

cDNA Cloning, Expression and Characterization of a Cathepsin D of the Silkworm, *Bombyx mori*

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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 40-kDa polypeptide in the baculovirus-infected insect Sf9 cells and the recombinant cathepsin D showed protease enzyme activity. 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.