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Tissue specificity of a baculovirus-expressed, basement membrane-degrading protease in larvae of *Heliothis virescens*

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Abstract

ScathL is a cathepsin L-like cysteine protease from the flesh fly, *Sarcophaga peregrina*, which digests components of the basement membrane during insect metamorphosis. A recombinant baculovirus (AcMLF9.ScathL) expressing ScathL kills larvae of the tobacco budworm *Heliothis virescens* significantly faster than the wild type virus and triggers melanization and tissue fragmentation shortly before death. The tissue fragmentation was assumed to be a direct consequence of basement membrane degradation by ScathL. The goal of this study was to investigate the tissue specificity of ScathL when expressed by AcMLF9.ScathL using light, transmission and scanning electron microscopy. Baculovirus expression of ScathL resulted in damage to the basement membrane overlying the midgut, fat body and muscle fibers in larvae infected with AcMLF9.ScathL, but not in larvae infected with the control virus AcMLF9.ScathL.C146A or wild type virus AcMNPV C6. Injection of recombinant ScathL and high levels of baculovirus-mediated expression of ScathL resulted in complete loss of the gut. Extensive damage to the basement membrane mediated by ScathL likely resulted in loss of viability of the underlying tissue and subsequent death of the insect. These results confirm the conclusion of an earlier study (Philip, J.M.D., Fitches, E., Harrison, R.L., Bonning, B.C., Gatehouse, J.A., 2007. Characterisation of functional and insecticidal properties of a recombinant cathepsin L-like proteinase from flesh fly (*Sarcophaga peregrina*), which plays a role in differentiation of imaginal discs. Insect Biochem. Mol. Biol. 37, 589–600) of the remarkable specificity of this protease.

Keywords: *Heliothis virescens*; Basement membrane; *Autographa californica* multiple nucleopolyhedrovirus; Cathepsin L; Ultrastructure

1. Introduction

Baculoviruses are arthropod-specific viruses that infect many agriculturally significant pests, primarily within the Lepidoptera. Baculoviruses have double-stranded circular DNA genomes contained within an enveloped, rod-shaped nucleocapsid, and have two phenotypes: occlusion-derived virus (ODV) and budded virus (BV). Following ingestion of virus, ODVs released from polyhedra (occlusion bodies) within the alkaline environment of the insect midgut cause the initial infection of the midgut epithelial cells (Bonning, 2005); BVs are produced and released from the infected cells and are responsible for the secondary infection of other tissues within the host (Trudeau et al., 2001). Polyhedra are produced in massive amounts in the host and are released into the environment following death and lysis of the host insect. Although baculoviruses have potential for insect pest control, they have not been widely used in part because they are poorly competitive with chemical pesticides in terms of speed of action. Recombinant baculoviruses expressing neurotoxins, enzymes, and insect peptide hormones have been constructed for enhanced insecticidal efficacy (Van Beek and Hughes, 1998; Kamita et al., 2005).

Host basement membrane has been identified as a potential target for improving baculovirus insecticidal efficacy (Keddie

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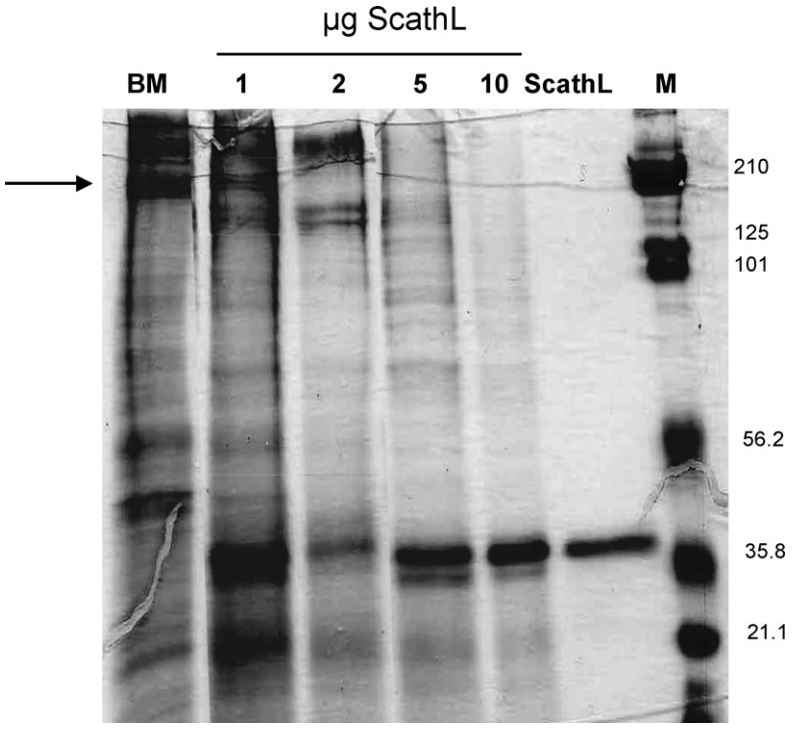

Fig. 1. *In vitro* digestion of the basement membrane (BM) of *H. virescens* larvae by recombinant ScathL. SDS-PAGE showing incubation of 25 μg BM protein with various amounts of purified, yeast-expressed ScathL in 0.5 M sodium acetate buffer (pH 5.0). Samples were incubated at 37 °C for 3 h. Arrow indicates the missing proteins around 210 kDa in ScathL-digested BM samples. BM, BM only; 1, BM plus 1 μg ScathL; 2, BM plus 2 μg ScathL; 5, BM plus 5 μg ScathL; 10, BM plus 10 μg ScathL; ScathL, 10.0 μg ScathL only; M: molecular mass markers (kDa).

et al., 1989). Basement membrane are extracellular sheets of protein, composed primarily of laminin, type IV collagen, and proteoglycans that surround all tissues providing structural support, and a surface for cell attachment (Rohrback and Rohrback, 1993; Yurchenco and O'Rear, 1993). There is high homology between the basement membrane of invertebrates and vertebrates in composition, structure, and function (Fessler and Fessler, 1989). Within infected insects, basement membranes appear to act as a barrier to dissemination of baculoviruses as well as other viruses (Romoser et al., 2005). BVs are too large to freely diffuse through the pores in the basement membrane that surround tissues of the host insect (Reddy and Locke, 1990). Coinjection of BVs and clostridial collagenase, a protease known to degrade basement membrane, resulted in enhanced infection of host tissues (Smith-Johannsen et al., 1986). An ultrastructural study of infection by the baculovirus *Cydia pomonella* granulovirus revealed substantial accumulation of BVs in the extracellular spaces between basement membranes and the plasma membranes of midgut and fat body cells (Hess and Falcon, 1987). Collectively, these observations suggest that insect basement membrane inhibits the movement of BVs.

ScathL, a cathepsin L-like cysteine protease from the flesh fly *Sarcophaga peregrina* Robineau-Desvoidy, is a potent basement membrane-degrading protease. In the flesh fly, this cathepsin L degrades two components of the BM (Homma and Natori, 1996). To determine whether disruption of the BM could accelerate dissemination of BV within an infected host, a recombinant baculovirus AcMLF9.ScathL expressing ScathL was constructed (Harrison and Bonning, 2001). The recombinant virus killed *Heliothis virescens* larvae approximately 30% faster than a virus expressing a scorpion venom-derived neurotoxin, and over 50% faster than the wild type virus AcMNPV C6 (Harrison and Bonning, 2001). In addition, AcMLF9.ScathL caused fragmentation of internal tissues and melanization of infected *H. virescens* larvae prior to death. Wild-type baculovirus-infected larvae typically melanize after death.

We have tested several hypotheses to elucidate the mechanisms underlying the insecticidal activity of ScathL. In this paper we describe experiments conducted to test the hypoth-

Fig. 2. Gut damage and cuticle melanization of fifth instar larvae of *H. virescens* at 3 h post injection (hpi) of ScathL enzyme (B–D), or infected with ScathL-expressing viruses (F, G). (A) injected with PBS buffer (pH 7.4) as a control; (B) and (C) injected with ScathL enzyme at 20 and 100 μg, respectively; (D) ScathL-injected larvae melanized; (E) PBS buffer-injected larvae without melanization; (F) and (G) gut of insects orally inoculated with AcMLF9.ScathL.hsp70/LacZ or AcMLF9.ScathL.C146A.hsp70/LacZ respectively at 5×10^5 polyhedra/larva, at 48 hpi. The extent of virus infection is visualized with X-gal. Note the absence of the midgut in (F) (arrow head). MG, midgut; T, tracheal branch. All bars = 1 cm.