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Anti-platelet aggregation activity of two novel acidic Asp49-phospholipases A_2 from *Bothrops brazili* snake venom

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ABSTRACT

Phospholipases A_2 (PLA_2s) are important enzymes present in snake venoms and are related to a wide spectrum of pharmacological effects, however the toxic potential and therapeutic effects of acidic isoforms have not been fully explored and understood. Due to this, the present study describes the isolation and biochemical characterization of two new acidic Asp49-PLA_2s from *Bothrops brazili* snake venom, named Braziliase-I and Braziliase-II. The venom was fractionated in three chromatographic steps: ion exchange, hydrophobic interaction and reversed phase. The isoelectric point (pI) of the isolated PLA_2s was determined by two-dimensional electrophoresis, and 5.2 and 5.3 pIs for Braziliase-I and II were observed, respectively. The molecular mass was determined with values of 13,894 and 13,869 Da for Braziliase-I and II, respectively. Amino acid sequence by Edman degradation and mass spectrometry completed 87% and 74% of the sequences, respectively for Braziliase-I and II. Molecular modeling of isolated PLA_2s using acid PLA_2BthA-I-PLA_2 from *B. jararacussu* template showed high quality. Both acidic PLA_2s showed no significant myotoxic activity, however they induced significant oedematogenic activity. Braziliase-I and II (100 μg/mL) showed 31.5% and 33.2% of cytotoxicity on *Trypanosoma cruzi* and 26.2% and 19.2% on *Leishmania infantum*, respectively. Braziliase-I and II (10 μg) inhibited 96.98% and 87.98% of platelet aggregation induced by ADP and 66.94% and 49% induced by collagen, respectively. The acidic PLA_2s biochemical and structural characterization can lead to a better understanding of its pharmacological effects and functional roles in snakebites pathophysiology, as well as its possible biotechnological applications as research probes and drug leads.

1. Introduction

The venomous snake *Bothrops brazili* [1] known as "Jergón íShushupe" is distributed throughout South America and is found mainly in Brazil (the Amazon region), Colombia, Ecuador, Guyana, Peru, Suriname and French Guiana [2]. It has an average size of approximately 1.2 m in length and is found mainly in the primary forest [3–5].

One of the main local effects is myonecrosis, which is mainly caused by phospholipases A_2 (PLA_2) present in the venom [6–8]. Other actions triggered by venom PLA_2s are oedema, neurotoxicity, cardiotoxicity and hemostatic disorders [9–11]. Snake venom PLA_2s are secreted enzymes belonging to groups IA (Elapidae) and IIA (Viperidae). PLA_2s from Viperidae venoms (including the genus *Bothrops*) have been divided into two main subgroups: the enzyme Asp49 (D49), catalytically active and the Lys49 (K49) PLA_2-homologue, catalytically inactive [12,13]. Most PLA_2 described

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