Age-Dependent Arylsulphatase and Sulphotransferase Activities in the Southern Armyworm: A Possible Insect Endocrine Regulatory Mechanism?

By RAYMOND S. H. YANG, JOSEPH G. PELLICCIA and C. F. WILKINSON
Department of Entomology, Cornell University, Ithaca, N. Y. 14850, U.S.A.

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Arylsulphate and sulphotransferase activities were determined during the late larval development of the southern armyworm. Arylsulphatase titre attains a maximum during the moult and is at a minimum between moults. Sulphotransferase titre appeared to follow the inverse course. A possible enzymic regulatory mechanism for insect steroid hormones is discussed.

In a previous study in this laboratory (Yang & Wilkinson, 1972), an active sulphotransferase system which catalyses the sulphation of 4-nitrophenol, cholesterol, α -ecdysone, β -sitosterol, dehydroepiandrosterone, oestrone and four other steroids of plant and insect origin was isolated and characterized from the gut tissues of the southern armyworm (*Prodenia eridania*). Subsequently the presence of a similar enzyme system was demonstrated in seven other species of insects representing four major orders (Yang & Wilkinson, 1973). These findings, combined with earlier studies *in vivo* indicating the existence of a sulphotoconjugation pathway in 14 other species of insects (Smith, 1955; Butenandt *et al.*, 1960; Viscontini & Schmid, 1963; Smith & Turbert, 1964; Binning *et al.*, 1967), strongly suggest that sulphotransferases have a broad distribution in the class Insecta.

It has been suggested that insect sulphotransferases operating in close concert with arylsulphatases might have an important physiological role in regulating the concentrations of insect steroids in general and more specifically insect moulting hormones (Yang & Wilkinson, 1972). Thus, through a process of enzymic sulphotoconjugation and sulphotohydrolysis, the insect moulting hormones could be deactivated, stored and subsequently reactivated as required at different stages of insect development. The possible involvement of ecdysone conjugates in the regulation, transport and storage of this hormone has also been suggested by other investigators (Kaplanis *et al.*, 1973; Thompson *et al.*, 1973).

There is little information presently available about insect arylsulphatases. Although arylsulphatase activity has been detected in the crop fluid and gut content of *Schistocerca gregaria* and the crop fluid and haemolymph of *Locusta migratoria* (Robinson *et al.*, 1953; Kikal & Smith, 1959) no physiological function of this activity has been suggested.

The present study was undertaken to investigate arylsulphatase and sulphotransferase activities in larvae of the southern armyworm and to pay special attention to changes in the titre of the enzymes in relation to insect development.

Materials and methods

Insects. The southern-armyworm larvae used in this study were from a culture maintained under greenhouse conditions as reported by Yang & Wilkinson (1972). For each experiment, 250 larvae during fourth moult or 150 larvae during fifth moult were isolated over a period of less than 2 h. The selection of larvae of the appropriate age was based on the size and the first sign of the swollen 'double-imaged' head capsule, which signals the beginning of the moult. At this time and at approximately the same hour of each succeeding day, 15–50 larvae, depending on the size of the insects, were used for enzyme preparation and assay.

Enzyme preparations. For arylsulphatase studies, the enzyme source was the haemolymph of the larvae. The insects were rinsed twice in distilled water and blotted to remove soil particles and/or regurgitated gut contents. After excision of one of the abdominal prolegs, the haemolymph was drained into a chilled graduated tube. An equal volume of 0.15 M-KCl containing 1 mM-EDTA was added and the resulting mixture was used as the enzyme source.

For sulphotransferase studies, the gut-tissue high-speed supernatant constituted the enzyme source. This was prepared as described by Yang & Wilkinson (1972) except that the tissues were homogenized in 9 vol. of 0.15 M-KCl containing 1 mM-EDTA and only one centrifugation step (100000g, 1 h) was used.

All operations were carried out at 0–4°C and fresh enzyme preparations were used throughout the investigation.

Chemicals and reagents. ATP, GSH, 2-hydroxy-5-nitrophenyl sulphate (dipotassium salt), 4-nitrocatechol and 4-nitrophenol were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. All other chemicals and reagents were of analytical-reagent grade. The preparation and storage of buffers, cofactors and substrates were as previously reported (Yang & Wilkinson, 1972).

Enzyme assays. Arylsulphatase activity was measured by a method slightly modified from that originally reported by Roy (1960). The reaction mixture contained, in a total volume of 0.5 ml, 50 μ mol of sodium acetate buffer, pH 5.0, 1.5 μ mol of 2-hydroxy-5-nitrophenyl sulphate and 0.1 ml of enzyme. After incubation for 1 h at 38°C the reaction was terminated by the addition of 1.5 ml of 0.1 M-trichloroacetic acid. The protein was removed by centrifugation and 1.5 ml of the resulting supernatant was added to 2.5 ml of 2.5 M-NaOH. The extinction of the solution was measured at 515 nm and the amounts of 4-nitrocatechol present were determined from a suitable calibration curve.

Sulphotransferase activity was measured by the decrease in E_{400} of 4-nitrophenol as reported previously (Yang & Wilkinson, 1972).

Protein was determined by the modified Lowry method described by Chaykin (1966). All spectrophotometric measurements were made with a Bausch and Lomb Spectronic 70 spectrophotometer. The results reported are the average of two or more experiments and within each experiment the enzyme activities were assayed at least in triplicate.

Results

Arylsulphatase and sulphotransferase activities were determined daily with tissue preparations from the southern-armyworm larvae between the fourth moult and the burrowing stage, which served as an indication of the onset of pupation. The results (Fig. 1) clearly indicate that both enzyme activities are age-dependent. The arylsulphatase titre attains a maximum during the moult and is at a minimum in the period between moults. In contrast sulphotransferase titre appears to follow an inverse course.

Despite some seasonal variations in the developmental period of the larvae, arylsulphatase and sulphotransferase activities consistently followed the same pattern as that shown in Fig. 1.

Each of the enzymes appeared to be specifically associated with the tissues employed. Thus sulphotransferase activity was found almost exclusively in the gut tissues and arylsulphatase in the haemolymph. Neither tissue contained a significant titre of the enzyme not specific to it.

As shown in Table 1, the protein content per unit volume of insect haemolymph fluctuated during

larval development and was modified to some extent by changes in the volume of haemolymph per larva. Highest protein concentrations per unit volume occurred during the moult and there was a marked increase immediately before pupation. In contrast the protein content of the gut-tissue preparations remained relatively constant throughout the period of the investigation despite the progressive increase in the amount of tissue per larva during the later developmental stages.

Discussion

The results of this investigation have demonstrated age-dependent changes in arylsulphatase and sulphotransferase activities during late larval development of the southern armyworm (Fig. 1). Although small size precluded similar experiments with the larvae of earlier instars, it is probable that a similar pattern of enzyme activity exists throughout the entire period of larval development.

On the basis of studies with various animal species, arylsulphatases with low pH optima appear to be

Fig. 1. Age-dependent arylsulphatase and sulphotransferase activities in the tissues during the late larval development of the southern armyworm

●, Arylsulphatase activity in the haemolymph; the activity is expressed as nmol of 4-nitrocatechol liberated/h per 0.1 ml of enzyme preparation. □, Sulphotransferase activity in the gut tissues; the activity is expressed as nmol of 4-nitrophenyl sulphate formed/h per 0.1 ml of enzyme preparation. Methods of enzyme preparation and assay are as described in the text.

Table 1. *Relative quantity of tissues and their protein content during the late larval development of the southern armyworm*

The data represent the mean values of determinations with 30–140 insects at any given stage of development. The gut tissues consisted mainly of the mid-gut portion and were free of contamination with Malpighian tubules. The enzyme preparation from gut tissues was the high-speed supernatant (100000g, 1h) from a homogenate of gut tissue in 9vol. of 0.15M-KCl containing 1 mM-EDTA. Haemolymph preparations consisted of the crude larval haemolymph with an equal volume of 0.15M-KCl containing 1 mM-EDTA. Detailed methods of isolation and preparation of tissues are as described in the text.

Stage of development	Haemolymph		Gut tissues	
	(μ l/insect equivalent)	(mg of protein/0.1 ml of enzyme preparation)	(mg/insect equivalent)	(mg of protein/0.1 ml of enzyme preparation)
4th moult	7.7	0.82	3.96	0.28
Day 1	13.1	0.64	5.84	0.25
2	41.1	0.73	9.91	0.29
5th moult	32.9	0.99	13.06	0.32
Day 1	55.0	0.71	21.97	0.31
2	106.7	0.90	29.33	0.33
3	143.3	1.19	32.22	0.38
4	186.7	1.53	32.10	0.38
5	183.3	2.17	27.00	0.38
(burrowing)				

principally derived from lysosomes (Dodgson & Rose, 1970; Roy & Trudinger, 1970; Nicholls & Roy, 1971; Roy, 1971). It is possible therefore that the arylsulphatase activity observed in the haemolymph of the southern armyworm (Fig. 1) is released from the lysosomes of tissues such as the insect fat-body, and this is supported by the known increase in lysosomal activity during the moulting period (Lockshin, 1969).

The results of this study are also consistent with our earlier hypothesis concerning the possible enzymic regulatory mechanism for insect moulting hormones (Yank & Wilkinson, 1972). On the basis of present knowledge, it is tempting to suggest that at least in lepidopterous larvae the insect moulting hormones may be sulphoconjugated (and thus deactivated) by enzymes in the gut tissues. The hormone may be stored in this conjugated form and when required at the moulting stage may be reactivated to the active free form by arylsulphatases of lysosomal origin. At the present time much of this hypothesis is speculative in nature and presents an alternative to the hypothesis proposed by Karlson & Bode (1969) that ecdysone titre might be controlled directly by ecdysone-inactivating enzymes. However, in view of the fact that recent studies (Moriyama *et al.*, 1970; Gorell *et al.*, 1972) have established the transport and accumulation of α -ecdysone and its metabolites in various tissues of insects, further experimentation in this area is warranted.

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