ISOLATION AND CHARACTERIZATION OF JUVENILE HORMONE ESTERASE FROM GYPSY MOTH (*LYMANTRIA DISPAR*).

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ABSTRACT

Insect metamorphosis is under precise hormonal control. During the last larval stadium, the degradation of juvenile hormone by juvenile hormone esterase (JHE) is essential for the initiation of pupation. Therefore, we have targeted this system for disruption with a strategy to produce a recombinant gypsy moth virus which expresses JHE. In order to clone and insert the JHE gene into the virus, purification of the enzyme, amino acid sequence information, and polyclonal antibody were needed.

Developmental analysis of JHE activity revealed a single major peak during the last larval stadium and another peak of JHE 3-5 days after pupation. JHE was purified from larval and pupal hemolymph by classical procedures. The specific activity of the purified enzyme approached 1000 units/mg. Gypsy moth JHE was found to have an apparent size of 62 kilodaltons, was insensitive to diisopropylphosphofluoridate, and was activated by polyethylene glycol. Partially purified enzyme displayed two closely-spaced bands on SDS PAGE. Polyclonal antiserum raised against the larval enzyme also reacted with the pupal JHE. This antiserum did not cross-react with hemolymph JHE from other Lepidoptera by western blot analysis. Two forms of JHE, JHE-A and JHE-B, were isolated by reverse-phase HPLC. Both appeared similar in size, had very similar amino acid compositions, were indistinguishable by HPLC tryptic peptide mapping, and had an identical N-terminal amino acid sequence. Since JHE-A and JHE-B are structurally very similar, these two forms may reflect minor differences in post-translational modification of the gypsy moth enzyme. Whether these forms differ with respect to their function remains to be determined.

Comparison of the gypsy moth enzyme with that from other Lepidoptera showed that they were antigenically distinct. In addition, the N-terminal and peptide amino acid sequences revealed marked differences in the structures of JHE from different Lepidoptera. Whether these enzymes also differ in their properties remains to be determined.