identified in the venom of animals including snakes, bees, scorpions, fish, spiders, ants, wasps, caterpillars etc. [11–16]. Clinical studies have demonstrated that HAase is an "allergic factor" due to its ability to initiate pathogenic reactions in the majority of venom allergic patients [17–19]. It is also able to induce several anaphylactic IgE-mediated reactions in humans and has been suggested to be involved in the difficulties in the clinical diagnosis of venom allergic individuals [20–22]. The wasp venom HAase belongs to the hyaluronate glycanohydrolase family (EC 3.2.1.35), which degrades hyaluronic acid (HA) [23, 24]. Wasp venom HAase is responsible for the cross-reactivity of wasp and bee venom sera in patients as well [2, 25].

The greater banded wasp (*Vespa tropica*) is mostly distributed in the forest throughout Indochina peninsula including Thailand. It has a body length of up to 5 cm and its nest is usually found underground [26]. *V. tropica* is among the most venomous known insects. The lethal dose of its pure venom in experimental animals (LD_{50} of approximately 2.8 mg/kg in mice) is more potent than that of *V. affinis* venom [26, 27]. The potency of *V. tropica* venom has been reported to nearly stop the end plate potentials of *Drosophila* larvae in nerve-muscle preparation in response to treatment with this venom [28]. HAase was reported to be a major protein in *V. tropica* venom, where it is found by 2.5-fold the proportion observed in *V. affinis* venom [26]. The understanding of HAase in terms of biochemical and structural characterization of these wasps is important for the development of new tools for treating multiple stings and for diagnosis and therapy of allergic reactions caused by this venom. Therefore, the present study aimed to characterize HAase isoforms in the venom of *V. tropica* by analyzing its sequence and 3D modelling.

Methods

Animals

The wasps were collected from Siang Sao Village, Sri Songkram district, Nakorn Panom Province, northeastern Thailand [26]. The worker wasps were immediately shocked on ice. The venom reservoirs were removed from the sting apparatus by removing them from the bodies with forceps and squeezing. The droplets of venom and specimens of *V. tropica* were collected in a 1.5-mL microcentrifuge tube and then keep at −80 °C until use.

RT-PCR and rapid amplification of cDNA ends (5′ and 3′ RACE)

Total RNA was extracted from the venom gland of *V. tropica* with TRIzol® reagent (Invitrogen, Life technologies, USA). RT-PCR was performed using the RevertAid First strand cDNA synthesis kit (Thermo Scientific, USA) as described in the instruction manual. PCR primers for the amplification of VesT2 were designed based on the sequence similarity of the conserved region of HAase from vespid venom and conserved nucleotide sequences corresponding to peptide sequences obtained from LC-MS/MS analysis (Table 1) [26]. The PCR was performed using green master mix reagent kits with Taq DNA polymerase (Promega, Singapore). The reaction contained 2 μg of cDNA, 1 UTaq DNA polymerase, 2.0 mM dNTP, 2.0 mM $MgCl_2$ and 2 μM of primerin to a final volume of 25 μL under the following conditions: initial denaturation for 5 min at 94 °C, followed by 35 cycles at 94 °C (30 s); 55 °C

Table 1 Primer design of gene-specific primers and PCR product size

Forward primer	Reverse primer	Product size (bp)
Full nucleotide sequence active form		
F4 GCCAGACTTTTCATGGAGGA (GSP1 for active)	R3 (7) ATCAGGGGTCAGTTCACGTC (GSP1 for active)	225
Adaptor primer (AP) 5′GGCCACGCGTCGACTAGTAC (T) 16 (GSP for cDNA synthesis of 3′ RACE system)	R4 (8) CGTCGGTCTCGGTAAGAAAA	
Abridged universal amplification primer (AUAP)	R5 (9) GTTCTCGTGCATCGCTGTAA	
VesT2a (F) *Nco*I **CCATGG**CTTCCGAGAGACC	VesT2a (R) *Xho*I **CTCGAG**TTAGTTAACGGCTTCTG	
Full nucleotide sequence inactive form		
F1 CGAAAAGGAAGCGTCGAATA (GSP for RT-PCR inactive form)	R1 CATCTTGTCGTTCTCGCTCA (GSP for RT-PCR inactive form)	190
F2 CTTCGGCGTCTATTTCAAGG (GSP for RT-PCR inactive form)	R2CCGCTAAGACAGTGGGGATA (GSP for inactive form)	229
Adaptor primer (AP) 5′GGCCACGCGTCGACTAGTAC (T) 16 (GSP for cDNA synthesis of 3′ RACE system)	R2 (1) CATCTTGTCGTTCTCGCTCA (GSP for RT-PCR inactive form)	
Abridged universal amplification primer (AUAP)	R1 (2) CCGCTAAGACAGTGGGGATA (GSP for inactive form)	

The bold letters represent the restriction sites