

Cytotoxic action in myoblasts and myotubes (C2C12) and enzymatic characterization of a new phospholipase A₂ isoform (Bj-V) from *Bothrops jararacussu* venom.

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A new PLA₂ Bj-V from *Bothrops jararacussu* (14039.49 Da determined by MALDI-TOF mass spectrometry) was isolated in only one chromatographic step by HPLC ion-exchange and its purity was confirmed by reverse phase. Amino acid analysis showed a high content of hydrophobic and basic amino acids as well as 14 half-cysteine residues. The N-terminal sequence (DLWQFGQMILKETGKIPFPY YGAYGCYCGW GGRGGKPKDG TDRCCYVHD...) showed a high degree of homology with basic D49 PLA₂ myotoxins from other *Bothrops* venoms. Bj V showed discrete sigmoidal enzymatic behavior, with maximal activity at pH 8.4 and 35-40 degrees C. Full PLA₂ activity required Ca²⁺ (10mM) and there was little catalytic activity in the presence of 1mM Ca²⁺. The addition of Mn²⁺ or Mg²⁺ (10mM) in the presence of low (1mM) Ca²⁺ slightly increased the enzyme activity, whereas Zn²⁺ and Cu²⁺ (10mM) diminished the activity. The substitution of Ca²⁺ for Mg²⁺ or Cu²⁺ also reduced the enzymatic activity. Bj V had PLA₂ activity and produced cytotoxicity in murine C2C12 skeletal muscle myoblasts and myotubes. The isolation of these isoforms Bj-IV and Bj-V found in a fraction previously described as homogeneous shows us the importance of optimization in purification techniques in order to better understand their biological behavior.

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