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REVIEW ARTICLE

Potential of Juvenile Hormone Esterase as a Bio-Insecticide: an Overview

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ABSTRACT

Juvenile hormone (JH) is a key hormone in regulation of the insect's life cycle. This role is carefully regulated in insects to successfully develop. Conversely, this careful regulation of JH titer opens a window of attack where a recombinant baculovirus expressing an appropriate protein(s) could disrupt the fine balance in JH titer and consequently the insect life cycle. Juvenile hormone esterase (JHE), a member of the carboxylesterase family (EC 3.1.1.1), contributes to the rapid decline in the JH titer. Therefore, if JHE was introduced prematurely into insect larvae, the enzyme should induce physiological and morphological anti-JH effects by degrading JH at a time when JH biosynthesis is active. Recombinant baculoviruses with JHE proteins represent valuable technology that may has great potential for effective integration into pest-management system. Such a demonstration would indicate that JHE, as a novel anti-JH agent, may be potentially useful for insect control and may represent a major step toward a more sustainable agriculture.

Key words: Juvenile hormone esterase, recombinant nucleopolyhedroviruses bio-insecticide, pest control.

INTRODUCTION

Over the past five decades, use of synthetic chemical pesticides has significantly increased the yields of food, fiber, and feed. These increases in agriculture production make producers able to generate from a given amount of land. Although chemical pesticides have without a doubt improved the efficiency of agricultural output and reduced the incidence of disease by killing disease vectors, they are also a source of environmental pollution associated with acute and chronic problems to human health. Moreover, intensive uses of broad-spectrum insecticides have elected high levels of resistance in several insect populations (Miles and Lysandrou, 2002; Abo Elghar *et al.,* 2005 and Mushtaq, 2008). Therefore alternative control techniques need to be evaluated.

To overcome problems associated with chemical pesticides, alternative methods of control were given more attention. Baculoviruses are receiving renewed attention as insect pest control agents following the development of fast-acting recombinant baculoviruses. They have been considered as viral insecticides and are safe for the environment (Cheng and Lynn, 2009). One of the biotechnological methods for using alternative in pest control is constructing a recombinant baculovirus derived from the nucleopolyhedrovirus of *Autographa californica* (Lepidoptera: Noctuidae) which expresses a form of juvenile hormone esterase (JHE).

Juvenile hormone esterase as anti-juvenile hormone agent

Wigglesworth (1935) was the first to identify a "juvenile factor" produced by the *corpora allata* that keeps larval insects in the juvenile state. Subsequently, Röller *et al.* (1967) and Meyer *et al.* (1970) showed the chemical structure of juvenile hormone (JH). Six JHs (JH-0, JH-I, JH-II, JH-III, 4-methyl JH-1, and JHB_3) have been identified to date, all of which are terpenoids derived from farnesenic acid (or its homologs) with an epoxide group at the 10, 11 position of one end and a conjugated methyl ester at the other end. JH-III appears to be the most common of the JHs being found in all of the insect orders examined to date. In addition to their function as a juvenile factor, JHs and/or their metabolites are involved in a diverse array of other functionalities including roles in development, metamorphosis, reproduction, diapause, migration, polyphenism, and metabolism (Riddiford, 1994; Gilbert *et al.*, 2000; Truman and Riddiford, 2002 and Riddiford, 2008).

Two pathways for the degradation of JH have been intensively studied in insects (Hammock, 1985; Roe and Venkatesh, 1990; de Kort and Granger, 1996; Gilbert *et al.,* 2000). One involves a soluble esterase, JH esterase (JHE), that hydrolyzes the methyl ester moiety at one end of the JH molecule resulting in a

carboxylic acid moiety (Kamita *et al.*, 2003). The other involves a microsomal epoxide hydrolase, JH epoxide hydrolase (JHEH) that hydrolyzes an epoxide moiety at the other end of the JH molecule to produce a diol (Fig. 1). JHEs show high specificity for JH but only a moderate turnover rate (Kamita and Hammock, 2010).

Anti-JH agents have been of interest as chemicals to control insect development. Various chemical agents with different biochemical actions and targets have been developed to block JH biosynthesis and JH-receptor interactions as effective anti-JH agents.

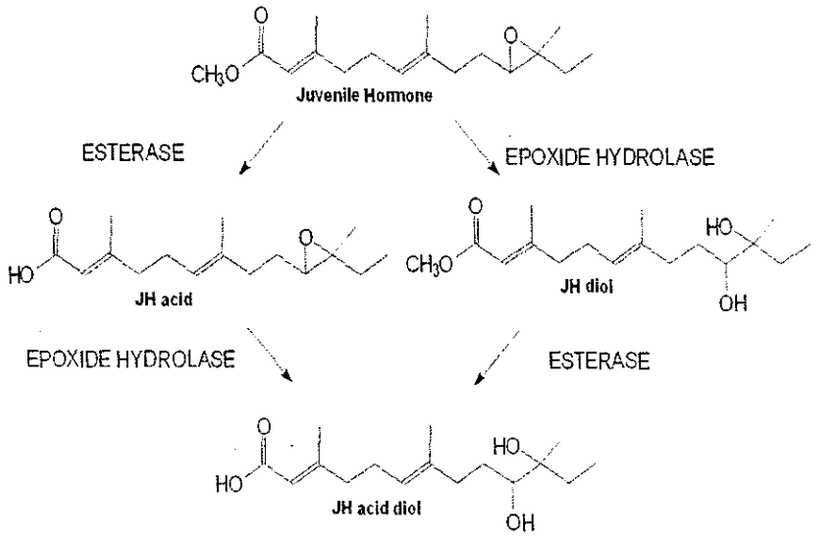

Fig. (1): Pathways of juvenile hormone degradation.

Esterases exist in at least three orders of insects with a high degree of specificity towards JH (Hammock, 1985). These juvenile hormone esterases (JHEs) hydrolyze JH to the biologically inactive acid. In the Lepidoptera, JHE appears in the final larval stadium as two distinct peaks of hydrolytic activity, contributing to the rapid decline in the JH titer that signal the onset of pupation (Hammock, 1985). In the early instars, JHE activity is usually very low, while the JH titer remains relatively high.

Bonning *et al.* (1997a) showed significant reduction in the number of eggs laid compared to the PBS-injected control crickets, *Acheta domesticus*, after injection with JHE (Fig. 2). Number of eggs laid was reduced by 97%. This effect was partially reversed (to 41%) by application of the JHE inhibitor, 3-n-octylthio-1, 1, 1-trifluoro-2-propanone (OTFP). The anti-JH effect of the JHE on the numbers of eggs laid was partially reversed by topical application of low concentration of OTFP. Complete reversal of the anti-JH effects may be possible by application of more OTFP. However, reactivation of OTFP-inhibited JHE can occur within the insect (Ichinose *et al.*, 1992a). Their data showed that JHE is a powerful and specific anti-JH reagent, when injected into *A. domesticus*. This technology may prove to be a useful supplement to allatectomy in testing the role of JH in physiological processes.

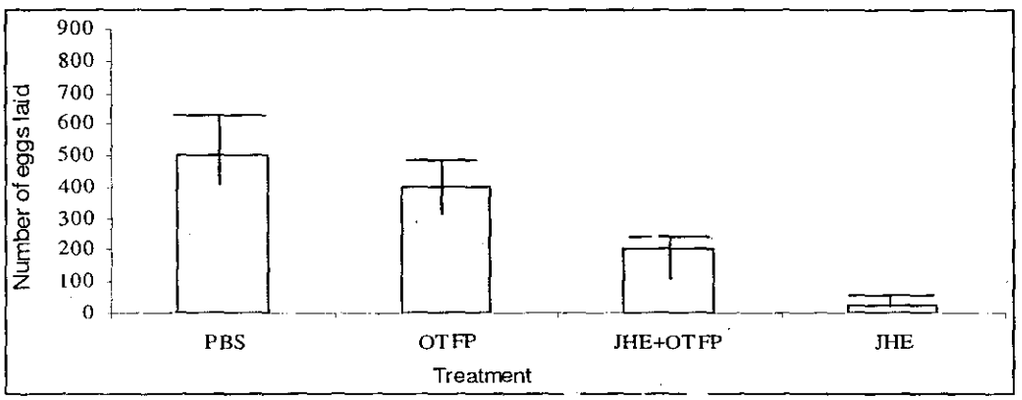

Fig. (2): Reversal of the anti-JH effects of JHE with the JHE inhibitor OTFP. Crickets were injected with 1.5 units of JHE, with or without topical application of a total of 18 µl of 0.01 M OTFP. Control crickets were injected with PBS or dosed with 21 µl of 0.01 M OTFP (modified from Bonning *et al.*, 1997a).

Increase in vivo stability of JHE

Studies have shown that JHE from different lepidopteran species, when injected into larvae of *Manduca sexta*, *Heliothis virescens* and *Agrotis ipsilon*, is rapidly recognized and taken up into the pericardial cells from the hemolymph (Booth *et al.*, 1992; Ichinose *et al.*, 1992a,b; Bonning *et al.*, 1997a; El-Sheikh *et al.*, unpublished data). This removal of JHE from the hemolymph occurs by a receptor-mediated, endocytotic, saturable mechanism that does not involve passive filtration (Ichinose *et al.*, 1992 a, b). The JHE is presumed to be degraded in lysosomes (Booth *et al.*, 1992). At least two putative JHE binding proteins may be involved in transport and/or degradation of JHE in the pericardial cells, including a putative heat shock binding protein (hsp) (Bonning *et al.*, 1997a) and P29 (Shanmugavelu *et al.*, 2000; Shanmugavelu *et al.*, 2001). Receptor-mediated endocytosis of JHE has been demonstrated in early and late larval instars of *M. sexta* (Ichinose *et al.*, 1992a). Although authentic JHE is normally stable in hemolymph, the half-life of JHE injected into the hemolymph can be as little as 20 minutes under conditions where endogenous and exogenous proteins including bovine serum albumin, ovalbumin, and hemolymph JH binding protein have half-lives of days (Ichinose *et al.*, 1992a). Thus, the mechanism by which JHE is specifically recognized and removed by the pericardial cells could be a very important target for increasing the half-life of JHE in the hemolymph. There are three ways in which the uptake and degradation of JHE can be disrupted (i) prevention of receptor-mediated uptake by the pericardial cells, (ii) disruption of transport to the lysosomes, and (iii) prevention of lysosomal degradation.

On the basis of a homology model of the JHE of *H. virescens and M. sexta*, Thomas *et al.* (1999) and Wogulis *et al.* (2006) identified an amphipathic helix on the face of the enzyme opposite the catalytic site (Fig. 3 a and b). Although all known members of the esterase family possess a corresponding 4 to 5 turn helix, the amphipathic character is only found in JH esterases and steroid carrier proteins that are actively transported by the mammalian liver. Thomas *et al.* (1999), thus, hypothesized that the amphipathic property of the helix is involved in the receptor-mediated uptake of JHE by the pericardial cells. In order to test this hypothesis, the degradation rates of JHEs from distantly related species as well JHEs with polymorphisms of the amphipathic helix have been determined. Additionally, the surface of the JHE of *H. virescens* has a line of positively charged amino acid residues (Arg-174, -181, and -185) termed RRR that are present on the buffer face of each turn of the helix (Fig. 3a). The JHE of *M. sexta* predicts a similar structure with the middle arginine residue being replaced by another positively charged amino acid residue, lysine (termed RKR) (Fig 3b). In the corresponding regions of the JHE of *Tenebrio molitor* the first two arginine residues are replaced by lysine residues and the third arginine residue is replaced by a negatively charged glutamic acid residue (KKE). Analysis of the half-lives of each of these JHEs shows that they are roughly the same suggesting that the amphipathic property may not be important for uptake by the pericardial cells. Currently, other mutations of the amphipathic helix of the JHE of *H. virescens* including KKR, RHR, KRR, and QQR are widely testing for their efficiency against insects. All of these JHE mutants show similar catalytic activity to the authentic JHE and the QQR mutant had similar uptake to the authentic JHE suggesting that mechanism of the recognition and uptake of JHE by pericardial cells is more complex than initially thought.

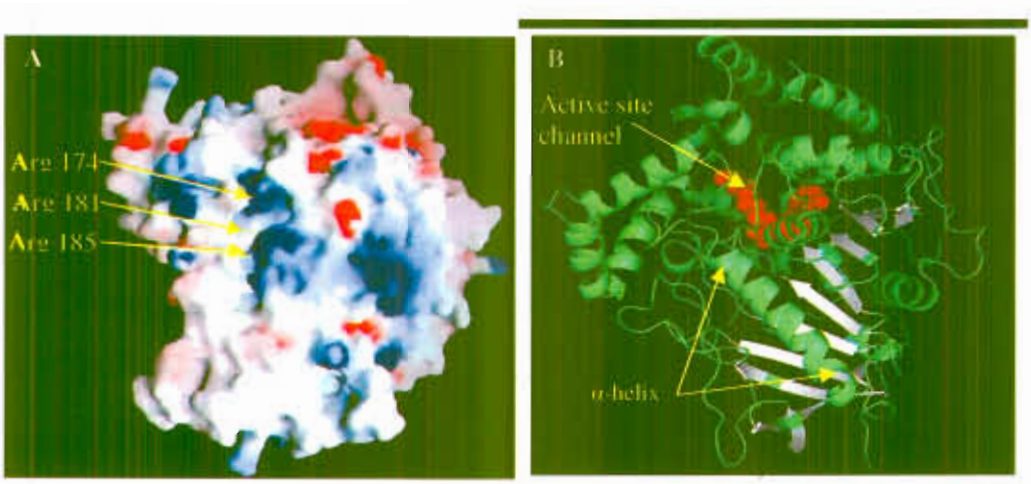

Fig. (3): Crystal structures of electrostatic potential surface of *H. virescens* JHE (A; modified from Thomas *et al.*, 1999), and *M. sexta* JHE (B; El-Sheikh *et al.*, unpublished data) showing active site and α-helix containing Args residues that are believed to play a role in receptor recognition.

Bonning *et al.*, (1997b) identified two lysine residues that are likely to be on the surface of the JHE protein of *H. virescens* and potentially involved in uptake or degradation of JHE. These lysine residues are Lys-29 near the N terminus, which is potentially involved in ubiquitin conjugation and Lys-524, which is located within a putative lysosome targeting sequence. A mutated JHE protein (JHE-KK) in which both of these lysine residues were mutated to arginine residues showed decreased efficiency of lysosomal targeting (Bonning *et al.*, 1997b), and binds to the putative JHE binding protein (P29) with significantly less affinity than authentic JHE (Shanmugavelu *et al.*, 2000). However, JHE-KK as well as mutant JHEs in which only one of the lysine residues were mutated to arginine residues showed similar catalytic activities and removal rates of JH from the hemolymph as the authentic JHE. A high-resolution crystal structure should assist with determining the basis of the interaction of JHE and the putative JHE binding protein P29 described by Shanmugavelu *et al.* (2000). The authors hypothesized that P29 interacts with JHE of *H. virescens* and facilitates targeting of JHE to lysosomes within pericardial cells. Other putative JHE binding proteins have been identified in *M. sexta*, and their possible roles in the degradation of JHE remain to be elucidated *(Shanmugavelu et al.*, 2001).

Recombinant baculoviruses expressing JHE

Baculoviruses are arthropod specific viruses which have long been in use as insect pest control agents for protection of numerous crop plants (Entwistle and Evans, 1985). According to environmental conditions, death can occur anywhere from several days to weeks post infection. A reduction in the lethal time of the virus would enable broader use of the virus for protection of crops less able to sustain foliar damage without economic loss. Recent advances in recombinant DNA technology have facilitated genetic engineering of baculoviruses to reduce the time taken for the virus to kill its larval host (Bonning and Hammock, 1992). Several recombinant baculoviruses derived from *A. californica* nucleopolyhedrovirus (AcNPV) with reduced lethal times have been constructed (Maeda, 1989; O'Reilly and Miller, 1989; McCutchen *et al.*, 1991; Stewart *et al.*, 1991; Tomalski and Miller, 1992 and Bonning and Hammock, 1994).

Juvenile hormone (JH) and juvenile hormone esterase (JHE) are key components in the regulation of development in lepidopteran larvae (Hammock, 1985; Riddiford, 1994). As such they have been exploited for use in pest control with development of the juvenoids (Staal, 1982) and anti-JH agents (Staal, 1986) and insertion of JHE into recombinant baculoviruses (Hammock et al., 1990; Bonning *et al.*, 1992; Bonning and Hammock, 1994). A series of mutant forms of JHE were made by site-directed mutagenesis for analysis of key residues involved in catalysis of JH (Ward *et al.*, 1992), and mutations in amphypathic helix of JHE (El-Sheikh *et al.*, unpublished Data) for increasing stability of protein *in vivo*.

Hammock *et al.* (1990) first hypothesized that the natural insecticidal activity of the baculovirus AcMNPV could be improved by the expression of a gene encoding JHE. The rationale behind this was that the recombinant AcMNPV would be ingested by early larval instars, and subsequently JHE would be produced at a point in development that is inappropriate for the insect. Two approaches to improve this technology have included (i) improving the *in vivo* stability (i.e. reducing removal and/or degradation from the hemolymph) of the JHE enzyme by genetic modification of the JHE gene and (ii) increasing or altering the timing of gene expression by using alternative promoters to drive JHE expression. Recombinant baculoviruses expressing JHE have also been used as tools for hypothesis testing with regard to the biological activity of JHE within the insect host (van Meer *et al.*, 2000). JHE proteins from at least five different insect species: *H. virescens* (Hammock et al., 1990; Bonning *et al.*, 1992), *Choristoneura fumiferana* (Feng *et al.*, 1999), *B. mori* (Hirai *et al.*, 2002), *M. sexta* (Hinton and Hammock, 2003a), and *T. molitor* (Hinton and Hammock, 2003b)) have been expressed using recombinant AcMNPVs.

Efficiency of recombinant baculoviruses with wild type and mutated JHE genes in insect pest control

Many insect regulatory enzymes are proteinaceous, and control critical physiological processes such as metamorphosis, or reproduction. Several insect neurohormones have been expressed in baculoviruses. However, the only recombinant virus with insecticidal activity was the *B. mori* virus (BmNPV) expressing a putative diuretic hormone from *M. sexta* which caused a 20% reduction in the time to kill when compared to the wild-type virus (Hoover *et al.*, 1996).

The first insect enzyme expressed in a baculovirus was juvenile hormone esterase (JHE), being partly responsible for the degradation of JH and regulation of metamorphosis (Hammock *et al*, 1990). Newly hatched *Trichoplusia ni* larvae infected with the virus expressing this enzyme consumed less diet than larvae infected with the wild-type virus, but this effect was not seen in older larvae. Many mutant forms of JHE

were cloned into the baculovirus. The resultant viruses significantly reduced the time required to kill host insects by 30% compared to wild-type viruses and reduced foliar damage to plant hosts by up to 50% as shown in Table (1).

The serine at the catalytic site of the JHE has been mutated to a glycine residue so that the protein does not degrade JH. The recombinant baculovirus expressing the modified form of JHE with the catalytic site serine (Ser_{201}) changed to Glycine, named AcJHE-SG, has enhanced activity against lepidopteran larvae. Lethal times of the recombinant are 20 to 30% lower than for the wild type virus, and a 66% reduction in feeding damage caused by infected larvae is observed. This finding is comparable to the best recombinant baculovirus developed to date, AcAaIT, which expresses an insect-selective scorpion toxin (Bonning *et al.*, 1995). The potential of these recombinant viruses for commercialization as insecticides was discussed (McCutchen *et al.*, 1991 and Stewart *et al.*, 1991). Bioassays of AcJHE-SG in conjunction with anti-JH agents indicate that the virus is not lethal by an anti-JH mechanism. Larvae apparently die from contraction-paralysis, or disruption of the normal sequence of events at the molt.

Table (1): Recombinant baculovirus with wild type and mutated JHE gene for enhanced insecticidal activity

Protein	Name of virus*	Physiological target	Physiological or behavioral effect	Efficacy	Host used for testing efficacy	Reference
JHE wild type	AcJHE	JH	disrupt metamorphosis and decrease feeding damage	reduce larval wight by 50%	*Manduca sexta*	El-Sheikh *et al.*, unpublished**
JHE mutant forms	AcJHE-KK	JH		reduce LT_{50} by 30%	*Heliothis virescens*	Bonning *et al.*, 1997b
	AcJHE-SG	JH		reduce feeding by 36-50%	*Trichoplusia ni*	Bonning *et al.*, 1995
	AcJHE-HH	JH		reduce larval weight by 70%	*Manduca sexta*	El-Sheikh *et al.*, unpublished

* Recombinant baculoviruses were generated by inserting wild type JHE gene (AcJHE); mutated JHE gene with replacing lysine residues at positions 29 and 524 to argentine residues (AcJHE-KK); mutated JHE gene with changing the catalytic site serine-201 to glycine (AcJHE-SG) and mutated JHE gene where lysine-204 and argentine-208 of the amphypathic helix were mutated to histidine residues (AcJHE-HH) .

** Under publication.

In order to gain insight into the mechanism of action of AcJHE-SG, a series of bioassays were carried out using viruses in conjunction with various chemical agents designed to interfere with regulation of JH. The anti-JH agent ethyl 4-(2-(tert-butyl-carbonyloxy) butoxy) benzoate), ETB, acts as an anti-JH agent at low doses at the tissue level. ETB may reduce JH titers by acting on the feedback regulation of JH production by the corpora allata (Staal, 1986). At high doses, ETB acts as a juvenoid (Sparks *et al.*, 1979; Kramer and Staal, 1981 and Kiguchi *et al.*, 1984). Fluoromevalonolactone, tetrahydro-4-fluoromethyl-4-hydroxy-2H-pyran-2-one, FMev, is an anti-JH agent which acts directly on the corpora allata. FMev acts as a competitive inhibitor of mevalonic acid in the conversion of mevalonate to JH (Quistad *et al.*, 1981). The juvenoids, epofenonane, and fenoxycarb were used in bioassays along with the JHE inhibitors *O*-ethyl-*S*-phenylphosphoramidothiolate (EPPAT) (Sparks *et al.*, 1983) and 3-oclylthio-l, 1, 1-trifluoropropan-2-one (OTFP) (Abdel-Aal and Hammock, 1985). These compounds are very potent inhibitors of JHE but also inhibit other esterases to varying degrees. Previous is a screen on the action of either wild type or mutated (JHE-SG) JHEs inserted in baculovirus genome and their potential for insect control.

First instar larvae of *H. virescens* that were infected with a recombinant AcMNPV expressing JHE-KK (AcJHE-KK) under a very late viral promoter died approximately 22% faster (Survival time (ST_{50}) = 116 h) than control larvae infected with a recombinant AcMNPV expressing the authentic JHE (AcJHE) (Bonning *et al.*, 1999). In similar experiments, first instar of *T. ni* that were infected with AcJHE-KK died approximately 27% faster (ST_{50} = 113 h) compared to control larvae infected with AcJHE. Third to fifth instar *T. ni* that were infected with AcJHE-KK to analyze ST_{50}. In these older insects, the ST_{50}s of AcJHE-KK-infected third, fourth, and fifth instars were reduced by roughly 4% (124.8 vs. 130.2 h), 9% (126.6 vs. 138.6 h), and 8% (135.0 vs. 147.0 h), respectively, in comparison to control AcMNPV-infected larvae. In similar bioassays of second to fifth instars of the soybean looper, *Pseudoplusia includens*, AcJHE-KK-infected larvae did not show a reduced survival time in comparison to AcMNPV-infected control larvae. The reasons for these differences in the survival times between species and instars are unknown, however, Bonning *et al.* (1999) speculated that host- and/or age-specific effects may be involved.

Future outlook

Wild type baculoviruses are considered as integral component of the natural biological control of many pest species. However, in most cases the wild type viruses have failed to compete with classical insecticides because of several factors including application technology and low field persistence. The slow kill by these viruses makes them poorly competitive. Recombinant DNA technology was used to overcome problems associated with wild type viruses through introducing different genes in virus genome. Public acceptance of recombinant baculoviruses with insect toxins is a new problem facing this technology. Determining x-ray of insect proteins used for insect development disruption like JHE from different insect species will help more in this technology. This x-ray will help scientists for their hypothesize that prevent JHE from uptake and degradation by pericardial cells. Upon the stability of JHEs *in vivo* for a long time will lead to insect development disruption through JH degradation. By modification of proteins for enhancing *in vivo* stability, recombinant baculoviruses may improved for insect control, especially those that show minimal increase in speed of kill.

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