

INHIBITION OF CHITIN SYNTHESIS IN *PLODIA INTERPUNCTELLA*

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ABSTRACT: Chitin synthesis was studied in an *in vitro* tissue culture system. Wing imaginal disks from last instar larvae of the Indian meal moth, *Plodia interpunctella* (Hubner), synthesized chitin and deposited a tanned pupal-cuticle when incubated with beta-ecdysone. Cecropia juvenile hormone (methyl-10, 11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate) prevented the ecdysone-induced cuticle deposition. As part of an effort to determine the mode of action of juvenile hormone on imaginal disks, the effects of the hormone on uptake and incorporation of tritiated-glucosamine into chitin were examined. In addition the action of juvenile hormone was compared with the following agents: Cytochalasin B, an inhibitor of sugar transport in a variety of systems; Polyoxin D, an inhibitor of the chitin synthetase reaction in fungi; and TH6040, a new insecticide that apparently inhibits chitin synthesis (although its site of action has yet to be determined). Cytochalasin B. and Polyoxin D, but not TH6040, inhibited chitin synthesis and cuticle deposition *in vitro*. The significance of inhibition of chitin synthesis for the development of insecticides against stored-product insects will be discussed.

Concern for the environment has focused the attention of entomologists on pest control measures that are highly selective. Hence, there is great interest in compounds that prevent insect metamorphosis (mimics of insect juvenile hormone) and in means of disrupting the insect's communication system (saturation with pheromone). There is another area worthy of investigation that may prove useful; the control of chitin biosynthesis. Chitin is not found in higher plants or vertebrates, but is an important structural element in many fungal cell walls and in arthropod cuticle. At least three fungicides work by interfering with chitin biosynthesis[1]. Although no insecticide currently in use is known to act in this manner, several laboratories recently have worked with compounds that inhibit chitin formation in insect tissues cultured *in vitro*. An understanding of the mode of action of these inhibitors may help develop a new class of "Insect Growth Regulators" that interfere with chitin biosynthesis.

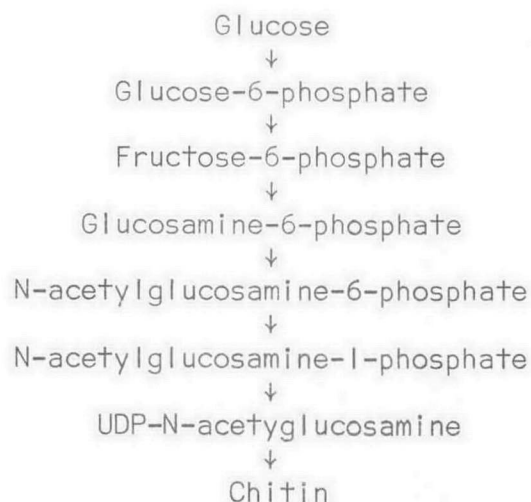
The *in vitro* model systems for testing chitin synthesis inhibitors include the following: Cockroach leg regenerates[2]; and imaginal disks of dipteran[3] and of lepidopteran[4,5] larvae.

In each case, the cultured tissues respond to ecdysone by producing cuticle *in vitro*. Both the cockroach leg regenerates[6] and *Plodia interpunctella* (Hubner) [7] imaginal disks produce cuticle that contain chitin.

A scheme for the biosynthesis of insect chitin is shown in Figure 1. Manifestly, there are a number of steps that could be targets for chitin inhibitors.

FIGURE 1.

Chitin Biosynthesis



The model system that we employ to investigate chitin synthesis is based upon the response of undifferentiated wing primordia (imaginal disks) to ecdysone. Wing disks are dissected from 5th-instar *P. interpunctella* larvae and cultured in petri dishes in a modified Grace's tissue culture medium. After treatment with beta-ecdysone, the disks evaginate and produce a tanned pupal cuticle[5]. We have established by histochemical means that the disk cuticle contains chitin. Furthermore, isotopic D-glucosamine, a precursor of chitin, is incorporated into the disk cuticle in response to ecdysone. The label is removed by treatment with chitinase[7].

The first questions that should be answered with this *in vitro* system include the following: Do the disks at the time of dissection contain sufficient hexose or hexosamine precursors (of chitin), or do they require sugar from the tissue culture medium? If the latter is the case, then this system would be useful for examining the control of hexose uptake. Secondly, can ecdysone-induced cuticle formation be inhibited by limiting precursor availability or only by direct interference with biosynthesis? Do inhibitors of chitin synthesis in fungi affect the same biosynthetic pathway in insects?

To answer these questions, we have examined the effects

on cultured disks of several putative chitin inhibitors: cytochalasin B, polyoxin D, and Thompson-Hayward (TH) 6040.

Cytochalasin B is a mold metabolite that since 1967 has been used in studies of animal cytokinesis. Apparently, cytochalasin B, prevents cellular motility by binding to microfilaments. Moreover, evidence recently has been obtained that this agent also inhibits hexose transport[8]. Hence, we were provided with a compound that would help us test whether the cultured wing disks required an extrinsic supply of chitin precursors, or whether they already contained sufficient intracellular hexose and hexosamine molecules.

At a concentration of 4 $\mu\text{g/ml}$, cytochalasin B prevented the increase in D-glucose-6- ^3H uptake by disks that would otherwise result from treatment with ecdysone. This was the first demonstration of inhibition of hexose uptake by cytochalasin B in insects. Cytochalasin B also inhibited cuticle formation by the disks, though the treated disks appeared healthy and continued to take up D-glucose-6- ^3H at near the normal basal level. Therefore, wing disks from 5th instar larvae that were cultured *in vitro* required extrinsic chitin precursors. Whether more mature disks late in the 5th instar are also dependent on extrinsic hexose has not yet been determined (Table I).

TABLE I. Effects of inhibitors of chitin synthesis on cultured *P. interpunctella* imaginal disks.

Compound	% cuticle deposition	D-glucose-6- ^3H uptake (cpm at 48 hr)
(no ecdysone) ¹	0	100
	80	251
Cytochalasin B (4 $\mu\text{g/ml}$)	0	65
Polyoxin D (1 mg/ml)	40	215
TH 6040 (100 $\mu\text{g/ml}$)	80	185

¹In all instances except the first, beta-ecdysone (ca. 0.5 $\mu\text{g/ml}$) was present in the culture medium.

Polyoxin D is produced by *Streptomyces cacaoi* var. *asoensis* and inhibits the growth of some filamentous fungi[1]. The mode of action involves inhibition of chitin N-acetylglucosaminyl transferase (chitin synthetase)[9]. The similarity in structure to UDP-N-acetylglucosamine may account for its competitive inhibition of chitin synthetase.

We have found that polyoxin D (1 mg/ml) can prevent cuticle formation in cultured imaginal disks, though the inhibition is less than with cytochalasin B (Table I). Moreover, Marks[10] recently demonstrated that polyoxin D blocks incorporation of D-glucosamine- ^{14}C into cultured cockroach leg regenerates, though cuticle is still produced. In our cultured D-glucose-6- ^3H uptake was not blocked by polyoxin D. Therefore, we may tentatively conclude that the chitin synthetase that operates in fungi also

functions in insects, and can be inhibited with the same compounds.

TH 6040, [(1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)-urea)], is being tested in a number of laboratories because of its insecticidal properties. It has been suggested that TH 6040 interferes with endocuticle deposition by inhibiting chitin synthesis [11]. We found that TH 6040 did not inhibit cuticle deposition in the *P. interpunctella* tissue culture system (Table I). Marks [10] made a similar observation on cockroach leg regenerates but did note that TH 6040 inhibited incorporation of D-glucosamine-¹⁴C. We found only a small inhibition of uptake (Table I). In light of these observations, we decided to determine whether TH 6040 had any effect on *P. interpunctella* *in vivo* before pursuing its mode of action in the tissue culture system.

TH 6040 was mixed into the *P. interpunctella* diet by our standard procedure for testing insect growth regulators (Silhacek, personal communication). The lowest concentration of TH 6040 that reduced adult emergence was 4 ppm. However, 400 ppm was required for 90% inhibition of eclosion. Some insects died as larvae or pupae, and some had the appearance of pupal-adult intermediates (Table II). Hence, in this bioassay TH 6040 inhibited development at concentrations that did not prevent cuticle deposition *in vitro*. The low solubility of TH 6040 in the aqueous medium may have been a factor in the lack of inhibition *in vitro*.

TABLE II. Adult emergence of *P. interpunctella* larvae reared on TH 6040 treated diet.

TH 6040 (ppm)	No. adults (240 eggs added to diet)	No. adults (24 eggs added to diet)
0	164	10
0.4	139	11
4.0	91	2
40.0	84	3
400.0	-	1

The action of the inhibitors discussed may help us understand the mode of action of juvenile hormone. Synthetic juvenile hormone (mixed isomers of methyl-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate) inhibits the development of *P. interpunctella* imaginal disks both *in vivo* [12] and *in vitro* [13]. Because deposition of a pupal cuticle is diagnostic of differentiation of these imaginal disks, juvenile hormone is, in effect, inhibiting cuticle deposition in this tissue. Whether juvenile hormone inhibits chitin synthesis in imaginal disks by preventing precursor uptake or biosynthesis is presently under investigation. The initial results suggest that juvenile hormone does not prevent ecdysone-induced hexose uptake by cultured disks.

These new investigations of the control of chitin synthesis in insects provide a meeting ground for scientists interested in mechanisms of action of growth regulators as well as those directly concerned with using such molecules in pest control.

Hopefully, the joint effort will be fruitful.

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