Journal of Molecular Structure 1158 (2018) 14–18

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Effect of amino acid substitution on biological activity of cyanophlyctin-β and brevinin-2R

Adel Ghorani-Azam [a], Mahdi Balali-Mood [a], Ehsan Aryan [b], Gholamreza Karimi [c], Bamdad Riahi-Zanjani [a, *]

[a] Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Pharmaceutical Research Center, Pharmacy School, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article history:
Received 20 December 2017
Received in revised form
7 January 2018
Accepted 7 January 2018
Available online 8 January 2018

Keywords:
Antimicrobial peptides
Cyanophlyctin
Structural prediction
HPLC
Homology modeling

ABSTRACT

Antimicrobial peptides (AMPs), as ancient immune components, are found in almost all types of living organisms. They are bioactive components with strong antibacterial, antiviral, and anti-tumor properties. In this study, we designed three sequences of antimicrobial peptides to study the effects of structural changes in biological activity compared with original peptides, cyanophlyctin β, and brevinin-2R. For antibacterial activity, two Gram-positive (*Staphylococcus aureus* and *S. epidermidis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeroginosa*) were assayed. Unlike cyanophlyctin β and brevinin-2R, the synthesized peptide (brevinin-M1, brevinin-M2 and brevinin-M3) showed no considerable antibacterial properties. Hemolytic activity of these peptides was also ignorable even at very high concentrations of 2 mg/ml. However, after proteolytic digestion by trypsin, the peptides showed antibacterial activity comparable to their original template sequences. Structural prediction suggested that the motif sequence responsible for antibacterial activity may be re-exposed to bacterial cell membrane after proteolytic digestion. Also, findings showed that only a small change in primary sequence and therefore structure of peptides may result in a significant alteration in biological activity.

1. Introduction

Antimicrobial peptides (AMPs) are a group of naturally occurring peptides with wide ranges of biological activity [1,2]. AMPs are widely distributed in all forms of life and play important role in the immune system of mammalian, insects, plants, and bacteria [3]. These compounds may have a range of biological activities such as antimicrobial, antifungal, and antiviral properties [4–6]. These biologically active peptides that may have 7 to 50 amino acids in length are also involved in regulation of cellular processes such as apoptosis as well as regulation of endocrine and neuroendocrine system [7,8]. AMPs have important role in inducing apoptosis as well as cancer therapy in vitro.

Relationship between primary sequence and structure and function of proteins and peptides has always been discussed. It is long known that amino acid composition and primary sequence of proteins are responsible for their biological activity. It is now believed that information about target organism, mood of action and final subcellular localization of peptides and proteins are coded in their primary sequence [9,10]. Although numbers of peptides with variety of biological activity have been introduced in the recent years, yet it is not easy to introduce peptide motifs that can result in a specific biological activity. Hence, it would be valuable to consider the relationship between amino acid sequences with biological properties through their amino acid composition. Recently, scientists have tried to find association between amino acid compositions, amino acid n-tuples, peptide length, and number of charged amino acids with biological activity, cellular localization, and therefore possible mechanism of action [11,12]. In the recent years, scientists are much interested in using computational data and analysis of amino acid sequences to recognize the necessity of each amino acid in biological activity of peptides and proteins. These simulations may deepen the knowledge about interactions between peptides and cellular organelles especially cell membrane, and hence have advantage to design new peptides with enhanced biomedical values.

In this paper, we have focused on exploring the possible

* Corresponding author.
E-mail address: riahib@mums.ac.ir (B. Riahi-Zanjani).

https://doi.org/10.1016/j.molstruc.2018.01.015
0022-2860/

discriminative aptitude of natural peptide according to the primary sequences, amino acid composition, and their 2D and 3D modeled structure.

2. Experimental

2.1. Chemicals

Acetonitrile, trifluoroacetic acid, methanol HPLC grade and 2-mercaptoethanol were obtained commercially from Merck Company. Trypticase soy broth (TSB), Mueller Hinton Broth (MHB), and blood agar media cultures were purchased from HiMedia Leading BioSciences Company. C_{18} semi-preparative column was purchased from Macherey Nagel GmbH Co. (Düren, Germany). All other chemicals were of analytical grade.

2.2. Sequence design and peptide synthesis

For peptide design, cyanophlyctin, cyanophlyctin β, and brevinin-2R were used as template sequence. In this study, we investigated whether substitution of only a few amino acid residues can change the structure and therefore biological activity. For this purpose, 4 amino acids in the template (Brevinin-2R) were substituted with their counterparts or amino acids with different features [13]. Also, the S–S bound in the template peptide was transferred to the middle of the peptide chain. Since the synthesized peptides were the modified sequences of the brevinin-2R, they were named as brevinin-M1, brevinin-M2 and brevinin-M3. The sequences of templates and modified peptides are shown in Fig. 1. After sequence designing, the peptides were synthesized at Peptide Sciences CO. (Shanghai; China).

2.3. Antimicrobial activity assay

Antimicrobial activity of the peptides was assayed on two Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeroginosa*) bacteria according to the radial diffusion assay (RDA) [14]. For this purpose, 4×10^6 CFU bacteria were poured into 5 ml of 10 mM cold phosphate buffer and were mixed with Mueller-Hinton agar media culture (Sigma-Aldrich) and were then poured into the plates. Afterwards, the peptides were dissolved in PBS and poured into the previously punched wells of the plates, and incubated for 12 h at 37 °C. Antibacterial activity of the peptides was determined by measuring the mean radius zone of inhibitions in millimeter and defined as percent activity. For this purpose, bactericidal power of peptides were evaluated and compared to the standard streptomycin and penicillin antibiotics as positive control (Pattan Teb Company, Tehran, Iran). Each experiment was repeated three times.

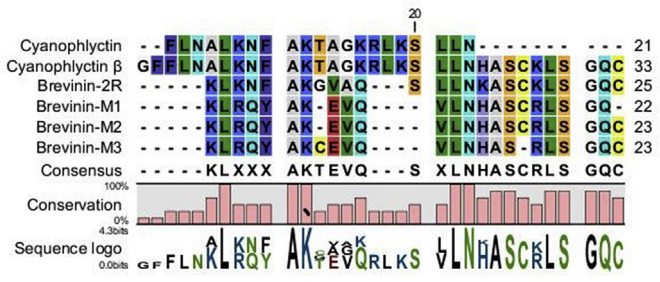

Fig. 1. Multiple alignment of the modified peptides with Brevinin-2R, Cyanophlyctin-β, and Cyanophlyctin as the template sequences.

2.4. Hematotoxicity assay

Hemolysis assay for the synthesized peptides was performed at the highest concentration of 2 mg/ml in phosphate-buffered saline (PBS) in which 20 μl of the peptide samples were poured into the punched wells in blood agar plate and incubated for 8 h at 35 °C. Deionized water was used as a positive control with 100% hemolytic activity.

2.5. Proteolytic digestion and RP-HPLC analysis

The proteolytic digestion of synthesized peptides was performed by trypsin (EC 3.4.21.4) at 37 °C for 6 h. For this purpose, the peptides were first incubated with 2-mercaptoethanol in 50 mM Tris buffer (pH 8) for 4 h. Afterwards, the trypsin was added to the solution with enzyme substrate ratio of 1/10 and incubated for additional 6 h. The solution was then lyophilized for subsequent experiment.

Reverse phase-HPLC was also used to confirm the peptide purity as well as the efficiency of enzymatic digestion, before and after proteolysis. For this purpose, the lyophilized samples were dissolved in 100 μl of 0.1% (v/v) Trifluoroacetic acid (TFA) in water, and 30 μl of the sample was loaded onto a C_{18} column (10 × 250 mm). Elution was performed using 0.1% TFA in water combined with a 10%–70% increasing gradient of 0.098% TFA in acetonitrile in a period of 45 min. The flow rate of elution was 2 ml/min.

2.6. Sequence and structural analysis

The peptides were aligned against all sequences in NCBI and the SWISS-PROT databases using blast program (http://www.ncbi.nlm.nih.gov/BLAST). CLC main workbench software was used for secondary structure prediction, creating hydrophobicity plot and determining possible binding sites of peptides on pathogenic organisms. ICM-Chemist-Pro 3.8 and ZMM were also used for prediction of tertiary structure.

3. Results and discussion

3.1. Antimicrobial activity

Antibacterial assay showed that unlike cyanophlyctin β, and brevinin-2R at 1 mg/ml of each, none of the modified peptides have considerable antimicrobial properties up to 5 mg/ml. After protein cleavage by trypsin, antimicrobial assay of digested peptides was performed and the results showed satisfactory antibacterial properties almost near to their template sequence of cyanophlyctin β and brevinin-2R at 1 mg/ml. Antimicrobial activity of the brevinin-2R, which had more antibacterial properties than other peptides and antibiotics, was considered as 100% activity and was compared to that of the modified peptides before and after enzymatic digestion. Findings showed that the digested peptides had relatively more bactericidal potency on gram positive than gram negative bacteria (Table 1).

3.2. Hemolysis assay

Hemolysis assay is a standard biological method to investigate cytotoxic effects of an agent on red blood cells. Hemolytic activity for cyanophlyctin β was 0.7% at 500 μg/ml, while no hemolytic activity was observed for brevinin-M1, brevinin-M2 and brevinin-M3 even at high dose of 2 mg/ml. Low hemolytic activity makes these peptides potential candidates for further studies in drug discovery, and targeted drug delivery.