JUVENILE HORMONE ESTERASE AS TRANSGENES FOR VIRAL INSECTICIDES.

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Summary

Alternative technologies for insect pest control are becoming increasingly important as a result of increasing insecticide resistance in the cotton pest *Helicoverpa armigera* the move towards ecologically sustainable agriculture. Two biotechnological approaches under investigation are insect resistant transgenic crops and insect viruses engineered for increased speed of kill. An important limiting factor for both approaches is the availability of efficacious insecticidal insert genes that can be inserted into the crop or virus. Juvenile hormone esterase (JHE) is an attractive candidate, especially for transgenic insect viruses. JHE acts to control juvenile hormone and hence is a key controller of metamorphosis and maturation of reproductive tissues in a range of invertebrates. A form of JHE specific for lepidopterans has already been isolated and engineered into an insect virus, demonstrating insecticidal properties against *Helicoverpa* species. Infection of lepidopteran larvae with a baculovirus engineered to produce a large increase in JHE levels causes a reduction in juvenile hormone level, leading to aberrant moults and the end of feeding behaviour. Here we describe a project that aims to isolate a more versatile JHE, which should be effective against a range of chewing and sucking pests.

Introduction

Nuclear polyhedrosis viruses of the family Baculoviridae are used in engineering recombinant insect viruses for pest control (Hoover *et al.*, 1996). Their mode of infection is a simple one that allows aerosol application. The virus is ingested by the insect pest where it binds to gut cells of the larvae allowing the viral DNA to enter the nucleus of the gut cell. Once the viral DNA is in the insect cell nucleus, replication of the viral DNA takes place allowing more viral particles to be manufactured that will then infect more host cells (Hoover *et al.*, 1996). In infections with wild type virus this process can take days to weeks to kill pest insects (Bonning *et al.*, 1995). Therefore transgenes whose expression would decrease the time to kill the target insects or to stop them feeding are required.

Several recombinant versions of *Autographa californica* nuclear polyhedrosis virus have now been studied. Most of these have involved expression of genes encoding insect specific neurotoxins isolated from scorpions or mites (Hoover *et al.*, 1995). These recombinants can reduce times to kill or stop feeding by over 50%, which makes them potentially very useful control agents for a variety of pest infestations. However there is a perception that recombinant viruses expressing neurotoxins

may face public acceptance problems, even although the neurotoxins are insect-specific in their effects. This has stimulated research to isolate transgenes that are not toxins but simply interfere with key invertebrate-specific hormones, like those that regulate metamorphosis.

JHE in *Helicoverpa virescens*

Two key hormones, ecdysone and juvenile hormone, control metamorphosis and moulting in insects. Generally a dramatic decrease in levels of juvenile hormone in the larvae is required to allow metamorphosis to the adult form. This decrease occurs as a result of both decreased synthesis of juvenile hormone and an increase in the level of JHE. The JHE protein from the American crop pest *H. virescens* has been purified (Abdel-Aal *et al.*, 1988) and the gene cloned and sequenced (Hanzlik *et al.*, 1989). The protein has been shown to efficiently degrade juvenile hormone under biological conditions with a high affinity and a rapid rate of turnover. It is present in the haemolymph at very low levels (less than 0.1% of total protein in the final stadium of *H. virescens*). This finding suggests that only small amounts of JHE would be required to degrade the amount of juvenile hormone produced by the insect and reduce whole body amounts of juvenile hormone, initiating metamorphosis and the end of feeding behaviour (Hammock and Philpott, 1992).

The *H. virescens* JHE has been studied intensively as a transgene in recombinant viruses in both its naturally occurring state and with several alterations. The results of these studies suggest that JHE might be a useful gene in recombinant virus technology. Initially, the use of the wild type gene in a recombinant baculovirus led to disappointing results. A recombinant virus which expressed the wild type JHE showed only marginally (about 20%) increased speed of kill compared to the control viruses. However, a large increase in JHE activity was seen in infected insects suggesting that the recombinant virus was producing JHE (Eldridge *et al.*, 1992). The limited effect on speed of kill proved to be the result of the removal of the expressed JHE from the haemolymph of the insect, keeping the amount of potentially reactive JHE constant (Ichinose *et al.*, 1992). Two amino acids implicated in the removal of JHE from the haemolymph were therefore selectively modified. The resulting enzymes did not differ significantly in kinetics for juvenile hormone hydrolysis or in baculovirus expression levels from the wild type JHE. However, expression of one of these modified enzymes resulted in a significant reduction in JHE in lysosomes, storage and degradation organelles. This correlated with a 50% reduction in feeding damage when insects were infected with the modified virus compared to those infected with the wild type virus (Bonning *et al.*, 1997). The increased insecticidal activity was most likely the result of increased levels of JHE being retained in the haemolymph of the insects.

Another independent approach has also yielded a different mutant of the lepidopteran JHE which improves baculovirus speed of kill to levels comparable with those of the recombinants expressing scorpion toxin. Several modified JHE enzymes have been made in which the catalytic residues (the amino acids directly involved in JH binding and degradation) have been altered, resulting in enzymes with no detectable catalytic activity towards juvenile hormone (Ward *et al.*, 1992). Despite the lack of catalytic activity, one of the modified forms of JHE (in which the catalytic serine had been

changed to a glycine; Ward *et al.*, 1992) has increased insecticidal activity compared with either the wild type virus or the recombinant virus expressing the wild type JHE (Bonning *et al.*, 1995). This surprising result could not be due to the degradation of juvenile hormone in the larvae, as the catalytic activity of the modified enzyme was not above background levels. While the mechanism that results in this increased speed of kill is unknown it has been suggested that it might be the result of a different, as yet unknown, function of the enzyme (Bonning *et al.*, 1995).

There are no reports to date of the insecticidal activity of a double mutant JHE carrying both serine to glycine active site change and the mutation retaining JHE in the haemolymph.

JHE in *Drosophila melanogaster*

The JHE protein of the fruit fly, *D. melanogaster*, has been purified and characterised. When compared to the lepidopteran form of the enzyme, activity and expression patterns appear similar (Campbell *et al.*, 1992; 1998). There are four naturally occurring forms of juvenile hormone, the most common isoform being juvenile hormone III which is found in all nine insect orders investigated (Schooley *et al.*, 1984). The JHE, purified from *H. virescens* is specific for one form of juvenile hormone found in moths, namely juvenile hormone III. The JHE purified from *D. melanogaster* also has a high specificity constant for juvenile hormone III, meaning that it has both a high affinity and high turnover capacity for this substrate. Inhibition studies using alternative forms of juvenile hormone to inhibit the hydrolysis of juvenile hormone III provide indirect evidence that JHE from *D. melanogaster* is able to hydrolyse both juvenile hormone I and juvenile hormone II, albeit at a lower rate than the hydrolysis of juvenile hormone III (Campbell *et al.*, 1998). We suggest that the *D. melanogaster* form of JHE may represent an ancestral form of the enzyme and have catalytic activity against most, if not all, forms of juvenile hormone. This could make it a more versatile and effective transgene for insecticidal viruses than the lepidopteran enzyme.

The first aims of this postgraduate project are to clone the JHE gene from *D. melanogaster* and to compare the kinetic data of the cloned gene product with those of the purified protein. Cloning has been achieved but the kinetic data are as yet incomplete. The cloned gene product will then be characterised for its potential as a transgene. Time permitting, the mutations found to increase the insecticidal activity of the lepidoteran JHE will also be tested in the dipteran enzyme. A surprising result from the cloning work has been the finding, and cloning, of another esterase gene very closely related to the JHE gene (Claudianos *et al.*, 2000). Kinetic performance and possibly insecticidal potential of this related gene will also be evaluated.

Conclusion

Juvenile hormone and JHE play an extremely important role in the proper development of insects and certain mutants of lepidopteran JHE can be engineered into baculoviruses to substantially enhance their insecticidal potential. We are seeking to achieve further improvements using JHE genes and certain mutants from other insects which evidence to date suggests may have more versatile catalytic capabilities.

Acknowledgements

We are grateful for the support of the Cotton Research and Development Corporation (grant no. CSE 82C).

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