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Short communication

Characterization of a serine carboxypeptidase in the salivary glands and fat body of the orange wheat blossom midge, *Sitodiplosis mosellana* (Diptera: Cecidomyiidae)

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Abstract

A full-length cDNA encoding a serine carboxypeptidase (designated *SmSCP-1*) was recovered from an ongoing salivary gland EST project of the wheat midge. The deduced 461-amino acid sequence had a putative signal sequence at the amino terminus, indicating it was a secreted protein. The protein shared homology with serine carboxypeptidases from other insects, mammals, plants, and yeasts. *SmSCP-1* mRNA was expressed in all stages of development and detected in salivary gland and fat body tissues but not in midgut tissue. Expression analysis and quantitative real-time PCR assays in male and female wheat midges and the fat body tissue of adult midges revealed that *SmSCP-1* was up-regulated nearly four-fold in the female midges compared to males and nearly two-fold in female fat body compared to male fat body. The wheat midge serine carboxypeptidase (SmSCP-1) most likely has a dual function. As a secreted digestive enzyme, it could play a role in mobilizing host-plant seed reserves for feeding larvae and as expressed in fat body could function as an exopeptidase in degradation of vitellogenin and/or in post-translational processing of other enzymes.

Keywords: Wheat midge; *Sitodiplosis mosellana*; Salivary glands; Fat body; Serine carboxypeptidase; Wheat

1. Introduction

The orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin), is the most important insect pest of spring wheat, *Triticum aestivum* L., in western Canada (Lamb et al., 2000) and is also a pest of wheat in the United States, Europe, Russia, and China (Berzonsky et al., 2003). Alternate hosts of the wheat midge in the family Gramineae include rye, *Secale cereale* L., and barley, *Hordeum vulgare* L. Adult female flies oviposit on the surfaces of wheat heads ('spikes' or 'ears') from head emergence until anthesis. The hatched larvae feed on developing seeds for 10–12 days, resulting in significant yield and crop grade losses annually (Lamb et al., 2000). Damage to the developing wheat seed is due entirely to the first two feeding larval instars, while the third instar is a non-feeding stage. Adults live for only a few days, do not feed (Pivnik and Labbe, 1993) and are thought to not have functional salivary glands (I.L. Wise, unpublished data). The short lifespan of adults is directed towards reproduction.

Wheat midge is controlled in western Canada (Saskatchewan and Manitoba) and the United States (North Dakota) mainly by insecticide applications. Ding and Ni (1994) in China and Barker and McKenzie (1996) in Canada initially identified genetic resistance to wheat midge in winter wheat. Resistant wheats have few damaged seeds and their infested seeds often contain dead larvae (antibiotic resistance) (Barker and McKenzie, 1996; Lamb et al., 2000). The host plant resistance is associated with elevated levels of phenolic acids (p-coumaric and ferulic),

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which are rapidly induced by feeding larvae, on infested seed surfaces (Ding et al., 2000). The phenolic acid levels soon return to normal, low levels after the chemical defense has been effective and the seeds mature (Lamb et al., 2000). To date, only one wheat midge resistance gene *Sm1* has been identified and characterized (McKenzie et al., 2002). Efforts to incorporate *Sm1* into spring wheat cultivars in Canada and to safeguard its breakdown by virulent wheat midge populations are being undertaken (Smith et al., 2004).

Identifying and characterizing genes that are important in the biology of the wheat midge will provide a more comprehensive understanding of this pest at the molecular level. Serine carboxypeptidases participate in several physiological and cellular processes, including protein digestion in the guts of animals (Bown et al., 1998), mobilization of seed storage proteins in wheat plants (Mikola, 1986), degradation of yolk proteins in mosquito oocytes (Cho et al., 1991), and post-translational processing of other enzymes (Galjart et al., 1990). These enzymes are exopeptidases and function by cleaving a single amino acid residue from the C-terminus of a protein or peptide substrate. All known serine carboxypeptidases are characterized by the presence of a conserved catalytic triad of Ser-Asp-His similar to serine proteases (Lehfeldt et al., 2000).

Here, we provide the first report of a wheat midge gene encoding a secreted serine carboxypeptidase. The gene, designated *SmSCP-1*, was identified from an on-going EST project for the salivary glands of the wheat midge. The deduced amino acid sequence of *SmSCP-1* contained a conserved catalytic triad and shared homology with other Dipteran, mammalian and plant serine carboxypeptidases. The potential relevance of the serine carboxypeptidase in the biology of the wheat midge is discussed.

2. Materials and methods

2.1. Insect material

Adult wheat midges were obtained from cultures that had been established by collecting mature larvae from the field or laboratory (Lamb et al., 2000). The mature larvae were maintained in moist soil at 2.5 °C for at least 180 days to allow them to complete their diapause. Emerged adult midges were added to wheat, cv. 'Roblin', with newly emerged spikes, inside meshed cages (Wise et al., 2001). After 2–3 days, the plants, grown hydroponically in tubular plastic pots, were transferred to a greenhouse at 20 °C with an 18-h photoperiod. Larvae were extracted from the spikes at the desired larval instar.

2.2. Larva and adult midge dissections and RNA isolation

Two hundred salivary gland pairs were dissected from early and mid-2nd-instar larvae (5–8 days old) immersed in ice-cold 1 × phosphate buffered saline (PBS). Salivary glands were removed by first pinching off the anterior tip of a larva and then gently compressing the body at the posterior end to expel the entire alimentary tract from the body. Each salivary gland was gently removed from the anterior end of the foregut with a pair of fine-tipped forceps. Midguts were dissected from the remaining part of the alimentary tract by carefully separating the midgut with its contents intact from the foregut and hindgut. All dissected tissues, including salivary glands, midguts and larval carcasses (mainly fat body), were immediately transferred to separate 1.5-ml Eppendorf microcentrifuge tubes containing 200 µl of ice-cold 1 × PBS, flash frozen in liquid nitrogen and stored at −80 °C until RNA was isolated. Fat body from adult midges was isolated by first separating the abdomens from the remaining body and then the fat body tissue was collected by dissecting the abdomens. Total RNA was extracted from the isolated tissues and from all development stages with the RNAqueous®-4PCR kit from Ambion following the manufacturer's protocol.

2.3. Comparison of SmSCP-1with other serine carboxypeptidases

Results for each genomic clone were aligned, and a consensus was formed with the SeqWeb sequence analysis program (http://silverjack.genomics.purdue.edu.) to correct sequencing errors or errors introduced by the *Taq* polymerase. Sequence similarity and annotations were determined with available BLAST programs (Altschul et al., 1990) on the National Center for Biotechnology Information (Bethesda, MD) website (http://www.ncbi.nlm.nih.gov/).

2.4. Salivary gland cDNA library construction

A cDNA library was constructed from the total RNA isolated from all 200 pairs of salivary glands with a 'SMART™, cDNA library construction kit from BD Biosciences Clontech (Palo Alto, CA, USA) following the manufacturer's protocol with one modification. Instead of cloning the PCR fragments into the original phage vector provided with the kit, the fragments were cloned directly into the pCR®4-TOPO® vector included in a TOPO TA cloning® for sequencing kit from Invitrogen. Plasmid DNA was isolated with a Qiagen BioRobot 3000 and sequenced from both ends with T7 forward, T3 reverse and sequence-specific primers in an ABI 3700 DNA analyzer.

2.5. Fluorescence in situ hybridization (FISH)

Salivary glands of late 2nd-instar wheat midge larvae were dissected in ice-cold Schneider's insect medium (Sigma-Aldrich) and fixed in 40% acetic acid. Polytene chromosome spreads were prepared as described by Pardue (1986) for the salivary polytene chromosomes of