ELSEVIER

Comparative Biochemistry and Physiology, Part B 141 (2005) 373 – 381

CBP

www.elsevier.com/locate/cbpb

Hydrolase activity in the venom of the pupal endoparasitic wasp, *Pimpla hypochondriaca*

M.P. Dani*, J.P. Edwards, E.H. Richards

Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK

Received 21 December 2004; received in revised form 24 April 2005; accepted 25 April 2005
Available online 4 June 2005

Abstract

Venom from the pupal endoparasitoid, *Pimpla hypochondriaca* has previously been shown to contain a mixture of biologically active molecules. Currently, *P. hypochondriaca* venom was examined for the presence of hydrolase activity. Six hydrolases were consistently detected using the API ZYM semiquantitative colourimetric kit. The main hydrolases detected were; acid phosphatase, β-glucosidase, esterase, β-galactosidase, esterase lipase, and lipase. The most rapid and intense colour reaction was detected for acid phosphatase. The pH optimum and the specific activity of venom acid phosphatase was determined using *p*-nitrophenol phosphate as a substrate and were 4.8 and 0.47 nmol *p*-nitrophenol/min/μg of venom protein, respectively. The acid phosphatase activity was inhibited in a dose dependent manner by sodium fluoride (IC_{50} 4.2×10^{-4} M), and by cocktail inhibitor 2 (CI 2). *P. hypochondriaca* venom has previously been shown to display potent cytotoxic activity towards *Lacanobia oleracea* haemocytes maintained in vitro. The contribution of acid phosphatase in venom to this cytotoxic activity was investigated by titrating venom against CI 2 prior to the addition of *L. oleracea* haemocytes. The results suggest that, despite the relatively high levels of acid phosphatase activity in venom, venom acid phosphatase plays no role in the antihaemocytic activity of *P. hypochondriaca* venom in vitro.

Keywords: Acid phosphatase; Antihaemocytic activity; Endoparasitoid; Hydrolase; *Lacanobia oleracea*; Phosphatase inhibitors; *Pimpla hypochondriaca*; Venom

1. Introduction

Endoparasitoids that successfully develop within the haemocoel of their host insect do so by avoiding or overcoming elimination by the immune defence responses of the host. This may be achieved by the introduction, during oviposition, of secretions such as polydnaviruses, calyx fluid, ovarian proteins and/or venom from the female reproductive system (Vinson and Iwantsch, 1980; Beckage, 1998). Depending on the parasitoid species concerned, some of these secretions have been reported to inhibit essential immune defence responses of the host insect (Vinson, 1990; Lavine and Beckage, 1995; Strand and Pech, 1995). For instance, in certain parasitoid species, venom plays a major role in ensuring the survival and development of the parasitoid progeny in vivo (Kitano, 1982, 1986; Tanaka, 1987).

The solitary pupal endoparasitoid, *Pimpla hypochondriaca* Retzius (Hymenoptera: Ichneumonidae) parasitizes a number of lepidopteran species including the tomato moth, *Lacanobia oleracea* L. (Lepidoptera: Noctuidae) (Thompson, 1946; 1957). Venom of *P. hypochondriaca* consists of a mixture of high and low molecular weight proteins, some of which have been reported to possess enzyme activity. For example, using L-DOPA as a substrate, Parkinson and Weaver (1999) demonstrated the presence of phenoloxidase activity that could be inhibited by a known phenoloxidase inhibitor, phenylthiocarbamide (PTC). The venom also contains a low molecular weight heat-stable phenoloxidase inhibitor. In addition, *P. hypochondriaca* venom induced paralysis in several insect species and exhibited a cytotoxic effect on a *Spodoptera frugiperda* insect cell line. The cytotoxic activity can be distinguished from the phenolox-

* Corresponding author. Tel.: +44 1904 462686; fax: +44 1904 462111.
E-mail address: p.dani@csl.gov.uk (M.P. Dani).

1096-4959/$ - see front matter.
doi:10.1016/j.cbpc.2005.04.010

idase activity detected (Parkinson and Weaver, 1999). More recently, phenoloxidase genes have been isolated from a *P. hypochondriaca* venom gland cDNA library (Parkinson et al., 2001), in addition to the identification of cDNAs coding for a serine protease, a reprolysin-like metalloprotease and a trehalase (Parkinson et al., 2002a,b, 2003). Angiotensin-converting enzyme (ACE)-like activity, sensitive to the specific mammalian inhibitor captopril, has also been detected in *P. hypochondriaca* venom (Dani et al., 2003). Furthermore, polyclonal antiserum generated against recombinant *Drosophila melanogaster* ACE (AnCE) cross-reacted with a protein in *P. hypochondriaca* venom (Dani et al., 2003). Using synthetic fluorogenic substrates, the presence of proteolytic activity has also been examined for *P. hypochondriaca* venom (Dani et al., 2003). The above studies suggest that venom is composed of a mixture of active molecules, including a number of enzymes.

Previously, detailed studies have been performed demonstrating the detrimental effect of *P. hypochondriaca* venom on *L. oleracea* larval and pupal cellular immune responses both in vivo and in vitro (Richards and Parkinson, 2000; Parkinson et al., 2002a). For example, following injection of the venom into larval and pupal *L. oleracea*, the encapsulation of Sephadex A-25 beads was reduced (Richards and Parkinson, 2000; Parkinson et al., 2002a). The venom of this wasp also reduced the phagocytosis of FITC-labelled *Escherichia coli* by larval *L. oleracea* haemocytes (Richards and Parkinson, 2000). Moreover, a prior injection of *P. hypochondriaca* venom was found to increase the susceptibility of *L. oleracea* larvae to micro-organisms (Dani et al., 2004). The aim of the current study was to investigate further the properties of *P. hypochondriaca* venom with a view to identifying factors, particularly enzymes, that might play a role in altering host insect immune defence responses.

2. Materials and methods

2.1. Chemicals

Chemicals were purchased from Sigma-Aldrich (Poole, Dorset, UK) unless stated otherwise.

2.2. Insects

L. oleracea were reared as described by Corbitt et al. (1996), on noctuid artificial diet (Bioserv, New Jersey) at 20 °C, 65% relative humidity and under a 16 h light–8 h dark photoperiod. *P. hypochondriaca* were reared as described previously by Richards and Parkinson (2000). Venom was harvested from adult female *P. hypochondriaca* as outlined by Parkinson and Weaver (1999). In brief, each venom sac dissected from adult female wasp, was rinsed in sterile Dulbecco's phosphate buffered saline (DPBS) and then placed into 20 μL of DPBS. The venom sac was carefully torn apart to release the venom into the buffer and then the sac was discarded (Fig. 1). Venom harvested at any one time was pooled and stored at −20 °C. The stored batches of venom were homogenised by passing the venom many times through pipette tips of decreasing bore size. Finally, the venom was centrifuged at 10,000 ×*g* for 5 min to pellet the 'gel-like' component, and the supernatant was collected. This supernatant (referred to herein as "venom") was used in all studies and generally contained venom obtained from approximately 60 to 150 venom sacs.

2.3. Detection of hydrolase activity

Hydrolase activity in *P. hypochondriaca* venom was investigated using the colourimetric API ZYM kit (Bio-Mérieux UK limited, Basingstoke, Hampshire). The kit was used according to the manufacturer's instructions. In brief, venom diluted in sterile distilled water (SDW) was added to each well of the test strip (40–62 μg venom protein/well, or 0.22–0.34 venom sac equivalent (vse) per well). The test strip was then placed in a humid chamber and incubated at 37 °C for 4 h. The controls consisted of heat-treated venom (70 °C for 30 min, followed by centrifugation at 10,000 ×*g* for 30 min), and SDW. Following incubation, two kit reagents were added to each well and the colour reaction was monitored. Each well was scored from 0 to 5; 0 representing no colour change and 5 representing a strong colour reaction. Venom that had been pre-incubated for 20 min with a 1 in 75 dilution of cocktail inhibitor 2 (CI 2; a phosphatase inhibitor), was also applied to a test strip. The term "vse" is used to define the mean protein content from

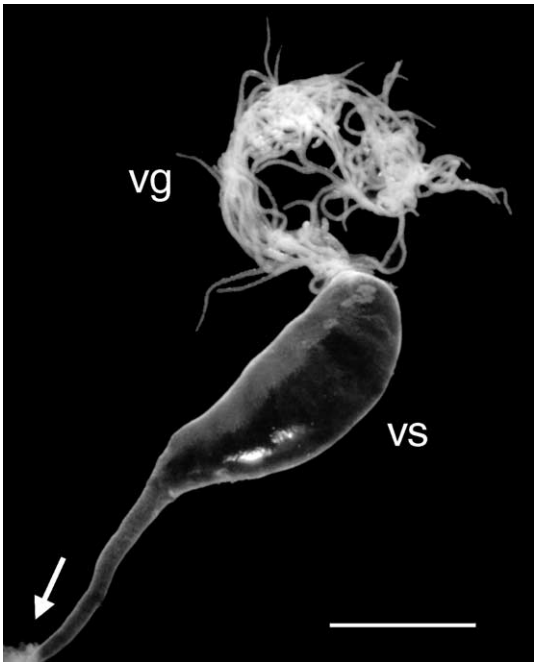

Fig. 1. Photograph of venom sac (vs) and venom gland (vg) dissected from adult female *P. hypochondriaca*. The arrow indicates the duct leading to the ovipositor. Bar=1 mm.