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Differential Expression of Silkworm Cathepsin D

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We have cloned a cDNA encoding the cathepsin D (EC 3.4.23), a member of the aspartic proteases, from the silkworm, Bombyx mori. The B. mori cathepsin D cDNA contains an open reading frame of 1,155 bp encoding 385 amino acid residues. Two catalytic aspartyl residues were conserved in the deduced amino acid sequences of B. mori cathepsin D at positions Asp 84 and 269. The deduced amino acid sequence of the B. mori cathepsin D cDNA was closest to the Aedes aegypti (63% protein sequence identity) and next to both Aperiona germari and Drosophila melanogaster (60% protein sequence identity), but low sequence identity (27%) to Blattella germanica cathepsin D. Phylogenetic analysis confirmed a closer relationship of the deduced amino acid sequences of the B. mori cathepsin D gene to the A. aegypti, A. germari and D. melanogaster within the insect cathepsin D group. The B. mori cathepsin D cDNA was expressed as a 40kDa polypeptide in the baculovirus-infected insect Sf9 cells and Nglycosylation of the recombinant cathepsin D was revealed by tunicamycin to the recombinant virus-infected Sf9 cells, demonstrating that the silkworm cathepsin D is glycosylated. The expression profile of B. mori cathepsin D revealed by Northern blot and Western blot analyses that the high-level expression of B. mori cathepsin D was detected in fat body on the end of the fifth instar and in midgut on the first day to third day of pupal stage, demonstrating that B. mori cathepsin D is differentially expressed in fat body and midgut with growth stage. This result suggests that differential expression of B. mori cathepsin D is involved in both cellular remodeling associated with larval-pupal metamorphosis and gut deterioration during the pupal stage.