Structural and Functional Characterization of Myotoxin, Cr-IV 1, a Phospholipase A_2 D49 from the Venom of the Snake *Calloselasma rhodostoma*

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A new D49 PLA_2 was purified from the venom of Calloselasma rhodostoma after two chromatographic steps. Molecular exclusion chromatography was done through a Protein-Pack 300 SW column (0.78 cm x 30 cm), eluting with 0.25 M ammonium bicarbonate, pH 7.9, at a flow rate of 0.3 ml/min. Reverse-phase HPLC was then performed on m-Bondapack C-18. The sample was determined to have a molecular mass of 13,870.94 Da MALDI-TOF by mass spectrometry, and the amino acid composition showed that Cr-IV 1 presented a high content of Lys, Tyr, Gly, Pro, and 14 half-Cys residues, typical of a basic PLA_2. Cr-IV 1 presented a sequence of 122 amino acid residues: DLWEFGQMIL KETGSLPFPY YTTYGCYCGV GGRGGKPKDA TDRCCFVHDC CYGKLTGCPK TNDRYSYSRL DYTIVCGEGG PCKQICECDK AAAVCFRENL RTYNKKYRYH LKPFCKEPAE TC and a calculated pI value of 8.0. Cr-IV 1 had PLA2 activity in the presence of a synthetic chromogenic substrate (4-nitro-3-(octanoyloxy)benzoic acid) and showed a rapid cytolytic effect on mouse skeletal muscle myoblasts and myotubes in culture. In mice, Cr-IV 1 induced myonecrosis and edema upon intramuscular and intravenous injections, respectively. The LD_{50} of Cr-IV 1 was determined to be 0.07 mg/k body weight by intracerebroventricular (i.c.v.) injection. The combination of structural and functional information obtained herein classifies Cr-IV 1 as a new member of the D49 PLA_2 family, as it presents the typical behavior of a phospholipase A_2 from this family.

Keywords: Snake venom; *Calloselasma rhodostoma*; Myotoxin; Structural and functional characterization.