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Prediction of the best signal peptide for periplasmic expression of melittin peptide in *Escherichia coli*

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A B S T R A C T

Periplasmic expression of antimicrobial peptides is one of the most important issues in cloning and protein expression systems. In the present study, bioinformatics methods were used to predict the best signal peptides for periplasmic expression of the melittin peptide in *Escherichia coli*. Therefore, the sequence of 12 signal peptides was prepared from signal peptide databases. In order to choose the best signal peptide, periplasmic expression, sub-cellular localization site, solubility, physical and chemical properties of signal peptides were analyzed by Signalp6, Psort, Protein-sol, PRED-TAT, and Portparam servers. Six of the 12 signal peptides passed the Signalp6 filter, and only two passed Psort. After examining the solubility of signal peptides and investigating their physical and chemical properties, it was determined that zraP has the most favorable characteristics. Finally, zraP could be considered the best signal peptide for the melittin expression. Our results can be used for periplasmic expression of melittin in *Escherichia coli* host.

Keywords: *Signal peptide, periplasmic expression, melittin, in silico.*

1 Introduction

Genetic engineering techniques have revolutionized veterinary medicine's diagnostic, therapeutic, and preventive strategies, offering unprecedented opportunities to enhance animal health and welfare (1). A severe concern to world health is antibactrial resistance, which makes many traditional medications useless against bacterial diseases. The overuse and misuse of antibiotics have accelerated the emergence of resistant strains, leading to increased morbidity, mortality, and healthcare costs worldwide (2-4).

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New approaches are needed to treat bacterial infections and reduce selective pressure (5).

Antimicrobial peptides (AMPs) are a potentially effective way to overcome antibiotic resistance. These small, naturally occurring compounds have a wide range of antibacterial activity and several modes of action, which reduces the likelihood of resistance evolution. AMPs are essential elements of the innate immune system in various organisms, acting as the first line of defense against pathogens (6). In addition, AMPs can stimulate cells to produce chemokines, stimulate angiogenesis, accelerate wound healing, and affect programmed cell death in multicellular organisms (7). During the past ten years, peptides have been recognized as a promising treatment for various diseases, such as cancer, diabetes, cardiovascular, and infectious diseases (8). Most antimicrobial peptides have a positive charge and usually consist of 12 to 50 amino acids, whose positive charge is mainly due to the presence of basic amino acids such as lysine and arginine (9-11).

Bee venom contains various bioactive compounds such as melittin, apamin, adolapin, phospholipases A2 and B, hyaluronidase, serotonin, histamine, dopamine, and noradrenaline (12). Melittin is a basic 26-amino acid polypeptide weighing 2840 daltons, which makes up 40–60% of bee venom. Melittin has various biological and pharmacological effects, including strong surface activity on cell lipid membranes and antibacterial, antifungal, and antitumor activities. Further, melittin is known as a natural pore-forming peptide that can insert itself into phospholipid bilayers (13, 14). Currently, melittin is being studied to develop new therapeutic agents with specific actions against antibiotic-resistant pathogens (15).

In order to produce antimicrobial peptides through genetic engineering methods, it is very important to design an appropriate signal peptide for periplasmic expression (16, 17). Signal peptide is a very important component in genetic engineering and should be able to cause peptide secretion at high levels. This study uses bioinformatics approaches to predict the most suitable signal peptide for the expression and secretion of melittin in *Escherichia coli* bacteria.

2 Materials and Methods

2.1 Melittin sequence

The amino acid sequence of melittin (GIGAVLKVLTTGLPALISWIKRKRQQ) was obtained from a study by Guha et al. (18).

2.2 Collection of signal peptides

The signal peptide database was used to obtain signal peptide sequences. It should be noted that the indicators selected from the database were used to validate the signal peptide for expression in *Escherichia coli*. In other words, signal peptides were selected based on experiment studies. Then, a comprehensive study was performed on 12 signal peptide sequences, focusing on their molecular and physicochemical properties using online servers.

2.3 Prediction of an appropriate signal peptide

Initially, the SignalP6 server was used to analyze 12 selected signal peptides considered to be expressed in *Escherichia coli*. This analysis aimed to determine the n, h, and c cutoff regions for the peptide signal. Overall, this web-based server predicts the presence of signal peptides and identifies their cleavage sites in gram-positive and gram-negative bacteria, archaea, and eukaryotes.

Since this research aimed to identify the best signal peptides for the expression of melittin in the periplasm, the active site of the peptide was evaluated. Several algorithms have been designed to predict the active sites of peptides, including PSORT, CELLO, Gneg-PLoc, and ProtComp servers (19). Psort server was used with acceptable accuracy. Regarding the proteins in the gram-positive bacterial system, three main routes have been identified: the cytoplasmic, membrane, and extracellular compartments. In contrast, five distinct regions for proteins found in gram-negative bacteria include the cytoplasmic, membrane (outer and inner), periplasmic, and extracellular (secreted) compartments.

In the next step, the solubility of signal peptides was evaluated using the online software Protein-sol (http://protein-sol.manchester.ac.uk). Protein-Sol is an open-access web server that provides predicted solubility on a scale of zero to one, simplifying the interpretation of results (20). Then, unstable and insoluble proteins were removed. The PRED-TAT server was used to classify signal peptides based on their secretion characteristics. Portparam server was used to predict physical and chemical properties related to amino acid composition, molecular weight, isoelectric point (PI), instability index, aliphatic index, charged amino acids, and average hydrophilicity values (GRAVY) of signal peptides (21).

3 Results

As shown in Table 1, out of 12 signal peptides evaluated using SignalP6 server, only six cases were cut in the desired area with acceptable accuracy with a probability higher than 75% and then selected for the next step. Among several algorithms that exist for predicting the subcellular location of proteins, such as PSORTb, CELLO, Gneg-PLoc, and ProtComp (19), it has been reported that PSORTb has the highest accuracy. Out of the six signal peptides entered into the PSORTb server, only two (zraP and sufI) had favorable features for expression in the periplasmic region (Table 1). In the next step, the solubility value for both signal peptides were predicted to be greater than 0.45, which is acceptable.

Table 1. The result of signal peptides evaluation using signalp6, Psort, and protein Sol servers. Source of all signal peptides is E.coli.

N.	Protein	Signal peptide	Sequence	Cut site	Possibility (signalp6)	Periplasmic region (Psort)	Solubility (protein sol)
1	Protein sufI	sufI	MSLSRRQFIQASGIALCAGAVP LKASA	27 & 28	0.889670	6.53	0.676
2	Zinc resistance-associated protein	zraP	MKRNTKIALVMMALSAMAM GSTSAFA	26 & 27	0.783085	6.58	0.710
3	Outer-membrane lipoprotein carrier protein	LOLA	MKKIAITCALLSSLVASSVWA	21 & 22	0.850167	0.12	
4	Protein GltF	GltF	MFFKKNLTTAAICAALSVAAF SAMA	21 & 22	0.838638	0.12	
5	Threonine-rich inner membrane protein GfcA	GfcA	MKHKLSAILMAFMLTTPAAFA	22 & 23	0.934402	0.12	
6	Uncharacterized protein YpeC	YpeC	MFRSLFLAAALMAFTPLAANA	16 & 17	0.751500	0.12	
7	Maltose-binding periplasmic protein	malE	MKIKTGARILALSALTTMMFS ASALA	26 & 27	0.663157		
8	Protein prsK	prsK	MIKSTGALLLFAALSAGQAMA	21 & 22	0.486316		
9	Periplasmic appA protein	appA	MKAILIPFLSLLIPLTPQSAFA	22 & 23	0.676652		
10	Outer membrane protein N	OMPN	MKSKVLALLIPALLAAGAAHA	21 & 22	0.706237		
11	Outer membrane protease ompP	OMPP	MQTKLLAIMLAAPVVFSSQEA SA	23 & 24	0.714308		
12	Glucose-1-phosphatase	Agp	MFRSLFLAAALMAFTPLAANA	21 & 22	0.674981		

The results showed that both signal peptides attached to melittin can generate a soluble protein with a high confidence score. The prediction results for peptide signal secretion showed that both peptides belong to the type II protein secretion system. The results of examining the physical and chemical characteristics, molecular weight, PI, instability index, aliphatic index, and the overall hydropathic average (GRAVY) of the signal peptides were presented in Table 2.

Table 2. Physicochemical properties of the signal peptides.

N.	protein	Signal peptide	Protein secretion system	Molecular weight	PI	Gravity	Aliphatic index	stability	Half life
1	Protein sufI	sufI	TAT signal peptide (Tat/SPI)	5707.93	12.02	0.483	117.59	Unstable	>10h
2	Zinc resistance-associated protein	zraP	Signal Peptide (Sec/SPI)	5694.04	12.32	0.536	105.09	Stable	>10h

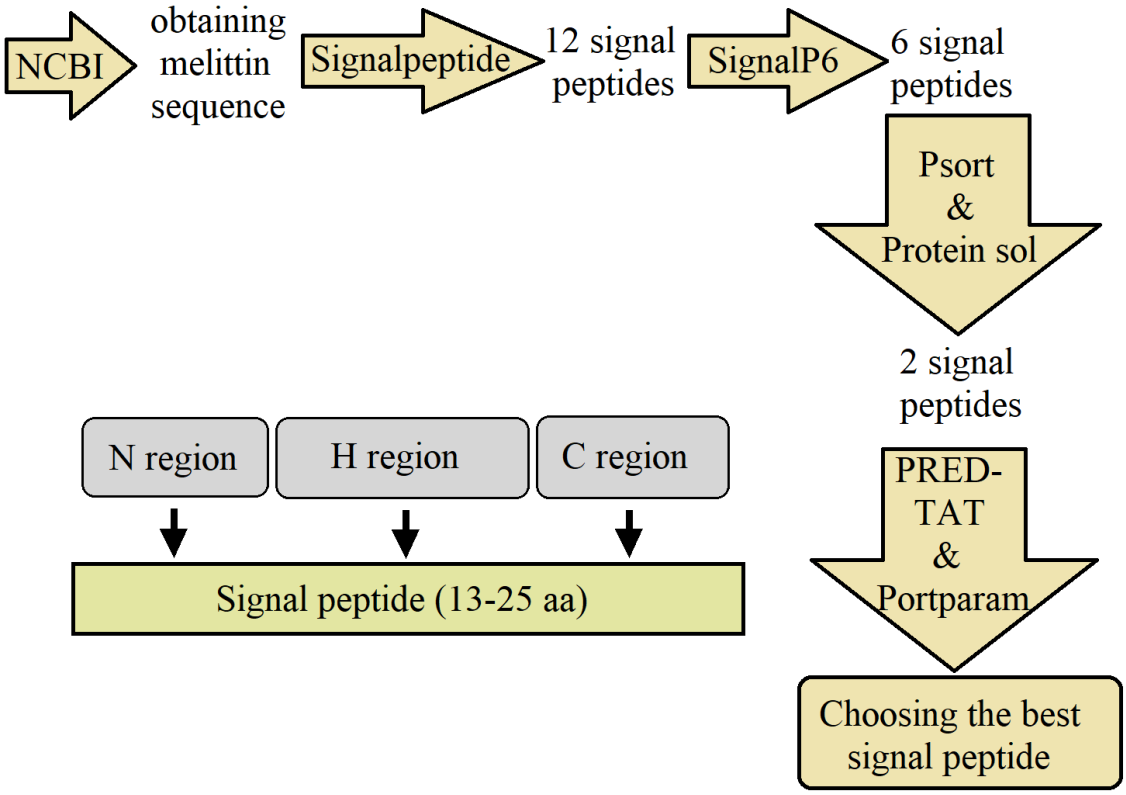

Figure 1. Physicochemical properties of the signal peptides.

4 Discussion

Genetic engineering is the term for various methods used to modify an organism's genome, from conventional selective breeding to advanced molecular tools. These approaches have been crucial in veterinary medicine in determining the pathophysiology of infectious diseases, determining the genetic basis of inherited disorders, and creating new treatments and vaccinations (22). Researchers conducted a study to select the best signal peptide for the expression of the antimicrobial peptide buforin I in *Escherichia coli*. These researchers showed that MalE, ptrA, and zraP signal peptides were suitable for expression of buforin I. In addition, all the selected signal peptides had proper n, h, and c regions except torA. In agreement with the results of the present study, sufI, ptrA, LOLA, GltF, GfcA, and YpeC obtained the required confidence score (21). With the development of technology and the creation of recombinant DNA techniques, the solubility of heterologous proteins became important.

The accumulation of expressed insoluble proteins and incorrect folding can lead to the formation of inclusion bodies and, on the other hand, reduce the efficiency of correctly folded proteins (23). To solve this problem, the expression of heterologous proteins in the periplasmic space of *Escherichia coli* is suggested (24). The transfer of protein to the periplasmic space leads to ease of purification because there is less protein. Other advantages of using the periplasmic expression system include preventing protease attack and N-terminal Met extension and having a more suitable protein folding (23).

In agreement with the present findings, Roshanak et al. worked on MdoD, YcdO, torA, MalE, hofQ, papK, ptrA, ugpB, zraP, sfmC, rbsB, efeO, and pbpG signal peptides and demonstrated that zraP is the best option for periplasmic expression in *E.coli* (21).

Various web servers have been generated to predict the solubility of proteins in *Escherichia coli* and (8) Protein sol server is one of the most accurate solubility prediction servers. The numerical value reported in the Protein sol server is between zero and one (20, 25).

As shown in Table 2, among the types of protein secretion systems (types I, II, and III), type II is the most commonly used. The type II system consists of three secretory pathways (SecB-dependent (Sec), signal recognition particle (SRP), and dual arginine transport (TAT) pathways) and is widely distributed among gram-negative bacteria (26).

During the present study, ProtParam, as part of EXPASY and the European Institute of Bioinformatics (EIB), was used to measure proteins' physical and chemical properties with high reliability (27).

The present study showed that the highest and lowest molecular weights (MW) were related to sufI (5707/93) and zraP (5694/04), respectively. In addition, the results showed that PI was in the range of 12 for both cases. Average overall hydropathy (GRAVY) was used to compare the hydropathy of signal peptides (28). The lowest GRAVY belongs to sufI (0.483), and the highest belongs to zraP (0.536). The aliphatic index, as the relative volume occupied by the aliphatic side chain in an amino acid sequence, is a positive factor for increasing the thermal stability of globular proteins. The changes in this index for the signal peptides studied, sufI and zraP, were 117.59 and 105.09, respectively. Generally, when the instability is greater than 40, the proteins are considered unstable, and in this study, sufI was reported to be unstable and zraP was reported to be stable (28). Therefore, our in silico analysis revealed that zraP could be considered a suitable signal peptide for expression of melittin in periplasmic compartment, which agrees with previous observation (21).

5 Conclusion

This study aimed to predict the most appropriate signal peptide for the periplasmic expression of melittin in *Escherichia coli*. After evaluating various signal peptides using different servers, the zraP was identified as the best signal peptide.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

All authors have contributed in writing, critically reviewed, and approved the final version of the manuscript.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

Ethical Considerations

There were no ethical considerations in this research.

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