Abstract

Antimicrobial peptides (AMP) are evolutionarily conserved components of the innate immune system. They have a broad spectrum of action against bacteria, fungi and viruses. Therefore, AMP are studied as probable substitutes of the traditional antibiotics, for which most pathogens have developed resistance.

The main objective of this work was the design of novel linear peptides capable to interact with the cellular membrane of the common pathogens.

In this work, sequences of active AMP were carefully obtained from the scientific literature and collected in Yadamp (http://yadamp.unisa.it/), a database of AMP created recently in the laboratory where this project was carried out. In Yadamp, there are information about peptides name, amino acid sequence, length, presence of disulfide bridges, date of discovery, activity and taxonomy. The most relevant chemical-physical properties are also listed. This database is mainly focused on the peptides activities. Experimental MIC values (the lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism) are constantly obtained from careful reading the original papers. In this work, a great contribution was made in the enrichment of the database. In fact, 1009 sequences were added to Yadamp. It currently contains 3142 AMP sequences. For these AMP, 573 molecular descriptors were calculated. In addition, this project also involved the search for new molecular descriptors. Yadamp is a resource for QSAR investigations on AMP. It allows to create subsets of AMP, homogeneous in one, two or more parameters. The working hypothesis was that AMP with similar chemical physical features can share the same mechanism of action. Therefore, during this work, genetic algorithms (GA), artificial neural networks (ANN) and classification analyses were performed on homogeneous subsets of AMP. AMP with activity against five different microorganisms were studied: *Staphylococcus aureus* and *Bacillus subtilis* (Gram + bacteria), *Escherichia coli* and *Pseudomonas aeruginosa* (Gram - bacteria), and *Candida albicans* (saprophytic fungus). Numerous prediction models of activity were obtained, each

of them validated through effective statistical techniques. These obtained models gave a preliminary idea of the probable mechanism of action that the studied AMP have. For example, the results suggest that the charge and the hydrophobicity of the amino acid residues are important factors for the binding of the AMP to the target membranes.

However, the descriptors 1D and 2D currently available fail to capture all of the peptides properties. The peptides are extremely flexible molecules and when they interact with the target membranes, they undergo conformational changes. Consequently, one of the goal of this project was also to find new molecular descriptors of AMP. For example, a new molecular docking software (www.yada.unisa.it) was developed in our laboratory. The idea was to use YADA to calculate the binding energy of the interaction between the AMP and other peptides, protein receptors and target membranes.

All the models obtained by computational studies were implemented in the "Yadamp predict" tool (http://yadamp.unisa.it/predict.aspx). It allows researchers to submit sequences of unknown molecules and to see if and to which organisms these molecules are potentially active.

In this work, 10000 amino acidic sequences were generated through a combinatorial calculation. The "Yadamp predict" tool allowed the prediction of the interaction between these peptides and the lipid membranes of specific pathogens. The results of the "Yadamp predict" tool suggested a specificity of three sequences toward Gram positive bacterial membranes. These peptides, called p458 (WMLKKFRWMF), p459 (KILGKLWKWVK) and p460 (KILKKIKKLLW), were synthesized for further analysis. Since the 3 peptides contained tryptophan, an aromatic amino acid with a maximum absorption and emission of 280 nm and ~ 360 nm, the peptides binding was monitored via spectrophotometric assays. This interaction was tested in vitro on unilamellar vesicles of 400 nm having different lipid composition. According to in silico studies, the fluorescence and absorbance results suggest that the three peptides predominantly bind Gram + bacteria. They probably bind the target membranes through a mechanism of action that does not depend only on electrostatic interaction, but also on structural changes that occur in the lipid membrane after

the binding process. Highlights on the mechanism of interaction were provided by all atoms molecular dynamics simulations (data not shown) carried out in the lab of Prof. Piotto.

All together, these findings support the proposed mechanism of action of the 3 peptides and pave the way for novel and more focused design of antimicrobial peptides.