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Interfacial surface charge and free accessibility to the PLA_2-active site-like region are essential requirements for the activity of Lys49 PLA_2 homologues

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Abstract

Lys49 phospholipase A_2 homologues are highly myotoxic and cause extensive tissue damage but do not display hydrolytic activity towards natural phospholipids. The binding of heparin, heparin derivatives and polyanionic compounds such as suramin result in partial inhibition (up to 60%) of the myotoxic effects due to a change in the overall charge of the interfacial surface. In vivo experiments demonstrate that polyethylene glycol inhibits more than 90% of the myotoxic effects without exhibiting secondary toxic effects. The crystal structure of bothropstoxin-I complexed with polyethylene glycol reveals that this inhibition is due to steric hindrance of the access to the PLA_2-active site-like region. These two inhibitory pathways indicate the roles of the overall surface charge and free accessibility to the PLA_2-active site-like region in the functioning of Lys49 phospholipases A_2 homologues. Molecular dynamics simulations, small angle X-ray scattering and structural analysis indicate that the oligomeric states both in solution and in the crystalline states of Lys49 phospholipases A_2 are principally mediated by hydrophobic contacts formed between the interfacial surfaces. These results provide the framework for the potential application of both clinically approved drugs for the treatment of *Viperidae* snakebites.

Keywords: Lys49 phospholipases A2 homologues; Myonecrosis; Suramin; Polyethylene glycol; Small angle scattering; Crystal structure and molecular dynamics

Abbreviations: PLA_2, phospholipase A_2; BthTX-I, *Bothrops jararacussu* bothropstoxin-I; ACL myotoxin, *Agkistrodon contortrix laticinctus* myotoxin; Basp-II, *Bothrops asper* myotoxin II; CK, creatine kinase; NSD, normalized spatial discrepancy; SAXS, small angle X-ray scattering; PEG-400, polyethylene glycol 400; MD, molecular dynamics; RMSD, root mean square deviations; IIP, intermolecular interaction potential; PISA, Protein, Interfaces, Surfaces and Assemblies.

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1. Introduction

Envenomation by Viperidae snakes results in acute myonecrosis that provokes permanent tissue loss resulting in disability, amputation and in some cases, death (Gutiérrez and Lomonte, 1995). Myonecrosis is mainly caused by the direct action of catalytically inactive Lys49 phospholipases A_2 (PLA_2) homologues upon the plasma membrane of muscle cells and by the indirect action of metalloproteinases and serine proteinases on the haemostatic system (Gutiérrez and Lomonte, 1995; Ownby et al., 1999). Since the widely used antivenom serum therapy does not address myonecrosis, the inclusion of a supplementary agent for the treatment of Viperidae snakebites is therapeutically relevant.

Heparin and related polyanions are able to inhibit myonecrosis induced by snake venoms that contains myotoxins such as *Bothrops jararacussu* bothropstoxin-I (BthTX-I) (Homsi-Branderburgo et al., 1988), *Agkistrodon contortrix laticinctus* myotoxin (ACL myotoxin) (Johnson and Ownby, 1993) and *Bothrops asper* myotoxin II (Basp-II), both in vivo and in vitro (Lomonte and Gutiérrez, 1989). Suramin, a polysulphonated naphthyl urea derivative used clinically in the treatment of onchocerciasis (Burch and Ashburn, 1951), African trypanosomiasis (Williamson and Desowitz, 1956) and several kinds of cancers (LaRocca et al., 1993; van Oosterom et al., 1991), is also a potential inhibitor of myotoxins both in vivo and in vitro and represents an important therapeutic agent for the treatment of Viperidae snakebites (Arruda et al., 2002; Murakami et al., 2005).

Catalytically inactive Lys49 PLA_2s homologues cause membrane leakage in the absence of Ca^{2+} ions, without concomitant hydrolysis when tested against negatively charged liposomes (Gutiérrez and Lomonte, 1995; Ownby et al., 1999). A number of studies involving different techniques, such as chemical modification, sequence comparison analyses, interaction with neutralizing molecules, synthetic peptide studies, and site-directed mutagenesis, have been used in an attempt to elucidate the structural determinants for myotoxicity of Lys49 PLA_2s homologues (Ownby et al., 1999; Murakami et al., 2005).

Our studies combining small angle X-ray scattering, crystallography, molecular dynamics simulations, inhibition by suramin and polyethylene glycol and in vivo tests, reveal new features of the action mechanism of myotoxins, suggesting a promising supplement of the current serum treatment by the inclusion of potent inhibitors that simultaneously bind both in the putative catalytic and at the interfacial recognition sites.

2. Materials and methods

2.1. Purification and biochemical characterization

Crude dessicated *B. jararacussu* venom was obtained from a local serpentarium and BthTX-I was purified with minor modifications following the published protocol (Spencer et al., 1998). The purity of the samples was confirmed by SDS-PAGE (Laemmli, 1970) and the protein concentrations were determined by the Bradford method (Bradford, 1976).

2.2. In vivo myotoxic assay

In vivo myotoxic assays were carried out as previously described in Murakami et al. (2005) with minor modifications. In accordance with each protocol, the quantity of toxin (50 μg/g) administered was adjusted taking into consideration the individual weight of each animal and different ratios of suramin and polyethylene glycol (1:0.25, 1:0.50, 1:1, 1:2.5, 1:5.0; BthTX-I:inhibitor). Enzyme activity was expressed as international Units per liter (U/L), where 1 U is defined as the amount that catalyzes the transformation of 1 μmol of substrate at 25 °C.

2.3. Scattering data acquisition and analysis

Small angle X-ray scattering (SAXS) measurements were conducted at room temperature utilizing the D11A-SAXS beamline at the Brazilian National Synchrotron Light Source where the wavelength was set to 1.488 Å. A sample concentration of 4–10 mg/mL in a 20 mM, pH 7.0 Tris–HCl buffer was used and serial dilutions were prepared to permit the extrapolation of the SAXS curves to zero concentration. Data acquisitions were performed by taking several 600 s frames of each sample. Data fitting was performed using the GNOM program (Semenyuk and Svergun, 1991) and the radius of gyration (R_g) of the protein in solution was determined from the lowest q values using the Guinier approximation (Guinier and Fournet, 1955). The ab initio shape determination was