Characterization of the Peptidase Activity Present in the Skin Secretion of the Frog Leptodactylus labyrinthicus

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Peptidases are proteolytic enzymes involved in diverse biological processes. Peptides fragments found in frogs' skin secretion suggest the presence of these enzymes. In this way, this work aimed at the characterization of the peptidase activity present in the skin secretion of Leptodactylus labyrinthicus. The zymographic assay revealed the activity of two gelatinolytic bands with about 65 kDa, and 70 kDa, calcium dependents, and inhibited either by EDTA, or zinc excess, characteristics of metallo-peptidases. Furthermore, the identified proteolytic enzymes were active just in neutral to alkaline pH, and they worked better with the increase of ionic strength. The experiments with fluorogenic substrates identified peptidases able to cleave after leucine, proline, and alanine residues of free N-terminal peptides. This activity was inhibited in the presence of metallo, and serine peptidases inhibitors. Besides, the optimum pH remained around 7.5. However, this profile suggests the presence of more than one peptidase. Moreover, the results of the SDS-PAGE gel showed that the utilized substrates are cleaved by the same band of 50 kDa. Nevertheless, it doesn't mean that the same enzyme is responsible for the cleavages. Finally, our research line will help to elucidate the implication of those proteins in the processing of bioactive peptides present in the venom, expanding the knowledge of the amphibian biology.

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