Biological and structural characterization of crotoxin and new isoform of crotoxin B PLA_2 (F6a) from *Crotalus durissus collilineatus* snake venom

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A new crotoxin B isoform PLA_2 (F6a), from *C.d.collilineatus* was purified from by one step reverse phase HPLC chromatography using μ-Bondapack C-18 column analytic. The new crotoxin B isoform PLA_2 (F6a), complex crotoxin, the catalytic subunit crotoxin B isoform PLA_2 (F6a) and two crotapotin isoforms (F3 and F4), were isolated from the venom of *C.d.collilineatus*. The two proteins different in their ability to inhibit of isoforms of PLA_2 (F6 and F6a). The molecular masses estimated by MALDI-TOF mass spectrometry were: crotoxin B: 14943.14 Da, crotapotin F3 8693.24 Da, and crotapotin F4: 9314.56 Da. The isoform PLA_2 (F6a) contained 122 amino acid residues and a pI of 8.58 and contained 122 amino acid residues, with a primary structure of HLLQFNKMIK FETRRNAIPP YAFYGCYCGW GGRGRPKDAT DRCCFVHDCC YGKLAKCNTK WDFYRYSLKS GYITCGKGTW CEEQICECDR VAAECLRRSL STYRYGYMIY PDSRCRGPSE TC. A neuromuscular blocking activity was induced by crotoxin and new crotoxin B isoform PLA_2 (F6a) in the isolated mouse phrenic nerve diaphragm and the biventer cervicis chick nerve-muscle preparation. Whole crotoxin was devoid of cytolytic activity upon myoblasts and myotubes *in vitro*, whereas new crotoxin B isoform PLA_2 (F6a) was clearly cytotoxic to these cells.

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