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OPTIMIZATION OF ASSAY CONDITIONS FOR EPOXIDE METABOLIZING ENZYMES IN *TRICHOPLUSIA NI*

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Abstract—The optimization of assay conditions for glutathione *S*-transferase and epoxide hydrolase activities in the cabbage looper (*Trichoplusia ni* [Hübner], Lepidoptera; Noctuidae) using *trans* and *cis*-stilbene oxides as substrates are reported. Glutathione *S*-transferase activity was predominantly cytosolic, and it was much greater in the fat body than the midgut. Differences in pH optima and rates of conjugation were seen between the *trans* and *cis* isomers of the substrate. In contrast, epoxide hydrolase activity was predominantly microsomal and was highest in the midgut. Patterns of substrate selectivity and pH optima differed between these two tissues. These data reflect the complexity of insect epoxide metabolism and suggest the involvement of multiple forms of these enzymes.

Key Word Index: Insect, epoxide, epoxide hydrolase, glutathione *S*-transferase, *trans*-stilbene oxide, *cis*-stilbene oxide, *Trichoplusia ni* (Hübner), Lepidoptera, Noctuidae

INTRODUCTION

The ability of organisms to metabolize foreign compounds to more easily excretable substances is essential to their survival. One such detoxication pathway involves the conversion of epoxides to more hydrophilic metabolites. Epoxides are cyclic ethers that are ubiquitous in the environment and may be present in high concentrations in our diet as well as that of insects. They are also formed *in vivo* by the oxidation of unsaturated compounds or the rearrangement of peroxides. The enzymatic transformation of epoxides is catalyzed by epoxide hydrolases (EHs; EC 3.3.2.3) and glutathione (GSH) *S*-transferases (GSTs; E.C. 2.5.1.18). EHs metabolize epoxides by introducing a molecule of water across the epoxide ring resulting in the formation of a diol, a more easily excretable compound (Oesch, 1973). In most (but not all) cases, this hydrolysis results in a decrease in biological activity (Sims *et al.*, 1974). GSTs catalyze the formation of a relatively hydrophilic product by introducing a tripeptide, glutathione (GSH), while opening the epoxide ring (Chasseaud, 1979).

Despite many recent advances in our knowledge of mammalian epoxide metabolism (reviewed by Lu and Miwa, 1980; Wixtrom and Hammock, 1985), many questions are still unanswered (e.g. the endogenous roles of the EHs remain elusive). The fate of epoxides in insects is even less studied. While insect GSTs have received attention because of their involvement in the metabolism of insecticides (Yang *et al.*, 1971; Motoyama and Dauterman, 1980), their role in epoxide metabolism has not been examined in detail. Similarly, insect EH has been studied in relation to the hydration of cyclodiene insecticides (Brooks *et al.*, 1970) and with respect to its role in the metabolism of the juvenile hormones (Slade and Zibitt, 1972), but nothing is known of its intrinsic biological activity or its role in the metabolism of most insecticidal or dietary epoxides (Hammock, 1985).

Epoxide metabolism in insects is receiving increased attention for several reasons. The natural epoxides present in high levels in some plants, as well as compounds readily converted to epoxides, may represent defense mechanisms employed to discourage herbivory (Mullin, 1985). Since insects must detoxify these chemicals, there is a need to investigate the role of EH and GST in this process.

In addition, the disruption of epoxide metabolism may be a mechanism to effect insect specific control. A number of chemical mediators in insects, such as the juvenile hormones and various pheromones, contain epoxides. Moreover, epoxides are intermediates in important biosynthetic pathways (e.g. the conversion of phytosterols to cholesterol). Because these processes do not occur in mammals, disruption may represent a safe way to control insects.

Finally, insect epoxide metabolism may serve as a model for toxicological research. Since some of the most potent mutagens, carcinogens and toxins contain a reactive epoxide moiety (Miller and Miller, 1977), there is a need for an inexpensive and a reliable system for testing the biological properties of epoxides. Insects represent a level of intermediate complexity relative to procaryotes and mammals and offer numerous advantages in toxicity testing. Insects have been invaluable in the evaluation of a number of toxic chemicals (Vogel *et al.*, 1980; Tazima, 1980; Hällström *et al.*, 1981) and their use as models may increase as emphasis shifts to non-mammalian systems. If insects are to be used for toxicity testing of chemicals, it is critical that the metabolism of these xenobiotics in insects be appreciated.

To begin an examination of insect epoxide metabolism we have optimized the assay conditions for EH and GST using the cabbage looper, *Trichoplusia ni*,

as an enzyme source. Patterns of tissue and substrate selectivity observed indicate that a number of biochemically distinguishable activities are present.

MATERIALS AND METHODS

Insects

Larvae of *Trichoplusia ni* were reared on a 14 hr light–10 hr dark cycle at 27 C and fed diet 1 of Roe *et al.* (1982). Two hours after lights on, larvae in the third day of the fifth stadium were isolated for study. Insects of this age were easily discriminated on the basis of behavioural and morphological markers previously described (Jones *et al.*, 1981).

Enzyme preparation

Each experimental group consisted of 50 larvae. Fat bodies and midguts were dissected and homogenized in 4 ml, 0.25 M sucrose with 20 strokes of a Ten Broeck all-glass homogenizer (clearance 0.1–0.18 mm). Homogenates were centrifuged for 10 min at 10,000 *g*. The resulting pellets were discarded and the supernatants were centrifuged at 100,000 *g* for 60 min. Each microsomal pellet was resuspended in 4 ml 0.25 M sucrose. Unless otherwise stated, the resuspended microsomes and the cytosolic fractions were frozen at −70°C in 0.5 ml aliquots.

Enzyme and protein assays

Epoxide hydrolase and glutathione *S*-transferase activities were measured radiometrically using the procedures of Gill *et al.* (1983) for assays with *trans* and *cis*-stilbene oxides (TSO and CSO), and Mullin and Hammock (1980) for assays with *trans*-β-ethylstyrene oxide (TESO), with slight modifications (Wixtrom and Hammock, 1985). Enzyme aliquots were assayed before freezing and at various times after freeze–thaw. Enzyme aliquots (0–200 μl) were diluted in 1.5 ml cold buffer ($I = 0.2$), of varying pH. A typical reaction mixture contained 180 μl of enzyme solution (0–0.3 insect equivalents) and 20 μl of freshly dissolved GSH (5 mM final concentration in buffer). Reactions were carried out in 10 × 75 mm glass culture tubes. For EH assays, GSH was replaced by cold buffer. The mixtures were preincubated for 1 min at 30°C and the reactions were initiated by the addition of 2 μl of 5×10^{-3} M TSO or CSO (5×10^{-5} M final concentration), or 2 μl of 5×10^{-2} M TESO (5×10^{-4} M final concentration). These concentrations are just below the limit of solubility for these compounds. After 0–80 min, the reactions were terminated by the addition of 200 μl of either *n*-dodecane (for EH) or 1-hexanol (for GST) followed by vigorous vortexing and centrifugation (at *ca* 1500 rpm), in a table top, clinical centrifuge. Reaction products in 150 μl of the aqueous phase were quantitated in 3 ml of Aqueous Counting Scintillant (Amersham) by liquid scintillation counting in a LKB 1217 Rackbeta Scintillation Counter.

Lactate dehydrogenase (LDH; EC 1.1.1.27) activity was used as a cytosolic marker to determine the levels of cytosolic contamination of the microsomes. No corrections were made for possible contamination of the cytosolic or microsomal fractions by mitochondrial contents or membranes. The assay used was that of Bergmeyer and Bernj (1974). Protein concentrations were estimated by the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

RESULTS

Effect of freezing on enzyme activities

Epoxide hydrolase and glutathione *S*-transferase activities from each tissue and subcellular fraction were assayed immediately after thaw (time 0) and at 1, 3, 6 and 12 hr post-thaw when held at *ca* 0.5°C (i.e. in an ice bucket). The effects of these treatments were not significantly different among any of the activities assayed. Activities decreased less than 10% when kept

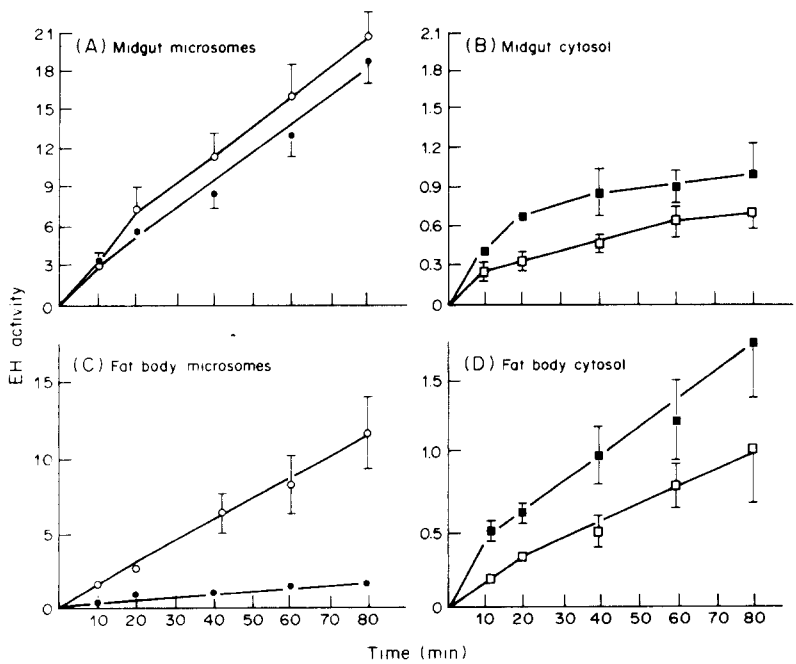

Fig. 1. Time dependence of EH activities using TSO (solid symbols) or CSO (open symbols) as substrate. Points represent mean activity at 30°C (nmol substrate hydrolyzed/mg protein) ± SE based on assays of five different homogenates with each assay run in triplicate. In all assays, 5×10^{-5} M (final concentration) stilbene oxides and sodium phosphate buffer ($I = 0.2$) of optimal pH for the respective enzyme were used.
Where standard error bars are absent, the error is smaller than the point indicated on the figure.

in ice for up to 6 hr. As much as 47% of the enzyme activities were lost between the 6 and 12 hr time points. There were no significant losses of activity following three freeze–thaw cycles.

Time dependence

The time courses of enzyme activities were determined to establish the optimal incubation time for assays. Activities were measured at 0, 10, 20, 40, 60 and 80 min after substrate was added. Enzymes were diluted 1:40 (v/v) in sodium phosphate buffer, pH 7.4 or 8.0 (*ca* 1.25 insect equivalents in 4 ml buffer). For all assays, resulting protein concentration was within the linear region of the corresponding protein/activity plot. Substrates were added ($t = 0$) and 200 μl aliquots were removed and vortexed in an equal amount of the appropriate solvent at the given times.

The time course of EH activity is shown in Fig. 1. In the midgut (Fig. 1A and B), activities with both TSO and CSO were 10 times greater in the microsomes than in the cytosol. In addition, differences in patterns of activity between the microsomal and cytosolic fractions were measured. In the midgut microsomes, there was only a slight, non-significant difference between the hydrolysis of TSO and CSO and activities were linear throughout the range of times tested. In the midgut cytosol however, TSO was hydrolyzed at a greater rate than CSO, and activities were linear with respect to time for a shorter period than the corresponding microsomal activities.

Similar differences in EH activities were measured in the fat body (Fig. 1C and D). In the microsomes of this tissue, TSO and CSO activities were linear from 0 to 80 min while rates of hydrolysis in the cytosol were slightly higher at short incubation times, and then apparently linear through 80 min. As in the midgut, fat body microsomal EH activity was greater with CSO than TSO while the reverse was seen in the cytosol of this tissue.

The time dependence of GST is shown in Fig. 2. No activity was measurable in the microsomes of the midgut with a limit of detection of 4 pmol conjugated/mg protein. In the midgut cytosol, similar levels of activity were observed using the two substrates. However, differences were observed between the conjugation of TSO and CSO with respect to time. In tests using TSO, linearity with time is lost between 20 and 40 min, while activity with CSO appeared linear throughout the range of times tested.

GST activities were measured from both microsomal and cytosolic fractions of the fat body (Fig. 2B). These activities were linear with time with the exception of the fat body cytosolic activity which began to curve between 20 and 40 min. In this tissue, differences in activity levels were observed for the two substrates: in the microsomes, no TSO activity was measured while in the cytosol, TSO activity was greater than that measured with CSO.

To ascertain the reason for the nonlinearity of some of these activities, the stabilities of the enzymes were examined following various pre-incubation times at 30°C. By 20 min, losses in cytosolic EH activity of the midgut were observed. By 60 min, over 50% of control (1 min pre-incubation) activity was eliminated. Similar results were obtained with the cytosolic GST activity from the fat body. At these

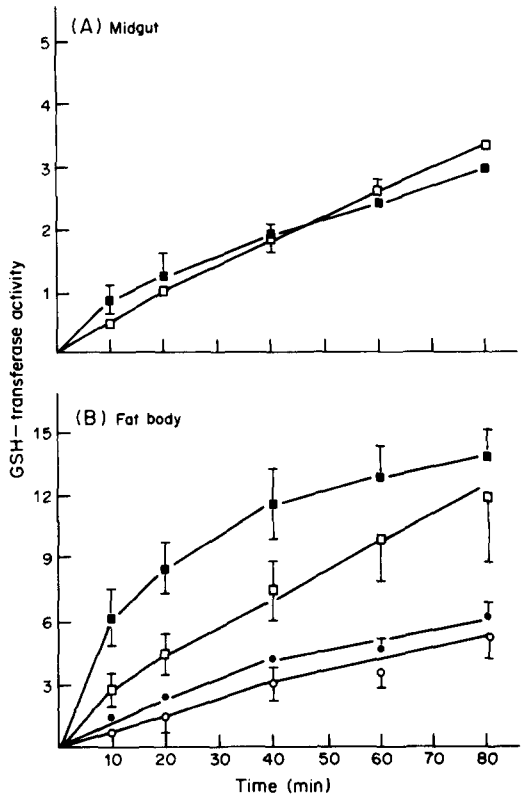

Fig. 2. Time dependence of GST activities using TSO (solid symbols) or CSO (open symbols) as substrate. Squares represent cytosolic activities while circles represent activities of microsomal fractions. All points represent mean activity (nmol substrate conjugated/mg protein) ± SE based on assays of five different homogenates with each assay run in triplicate. In all assays, freshly prepared GSH was used at 5 mM final concentration.

times, 80–85% of the substrate remained in the reaction mixture so it is unlikely that the distinct change in slope was due to substrate depletion. Hence, it appears that the loss of linearity in these particular tests is due to the instabilities of these activities at 30°C.

pH optimization

Buffers with pH 6–10 were used to determine the optimal pH for assay of activities, i.e. sodium phosphate, pH 6, 7.4 and 8.0; ammonia–ammonium chloride, pH 9 and 10. The ionic strength of the buffers was kept constant at 0.2.

The effect of pH on EH activity is shown in Fig. 3. In these tests, patterns of activity were the same for each determination. Thus, standard error bars represent variation within the patterns shown. In midgut microsomes, distinctively different curves were seen for TSO and CSO activities across the pH range tested. With TSO as substrate, a broad curve was observed with a peak at pH 7.4, while with CSO, a sharp peak at pH 8.0 was measured. In the midgut cytosol, activity toward both substrates was optimal at pH 8.

In tests with fat body preparations, pH/activity plots are broad and optima were observed at pH 8.0 for both tissues, both substrates and both subcellular

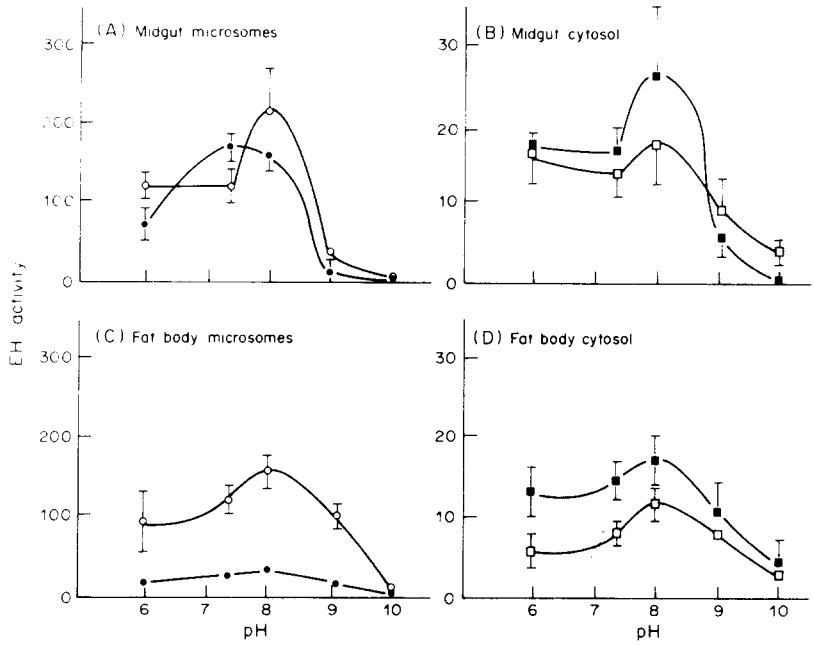

Fig. 3. Effect of pH on EH activities using TSO (solid symbols) or CSO (open symbols) as substrate. Each point represents mean activity (pmol substrate hydrolyzed/min per mg protein) ± SE based on assays of five different homogenates with each assay run for 10 min in triplicate.

fractions (Fig. 3C and D). Microsomal EH activity in this tissue was greater toward CSO than TSO while the corresponding cytosolic activities were similar.

pH optima for GST activities are shown in Fig. 4. It should be noted that high background levels at alkaline pH may limit what may be concluded in tests where GSH was used. At high pH, GSH is ionized and may non-enzymatically attack and open the epoxide ring. In addition, high pH accelerates the formation of glutathione disulfide (GS–SG) which cannot function as a substrate for the enzyme. The use of excess GSH in these assays reduced this concern. Background levels resulting from the non-enzymatic opening of the epoxide substrates were monitored at the incubation times and pH used for these assays and the results are presented in Fig. 5. In these tests, no differences were measured between boiled enzyme and buffer only. Under the assay conditions used and in the absence of GSH, background levels did not exceed 2%. In the presence of GSH, however, over 15% of the TSO added to the reaction was non-enzymatically conjugated at pH 8 after 80 min incubation (Fig. 5C). In addition, background levels exceed 40% after 10 min incubation at pH 10 (Fig. 5D). The corresponding background levels in tests with CSO under the same conditions remained below 10%. Thus, absence of activity toward TSO at pH 9 and 10 may be due to the depletion of both TSO and reduced GSH.

In the midgut, no GST activity is measureable in the microsomal fractions. In the cytosol, different patterns were measured for the conjugation of TSO and CSO. With TSO, optimal activity was observed at pH 7.4 and no activity was apparent at pH 9.0. In tests with CSO, optimal activity was measured at pH 8.0 and considerable levels of activity were seen at pH 9.0.

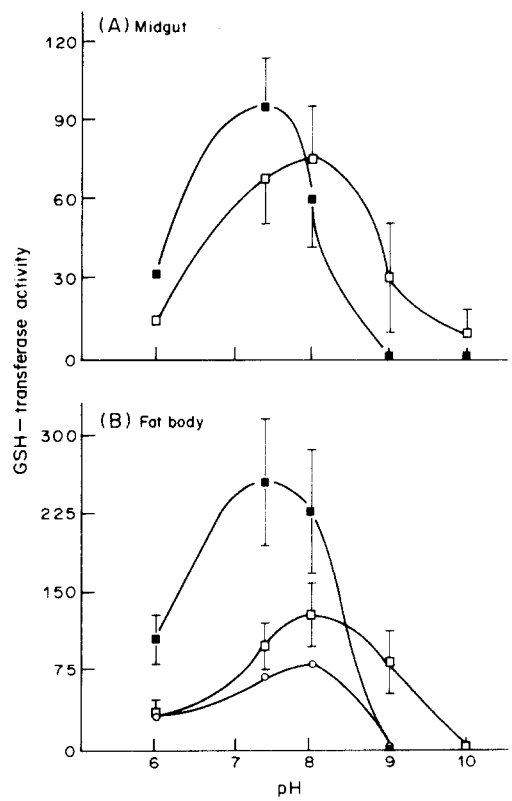

Fig. 4. Effect of pH on GST activities using TSO (solid symbols) and CSO (open symbols) as substrate. Each point represents mean activity (pmol substrate conjugated/min per mg protein) ± SE based on assays of five different homogenates with each assay run for 10 min in triplicate. Squares represent cytosolic activities while circles represent microsomal activities.

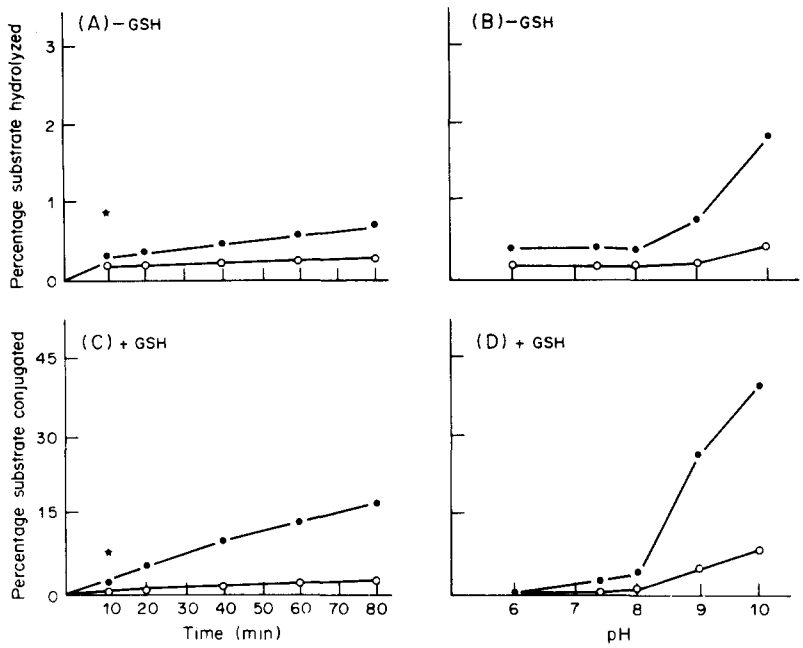

Fig. 5. Non-enzymatic hydrolysis (A, B) or conjugation (C, D) of TSO (solid symbols) and CSO (open symbols) as a function of incubation time (A, C) and pH (B, D). Time dependent experiments were performed at pH 8, while pH dependent studies were incubated for 10 min. Stars represent non-enzymatic hydration and conjugation of TESO at 10 min incubation time and pH = 8.0.

Fat body GST activity was measured in both microsomal and cytosolic fractions. In the microsomes, no activity was detectable with TSO. With CSO, the observed pH curve is broad with peak activity at pH 8.0. In the cytosol of this tissue, different patterns of activity were seen with TSO and CSO. In tests with TSO, optimal activity was measured at pH 7.4 and no activity was detectable at pH 9.0. In contrast, CSO activity was optimal at pH 8.0 and activity was apparent at pH 9.0.

Protein dependence of enzyme activities

The effect of protein concentration on enzyme activities was measured over the range of 0–60 μg microsomal, and 0–180 μg cytosolic protein. Optimal pH (7.4 or 8.0) and incubation time (10 min) were kept constant.

EH activities with both TSO and CSO were linear with protein concentration from 0 to 50 μg (microsomal) and 0 to 150 μg (cytosolic) protein. GST activities were also linear within these same ranges with the exception of the fat body cytosolic activity. In this case, both TSO and CSO activities began to curve between 60 and 90 μg protein.

The specific activities of EH and GST (measured as the slopes of regression lines calculated from protein/activity tests) are presented in Table 1. EH activity was greatest in the midgut microsomes where no substrate selectivity was observed. EH activity was also relatively high in the microsomes of the fat body but, in contrast to the midgut microsomes, clear selectivity toward CSO was observed. Comparable levels of activity and no substrate preference was observed in the cytosol from both tissues assayed.

GST activity was highest in the cytosol of the fat body. Relatively lower levels were also measured in the microsomes of this tissue using CSO as substrate while no activity was seen with TSO. In the midgut,

Table 1. Specific activities of epoxide metabolizing enzymes from tissue homogenates of *Trichoplusin. ni*

	Epoxide hydrolase*			GSH *S*-transferase*		
Tissue	TSO	CSO	TESO	TSO	CSO	TESO
Fat body						
Microsomes	28 ± 2	127 ± 22	97	ND†	80 ± 16	47
Cytosol	20 ± 5	17 ± 4	16	163 ± 7	125 ± 14	46
Midgut						
Microsomes	196 ± 39	185 ± 28	117	ND	ND	ND
Cytosol	24 ± 7	24 ± 6	15	68 ± 9	44 ± 13	52

*Enzyme activities (expressed as pmol metabolized/min per mg protein) ± SE were measured as the slopes of protein activity plots. For TSO and CSO, results represent at least five different enzyme homogenates prepared on different days. For TESO, the data represent triplicate assays of one homogenate at pH 8.0. Protein concentrations used in these tests were within optimal ranges for the respective enzymes, incubation time was 10 min and reaction pH were optimal. † Not detectable.

no microsomal activity toward either substrate was detectable while intermediate levels, and no substrate preferences were seen in the cytosol.

EH activity using TESO as substrate was similar to that measured with CSO (Table 1). In both fat body and midgut, activity was greatest in the microsomes. GST activity using TESO was low in the fat body when compared to levels of activity measured with TSO and CSO. In the midgut, activity was measured only in the cytosol.

No GST activity was measurable in the microsomes of either tissue tested when TSO was used as substrate. Because this activity is high in the cytosol, GST/TSO activity served as an internal marker for cytosolic contamination of microsomes. To accurately measure this contamination and insure that the microsomal GST activity measured from the fat body was not a result of cytosolic contamination during centrifugation, the microsomal fractions were assayed for LDH activity. In these tests, a 16% level of contamination was measured. Because the microsomal GST activity was 50% of that seen in the cytosol, this contamination cannot account for all the activity measured in the microsomes. In addition, activity remained in the microsomes after LDH contamination was eliminated by an additional wash (resuspension and 100,000 *g* centrifugation for 60 min).

DISCUSSION

We have optimized assay conditions for epoxide hydrolase and glutathione *S*-transferase activities from late fifth stadium *T. ni*. Insects of this age were chosen for study because they had been shown earlier to possess peak levels of EH (Mullin and Wilkinson, 1980; Wing *et al.*, 1981) and other detoxifying enzymes (Farnsworth *et al.*, 1981). In addition, this developmental age could be staged easily and precisely.

EH activities were measured in all tissues and subcellular fractions tested. These activities were predominantly microsomal with highest levels measured in the midgut. GST activities were highest in the cytosolic fraction of the fat body, but were also detected in the midgut cytosol and the fat body microsomes.

Differences in activity profiles both within and between the tissues assayed suggest the presence of more than one form of EH in *T. ni*. The different shapes of microsomal pH/activity curves for TSO and CSO indicate that at least two different microsomal forms are present in the midgut. Further, the presence of cytosolic activities which are labile at 30°C suggests differences in EH activities between these two subcellular fractions possibly resulting from the presence of different enzymes.

The substrate selectivity observed in the fat body microsomes indicates that EH activities present in this tissue are different from those measured in the midgut (where no such selectivity is observed), and may represent tissue-specific expression of EH activities. This selectivity is similar to activity patterns seen in mammals using these same two substrates (Gill *et al.*, 1983). As in the midgut, different activity profiles are observed between fat body microsomal and cytosolic fractions: no substrate selectivity is observed in the cytosol reflecting the presence of different forms of the enzyme in these two subcellular locales.

Similar observations were made indicating the presence of more than one GST activity. Different patterns of TSO and CSO activities were measured in pH/activity curves (between midgut and fat body), and time dependence plots (within the fat body), while differences in 30°C stabilities suggested different GST activities in the midgut and the fat body. A microsomal activity was measured in the fat body but not the midgut.

In general, activities of EH and GST using *trans* and *cis*-stilbene oxides are low relative to other substrates used previously in *T. ni* (Wing *et al.*, 1981) and other insects (Mullin and Wilkinson, 1980). However, TSO and CSO offer advantages as substrates in that they are easily made, they are relatively free of health hazards and they are less volatile compared to other, widely used substrates.

While kinetic data for the cytosolic GST indicate that the substrate concentrations used should saturate the enzyme, evidence for saturation of the cytosolic EH activity is less clear. Thus, these values should be taken as an indication of relative activities.

These data indicate that epoxide metabolism in insects is complex and probably involves multiple forms of GST and EH. We are currently attempting to corroborate these findings by differential induction and inhibition of these activities. Having distinguished these different enzymes activities, it may be possible to find biologically important substrates (e.g. lipid or dietary epoxides) and address the question of the intrinsic role of these enzymes in insects. It may also be useful to ask which, if any, of these enzyme activities are important in insecticide resistance.

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REFERENCES

Bergmeyer H. V. and Bernt E. (1974) Lactate dehydrogenase—UV assay with pyruvate and NADH. *Meth. Enzymat. Analysis* **2,** 574–579.

Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72,** 248–254.

Brooks G. T., Harrison A. and Lewis S. E. (1970) Cyclodiene epoxide ring hydration by microsomes from mammalian liver and houseflies. *Biochem. Pharmac.* **19,** 255–273.

Chasseaud L. F. (1979) The role of glutathione and glutathione *S*-transferase in the metabolism of chemical carcinogens and other electrophilic agents. *Adv. Cancer Res.* **29,** 175–274.

Farnsworth D. E., Berry R. E., Yu S. J. and Terriere L. C. (1981) Aldrin epoxidase activity and cytochrome *P*-450 content of microsomes prepared from alfalfa and cabbage looper larvae fed various plant diets. *Pestic. Biochem. Physiol.* **15,** 158–165.

Gill S. S., Ota K. and Hammock B. D. (1983) Radiometric assays for mammalian epoxide hydrolases and glutathione *S*-transferase. *Analyt. Biochem.* **131,** 273–282.

Hällström I., Sundvall A., Rannug U., Graftström R. and Ramel C. (1981) The metabolism of drugs and carcinogens in isolated subcellular fractions of *Drosophila melanogaster*, I. Activation of vinyl chloride, 2-aminoanthracene, and benzo(a)pyrene as measured by mutagenic effects in *Salmonella tymphimurium*. *Chem-Biol. Interact.* **34,** 120–143.

Hammock B. D. (1985) Regulation of juvenile hormone titer: degradation. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (Edited by Kerkut G. A. and Gilbert L. I.), Vol. 7, pp. 431–472. Pergamon Press, New York..

Jones D., Jones G. and Hammock B. D. (1981) Growth parameters associated with endocrine events in larval *Trichoplusia ni* (Hubner) and timing of these events with developmental markers. *J. Insect Physiol.* **27,** 779–788.

Lu A. Y. H. and Miwa G. T. (1980) Molecular properties and biological functions of microsomal epoxide hydrase. *A. Rev. Pharmac. Toxic.* **20,** 513–531.

Miller J. A. and Miller E. C. (1977) Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (Edited by Hiatt H. H., Watson J. D. and Winsten J. A.), pp. 605–627. Cold Spring Harbor, New York.

Motoyama N. and Dauterman W. C. (1980) Glutathione *S*-transferases: their role in the metabolism of organophosphorus insecticides. *Rev. Biochem. Toxic.* **2,** 49–69.

Mullin C. A. (1985) Detoxification enzyme relationships in arthropods of differing feeding strategies. In *New Concepts and Trends in Pesticide Chemistry* (Edited by Hedin, P. A. pp. 267–278. American Chemical Society, Washington D.C.

Mullin C. A. and Hammock B. D. (1980) A rapid radiometric assay for mammalian cytosolic epoxide hydrolase. *Analyt. Biochem.* **106,** 476–485.

Mullin C. A. and Wilkinson C. F. (1980) Purification of an epoxide hydrolase from the midgut of the southern armyworm (*Spodoptera eridania*). *Insect Biochem.* **10,** 681–691.

Oesch F. (1973) Mammalian epoxide hydrases: inducible enzymes catalysing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. *Xenobiotica* **3,** 305–340.

Roe R. M., Hammond A. M. and Sparks T. C. (1982) Growth of larval *Diatraea sacharalis* (Lepidoptera: Pyralidae) on an artificial diet and synchronization of the last larval stadium. *Ann. ent. Soc. Am.* **75,** 421–429.

Sims P., Grover P. L., Swaisland A., Pal K. and Hewer A. (1974) Metabolic activation of benzo(a)pyrene proceeds by a diol-epoxide. *Nature* **252,** 326–328.

Slade M. and Zibitt C. H. (1972) Metabolism of *Cecropia* juvenile hormone in insects and mammals. In *Insect Juvenile Hormones: Chemistry and Action* (Edited by Menn J. J. and Beroza M.), pp. 155–176. Academic Press, New York.

Tazima Y. (1980) Chemical mutagenesis in the silkworm. In *Chemical Mutagens* (Edited by Serres F. J. de and Hollaender A.), pp. 203–238. Plenum Press, New York.

Vogel E., Blijleven W. G. H., Klapwijk P. M. and Zijlstra J. A. (1980) Some current perspectives of the application of *Drosophila* in the evaluation of carcinogens. In *The Predictive Value of Short-Term Screening Tests in Carcinogenicity Evaluation* (Edited by Williams G. M.), pp. 125–147. Elsevier/North Holland Biomedical Press, Amsterdam.

Wing K. D., Sparks T. C., Lovell V. M., Levinson S. O. and Hammock B. D. (1981) The distribution of juvenile hormone esterase and its interrelationship with other proteins influencing juvenile hormone metabolism in the cabbage looper, *Trichoplusia ni*. *Insect Biochem.* **11,** 473–485.

Wixtrom R. N. and Hammock B. D. (1985) Membrane-bound and soluble-fraction epoxide hydrolases: methodological aspects. In *Biochemical Pharmacology and Toxicology* Vol. 1: *Methodological Aspects of Drug Metabolizing Enzymes* (Edited by Zakim D. and Vessey D. A.), pp. 1–93. Wiley, New York.

Yang R. S. H., Hodgson E. and Dauterman W. C. (1971) Metabolism *in vitro* of diazinon and diazoxon in susceptible and resistant houseflies. *J. agric. Fd Chem.* **19,** 14–19.