DaTrypsin, a novel clip-domain serine proteinase gene up-regulated during winter and summer diapauses of the onion maggot, Delia antiqua.

**Abstract**

Diapause prepares insects and other arthropods to survive in harsh environments. To explore the molecular basis of winter (WD) and summer diapauses (SD), we screened for diapause-specific genes in the onion maggot, *Delia antiqua*, that diapauses as a pupa in both summer and winter. A diapause-induced transcript, *DaTrypsin*, was identified through differential display, and examined by Northern blot, quantitative real-time PCR and sequence analyses. The full-length cDNA, 1379 bp long, encodes 384 a.a. with a molecular mass of 43,005 Da. The protein contains a 20-a.a. secretion peptide, followed by an amino-terminal clip domain and a carboxyl-terminal serine proteinase domain.
Ser, His and Asp as catalytic residues and Asp, Gly and Ser as specificity determinants, DaTrypsin is anticipated to be a trypsin-like enzyme. DaTrypsin transcription is up-regulated in both SD and WD pupae with higher mRNA levels during WD than SD. Heat shock further elevated gene transcription in both SD and WD pupae, whereas cold shock reduced DaTrypsin expression in SD pupae and had no significant effect on WD pupae. In SD pupae, DaTrypsin transcripts gradually build up during diapause, and after temperature shocks, whereas in WD pupae DaTrypsin mRNA levels are high at the beginning of diapause and immediately after a temperature shock and then gradually decrease with time. DaTrypsin represents the first serine proteinase gene expressed during diapause as well as the first gene up-regulated in both SD and WD. It may participate in the host's immune defense and/or maintain the developmental status in the diapausing pupae.

Abbreviations

SP, serine proteinase; SD, summer diapause; WD, winter diapause; DIG, digoxigenin; RAPD, random amplification of polymorphic DNA; PCR, polymerase chain reaction; Q-RT-PCR, quantitative real time-PCR; RACE, rapid amplification of cDNA ends; HSP, heat-shock protein

Keywords

Trypsin-like; Gene expression; Stress response; Protein structure; Insect immunity

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