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Hyaluronidase from the venom of the social wasp *Polybia paulista* (Hymenoptera, Vespidae): Cloning, structural modeling, purification, and immunological analysis

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ABSTRACT

In this study, we describe the cDNA cloning, sequencing, and 3-D structure of the allergen hyaluronidase from *Polybia paulista* venom (*Pp*-Hyal). Using a proteomic approach, the native form of *Pp*-Hyal was purified to homogeneity and used to produce a *Pp*-specific polyclonal antibody. The results revealed that *Pp*-Hyal can be classified as a glycosyl hydrolase and that the full-length *Pp*-Hyal cDNA (1315 bp; GI: 302201582) is similar (80–90%) to hyaluronidase from the venoms of endemic Northern wasp species. The isolated mature protein is comprised of 338 amino acids, with a theoretical pI of 8.77 and a molecular mass of 39,648.8 Da versus a pI of 8.13 and 43,277.0 Da indicated by MS. The *Pp*-Hyal 3D-structural model revealed a central core $(\alpha/\beta)_7$ barrel, two sulfide bonds (Cys 19–308 and Cys 185–197), and three putative glycosylation sites (Asn79, Asn187, and Asn325), two of which are also found in the rVes v 2 protein. Based on the model, residues Ser299, Asp107, and Glu109 interact with the substrate and potential epitopes (five conformational and seven linear) located at surface-exposed regions of the structure. Purified native *Pp*-Hyal showed high similarity (97%) with hyaluronidase from *Polistes annularis* venom (Q9U6V9). Immunoblotting analysis confirmed the specificity of the *Pp*-Hyal-specific antibody as it recognized the *Pp*-Hyal protein in both the purified fraction and *P. paulista* crude venom. No reaction was observed with the venoms of *Apis mellifera*, *Solenopsis invicta*, *Agelaia pallipes pallipes*, and *Polistes lanio lanio*, with the exception of immune cross-reactivity with venoms of the genus *Polybia* (*sericea* and *ignobilis*). Our results demonstrate cross-reactivity only between wasp venoms from the genus *Polybia*. The absence of cross-reactivity between the venoms

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of wasps and bees observed here is important because it allows identification of the insect responsible for sensitization, or at least of the phylogenetically closest insect, in order to facilitate effective immunotherapy in allergic patients.

1. Introduction

Hyaluronidases (Hyal) are a widely distributed group of enzymes that hydrolyze hyaluronic acid (HA), one of the primary components of the extracellular matrix in all vertebrates (Kreil, 1995). Hyal are also present in almost all venoms, acting as a "diffusion factor" by facilitating the penetration of the other harmful venom components and enhancing their action in various tissues into the bloodstream (Kemparaju and Girish, 2006; Senff-Ribeiro et al., 2008).

Hyal have been described as "allergenic factors" in scorpion, bee, and wasp venoms, and are able to induce severe and fatal anaphylactic IgE-mediated reactions in humans (Lu et al., 1995; Kolarich et al., 2005). Hyal have already been characterized as glycoproteins (Kemeny et al., 1984; Jin et al., 2008) and analysis by high performance liquid chromatography and mass spectrometry revealed that the α-1,3-fucose-containing N-glycan is the fundamental structure responsible for their allergenicity (Kubelka et al., 1995; Kolarich and Altmann, 2000; Kolarich et al., 2005).

Since allergenic Hyal are phylogenetically more conserved among the other Hymenoptera allergens (e.g. Ag5 and PLA1), a significant degree of homology is observed among the sequences and 3D structures of these proteins, whether they are from different vespids or honeybee *Apis mellifera* venom (Api m 2) (Jin et al., 2010). In addition, a large percentage of patients allergic to Hymenoptera venom show reactivity to both bee and wasp venoms (known as cross-reactivity) in tests for the presence of IgE-specific antibodies (Hemmer, 2008). This makes selection of the most suitable venom for immunotherapy difficult. However, it is unclear whether this cross-reactivity is due to (a) sequence homology between these hyaluronidases; (b) sensitivity to the specific IgE antibodies; or (c) cross-reactive N-glycans (cross-reactive carbohydrate determinants [CCDs]), which have been investigated in allergens from different sources (Jin et al., 2010; Eberlein et al., 2012; Al-Ghouleh et al., 2012).

In terms of the mechanism of action on the substrate, Hyal enzymes are classified into three types (Meyer, 1971): (a) the group of the endo-β-*N*-acetyl-D-hexosaminidases that hydrolize the high molecular weight substrate (HA) to tetrasaccharide as the main end product, being this group represented by the testicular enzyme; (b) the β-endoglucuronidases group represented by hyase from leeches and hookworm (Hotez et al., 1994); (c) and finally the group of lyases that act via β-elimination, yielding disaccharides as the main end products represented by the bacterial hyases. According to Laurent (1989), Cramer et al. (1994) and Takagaki et al. (1994) the enzymes of the first group also catalyzes transglycosylation reactions, producing hexa-, di-, and octa-saccharides during hydrolysis of HA. Hyaluronate-4-glycanohydrolase (EC 3.2.1.35), or Hyal type 1, is an endo-β-N-acetyl-D-hexosaminidase is also found in Hymenoptera venoms and mammalian spermatozoa. Unlike the other two types of hyaluronidases, this group acts not only on HA, but also on chondroitin 4-sulfate and chondroitin 6-sulfate (CS) (Fiszer-Szafarz, 1984; Fiszer-Szafarz et al., 1990; Kreil, 1995; Cherr et al., 1996; Stern and Jedrzejas, 2006).

The social wasp *Polybia paulista* (Hymenoptera, Vespidae) is endemic to Southeastern Brazil, especially São Paulo State, and is responsible for many accidents due to their venomous stings. Due to consequent and serious allergic reactions that may develop and lead to anaphylactic shock (Palma, 2006), the social wasp is thus of great medical importance.

Studies of crude extracts of *P. paulista* venom by chromatography, SDS-PAGE, and specific assays showed significant levels of hyaluronidase, phospholipase, and proteolytic, hemolytic and myotoxic activities (Silva et al., 2004). Recently, proteomic analysis by Pinto et al. (2012) detected four different glycoproteic forms of Hyal in *P. paulista* venom and subsequently sequenced and structurally modeled the most abundant form, Hyal III.

In order to examine the molecular characteristics and immunogenic potential of the *Pp*-Hyal venom allergen, the complete cDNA sequence of another form of this enzyme was obtained, cloned, sequenced and its 3D-protein structural model constructed by comparative modeling. Furthermore, the native form of this *Pp*-Hyal was purified through high performance chromatography and analyzed by mass spectroscopy. The protein was then used to produce a *Pp*-specific polyclonal antibody, which was tested by Western blotting to confirm its specificity and immune cross-reactivity with venoms from other Hymenoptera species.

2. Material and methods

2.1. Insects and crude venom extracts

P. paulista nests were collected in the city of Rio Claro, SP, Southeastern of Brazil. Insects were anesthetized at low temperature (−20 °C) and their venom reservoirs were extracted with tweezers. Crude venom extract was prepared from 1000 reservoirs, which were macerated at a 1:1 ratio (reservoir:solvent) with ultra pure water containing 1 mM PMSF (Sigma–Aldrich, USA). The suspension was centrifuged at 10,000 × *g* for 15 min at 4 °C and *Pp*-Hyal protein was purified from the freeze-dried supernatant. For immunological assays, venom extracts were prepared by the same method with 100 venom reservoirs from each of the following species of Hymenoptera: *P. paulista*, *Polybia sericea*, *Polybia ignobilis*, *Agelaia pallipes pallipes*, *Polistes lanio lanio*, *A. mellifera*, and *Solenopsis invicta*.

2.2. Protein determination

Quantification of total proteins in the extracts and fractions from chromatography was performed by the