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New Insights into Antimicrobial Peptides Isolated from Brazilian Natural Sources

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Introduction: Antimicrobial Peptides or Proteins (AMPs) are components of the humoral response of the innate immune system. Representing a fast and effective initial barrier against pathogens, these molecules gained visibility as several began to be described as potent bioactive molecules. AMPs are generally amphypathic and cationic and have high hydrophobic properties at physiological pH, leading to different mechanisms of actions. Some of them may interact with negatively charged microbial membranes; others can penetrate the membrane and have intracellular targets. As these mechanisms occur due to charge-based interaction, these molecules generally act in an independent protein-binding manner, hindering the development of resistance mechanisms. Nowadays there is an urgent need to produce new antibiotics that can act over resistant bacteria/fungi. Thus, AMPs are very good candidates to substitute commercial antibiotics due to these physical-chemistry features and mechanism of action. Thus, our field of study on Butantan Institute is to identify AMPs from different natural sources, including chicken eggs, pineapple, garlic and invertebrates, such as kissing bugs, spiders, centipedes and scorpions among others. A wide broad of molecules have been isolated and had their antimicrobial potential confirmed. Serrulin from the scorpion *Tityus serrulatus*, Lacrain from the centipede *Scolopendra viridicornis*, Sarconesin from the fly *Sarconesiopsis magellanica* and Juruin from the spider *Avicularia juruensis* are some of the examples. *Triatoma infestans,* member of the Triatominae subfamily, popularly known as kissing bugs, feeds with blood and has a major role as Chagas disease vector. Regarding new researches related to AMPs in triatomines, few molecules have been identified so far, and no research on this area have been developed with *T. infestans,* and due to the fact that this insect survives in a highly infectious habitat during its life cycle, we were able to obtain new information of AMPs used by this insect.

Methods and Findings: Following a workflow with the collection of the biological sample, acid extraction, fractionation with high performance liquid chromatography, peptide synthesis, liquid growth inhibition assay (antimicrobial assay), mass spectrometry (LC/MS) and database search, we were able to identify different sources from which *T. infestans* obtains AMPs.

Results and Discussion: Initially, on our previous work published on the journal Frontiers in Cellular and Infection Microbiology, the presence of a human fibrinopeptide with antimicrobial activity on the insects' hemolymph was described. Briefly, while analyzing the hemolymph of *T. infestans*, one potent antimicrobial peptide (active against *Micrococcus luteus)* presented 100% homology with human Fibrinopeptide A (FbPA). The main hypothesis was that the insect was able to absorb the entire molecule and that the peptide was still active inside the insect. Thus, as a matter of comparison, the native FbPA was obtained through blood coagulation and the synthetic peptide was also produced. These three FbPA from different sources presented the same antimicrobial activity, which demonstrated that the molecule isolated from the insect was indeed the FbPA. To confirm that it was absorbed, the synthetic FbPA was coupled with a fluorescein isothiocyanate (FITC) and was provided as food to the insects. The hemolymph of these insects was collected and it presented exhibited fluorescence at the same wavelength as FITC. With another fractionation step, it was possible to confirm that the fraction that Exhibited fluorescence was indeed the FbPA coupled with the FITC. These experiments show that beyond intrinsic AMP production, *T. infestans* is able to co-opt molecules through absorption and may use them as AMPs for protection, beyond nutrition. As we observed the presence of PAMs produced by the cleavage of large proteins inside the insect, a analysis of the intestinal content of *T. infestans* were made to verify the possibility of production of antimicrobial peptides during hemoglobin digestion. This second work was published on Biomolecules. The intestinal content of the insects were analyzed by the same workflow, where 10 fractions on the chromatogram presented potent antimicrobial activity and corresponded to hemoglobin fragments on the database search. As the fractions were not homogenous, more than one sequence corresponding to different portions of the hemoglobin where found for each fraction, and when all the sequences were aligned, it provided 67% coverage of the hemoglobin Alfa, beta 1 and beta 2 chains. Refinement of the mass spectrometry data led to a consensus sequence for each fraction, and it represented 2 fragments on each hemoglobin chain. When compared to the sequences from hemoglobin with antimicrobial activity already described on ticks. All of the active hemoglobin fragments produced in similar ways by ticks' digestion where found in our work, reinforcing the accuracy of our data refinement. On the insects' wild cycle, it has different natural reservoirs, such as opossum *Monodelphis domestica* (order Marsupialia), the armadillo *Dasypus novemcinctus* (order Xenarthra), the coati *Nasua nasua* (order Carnivora) and the pig *Sus scrofa* (order Artiodactyla). As the hemoglobin sequences of these species are highly conserved, sequence alignment showed that all the sequences found in our work are also present on every single species analyzed, demonstrating that they can be formed in every meal that the insect might have. This work represents a very important conclusion because it is the first in vivo description of a Hemiptera being capable of producing multiple hemoglobin fragments on the intestinal content, and that these molecules can present benefits beyond nutrition. And representing the third AMPs source, our most recently paper, published on Microbiology Insights, shows the presence of two active tachykinin-related peptides (TKRPs) on the *T. infestans* hemolymph. While analyzing the *T. infestans* hemolymph, two molecules presented relevant activity mainly against filamentous fungi, and when examined by mass spectrometry presented similarity with TKRPs. They were named as TRP1-TINF and TRP2-TINF as they are the first TKRP identified on this insect. TRP1-TINF is a random secondary structure peptide with 9 amino acid residues, susceptible to aminopeptidases degradation. TRP2-TINF is a 10-amino acid peptide with a 310 helix secondary structure, susceptible to carboxypeptidases degradation. Interestingly TKRP have neurotransmitter/neuromodulator activities as their main physiological functions. Seven TKRP sequences from *Rhodnius prolixus* were previously described, and the sequences 1 and 3 are conserved when compared to TRP1-TINF and TRP2-TINF respectively. All of these works together describe very relevant molecules as candidates as alternative drugs and a better understanding of *T. infestans* physiology by demonstrating that it can obtain AMPs from different sources.