Isolation and characterization of the Fruticulosin: a new type II ribosome-inactivating protein from *Abrus fruticulosus* Wall seeds.

Barroso, W.S.; Penha, S.S., Silva, V.R.C.; Leite, T.A.; Rocha, B.A.M.* and Silva, A.L.C.

1Laboratory of Molecular Biotechnology (LabBMol), Department of Biochemistry and Molecular Biology, Federal University of Ceará, Fortaleza-CE, Brazil.

2 Laboratory of Biocrystallography, Department of Biochemistry and Molecular Biology, Federal University of Ceará, Fortaleza-CE, Brazil. *E-mail: brunoanderson@gmail.com

Fruticulosin is a type 2 ribosome-inactivating protein (RIP) found in *Abrus fruticulosus* seeds. RIPs have been explored in chemical drugs construction as immunotoxins for targeted cancer therapy. This work aimed to develop a protocol for isolation of fruticulosin from *Abrus fruticulosus* seeds, intending to obtain pure and homogeneous protein fractions for biochemical and biological studies. Initially, the seeds were delipidated with hexane for 12 h and transformed into a fine and homogeneous powder, which was subjected to extraction (1:10 w/v) with 10 mM sodium phosphate buffer (pH 7.4), containing 150 mM NaCl, for 3 hours and centrifuged at 10,000 rpm for 30 min at 10°C. The supernatant was used in purification steps. The active fruticulosin was purified in a single step by affinity chromatography in a Sepharose-4B column. The pure protein yield was 140 μg/g of the powder seeds using this protocol. The purified protein showed a single band of 63 kDa (by SDS-PAGE) and two bands of 34 e 25 kDa (by SDS-PAGE with 2-mercaptoethanol), respectively, suggesting the presence of interchain disulphide-bonds as occurs in type 2 RIPs. The pure fruticulosin also haemagglutinated rabbit and human erythrocytes showing stability even after one hour of exposure to a different pH values (optimal between pH 6.0 and 8.0), but was inhibited after incubation with D-galactose. Circular dichroism analyses for fruticulosin sample showed a protein profile with predominance of α-helical elements: two negative bands at 222 and 208 nm and a positive peak at 196 nm. The fruticulosin presented a highly toxic activity *in vivo* when injected in peritoneal cavity of mice (LD₅₀ = 5 μg/Kg body weight). This fraction was also able to depurinate the ribosomal RNA from *Pichia pastoris* to a concentration of 4 ng, confirming the RNA-N-glicosidase activity of the fruticulosin isolated from seed.

Keywords: Toxin; Type 2 RIPs; RNA N-glicosidase.

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