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An anticoagulant serine protease from the wasp venom of *Vespa magnifica* ☆

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Abstract

Wasp is an important venomous animal that can induce human fatalities. Coagulopathy is a clinical symptom after massive wasp stings, but the reason leading to the envenomation manifestation is still not known. In this paper, a toxin protein is purified and characterized by Sephadex G-75 gel filtration, CM-Sephadex C-25 cationic exchange and fast protein liquid chromatography (FPLC) from the venom of the wasp, *Vespa magnifica* (Smith). This protein, named magnvesin, contains serine protease-like activity and inhibits blood coagulation. The cDNA encoding magnvesin is cloned from the venom sac cDNA library of the wasp. The deduced protein from the cDNA is composed of 305 amino acid residues. Magnvesin shares 52% identity with allergen serine protease from the wasp *Polistes dominulus*. Magnvesin exerted its anti-coagulant function by hydrolyzing coagulant factors TF, VII, VIII, IX and X.

Keywords: Wasp venom; *Vespa magnifica*; Serine protease; Blood coagulation; Anticoagulant

1. Introduction

The Vespidae family includes hornets (genera *Vespa* and *Dolichovespula*), yellow jackets (genus *Vespula*) and paper wasps (genus *Polistes*). They possess highly toxic venom, which is rich in toxins, enzymes and biologically active peptides (Habermann, 1972; Nakajima, 1984). They are important venomous animal endangering human life, causing fatalities in serious cases. Vespid venoms have similar venom compositions. The clinical symptoms induced by wasp stings include local reactions (such

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as pain, wheal, edema and swelling), immunological reactions usually leading to anaphylaxis with subsequent anaphylactic shock and systemic toxic reactions caused by large doses of venom, resulting in hemolysis, coagulopathy, rhabdomyolysis, acute renal failure, hepatotoxicity, aortic thrombosis and cerebral infarction (Chao and Lee, 1999; Chen et al., 2004; Evans and Summers, 1986; Korman et al., 1990; Sakhuja et al., 1988; Watemberg et al., 1995). Local reactions may result from biologically active peptides such as bradykinin-like peptides, chemotactic peptides and mastoparans. Allergic reactions induced by wasp venoms have been extensively focused on and studied. The major wasp venom allergens are phospholipase A_1, hyaluronidase, antigen 5 and serine proteases (Habermann, 1972; Nakajima, 1984, Asgari et al., 2003, Yamamoto et al., 2007, Yang et al., 2007). The anticoagulant effect of wasp venom sac extract (VSE) has been reported in several literatures. It is suggested that the anticoagulant effect of wasp VSE was attributed to a proteolytic process involving mainly coagulation factors VIII and IX (Kornberg et al., 1988; Haim et al., 1999). The venom component acting on the coagulation process from wasp has not been characterized still, although a proteolytic enzyme named protease I with anticoagulant activity has been purified from *Vespa orientalis* venom (Haim et al., 1999).

Serine proteinases belong to the trypsin family S1 of clan SA, the largest family of peptidases (Serrano and Maroun, 2005; Halfon and Craik, 1998). Many serine proteases have been found from animal venoms, especially from snake venoms. They can affect the hemostatic system by acting on a variety of components of the coagulation cascade, on the fibrinolytic and kallikrein–kinin systems and on cells (Seegers and Ouyang, 1979; Markland, 1997; Pirkle, 1998). Some serine proteases with allergic or melanization-inhibitory activity have been found from the wasp venom (Asgari et al., 2003; Winningham et al., 2004). No serine protease acting on the hemostatic system has been characterized from wasp venoms. In the current work, an anticoagulant serine protease was purified and characterized from the wasp venom of *Vespa magnifica* (Smith).

2. Materials and methods

2.1. Wasp venom

The wasps *V. magnifica* were collected in Yunan, China. The collected wasps were stimulated by alternative current (6 V) lasting for 6–10 s. The wasp venom was secreted onto a clean glass plate ($50 \times 50\,cm^2$), immediately collected and stored at $-20\,^{\circ}C$.

2.2. Protein purification

Lyophilized wasp venom samples of *V. magnifica* (0.3 g) were dissolved in 10 ml 0.1 M phosphate buffer solution (PBS), pH 6.0, containing 5 mM EDTA. The sample was applied to a Sephadex G-75 (Superfine, Amersham Biosciences, $2.6 \times 100\,cm^2$) gel filtration column equilibrated with 0.1 M PBS, pH 6.0. Elution was performed with the same buffer, collecting fractions of 3.0 ml as the purification procedure of the phospholipase A_1 from *V. magnifica* venoms (Yang et al., 2007). The absorbance of the elute was monitored at 280 nm. The anticoagulant activities of fractions were determined as indicated below. The protein peak containing anticoagulant activity was pooled (45 ml) and lyophilized, and re-suspended in 5 ml 0.05 M PBS, pH 6.5, and further dialyzed against 0.05 M PBS (pH 6.5). The product was consequently subject to the cation-exchange CM-Sephadex C-25 column (Amersham Biosciences, $1.6 \times 30\,cm^2$). The elution was achieved with a linear NaCl gradient in 0.05 M PBS, pH 6.0, at a flow rate of 1 ml/min as the purification procedure of the phospholipase A_1 from *V. magnifica* venoms (Yang et al., 2007). The peak with anticoagulant activity was collected and finally purified on an AKTA Mono S (1 ml volume, Amersham Biosciences) cationic exchange column equilibrated with 20 mM phosphate buffer solution, pH 6.5, using fast protein liquid chromatography (FPLC, Amersham Biosciences), equilibrated with 0.02 M PBS, pH 6.5. The elution was performed with a liner NaCl gradient in 0.02 M PBS, pH 6.5, at a flow rate of 1 ml/min as illustrated in Fig. 1.

2.3. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

About 5 μg of purified protein from FPLC was mixed with 10 μl of SDS sample buffer and boiled for 5 min before being loaded onto a 15% polyacrylamide gel. After electrophoresis, the gel was stained with Coomassie brilliant blue and destained to allow visualization of the protein (Laemmli, 1970).