

BIOLOGICAL EFFECT-DRIVEN BIOCHEMICAL ASSESSMENT OF THE SKIN SECRETION OF THE TREE-FROG *PHYLLOMEDUSA HYPOCHONDRIALIS*

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Amphibian skin secretions contain several bioactive compounds such as biogenic amines, alkaloids, steroids, proteins and peptides; being peptides a growing field of interest. Our work focus on the depiction of the main physiopathological events and the pattern of the local acute inflammatory response targeted by the skin secretion in mammals as well as the identification of six novel bioactive peptides present in this secretion. Complex toxic effects of the *P. hypochondrialis* skin secretion and proposed mechanisms of action are presented and supported by *in vivo* data. The inflammatory reaction in the mice footpad can be described as the release of IL-1, IL-6, TNF-alpha, KC, MCP-1, LTB 4 and PGE(2), corroborating the findings that crude skin secretion is able to induce local inflammation. Moreover, amphibian antimicrobial peptides, although well know can still serve as scaffolds for antibiotic-resistant bacteria. Two peptides were screened, isolated and sequenced from the crude skin secretion. These peptides were effective over pathogenic bacteria, presenting no hemolytic activity, and could be classified as one Phyllopsptin and one Dermaseptin. These peptides were sequenced by mass spectrometry and have had their sequences chemically confirmed. Furthermore, in the present study, we also describe the isolation and biological characterization of a novel bradykinin potentiating-peptide (BPP) and three bradykinin-like peptides isolated from this skin secretion. This new BPP, named Phypo Xa is able to potentiate bradykinin activities *in vivo* and *in vitro*, as well as efficiently and competitively inhibit ACE. This is the first canonical BPP (i.e. Pyr-Aaa(n)-Gln-Ile-Pro-Pro) to be found not only in the frog skin but also in any other natural source other than the snake venoms. All these finds are noteworthy for they not only present a series of new properties and molecules to be explored from the frog skin secretion, but also demonstrate the efficiency of the biological effect-driven biochemical and proteomic approach to the finding of new molecules. Once one possesses the target (i.e., the biological system to be screened), finding the players becomes much more a matter of choosing the appropriate techniques. Supported by FAPESP, CNPq and CAPES

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