 41
Formic acid	1	5
Glutamyl endopeptidase	1	36
LysC	1	13
LysN	1	12
NTCB (2-nitro-5-thiocyanobenzoic acid)	6	2 11 20 34 38 41
Pepsin (pH1.3)	5	5 6 10 13 14
Pepsin (pH>2)	5	5 6 10 13 14
Proteinase K	11	2 4 6 10 11 14 15 28 30 31 36
Thermolysin	10	3 9 13 14 19 27 29 30 31 32
Trypsin	11	9 13 16 18 22 24 27 29 34 37 41
Staphylococcal peptidase I	1	36

4.5 Stability prediction

Following phosphorylation or glycosylation, a protein's spatial conformation and structural stability may change, which in turn can affect physiological activities such as cellular localisation, metabolic activity and intracellular transport [18]. The results of this study indicate that the coral antimicrobial peptide AmAMP1 PT07 lacks O-glycosylation and N-glycosylation modifications, but may possess one potential phosphorylation site (>0.4), located at the serine residue at position 38, which is crucial for enhancing its structural stability and regulating its biological activity. Furthermore, the type of amino acids, their charge, structural characteristics and amphiphilicity are all key factors determining stability; good stability facilitates the consistent exertion of antimicrobial activity under varying temperatures, pH levels and proteolytic conditions. Various predictive results indicate that the coral antimicrobial peptide AmAMP1 PT07 is a relatively stable antimicrobial peptide, demonstrating good tolerance in practical applications and the ability to adapt to various environmental changes.

4.6 Prediction of transmembrane regions and signal peptides

Predicting the structure of transmembrane regions allows for a preliminary analysis of a protein's distribution within the cell. As membrane proteins are difficult to express in prokaryotic expression systems, if the

prediction indicates that the protein sequence contains transmembrane domains, a eukaryotic expression system should be selected for heterologous expression; if a prokaryotic expression system is nevertheless chosen, the transmembrane sequences must be excised to improve expression efficiency [19]. The results of this study indicate that AmAMP1 PT07 lacks transmembrane domains and is localised within the cytoplasm; consequently, its heterologous expression is not restricted by prokaryotic expression systems. Signal peptide prediction can further analyse whether a polypeptide enters the secretory pathway; fragments containing a signal peptide will traverse the cell membrane after synthesis and enter tissue cells [20]. Signal peptide prediction aids in the analysis of protein functional domains and cellular localisation. The results of this study indicate that AmAMP1 PT07 lacks a signal peptide sequence; therefore, should recombinant expression studies be required, a signal peptide sequence should be introduced to enhance expression levels.

4.7 Prediction of biosafety

Natural antimicrobial peptides are typically cytotoxic, which limits their application in the pharmaceutical and food sectors [21]. ToxinPred predicts that AmAMP1 PT07 is a non-toxic protein, indicating that it possesses good biosafety, thereby providing support for its further research and widespread application in the food sector.

5 Conclusions

The results of this study indicate that the coral antimicrobial peptide AmAMP1 PT07 is a cationic antimicrobial peptide comprising 43 amino acids, Its secondary structure comprises 53.49% α-helix, 41.86% random coil and 4.65% β-sheet; it has a theoretical isoelectric point of 11.09, lacks glycosylation sites and a signal peptide, possesses one phosphorylation site, contains no transmembrane domains, is located in the cytoplasm, and exhibits no cytotoxicity. Overall, AmAMP1 PT07 is a small-molecular-weight, hydrophilic cationic antimicrobial peptide suitable for heterologous expression, exhibiting good biosafety whilst maintaining high stability. The findings of this study provide a theoretical basis for optimising the sequence, recombinant expression and application of coral antimicrobial peptides, highlighting their potential for use in food preservation, aquaculture and medicine, and addressing existing issues such as drug residues and antibiotic resistance.

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