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Cloning and characterization of cDNAS encoding carboxypeptidase-like proteins from the gut of Hessian fly larvae [*Mayetiola destructor* (Say)]☆

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Abstract

Transcriptomic analysis of the gut from Hessian fly larvae [*Mayetiola destructor* (Say)] identified nine cDNA clones that encode different carboxypeptidase-like proteins. Sequence comparison revealed that five of the nine cDNAs encoded very similar proteins with amino acid sequence identity over 96%. The other four cDNAs encoded diversified proteins with amino acid sequence identity less than 60%. Further sequence comparison with well characterized carboxypeptidases from other organisms revealed that these cDNAs encoded MDCP (*M. destructor* carboxypeptidase)-A1, MDCP-A2, MDCP-B, MDCP-BL, and MDCP-D. All residues characteristic of metallocarboxypeptidases including the HXXE motif were conserved among members. Northern blot analysis revealed various expression patterns for different gene groups in different developmental stages of *M. destructor*, suggesting that individual carboxypeptidases perform specific functions or have different specificities. Enzymatic activity assays demonstrated that both carboxypeptidases A and B are predominant in the larval stage, the only feeding stage of *M. destructor*, indicating a role in food digestion. The digestive role is further supported by the fact that 80% of the enzymatic activity in larvae occurred in the gut. Among these two types of enzymes, the activity of carboxypeptidase A was at least four times higher than that of carboxypeptidase B under the same conditions, suggesting that carboxypeptidase A is the major digestive enzyme in the gut of *M. destructor* larvae.

Keywords: Hessian fly; *Mayetiola destructor*; Carboxypeptidase; Gut; Digestive enzyme

1. Introduction

Since proteinaceous inhibitors were first found as defense molecules in plants (Green and Ryan, 1972), digestive enzymes have been targets for developing biopesticides to control herbivorous arthropod pests. Numerous digestive proteases and amylases have been characterized from many agriculturally important insects (Bown et al., 1998; Gaines et al., 1999; Cristofoletti et al., 2001; Coffeen and Wolpert, 2004; Gruden et al., 2004; Diaz-Mendoza et al., 2005; Zhu et al., 2005). Subsequently, various proteinaceous inhibitors have been identified against these enzymes from different sources (Farmer et al., 1992; Zhao et al., 1996; Koiwa et al., 2000; Moura and Ryan, 2001). In vitro assays with artificial diets have demonstrated that elevated levels of proteinaceous inhibitors may retard the growth, increase the mortality, and lower the fecundity of insects (Wolfson and Murdock, 1987; Franco et al., 2004). However, the detrimental effect of elevated levels of inhibitors can be shortly overcome by insects through changes in the composition of gut digestive proteases by compensating

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for the loss of activity with induction of resistant proteases (Jongsma et al., 1995; Ishimoto and Chrispeels, 1996; Zhu-Salzman et al., 2003). Such adaptation enables insects to overcome plant defense mechanisms. Therefore, a better understanding of enzymatic composition and their expression profiling will improve strategies to control herbivorous arthropod pests.

Previous research on insect digestive proteases has been focused on endopeptidases that act on the initial phases of protein degradation, i.e. the cleavage of proteins into peptides. Nevertheless, most peptide products cleaved by endopeptidases cannot be absorbed by insect gut cells and need to be further hydrolyzed by exopeptidases and dipeptidases (Terra and Ferreira, 1994). Accordingly, a combination of inhibitors to endo-, exo-, and di-peptidases may be more effective and/or more durable than inhibitors to endopeptidases alone.

Mammalian carboxypeptidase, an exopeptidase, has been well characterized (Titani et al., 1975; Rees et al., 1983; Coll et al., 1991; Guasch et al., 1992; Aloy et al., 1998), but the studies of insect carboxypeptidases are limited to a few species including black fly *Simulium vittatum* (Ramos et al., 1993), corn earworm *Helicoverpa armingera* (Bown et al., 1998; Bown and Gatehouse, 2004), mosquitoes *Anopheles gambiae* and *Aedes aegypti* (Edwards et al., 1997, 2000), tsetse fly *Glossina morsitans* (Yan et al., 2002), bertha armyworm *Mamestra configurata* (Hegedus et al., 2003), cabbage looper *Trichoplusia ni* (Wang et al., 2004), and corn earworm *Helicoverpa zea* (Bayes et al., 2005). In the current research, we report the isolation and characterization of nine unique cDNAs that encode carboxypeptidases from the Hessian fly, *Mayetiola destructor* (Say), one of the most destructive pests of wheat (*Triticum aestivum* L.) (Hatchett et al., 1987; Buntin, 1999). We also analyzed the activity of different carboxypeptidases in different stages and tissues of this insect.

2. Materials and methods

2.1. Insects

The biotype of *M. destructors* used in this research was a Kansas population collected from Ellis County and maintained in the laboratory as described previously (Zhu et al., 2005).

2.2. cDNA library construction and sequencing

The gut was isolated from 200 of 3-day-old *M. destructor* larvae (first instar) by dissection under a microscope and immediately transferred into TRI reagent™ (Molecular Research Center, Inc., Cincinnati, OH, USA). Total RNA was extracted according to the procedure provided by the TRI reagent™ manufacturer. A cDNA library was constructed using the 'SMART™, cDNA library construction kit from Clontech (Palo Alto, CA, USA) according to the manufacturer's instruction with a slight modification. Instead of using the original phage vector, PCR fragments were cloned directly into plasmid pCR2.1-TOPO using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA). Recombinant plasmid DNA was isolated with a Qiagen BioRobot-3000 and sequenced using an ABI 3730 DNA analyzer.

2.3. Sequence analysis

Open-reading-frame (ORF) and sequence-similarity analysis were performed using the web-based program of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/, Bethesda, MD, USA). Molecular weight calculation and pI prediction of mature proteins were carried out using SDSC biology Workbench (http://workbench.sdsc.edu/). Signal peptides were predicted using SignalP 3.0 Server (http://www.cbs.dtu.dk/services/SignalP/). Multiple-sequence alignments and phylogenetic analysis were conducted using BCM Search Launcher (http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html) and Molecular Evolutional Genetics Analysis software (version 2.1).

2.4. RNA isolation and northern blot analysis

Total RNA was extracted from whole insects as above. For Northern blot, 5 μg of total RNA was separated on a 1.2% agarose gel containing formaldehyde and blotted onto a GeneScreen membrane (Perkin-Elmer Life Science Inc., Boston, MA, USA). Membranes were baked at 80 °C for 2 h to fix the RNAs and then hybridized separately to individual cDNAs. Probes were derived from full length cDNAs by labeling with ^{32}P-dCTP using the random labeling kit from Stratagene (La Jolla, CA, USA). Hybridization was performed as described by Chen et al. (2004). After hybridization, the membranes were washed twice with 2 × SSC at room temperature for 30 min, twice with 2 × SSC plus 1% SDS at 65 °C for 30 min, and twice with 0.1 × SSC plus 0.1% SDS at room temperature for 30 min. The membranes were then exposed to Kodak SR-5 X-ray film overnight.

2.5. Carboxypeptidase activity assay

Carboxypeptidase activities were assayed as previously described (Bown et al., 1998; Wang et al., 2004). To determine carboxypeptidase activity in different developmental stages, 0-, 2-, 4-, 6-, and 12-day-old larvae, pupae, and adults were weighted and 20 mg of insects were homogenized in 1 ml distilled water. The protein extract was collected by centrifugation at 13,000*g* for 10 min. One hundred microliters of the protein extract from each developmental stage was mixed with 400 μl of 0.1 M Borate–NaOH buffer (pH 8.0), and the mixture was incubated at 30 °C for 20 min before substrate was added. About 5 μl of 20 mM *N*-(3-[2-furyl] acryloyl)-Phe-Phe (FAPP) (Sigma, St. Louis, MO, USA) was used as the