

Mass Spectrometric studies on novel bradykinin and phyllokinin analogs from the skin secretion of *Phyllomedusa hypochondrialis*

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Amphibian skin is known as a storehouse of bioactive molecules, most of them involved in defensive functions against not only opportunistic microorganisms but also against a broad range of predators. Indeed, these secretions are known to contain not only antimicrobial peptides, but also hormone analogs that disturb physiological functions and produce noxious effects. A common strategy on behalf of venomous animals is the induction of alterations in cardiovascular functions by the delivery of supraphysiological quantities of bradykinin analogs, therefore, using the kallikrein-kinin system as a target. These are capable of increasing vascular permeability as well as the sensitivity of nociceptors to mechanical and thermal stimulation. In this work, we report the identification and characterization of 11 bradykinin and phyllokinin analogs in the skin secretion of *Phyllomedusa hypochondrialis* by Mass Spectrometric methods. Also, post-translational modifications (PTMs) common to these families have been addressed and determined. The lyophilized extract obtained from the skin by mild shock was purified by RP-HPLC (Shimadzu Co.), semi-preparative and analytical column C18. Monoisotopic masses and MS/MS sequencing were performed by both an ESI-Q-TOF Ultima API (Waters) and an ABI 4700 (Applied Biosystems) mass spectrometers. Peptides were categorized by similarity under the classification of mammalian bradykinins, snake bradykinins, phyllokinins and novel bradykinin analogs. Hydroxylated prolines were determined by using an automated EDMAN sequencer (PPSQ-23, Shimadzu Co.) combined with immonium mass analysis. Phyllokinin sulphonation was determined by the use of deuterated reagents following parental ion fragmentation. Tests were performed comparing the affinity of the human bradykinin with a newly described analog ([Val¹],[Thr⁶]-Bradykinin-QS) to the bradykinin BR2 receptor in culture. Supported by: EMBRAPA-CENARGEN, CNPq.