CYCASIN SYNTHESIS IN SEIRARCTIA ECHO (LEPIDOPTERA) LARVAE FED METHYLAZOXYMETHANOL

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Cycasin is a naturally occurring glucoside that has been isolated from plants of the genus Cycas (Nishida et al., 1955; Matsumoto and Strong, 1963). Cycasin, methylazoxymethanol-beta-D-glucoside, or its aglycone, methylazoxymethanol (MAM), has been shown to be carcinogenic, hepatotoxic (Laqueur, 1965; Laqueur and Matsumoto, 1966), mutagenic (Smith, 1966; Teas and Dyson, 1967), and radiomimetic (Teas et al., 1965), and an effective in vitro alkylating agent (Matsumoto and Higa, 1966).

The use of cycad plants as food by Seirarctia echo larvae and the accumulation within the insects of material giving an azoxyglycoside reaction has been reported (Teas et al., 1966). The occurrence of beta-glucosidase activity in homogenates of cycad-fed whole larvae raised the question of how larvae are able to avoid MAM intoxication from the combination of beta-glucoside and beta-glucosidase that they contain. The present communication reports the synthesis and accumulation of cycasin by S. echo larvae raised on synthetic diet fortified with MAM, and the concentration of beta-glucoside and beta-glucosidase in different organs or structures within the larval body.

METHODS

S. echo larvae were hatched from eggs laid in the laboratory. The parents had been collected in nature as caterpillars feeding on cycad plants

at Fairchild Tropical Garden, Miami, Florida. The artificial diet was a modified Vanderzant medium, available commercially, that contains agar, wheat germ, vitamins, minerals, and antibiotics. Approximately three-fourths grown larvae that had been fed artificial diet from the time of hatching (and whose sibs were negative for azoxyglycoside by the chromotropic acid test) were starved for 24 hours and then fed synthetic diet to which MAM had been added. After one day of feeding on the MAM diet, ethanol extracts of the larvae were chromatographed on silica gel thin layer plates, irrigated with butanol:acetic acid:water (4:1:1), and developed using a chromotropic acid reagent (Matsumoto and Strong, 1963) as a spray and heated at 105-110° C for 10-15 minutes. Carbohydrates were chromatographed on Whatman No. 1 paper using the same solvent and a silver nitrate developer (Block, et al., 1958). Evidence for beta-glucosidase was obtained by demonstration of an MAM spot on thin layer plates following incubation of extracts with cycasin. Beta-glucosidase estimations were carried out using the synthetic substrate p-nitrophenyl-beta-D-glucoside. Homogenates of tissues were incubated with the beta-glucoside substrate in 0.1 M triethanolamine buffer at pH 7. Released p-nitrophenol was determined at pH 8.5 by means of the absorption at 395 mμ. The beta-glucosidase used was almond emulsin. MAM was supplied by Dr. G. L. Laqueur or prepared from cycasin (Kobayashi and Matsumoto, 1965).

RESULTS AND DISCUSSION

Ethanol extracts of caterpillars fed synthetic diet containing MAM for 24 hours were found by chromatography to contain a material that migrated and colored as cycasin. The unconsumed synthetic diet and the feces showed no cycasin spot. Almond emulsin hydrolysis of a dried sample of caterpillar extract resulted in disappearance of the cycasin spot (R_f .42) and appearance of an MAM spot (R_f .70) on thin layer plates and a glucose spot (R_f .20) on paper. In as much as whole caterpillar homogenates showed strong beta-glucosidase activity with cycasin or p-nitrophenylglucoside as substrates, it

was decided to determine the distribution of enzyme and cycasin in dissected caterpillars. Ether anaesthetized larvae were dissected and the following fractions obtained: gut (entire, opened and rinsed in water); hemolymph; malpighian tubules; fat body; and body wall, including skin and musculature. These fractions were freed of contaminating organs, fluids, or tissues by rinsing and dissection. Other structures such as tracheae and gonads were not assayed. Azoxyglycoside levels were determined on pooled dissected fractions of larvae that had been fed from hatching on leaves of Zamia floridana, a native Florida cycad. The level of beta-glucosidase was determined in homogenized pooled fractions of larvae reared on artificial diet, in order to obviate the possibility of beta-glucosidase induction by azoxyglycoside in the food. Enzyme levels and cycasin content are tabulated in Table I.

TABLE I

beta-Glucosidase and cycasin content of S. echo larval fractions

Structure or fraction	Relative beta-glucosidase content	Cycasin, mg/gm, dry wt. basis
Gut	++++	20
Hemolymph	-	353
Malpighian tubules	-	158
Fat body	-	74
Body wall	$\pm$	9

Gut was the only portion that showed a strong beta-glucosidase activity. Although the gut was opened and repeatedly rinsed, the possibility that the observed reaction was due to the activity of microorganisms cannot be excluded; however, the penicillin and streptomycin content of the medium and the relatively brief transit time of food in the insect gut reduce this likelihood.

The questionable reaction in the body wall probably results from the yellow pigment of the skin that was extracted during incubation. The highest amount of azoxyglycoside was found in the hemolymph; the second highest concentration was in the malpighian tubules. It may be that cycasin concentration found in the malpighian tubules was high because cycasin is excreted by this organ.

It is interesting here to note that although the feces of larvae that had fed on Zamia or Cycas leaves contained very low levels of azoxyglycosides, and the larvae accumulated azoxyglycoside, the only azoxyglycoside found on chromatography of larval extracts had the R_f and color reaction of cycasin. This was the case even though the leaf material on which the larvae had fed in the case of Zamia contains a prominent azoxyglycoside component having a lower R_f than that of cycasin.

It seems likely that the S. echo larvae are protected from what must be injurious effects of a potent alkylating agent, MAM, by converting it into the beta-glucoside, cycasin. In view of the single chromatographic spot in extracts of Zamia-fed caterpillars, it is probable that azoxyglycosides are hydrolyzed in the gut and resynthesized into the relatively non-toxic cycasin which is accumulated in non-beta-glucosidase-containing parts of the insect. In view of the common finding that most beta-glucosidases are functionally hydrolases, although some are not (Cardini and Yamaha, 1958), it will be interesting to investigate the activity and distribution of beta-glucosidase within regions of the S. echo larval gut.

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REFERENCES

Block, R. J., Durrum, E. L. and Zweig, G., Paper Chromatography and Paper Electrophoresis. Academic Press, New York (1958), p. 178.

Cardini, C. E. and Yamaha, T., Nature, 182, 1446 (1958).

Kobayashi, A., and Matsumoto, H., Arch. Biochem. Biophys., 110, 373 (1965).

Laqueur, G. L. and Matsumoto, H., J. Natl. Cancer Inst., 37, 217 (1966).

Matsumoto, H. and Higa, H. H., Biochem J., 98, 20c (1966).

Matsumoto, H. and Strong, F. M., Arch. Biochem. Biophys., 101, 299 (1963).

Nishida, K., Kobayashi, A., and Nagahama, T., Bull. Agr. Chem. Soc. Japan, 19, 77 (1955).

Smith, D. S. E., Science, 152, 1273 (1966).

Teas, H. J. and Dyson, J. G., submitted for publication (1967).

Teas, H. J., Dyson, J. G., and Whisenant, B. R., J. Ga. Entomol. Soc., 1, 21 (1966).

Teas, H. J., Sax, H. J., and Sax, K., Science, 149, 541 (1965).