JUVENILE HORMONE ESTERASE AND THREE CLOSELY RELATED ESTERASES FROM *DROSOPHILA MELANOGASTER*

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Two key hormones, ecdysone and juvenile hormone, control metamorphosis and moulting in insects. Stated simply, the role of juvenile hormone in insect development is to determine the type of moult that is undertaken by the insect. The presence of juvenile hormone maintains juvenile characteristics and prevents development of the adult form (Kumaran, 1990). Very low levels of juvenile hormone are found in the insect immediately preceding a larval to pupal moult, while absence of juvenile hormone in the insect leads to a pupal to adult moult. There are at least six forms of juvenile hormone that occur alone or in combination across different insect orders and developmental stages. Juvenile hormone III is the most commonly detected form across insect orders. However, there is some evidence that in the higher Diptera such as *D. melanogaster* JHIII is a precursor foran alternative form of the hormone, juvenile hormone III bisepoxide (Richard *et al.*, 1989).

Juvenile hormone levels in the insect are controlled by its synthesis and release into the haemolymph, and degradation of the hormone by enzymes. Two enzymes are known to degrade juvenile hormone in insects, juvenile hormone epoxide hydrolase (JHEH) and juvenile hormone esterase (JHE). JHEH cleaves the epoxide ring of juvenile hormone and JHE hydrolyses the methyl ester bond of juvenile hormone.

We are interested in JHE (of *D. melanogaster*) as this enzyme has a high affinity for juvenile hormone and we are interested in finding enzymes that are able to degrade different forms of juvenile hormone. The JHE protein from *D. melanogaster* has been purified previously and shown to have a high specificity constant for juvenile hormone III (Campbell *et al.*, 1992; 1998; 2001). The high specificity constant indicates that JHE has a high affinity for juvenile hormone III and is able to hydrolyse the hormone rapidly under biological conditions. However, it is not known whether there is one JHE in *D. melanogaster* that is capable of degrading both forms of the hormone that maybe important in this species, or whether there is a different JHE for each form of the hormone. This is an important issue to consider for several chemical and biotechnological approaches to pest insect control now at various stages of development which aim to disrupt juvenile hormone metabolism (Bonning and Hammock, 1996).

The sequence of the *D. melanogaster* genome has been deduced by the Celera genome sequencing project (Adams *et al.*, 2000). Within this sequence we have identified four genes that encode enzymes which have the potential to degrade juvenile hormones; JHE, JHEdup, JHErel and JHEass. Previous research supports a role for one of these enzymes (JHE, Campbell *et al.*, 1992; 1998; 2001) and we are now able to characterise this further. The possible roles of the other three genes are currently being investigated with all four genes expressed using the baculovirus expression system.

As noted above, JHE purified directly from insects had earlier been shown to degrade juvenile hormone III with a high specificity constant (Campbell *et al.*, 1998). Because of difficulties in obtaining sufficient purified protein, direct measurements were not made of the ability of insect-expressed JHE to hydrolyse different forms of juvenile hormone. These measurements can now be made using the baculovirus-expressed JHE. We find that the baculovirus-expressed protein, like the insect derived protein, has a high specificity for juvenile hormone III. It also has good activity for several other generic esterase substrates like 4-methylumbelliferyl acetate and butyrate. Interestingly, the expressed protein is able to hydrolyse methoprene, which is a juvenile hormone analogue used to control the larvae of insects such as mosquitoes and fleas.

The second of the four identified genes, referred to as *Jhedup*, is a duplication of the *Jhe* gene. The sequence of the JHEdup protein shows several important differences from JHE, suggesting that its primary function may have diverged since the duplication event took place. The most important difference between the two proteins is a change in one of the amino acids in an active site motif that is commonly thought to be indicative of the physiological JHE across a range of insects (Thomas *et al.*, 1999). The protein is able to hydrolyse juvenile hormone III, although the degree of specificity for this substrate is yet to be determined. The ability of this protein to hydrolyse other juvenile hormones and analogues is also being determined.

A third gene related to the *Jhe* gene is *Jherel* (Claudianos *et al.*, 2001). The sequence of the JHErel protein, which is produced by this gene, shows significant similarity to the JHE protein sequence but not the active site motif. Like the other two proteins discussed above JHErel, displays good activity against a range of generic esterase substrates, indicating that it is an active esterase. This protein is also able to hydrolyse juvenile hormone III, although with much lower specificity than JHE. The activity of JHErel against a range of different juvenile hormones will be of particular interest because the *Jherel* gene appears to be located at or at least very near to the chromosomal position of a mutation which is predicted to have a role in mediating the response of the insects' tissues to juvenile hormone (Shirras and Bownes, 1989).

The final of the four esterase genes identified in the *D. melanogaster* genome to potentially play a role in juvenile hormone degradation is the juvenile hormone esterase associated gene, *Jheass*. The sequence of the JHEass protein shows significant similarity to that of the JHE protein and is the only other sequence in the *D. melanogaster* genome to share the active site motif. Preliminary experiments show that this protein is able to hydrolyse juvenile hormone III. The specificity for juvenile hormone III and the ability of the protein to hydrolyse different forms of juvenile hormone is under investigation.

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