PURIFICATION AND CHARACTERIZATION OF ANGIOTENSIN CONVERTING ENZYME OF *THALASSOPHRYNE NATTERERI* VENOM

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INTODUCTION Animal venoms are complex mixtures, including peptides, proteins and other compounds produced by animals in predation, digestion, and defense. These molecules have been investigated regarding their molecular mechanisms associated with physiological action and also possible pharmacological applications. Recently, we have described the presence of a type of angiotensin converting enzyme (ACE) activity in the venom of *Thalassophryne nattereri*. By removing dipeptide His-Leu from terminal C, the ACE converts angiotensin I (Ang I) into angiotensin II (Ang II) and inactivates bradykinin. **OBJECTIVES:** Purify and characterize the ACE of *Thalassophryne nattereri* venom. **MATERIAL AND METHODS:** We collected the venom from 20 fresh *T. nattereri* specimens through an opening in the spine by pressing the base. In order to isolate the ACE of *T. nattereri* venom, 150 µL of the venom was diluted in 4850 µL of Tris-HCl buffer 50 mM, pH 8. The total volume of the sample (5 mL) was injected in a liquid chromatograph type Fast Protein Liquid Chromatography (FPLC) AKTA Püre M1, with an ion exchange column CM-Sepharose of 50 mL. **RESULTS AND DISCUSSION:** The fractionation of *T. nattereri* venom in CM-Sepharose indicated a peak (CM2) with angiotensin-converting activity, converting Ang I into Ang II. Electrophoresis on polyacrylamide gel (12%) revealed one band for CM2 similar in size to natterins, which are toxins with proteolytic activity found in *T. nattereri* venom. Mass spectrometry indicated that the protein sequence of the ACE purified from *T. nattereri* venom corresponds to natterin 1. The isolated protein has also demonstrated inhibition through captopril and EDTA and is characterized as a classic ACE. The K_m and V_{max} values of the enzyme towards Ang I as substrate were 0.063±0.005 nmol and 9.317±0.20 nmol/min, respectively. **CONCLUSION:** Thus, the isolated enzyme, natterin 1, purified from *T. nattereri* venom is the first ACE isolated from fish venom.

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