



The role of α - and β -hydrolase fold enzymes as biopesticides in pest management

El-Sayed A. El-Sheikh¹ and Mary D. Mamatha²

ABSTRACT

Disrupting juvenile hormone (JH), an important hormone that has a major role in insect development and reproductive function, will disrupt insect development. JH was shown to be primarily metabolized by two hydrolytic enzymes in the α / β -hydrolase fold family known as JH esterase (JHE; EC 3.1.1.1) and JH epoxide hydrolase (JHEH; EC 3.3.2.3). The major routes of JH metabolism in Lepidoptera are ester hydrolysis by JHE and epoxide hydration by JHEH. These catabolic pathways generate three metabolites (JH-acid, JH-diol and JH-acid-diol) that are each believed to be physiologically inactive. The most direct use in disrupting insect life cycle is with baculoviruses through increasing the efficiency of them by introducing these two metabolic enzymes into baculovirus genome. This recombinant technology provides *in vivo* long-term stability and efficiency for pest control. Recombinant baculoviruses with JHE or JHEH proteins represent valuable technology that may have great potential for effective integration pest management system. Such a demonstration would indicate that JHE or JHEH, as novel anti-JH agents, make them as potent biopesticides and may represent a major step toward a more sustainable agriculture.

Key words: Juvenile hormone, juvenile hormone epoxide hydrolase, recombinant baculoviruses.

INTRODUCTION

To overcome problems associated with chemical pesticides, alternative methods of control were given more attention (El-Sheikh, 2009). In contrast to the nervous system, the endocrine system potentially offers targets that can be exploited in a very selective fashion. Disruption of a regulatory system is often amplified dramatically in terms of the effect on the target organism. The juvenoids (JH mimics) and diacylhydrazines (ecdysone mimics) include several commercial examples that show exceptional selectivity at the target site (El-Sheikh *et al.*, 2011). There are several ways to apply this technology in pest control. The most direct use is with baculoviruses which have been considered as viral insecticides and are safe for the environment (Cheng and Lynn, 2009).

Natural baculoviruses are being used as highly selective biological insecticides for the large-scale protection of soybean and cotton in Brazil and China (Moscardi, 1999; Kamita *et al.*, 2005). However, for many agricultural applications in developed countries, the natural viruses kill pest insects too slowly to be useful (Inceoglu *et al.*, 2001; Kamita *et al.*, 2005). A large number of approaches have been applied to improve the insecticidal efficacy of the wildtype baculovirus including the use of genes encoding

insect-selective scorpion toxins and enzymes (Kamita, 2005). Although the primary use of the recombinant baculoviruses will be for testing efficiency in basic endocrinology, some of the recombinant viruses generated are likely to have increased insecticidal efficacy against pest insects including the noctuid complex which causes major damage to crops worldwide. Additionally, if juvenile hormone (JH) metabolites turn out to be biologically active molecules, the enzymes like JH Esterase (JHE) and JH Epoxide Hydrolase (JHEH) that produce these metabolites become biosynthetic enzymes as well as degrading enzymes. Thus, the inhibition of these enzymes by either recombinant means or with chemical inhibitors could lead to alternative avenues for toxicity against pest insects (El-Sheikh *et al.*, 2011). The work from Vlask's laboratory with antisense strategies (Hajos *et al.*, 1999), the earlier work by Hanzlik with chemical JHE inhibitors (Hanzlik and Hammock, 1988) and the recent work by Tan *et al.* using transgenic insects (Tan *et al.*, 2005) indicate that blocking the JH metabolic pathway can result in immediate death or other severe consequence in early instar pest insects.

Juvenile hormone (JH) plays a role in almost all physiological, developmental and reproductive processes in insects. An early study for describing observations on the regulation of the esterase activity, which hydrolyzes JH during the last larval

stadium (Hammock *et al.*, 1984), shown that JH esterase hydrolysis is a major route of JH catabolism and that in the cabbage looper, *T. ni*, and in the tobacco hornworm, *M. sexta*. This hydrolysis is due largely to a single enzyme at developmentally critical times. This single protein in the early last larval stadium possibly is under the control of neurosecretory factors, which in the prepupal period JH directly results in JH esterase production. They advance the concept that in contrast to reports in vertebrate, where hormone titer is regulated by constant degradation and variations in biosynthesis, that titre of JH is, in part, controlled by a reduction in rate of biosynthesis and a large increase in the levels of catabolic enzymes.

A higher JH titer in adults occurs as a result of an increase in the rate of JH biosynthesis by the corpora allata (CA), the transfer of JH from the male to the female during copulation and a decrease in JHE activity. The initiation of JH synthesis in the CA appears to originate from factors synthesized in the male accessory glands and transferred to the female during copulation in *Drosophila melanogaster* (Richard *et al.*, 1998) or in the case of the tobacco hornworm, *Manduca sexta*, by brain allatropin produced in response to mating induced neural signals from the *bursa copulatrix* (Sasaki and Riddiford, 1984). There have been a number of reports of the activation of the female CA by mating (Herman and Barker, 1977; Stay and Tobe, 1977; Park *et al.*, 1998). Transfer of JH from the male to the female during copulation was reported in *Hyalophora cecropia* (Shirk *et al.*, 1980) and in the tobacco budworm, *Heliothis virescens* (Park *et al.*, 1998). Venkatesh *et al.* (1988) were the first to find a reduction in JHE activity in the haemolymph of adult female cabbage loopers, *Trichoplusia ni*, after mating. Ramaswamy *et al.* (2000) also reported the same for female adults of *H. virescens*. However, in the torticid moth, *Cydia pomonella*, Cole *et al.* (2002) found no differences in JHE activity between virgin and mated females and concluded that JHE was not important in reproduction. This conclusion appeared to be consistent with studies on other torticids (Cusson and Delisle, 1996; Cusson *et al.*, 1999; Delisle and Cusson, 1999). Although research is available on the role of JHE in the regulation of adult reproduction in the Lepidoptera, no studies have been conducted on the possible role of JHEH. In addition the role of EHs in JH metabolism has been in question because of a lack of *in vivo* JHEH inhibitors or successful *in vivo* EH expression studies (Gilbert *et al.*, 2000; Anspaugh *et al.*, 2005).

Importance of JHE and JHEH in JH metabolism

Juvenile Hormone (JH) has a major role in larval insect growth and development, as well as in reproductive function of adult insects. The JH concentration in insects is regulated by two dynamic processes, biosynthesis and degradation (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 (Figure 1). JHEs show high specificity for JH but only a moderate turnover rate (Kamita and Hammock, 2010). JH epoxide hydrolase (JHEH) (Share and Roe, 1988), and JH diol kinase (JHDK) (Maxwell *et al.*, 2002a,b), are involved in the JH degradation pathway. JHEH degrades JH acid to produce JH acid diol for which no activity has been discovered (Share and Roe, 1988). In addition, JHDK converts JH diol into JH diol phosphate, another inactive JH metabolite (Maxwell *et al.*, 2002a, b). Most of the research on JH degradation has concentrated on the mechanism of action of JHE (Kamita *et al.*, 2003). Although less is known about JHEH, it seems that JHEH is as critical as JHE in insect development. The extent of the roles that JHE and JHEH play in JH degradation depends on species and developmental stages of the insect (Keiser *et al.*, 2002; Kamita *et al.*, 2003).

Since a partition assay for the simultaneous determination of JHE and JHEH was developed (Share and Roe, 1988), rapid progress has been made on JHEH studies. For example, in *M. sexta*, a JHEH (Manse-JHEH) from eggs was purified and characterized (Touhara and Prestwich, 1993) and its cDNA cloned (Wojtasek and Prestwich, 1996). Subsequently the recombinant Manse-JHEH was expressed *in vitro* using the baculovirus system (Debernard *et al.*, 1998). In both Manse-JHEH and mammalian epoxide hydrolases (EH), the residues in the catalytic triad and those corresponding to the oxyanion hole are well conserved and are critical for enzyme activity. It was suggested that the catalytic mechanism of Manse-JHEH is similar to that of the mammalian EHs (Wojtasek and Prestwich, 1996; Debernard *et al.*, 1998; Severson *et al.*, 2002). In addition, JHEH cDNAs were also cloned from other insect species, such as *T. ni* (Harris *et al.*, 1999) and *Ctenocephalides felis* (Keiser *et al.*, 2002).

Efficiency of JH metabolic enzymes in insect pest control

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 (Staal, 1986). Esterases exist in at least three orders of insects with a high degree of specificity towards JH (Hammock, 1985). These 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,

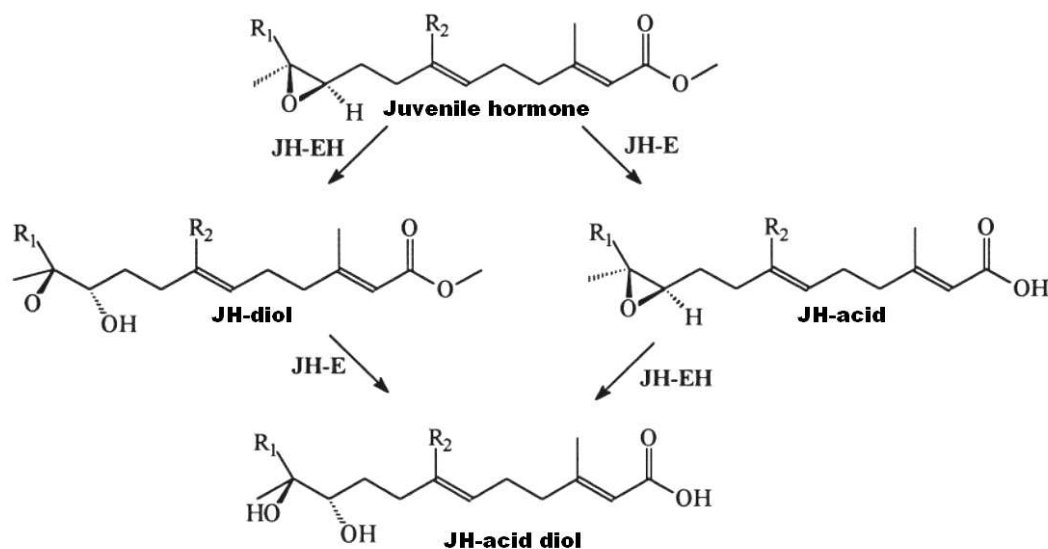


Figure 1. Pathways of juvenile hormone degradation.

contributing to the rapid decline in the JH titre 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. The infection of *M. sexta* with recombinant baculovirus expressing either wild type or mutant JHE show significantly reduction in larval mass (7% reduction) and (30% reduction) compared with wild type virus respectively (Table 1) (El-Sheikh *et al.*, 2011). Figure 2 shows the efficiency of recombinant baculoviruses with JHE over wild type which indicate the importance of JH hydrolytic enzymes for increasing the efficiency of natural baculoviruses in pest control.

Table 1. Effect of virus exposure using artificial diet on the larval mass of *M. sexta**.

Viruses	% reduction in larval mass
AcMsJHE-HH**	70
AcMsJHE	47
AcMNPV	40
Control	—

* This data adapted from El-Sheikh *et al.* (2011).

** Recombinant baculovirus with mutated JHE.

In the JH degradation process, it has been shown that JHE is dominant in many insects (Jesudason *et al.*, 1992; Kamita *et al.*, 2003). However, JHEH may be more important than JHE for the control of JH degradation in some developmental stages of some insect species. For example, in the eggs of *M. sexta*, JHEH is more active than JHE (Touhara and Prestwich, 1993). In several stages of larval *M. sexta*, the formation of diol by JHEH is the major *in vivo* route of JH degradation (Halarankar *et al.*, 1993). In *Culex quinquefasciatus*, JHEH activity exceeds JHE activity throughout most of the fourth (last) instar suggesting JHEH has a dynamic role in the initiation of metamorphosis (Lassiter *et al.*, 1995). During the pupal-adult development of *D. melanogaster*, JHE and JHEH play similar roles in JH degradation, whereas in adults of *D. virilis*, JHEH activity is higher than JHE activity (Khlebodarova *et al.*, 1996). Moreover, the significance of JHEH is also supported by the hormone function of JH acid during certain developmental stages of Lepidoptera.

CONCLUSION AND FUTURE OUTLOOK

Synthetic chemical pesticide problems are often intensified in developing countries because of poor governmental regulation and training in the appropriate use of these agents. Furthermore, the high costs of the newest generation of synthetic chemical pesticides are precluding their widespread use in many developing countries. These pressures in the agricultural industry are making biological control agents highly attractive as supplements or replacements for synthetic chemical pesticides in integrated pest management schemes

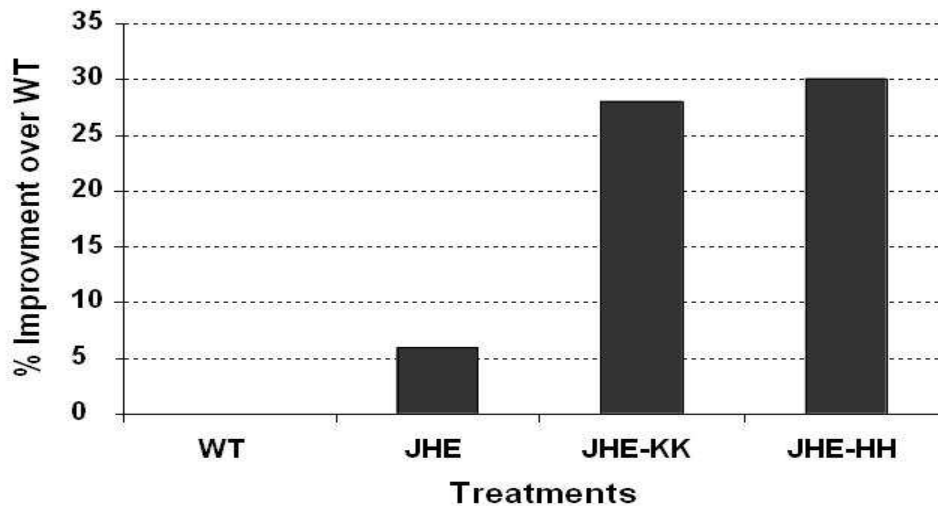


Figure 2. The effect of the wild type baculovirus can be dramatically improved by genetic modification. This figure shows insertion of a foreign gene either wild type gene or mutated one and percent improvement in speed of kill relative to the wild type virus. WT-wild type; JHE-juvenile hormone esterase (Hammock *et al.*, 1990); JHE-KK-stabilized JHE (Bonning *et al.*, 1997); JHE-HH, mutated JHE (El-Sheikh *et al.*, 2011).

Natural baculoviruses are providing an important part of biological pesticides. The major problem with these viruses is that they are slow in achieving the target. Industry and academic scientists have overcome most of the limitations associated with natural baculovirus pesticides, especially in terms of effective crop protection, through the use of recombinant DNA techniques and other technologies. The safety of baculoviruses has been thoroughly investigated and there is no evidence that natural or recombinant baculoviruses provide an increased threat to human or environmental health.

With the currently available genes like JHE and JHEH and parental viruses, the belief that the fastest speed of killing (median lethal time) that can be achieved is around 48 hours (i.e., roughly a 60% improvement over the wild type virus) because the virus will most likely have to undergo two replication cycles before the host insect is killed. Feeding cessation at 24 to 36 hrs post infection should be sufficient to make GM baculovirus pesticides competitive with synthetic chemical insecticides in terms of the protection of many types of crops. To achieve more improvement in this field, efforts should be directed towards more practical aspects of the field including increased extension and free access to the technology. Countries where GM baculoviruses are actively used could of course provide wisdom on how to successfully commercialize GM baculoviruses, and it would be a great waste to abandon this technology.

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El-Sayed A. El-Sheikh¹, and Mary D. Mamatha²

¹ Plant Protection Department, Faculty of Agriculture, Zagazig University, 44511, Egypt.

² Seribotechnology Department, Sri Padmavathi Mahila Visvavidyalayan (Women's University), Tirupati, 517502, India. *E-mail: eaelsheikh@yahoo.com.

Received: August 08, 2011

Revised: January 14, 2011

Accepted: February 24, 2012