ANÁLISIS DEL POTENCIAL DE DISRUPCIÓN DE MEMBRANAS Y ESTABILIDAD DE PÉPTIDOS CATIÓNICOS ANTIMICROBIANOS POR SIMULACIONES DE DINÁMICA MOLECULAR.

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Trabajo de grado presentado como requisito para optar al título de Biólogo.

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TÍTULO:
ANÁLISIS DEL POTENCIAL DE DISRUPCIÓN DE MEMBRANAS Y ESTABILIDAD DE PÉPTIDOS CATIÓNICOS ANTIMICROBIANOS POR SIMULACIONES DE DINÁMICA MOLECULAR.*

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PALABRAS CLAVES:
AMP, Péptidos antimicrobianos, Estabilidad, Estructura-3D, Dinámica Molecular

DESCRIPCIÓN:

Los péptidos catiónicos antimicrobianos, son una familia de proteínas altamente conservadas presentes en el sistema inmune de todos los organismos multicelulares que poseen un gran potencial como antibióticos de amplio-espectro gracias al bajo desarrollo de resistencia por parte de las bacterias. Se analizaron las estabilidades (radio de giro y desviación atómica estructural) de la estructura tridimensional de tres péptidos antimicrobianos (dos sintéticos y uno natural derivado del veneno de *Vespa magnifica*) reportados en la base de datos CAMP en solvatación bajo tres diferentes pH y temperaturas mediante simulaciones de dinámica molecular utilizando el paquete GROMACS. Los resultados obtenidos fueron comparados con el computo del índice de inestabilidad de proteínas propuesto por Guruprasad (1990), que evalúa la estabilidad de las proteínas en función de los dipéptidos que lo componen. Se encontró que uno de los péptidos sintéticos evaluados no forma una estructura tridimensional estable a ningún pH ni temperatura evaluado. Por tal motivo, no se espera una acción bactericida *per se*. Los otros dos péptidos mostraron una conformación de alfa-hélice bajo ciertas condiciones evaluadas. El péptido natural derivado del veneno de *Vespa magnifica* mostró ser más estable que los péptidos sintéticos. Adicionalmente, se encontró, que no existe correspondencia ni similitud entre el computo del índice de inestabilidad de proteínas propuesto por Guruprasad (1990) y los resultados del análisis de estabilidad evaluados por simulaciones de dinámica molecular.

*Trabajo de Grado

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ABSTRACT

TITLE:
ANALYSIS OF MEMBRANE POTENTIAL DISRUPTION AND CATIONIC ANTIMICROBIAL PEPTIDES STABILITY BY MOLECULAR DYNAMICS SIMULATIONS.*

AUTHOR:
DANIEL CAMILO OSORIO H.**

KEYWORDS:
AMP, Antimicrobial peptides, Stability, 3D-Structure, Molecular Dynamics

DESCRIPTION:

Cationic antimicrobial peptides are a family of highly homologous proteins conserved in all multicellular organisms with great potential as broad-spectrum antibiotics due the low resistance development by bacteria. In this paper, we analyze the stability (giration radii and RMSD) of the 3D structure of three antimicrobial (two syntetic and one natural derivated from the *Vespa magnifica* venom) peptides reported in CAMP database, working in solvation conditions at three pHs and three different temperaturas using molecular dynamics simulations. The results were compared with the computed instability index proposed by Guruprasad (1990) which evaluates the stability of proteins base don the dipeptides that compose. We found that one of the tested peptides did not form stable three-dimensional structure at any pH or temperature evaluated. For this reason, it is not expected a bactericidal action *per se*. The other peptides showed an alpha-helix conformation under certain conditions evaluated. Additionally it was found that the instability index proposed by Guruprasad (1990) evidenced no correspondence or similarity with stability analysis by molecular dynamics.

* Bachelor Thesis

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INTRODUCTION

Cationic antimicrobial peptides are a high conserved family of proteins having structural and physicochemical characteristics (charge, hydrophobicity, size, amino acid composition, etc.), which allow its antimicrobial activity (3). These biological molecules are currently subject of research due to its potential use as a new generation of broad-spectrum antibiotics, because they show a near zero rate of resistance by bacteria (5). These studies cover topics such as the design and processing of known peptide sequences, *in vitro* bioassay and *in vivo* analysis of the mechanism of action, 'docking' and interactions with molecular membrane by molecular dynamics. Due to the complexity in the formation and maintenance of the 3D structure of the peptides, which determines its antimicrobial activity (7), it is very important to predict conformation and folding of antimicrobial peptides. In this study, we analyzed the stability of the three dimensional structure of three antimicrobial peptides under solvation conditions. For this aim, we worked at both three pH values (5, 7 and 9) and temperatures (298K, 310K and 323K), carrying out simulations of peptides by molecular dynamics, and using as models two antimicrobial undecapeptides with sequences $KLKL_5K\text{-}NH_2$ and $RLKL_5RLK\text{-}NH_2$, which are derived from *sapecin* β reported by Alvares-Bravo et al. (2), and the peptide $FLPIPRPILLGLL\text{-}NH_2$ obtained by Xuequing et al. (9) from the venom of *Vespa magnifica* .

1 MATERIALS AND METHODS

Three-dimensional models of peptide structures were obtained through the 3D- JIGSAW (4) website portal by selecting the model with the lowest free energy value (Fig. 1). The molecular dynamics simulations were processed in GROMACS (6) 4.6 GPU-MPI-SP package using the OPLS force field-AA/L, configured under isothermal and isobaric conditions at salt concentrations (NaCl) of 0.13 mM. Depending on the pH to be evaluated in the computer simulation, the amino acids were protonated or deprotonated using the application *pdb2gmx*, which is included in the GROMACS package. We set three stages in the simulation: energy minimization to achieve a potential energy of 1000 (kJ/mol), the temperature stabilization (NVT) - pressure (NPT) and using a simulation time of 2ns. In order to assess the stability of the peptides, we evaluated for each simulation at the pH and temperature conditions studied, the root mean square deviation (RMSD) of the aligned proteins, and the progression of temperature, pressure and density in the simulated system. All simulations were render in *trjconv* program included in GROMACS package and visualized using VMD software and Radeon HD6750 graphic card.

2 RESULTS AND DISCUSSION

In Figure 1 are shown simulations of the three peptides stabilized at expected isobaric and isothermal configurations.

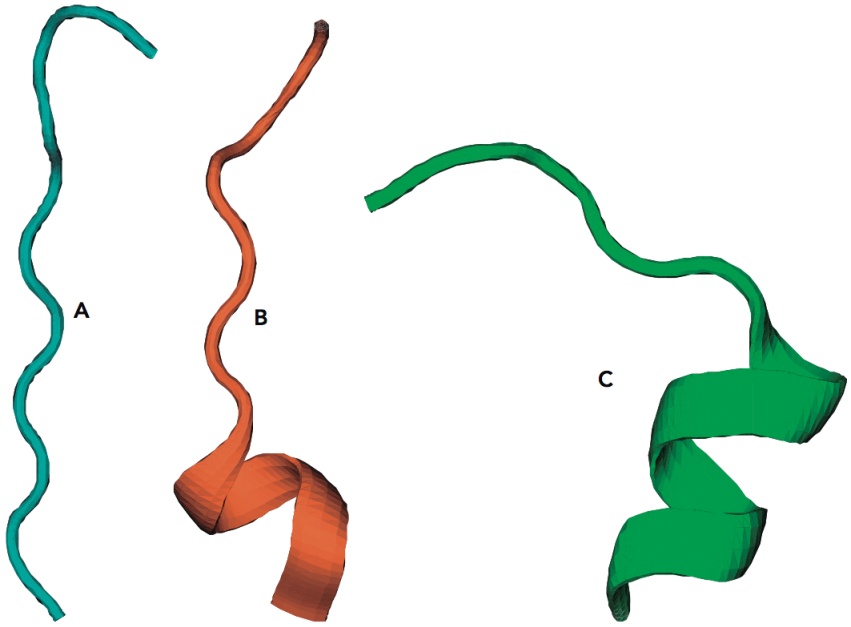

Figure 1: Three-dimensional structures obtained by homology modeling through 3D-JIGSAW of A: Peptide with KLKLLLLKLK-NH_2 sequence B: Peptide with RLKLLLLLRLK-NH_2 sequence C: peptide derived from the venom of *Vespa magnifica* with FLPIPRPILLGLL-NH_2 sequence

The first peptide analyzed (KLKL_5KLK-NH_2) did not show secondary structure formation at any pH or temperature evaluated in this study (vacuum and solvated). This effect can be explained in terms of an structural unbalance, because we obtained high deviation (0.25 $\pm$ 1.5 nm) of RMSD compared with the original structure (Fig. 2A) at all pHs and temperatures evaluated.
Initially, all structures for the second peptide showed an alpha-helix formed by the amino acids LKL-NH_2 with a free energy of formation of -7.12 kcal/mol. This structure (in vacuum) was kept after the protonation or deprotonation in all pHs studied, which indicates a conformational stability of the peptide. By modeling at 298K and 310K, and at pH5 and pH7, the α-helix added the R neighbor amino acid to its three dimensional structure, while simulations with pH 9 to 298K and 323K showed a denaturation of the peptide structure. The third modeled peptide obtained and kept (in solvation and vacuum at all pH and temperature simulated) an α-helix structure, which was formed by the amino acids RPILLGL-NH_2 with free energy of formation of -7.87 kcal/mol.
Considering that the peptide with sequence KLKL5KLK-NH_2 does not form a defined secondary structure at any temperature and pH evaluated, it is unlikely that it can display antimicrobial activity per se. However, this depends on the conformation that this peptide acquires during their interaction with the cell membrane of microorganisms. Furthermore, the stability of the secondary structure of the peptide RLKL5RLK- NH_2 is dependent on the temperature and pH, but it is stable at physiological conditions.

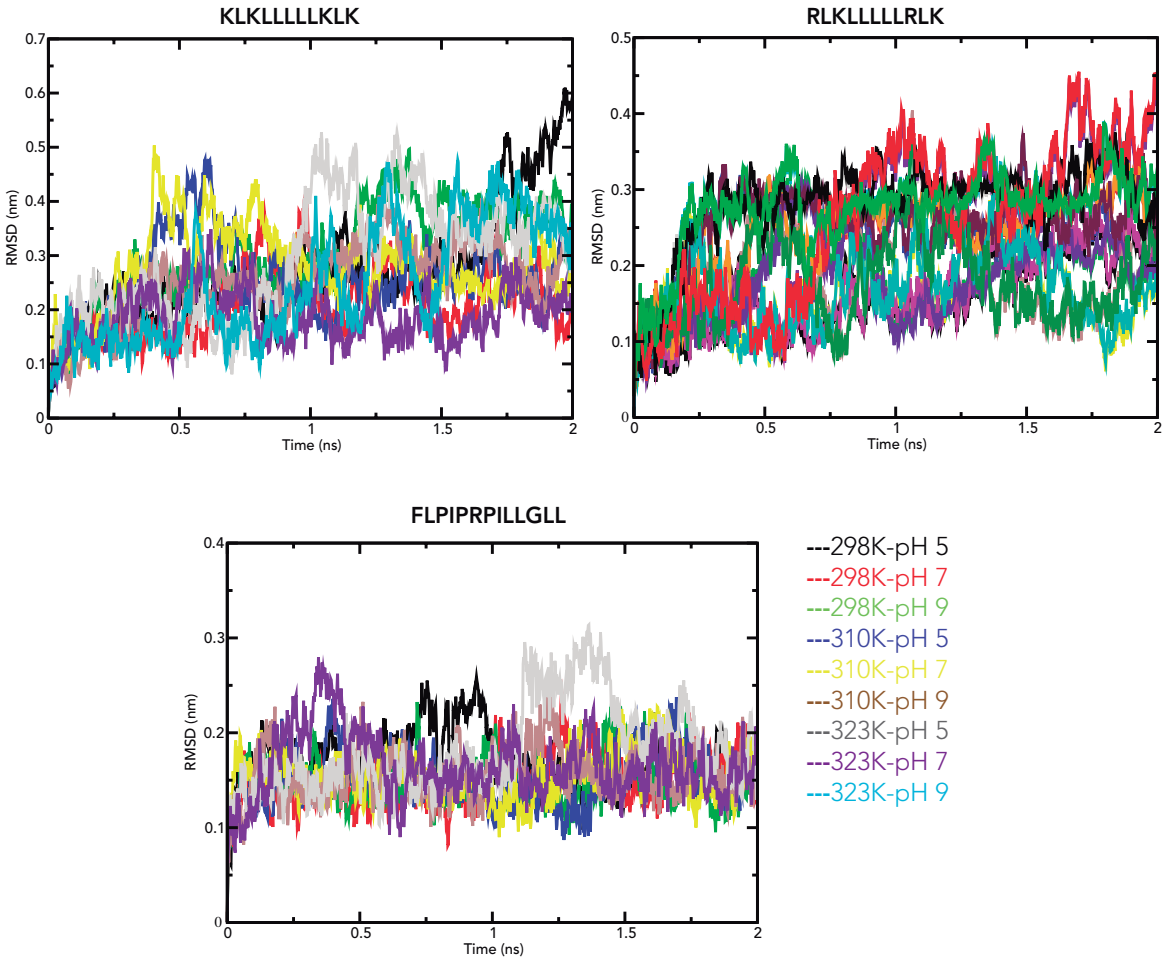

Figure 2: RMSD of the three antimicrobial peptides simulated at three different values of pH and temperature.

The α-helix formed by amino acid RLKL-NH_2 is consistent with results obtained from Alvares-Bravo et al. (2), and the mechanism of action proposed by the same authors (1). The secondary structure of the third peptide seems to be more stable regardless of pH and temperature than peptides derived from sapecin β, showing RMSD values around 1.5 ± 0.5 nm. These results are not consistent with those reported in the CAMP database (8), where these are scored with a higher instability index (45.66) than the other two peptides analyzed (-37.22 and 3.45).

3 CONCLUSIONS

Considering that simulations under solvation conditions of the 3D-structures of the peptides under different pHs and temperatures are similar than *in vitro* results, we can conclude that the approximations *in silico* by molecular dynamics methods provide information for a virtual screening of antibiotic peptides with potential to be tested for

antimicrobial activity in laboratory bioassays.
Finally, from results of the peptide derived from the venom of *Vespa magnifica*, we could consider that the stability index given by the CAMP database would not be a good indicator of the stability at physiological pH and temperature of these peptides, and it is necessary to assess this parameter in a experimental point of view.

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