

Molecular diversity of the cutaneous proteome and peptidome of the frog *Dendropsophus columbianus* (Boettger, 1892)Luz Elena Triana Vidal¹, Wagner Fontes², Marcelo Valle de Sousa², Carlos André Ornelas Ricart², Mariana Souza Castro² and Sébastien Charneau²¹University Libre of Colombia, Colombia²University of Brasilia, Brazil

Amphibian skin is a tissue specialized in the production of various molecules such as antimicrobial peptides, immunomodulators, biogenic amines and hormones. These bioactive compounds could be considered of biotechnological interest and new therapeutic alternatives. The aim of the present study was to identify and characterize peptides and proteins present in the cutaneous secretion of the amphibian *Dendropsophus columbianus* (Boettger, 1892), related to pathogen defense mechanisms. The first approach consisted of the classical fractionation of cutaneous secretion by RP-HPLC. All chromatographic fractions obtained were evaluated for their ability to inhibit bacterial proliferation and analyzed by MALDI-TOF/TOF. A single new antimicrobial neutral peptide was thus isolated and named dendropsophin 1 (Dc 1), with no sequence similarity with annotated peptides. Subsequently, two cationic analogues (Dc 1.1 and Dc 1.2) keeping the amphipathic structure of Dc 1 were designed. Dc 1 and its two analogues exhibited moderate antibacterial activities and no hemolytic and cytotoxic effects in mammalian cells. Dc 1.2 exhibited slightly improved antibacterial properties that could be related to the higher content of α -helix and thermal stability compared to Dc 1 and Dc 1.1 in hydrophobic experimental condition. The native peptide Dc 1 could be used as an alternative model to develop new AMPs. In a second modern high-throughput approach, the first comprehensive proteomic and peptidomic study of cutaneous secretion was performed by nanoLCMS/MS using Orbitrap Elite™ mass spectrometer. Data were processed using PEAKS and Blast2GO programs. 1248 proteins present in the cutaneous secretion of *D. columbianus* were identified, 127 proteins were categorized associated with the defense system and 19 related to the response against microorganisms. Three hydrolases from the total secretion were detected by zymography and subsequently identified, which could be related to the processing of peptides of defense. The peptidome results allowed determining a new group of peptides endogenous to *D. columbianus*, with high sequence similarity with Dc 1, which were named as dendropsophins. The dendropsophins Dc 2, Dc 7, Dc 13, Dc 17, Dc 18 and Dc 19 showed no activity against bacteria or fungi. However, Dc 2 and Dc 18 are able to activate human neutrophils. This activity could be associated to a modulation of the immune system in *D. columbianus*. This pathway of defense, highly conserved in vertebrates, opens further perspectives regarding the use of these new molecules in the therapy of infectious diseases.

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