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Comparative proteomic analysis of two wasps venom, *Vespa tropica* and *Vespa affinis*

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ABSTRACT

Vespid venom is composed of many bioactive compounds. The venom of the banded tiger wasp (*Vespa affinis*, or *VA*) and the great banded wasp (*Vespa tropica*, or *VT*)—which are locally found in the northeastern part of Thailand and are well known for their life-threatening venom potency—were comparatively studied in terms of potency, composition and biological activity. Clinical studies that included word-of-mouth information shared by traditional healers in local areas noted that the venom of *VT* is more potent than that of *VA*. Our previous study showed that the venom of *VA* is lower in potency (PD_{50} = 12.5 μg/g body weight) than that of *VT* (PD_{50} = 3 μg/g body weight). Analysis with the PAGE technique showed that these two venoms showed similar patterns of active proteins. Most protein spots were basic proteins at an isoelectric point (pI) ranging from 5 to 10, with molecular weights between 27 and 50 kDa. These spots were identified as hyaluronidase, phospholipase, antigen 5, dipeptidyl peptidase and albumin-like protein. The proportion of hyaluronidase was 2.5 times higher in *VT* than in *VA*. *VT* also showed higher hyaluronidase, phospholipase and dipeptidyl peptidase activities, suggesting that these components made *VT* venom more potent than *VA* venom.

1. Introduction

Wasp venom is well known for inducing strong allergic reactions and even causing fatalities in serious cases in humans and animals. Wasp venom secreted from the venom gland has a wide range of functions necessary for wasp survival (Yu et al., 2007). It is a complex mixture rich in toxins, enzymes and biologically active peptides. The major protein compositions of venom are protease, phospholipase, hyaluronidase, allergen, antigen 5 and mastoparan (Han et al., 2008; King et al., 1984; Lu et al., 1993, 1995; Sukprasert et al., 2013).

Two types of wasp in order Hymenoptera and genera Vespidae—the great banded wasp (*Vespa tropica*, abbreviated as *VT*) and the banded tiger wasp (*Vespa affinis*, abbreviated as *VA*)—are commonly found in the northeastern part of Thailand. They are social hymenoptera that closely encounter with humans, resulting in many cases of accidental stings annually (Barss, 1989; Kularatne et al., 2014). Sensitized victims stung by these two vespids may suffer from severe allergic reactions.

The potency of these 2 lethal wasps is well known.

Abbreviations: VA, *Vespa affinis*; *VT*, *Vespa tropica*.

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Envenomations of *VA* were clinically reported in Nepal, Sri Lanka, and Indonesia. Symptoms include intravascular hemolysis, hepatic dysfunction, oligo-anuria, azotemia, myocardial infarction, multiple organ failure—particularly acute renal failure—increased microvascular permeability, acute pulmonary edema, and anaphylactic shock (Das and Mukherjee, 2008; Kularatne et al., 2003, 2014; Lee et al., 2005). By contrast, there have been few reported cases of *VT* envenomations. This discrepancy can be explained by the fact *VT* live in distant areas or in inaccessible forests, whereas *VA* tend to adapt their behavior to live close to people. One *VT* sting case reported in Papua New Guinea led to acute renal failure (Barss, 1989). However, *VT* is famous for its lethal effects.

This study tried to comparatively report the characterization of venom from these 2 vespids, focusing on their size, shape and biological behavior, including venom composition in the biochemical aspects of the venom.

2. Material and methods

2.1. Wasp and venom collection

Wasps from Siang Sao Village, Sri Songkram District, Nakorn Panom Province in the northeastern part of Thailand were immediately shocked in ice and dissected. About 1000 of each worker wasp venom reservoirs were removed from the sting apparatus by forceps, pooled and stored at −30 °C (Santos et al., 2007). Protein concentration was determined by the Bradford method using bovine serum albumin as a standard.

2.2. Gel electrophoresis

SDS-PAGE and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) were slightly modified from Sukprasert et al. (2013). After being focused in the first dimension with 7 cm immobilized pH gradient strip pH 3–11 (Amersham Biosciences, Sweden) for 12 h and 9250 total Vhr using Ettan IPGphor (Amersham Bioscience), samples were resolved by 13% Tris-glycine SDS-PAGE for the second dimension. Protein was stained by Coomassie Blue G. Protein patterns in the gels were recorded as digitalized images using a Canon CanoScan8000F digital scanner (resolution 300 dpi) and saved as TIFF files. Gels were quantitated in profile mode using ImageMaster 2D Platinum software version 5.0 (GE Healthcare).

2.3. In-gel digestion and mass spectrometry

In-gel digestion and mass spectrometry were following a previous description (Prajanban et al., 2012). Briefly described, the spots were gel-excised, washed and digested with 20 ng/spot modified trypsin (Promega, Madison, USA) in 50% acetonitrile/10 mM ammonium bicarbonate at 37 °C for 3 h. Peptides were extracted by washing the gel pieces three times with 30 μl of 50% ACN/0.1% formic acid. The supernatant was dried at 37 °C for 3 h, dissolved in 0.1% (v/v) formic acid and kept at −30 °C for mass analysis. The sample was then subjected to the Ultimate 3000 LC System (Dionex), coupled with an ESI-Ion trap MS (HCTultra PTM Discovery System, Bruker Daltonik). A database search for peptide identification was generally performed using a local MASCOT server with the following search parameters: a specified trypsin enzymatic cleavage with one possible missed cleavage, ±0.6 Da mass tolerances for MS and MS/MS, a peptide tolerance of 1.2 Da, 1+, 2+, and 3+ ions, methionine oxidation variable modification, carbamidomethyl (C) fixed modification, monoisotopic mass, and 20 responses.

2.4. Venom potency

Paralytic dose 50 (PD_{50}) was used to determine the potency (Uawonggul et al., 2007). This dose affected 50% of crickets (*Gryllus* sp.) unable to recover from a turned-over position.

2.5. Hyaluronidase activity assay

For the zymographic method, proteins (50 μg) were resolved on 13% separating gel containing 4% hyaluronic acid of SDS-PAGE at 15 mA for 1 h. Gel was incubated in 3% triton X-100 for 1 h to remove SDS, transferred to the hyaluronidase assay buffer (0.15 M NaCl in 0.1 M formate buffer) at a suitable pH, and rotated for 16 h at 37 °C. After rinsing, gels were stained in Alcian Blue solution (0.5% Alcian Blue in 3% acetic acid) for 1 h and destained in 7% acetic acid until white clear bands appeared on a pale blue background (Mio and Stern, 2000).

For turbidity hyaluronidase activity, mixtures of samples, 0.5 mg/ml hyaluronic acid, and 0.15 M NaCl in 0.2 M acetate buffer, were incubated for 30 min at 37 °C. The reactions were stopped by 2 vol of 2% CTAB containing 2.5% NaOH. The turbidity was measured at 405 nm. The activity was calculated as [(100 − blank) − (test − blank)]/100. The turbidity reducing activity was expressed as the percentage of remaining hyaluronic acid by taking the absorbance of the tube at 100% in which no enzyme was added (Pukrittayakamee et al., 1988).

2.6. Phospholipase activity assay

Enzyme activity was detected on a 3% agar plate containing 3% lecithin to measure the size of the zone of precipitation by the slightly modified method of Chrisope et al. (1976). Protein 100 μg was mixed in 50 μl PBS buffer (135 mM NaCl, 1.5 mM KH_2PO_4, 2.5 mM KCl, and 8 mM Na_2HPO_4). The plate was incubated at 37 °C overnight prior to the appearance of white colonies. Enzyme activity was calculated using *Thermomyces lanuginosus* phospholipase A_1 as a standard (unit/mg liquid). Plot of the diameter (Y axis) was linearly proportional to the logarithm of standard enzyme concentration (X axis).

2.7. Dipeptidyl peptidase (DPP) activity

DPP activity was assayed as described by Blank et al. (2010). Venom (50 μg) was incubated with 0.5 mM glycine–proline–*p*-nitroanilide hydrochloride (Sigma-Aldrich), a synthetic substrate. The product was observed at 405 nm. Tris-HCl pH 7.5 in 100 mM NaCl was used as a negative control.

3. Results and discussion

This report intended to comparatively discuss the following three aspects of these two types of wasps: (1) biological aspects (sizes, shapes, habitats and behavior), (2) venom potency, and (3) proteomics (protein components in venom), including the proportion of contents, and the activity of enzymes in the venom.

3.1. Biological aspects

Banded wasps, *VA* and *VT*, are distributed throughout of Thailand. They are similar in their shape and appearance. Their black bodies are yellow banded around the abdomen; *VA* and *VT* have one and two bands, respectively (Fig. 1A and B). Both of these wasps are often referred to as “tiger wasps” because of these abdominal bands. *VA* is approximately 2–3 cm long, and *VT* is approximately 3–4 cm long. For their habitats, *VA* hang their nests