DNase II FROM *Bothrops alternatus* SNAKE VENOM: PURIFICATION AND TOXICITY IN MDCK CELLS

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Snake venoms contain a variety of enzymes that degrade nucleic acids and their constituents. Acidic deoxyribonucleases (DNase II) have been implicated in DNA fragmentation during apoptosis in mammals. In this work, we describe the purification of DNase II from *Bothrops alternatus* snake venom and its toxicity in Madin-Darby canine kidney (MDCK) cells. DNase II was purified from *B. alternatus* venom in five chromatographic steps (ion exchange, gel filtration and affinity chromatography); enzymatic activity towards salmon testes DNA was determined based on the increase in absorbance at 260 nm. The purified enzyme had an M_r of 26.4 kDa (SDS-PAGE), a pI of ~5.0 (2D-electrophoresis) and a specific activity of 3.4 $x10^3$ units/mg compared to 65 units/mg for venom (purification factor = 53.7), with an activity yield of 9.7% and protein recovery of 0.18%. MDCK cells treated with purified DNase II (200, 400 and 800 U/ml) for 24 h or 48 h showed DNA fragmentation. Feulgen staining of these cells showed condensed and fragmented nuclei, and toluidine blue staining showed cellular vacuolization, fragmentation, swelling and cell detachment. These results indicate that *B. alternatus* venom contains a DNase II that could contribute to DNA degradation and apoptosis following envenomation.

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