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Ecdysone Receptor Present in Insects is a Novel Target for Insecticide

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A B S T R A C T

Insects occupy more than 70% of entire animal kingdom and are the most successful group of organism living on earth. They are usually divided into three groups i.e., harmless, injurious and beneficial. A group of injurious insects referred as pests, annually destroy between 6-30% of agricultural harvest in developing countries. These losses become even more significant for stored cereal products than pre-harvest losses, because post-harvest costs are much higher than the cost of production.

Introduction

Lepidopteran insects are among the major pests of several economically important crops and their control requires a multi-pronged intervention. While insecticides have been used in several IPM (Insect pest management) programmes, pheromone traps have been frequently used to capture and kill insect pests in the field. The capacity of novel chemicals to disturb the mating and molting processes of insects has been capitalized for pest control. Insects on the basis of their ability to undergo metamorphosis are broadly classified into ametabolous (no metamorphosis), hemimetabolous (incomplete metamorphosis) and holometabolous (complete metamorphosis).

Insect metamorphosis

Metamorphosis is the characteristic feature of majority of the insects, including holometabola during the postembryonic development i.e., the ontogeny accomplished after hatching. Metamorphosis is marked by abrupt changes in the form and structure during the postembryonic development. The larval forms are the juveniles of holometabola that lack the external rudiments of wings and

genitalia but possess imaginal discs. The larvae are voracious feeders and have different habitat and niche from the adult stage. The non-feeding pupal stages are usually hidden or somehow protected stage. The tissue degeneration and rebuilding mainly occurs at the pupal stage, which also possesses the external rudiments of wings and genitalia.

The adult stage of holometabolous insect is morphologically very different from the previous stages and they are usually prolific breeders (Sehnal *et al.,* 1996; Truman and Riddiford, 1999, 2002; Buszczak and Segraves, 2000; Tissot and Stocker, 2000). Ecdysterone or 20E is responsible for insects development, Molting and metamorphosis. The molting hormone, 20-Hydroxyecdysone (ecdysterone or 20E), is a naturally occurring ecdysteroid hormone that controls the molting of arthropods (Thummel, 1995, 1996). During insect development, it binds to the ecdysone receptor, a ligand-activated transcription factor found in the nuclei of insect cells (Riddiford, 2000). This in turn leads to the activation of many other genes, as evidenced by chromosomal puffing at over a hundred sites. Ultimately the activation cascade causes physiological changes that result in molting (Henrich, 2005).

Ecdysone Biosynthesis

PTTH acts on the prothoracic glands (PGL) and stimulates ecdysteroid synthesis (Gilbert *et al.,* 1988). Thus 3-dehydroecdysone is released into the haemolymph where it is reduced by a ketoreductase to ecdysone. The prohormone ecdysone is converted to the principal molting hormone 20-hydroxyecdysone (20E) in the mitochondria and microsomes of peripheral tissues such as haemocytes, fat body, Malpighian tubules and epidermal cells (Riddiford *et al.,* 2001). 20E finally exerts its effect and causes apolysis and secretion of larval, pupal or adult cuticle (Gilbert, 1989).

Chemistry of ecdysteroids

Ecdysteroid is a well-defined term for all compounds structurally related to ecdysone. Further it includes true ecdysteroid and ecdysteroid related compounds. The biologically active ecdysteroid refers to the molting hormone. Chemically ecdysone is the trivial name of a specific compound (22R)-2β, 3β, 14α, 22, 25-pentahydroxy-5β-cholest-7-en-6-one, a derivative of cholesterol. 20-Hydroxyecdysone (20E) is the active form, which is a result of ecdysone 20-monoxygenase catalyzed hydroxylation (Grieneisen, 1994; Rees, 1995). The two molting hormones ecdysone and 20E were originally designated as α and β ecdysone respectively (Horn and Bergamasco, 1985). In arthropods, 20E is one of the most ubiquitously distributed ecdysteroid utilized by the molt cycle and is also associated with various physiological events (Gilbert *et al.,* 2002).

Regulation of ecdysteroid biosynthesis

The regulation of ecdysteroidogenesis has been studied continuously for the past several decades and recent discoveries using Drosophila molecular genetics have advanced our knowledge further.

The availability of genome sequences, the ease of genetic manipulation and the large collection of mutants all make Drosophila an attractive system for understanding the mechanisms regulating steroidogenesis. Regulation of ecdysteroid synthesis is complex, and is under the control of peptide hormones as well as the JH.

It has been known for some 85 years that a factor from the insect brain can stimulate the PGLs of both diptera and lepidoptera. PTTH stimulated ecdysteroid production in PGLs occurs via a cascade of events which is yet to be elucidated completely.

Ecdysteroids mode of action

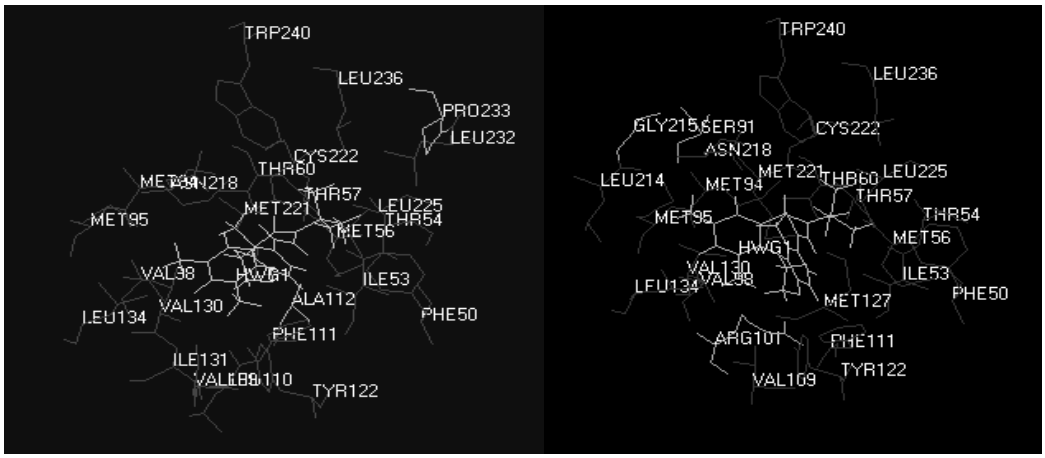
The isolation and purification of ecdysone and 20E by Butenandt and Karlson (1954) revolutionized the field of insect endocrinology. The pioneering research of Clever and Karlson (1960) revealed puffing patterns of the *Chironomous tentans* salivary gland polytene chromosome by ecdysteroid. This observation of puff regulation was later confirmed in genetic model organism, the fruit fly Drosophila melanogaster by several other groups (Ashburner *et al.,* 1974; Ashburner and Richards, 1976).

Based on these observations as well as through a series of detailed and elegant studies, Ashburner and group (1974, 1976) proposed a model for the regulation of gene expression by 20E. Since then this model became the basis of the knowledge of mechanism of steroid hormone action, which suggests that ecdysteroid could initiate a cascade of gene expression by directly acting on the nucleus. According to this model, the ecdysone upon binding to its specific receptor directly regulates two classes of genes, a small class of early regulatory genes and a large class of late genes. The protein products of the early genes in turn repress their own expression and induce the much larger set of late genes that play a more direct role in controlling the biological response of hormone. Extensive studies based on this model have provided insights into the molecular mechanism of 20E action (Henrich and Brown, 1995; Thummel, 1996; Henrich *et al.,* 1999; Riddiford *et al.,* 2001).

Ecdysone receptor in insects

The ecdysone receptor is a noncovalent heterodimer of two proteins the EcR protein and ultraspiracle protein (USP). These nuclear hormone receptor proteins are the insect orthologs of the mammalian farnesoid X receptor (FXR) and retinoid X receptor proteins (RXR) receptively.

Fig.1 LBD of EcR in a) *H. armegira* and b) *S. litura*



Indeed, based on sequence homology considerations, some researchers reserve the term USP for the EcR partner from lepidopteran and dipteran insects, and use RXR in all other instances. EcR and USP share multidomain architecture common to all nuclear hormone receptors, namely an N terminal transcriptional activation domain (A/B domain), a DNA-binding domain (C domain, highly conserved between receptors), a linker region (D region), a ligand-binding domain (E domain, moderately conserved)

and in some cases a distinct C-terminal extension (F-domain) (Koelle *et al.,* 1991) The DNA-binding domains of EcR and USP recognise specific short sequences in DNA, and mediate the binding of the hetero dimer to these ecdysone response elements (EREs) in the promoters of ecdysone responsive genes.

The ecdysteroid binding pocket is located in the ligand-binding domain of the EcR subunit, but EcR must be dimerised with a USP (or with an RXR) for high affinity ligand binding to occur. In such circumstances, the binding of an agonist ligand triggers a conformational change in the C terminal part of the EcR ligand-binding domain that leads to transcriptional activation of genes under ECRE control. There is also a ligand-binding pocket in the corresponding domain of USP. Its natural ligand remains uncertain, and USPs appears to be locked permanently in an inactive conformation (Clayton *et al.,* 2001)

Regulation of ecdysteroids action

A major area with regard to understanding the regulation of 20E action is that of tissue specificity. The diversity in function of the hormone to some extent could be due to the variation of responses among cell types that typifies the action of 20E. The 20E also shows differential effects on same tissue at different developmental stages (Riddiford *et al.,* 2001). Thus studies directed towards the molecular basis of differentiation during the development and the built in regulatory mechanisms at the tissue level largely aid in the understanding of 20E actions. In contrast to vertebrate systems, ecdysteroids perform a wide variety of functions in the entire insect class. Hence, it is often referred that almost the entire insect is target of ecdysteroids (Gilbert *et al.,* 1996). It stimulates the growth and development of imaginal discs, promotes the deposition of cuticle by epidermis, regulates the growth of motor neurons, regulates defensive secretions and controls choriogenesis (Gilbert *et al.,* 1996). The ecdysteroid also initiates the breakdown of larval structures during metamorphosis. As it is clear, there exists a vast amount of information regarding the regulation of ecdysteroids at the synthesis as well as at its titre level. However, knowledge regarding the mechanism of regulation of ecdysteroid dependent actions is not very clear and the field remains largely unexplored. In majority of holometabolous insects including lepidopterans, the 20E action is regulated to induce transition from the juvenile to adult forms.

Ecdysone receptor as a Target by insecticides

Lepidopteran insects are among the major pests of several economically important crops and their control requires a multi-pronged intervention. The molting hormone, 20-Hydroxyecdysone (ecdysterone or 20E), is a naturally occurring ecdysteroid hormone that controls the molting of arthropods (Thummel, 1995, 1996). During insect development, it binds to the ecdysone receptor, a ligand-activated transcription factor found in the nuclei of insect cells (Riddiford, 2000). This in turn leads to the activation of many other genes, as evidenced by chromosomal puffing at over a hundred sites. Ultimately the activation cascade causes physiological changes that result in molting (Henrich, 2005). In recent years research is focused on targeting the ecdysone receptor with the aim to disrupt the molting process of insects and facilitate insect control. Insect growth regulators control insect population, by primarily regulating molting, metamorphosis and many other physiological and developmental processes (Williams, 1956). Nonsteroidal di benzoyl hydrazines such as RH5849 and RH5992 exert their insecticidal effect by binding to the 20-hydroxyecdysone

binding site and activating the ecdysteroid receptors permanently. Their comprehensive effects and high selectivity as well as lower toxicity to non-target animals and the environment provide new tools for integrated pest management EcR is the target of the environmentally safe bisacylhydrazine insecticides used against pests which cause severe damage to agriculture. N-tert-butyl-N,N'- dibenzoylhydrazines (DBHs) were discovered as molting hormonal agonists, and causes incomplete molting in insects leading to death. A number of DBH analogues with various substitutes at benzene rings were synthesized and the structure-activity relationship (SAR) studies performed. Recently, four DBH compounds including tebufenozide, methoxyfenozide, chromafenozide and halofenozide have been commercialized. Chromafenozide is found to be significantly potent against various lepidopterous insects, but at the same time almost non-toxic to non-lepidopterous species, including pollinators, predators and parasitoids. Even though 20E is commonly used as molting hormone in most of insects and has similar potency among insects, SARs of non-steroidal ecdysone agonists varied among insect species. The reason for the difference of SARs between ecdysteroids and non-steroidal compounds is disclosed by the three dimensional structure analysis of ligand-bound EcR, showing that ponasterone A (PonA), one of the most potent ecdysteroids, does not necessarily overlap with a chromafenozide analog (BYI06830) in the binding pocket, and therefore, the interaction between EcR and DBHs can be species-dependent.

Comparison of 3D structures of hormone-binding pockets in EcR and LXR

As we found for TR and RAR, comparison of the 3D structure of the hormone-binding domains of EcR and LXR revealed that some hormone-binding residues are conserved (Figure 1). Specifically, Phe-397/Phe-329 (EcR/LXR) and Met- 380/Met-312 have hydrophobic interactions with the centre of the hormone, and Arg-387/Arg-319 interacts with the C3-OH group of both ponA and eCH. Trp- 526/Trp-457 has been proposed to be part of a tryptophan/histidine activation switch in LXR (Williams and others 2003). Carmichael and colleagues (2005), however, suggested that this tryptophan does not participate in an activation switch in EcR because this would not explain why ponA, which lacks the 25-hydroxyl group, is an agonist for EcR. Several amino acids of EcR that interact with the hydroxyl groups of ponA are not conserved in LXR, which likely is due to the absence of these hydroxyl groups in the ligands that bind LXR. For example, Tyr-408 in EcR, which forms a strong hydrogen bond to the 20-OH group of ponA, is a phenylalanine in LXR. Other key differences between the hormone binding pockets of EcR and LXR include Arg-383/ Glu-281 and Glu-309/Asn-239. Different types of inhibitors or insecticides bind on these binding sites where ecdysone bound, some inhibitors act as antagonist and other act as agonists. Agonists act as growth regulators and thus help pre metamorphosis because of that insects died. As compared to it antagonist retard the growth of insects and because of that metamorphosis not occur and adult insect are not formed. So various insecticides binds at these binding sites and help to control pest by retard the insects development, growth, molting, metamorphosis.

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