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DETOXICATION MECHANISMS IN PHYTOPHAGOUS INSECTS: ROLE OF GLUTATHIONE TRANSFERASES AND β-GLUCOSIDASES

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INTRODUCTION

Glutathione transferases are a group of detoxication enzymes which catalyze the conjugation of reduced glutathione (GSH) with various xenobiotics possessing an electrophilic center (Chasseaud, 1979). The reaction is the first step in the formation of mercapturic acids, a pathway through which lipophilic xenobiotics are metabolized and eliminated from the animal body. These transferases are important in the phase I metabolism of organophosphorus insecticides and DDT and are believed to play an important role in the resistance of insects to these insecticides (Motoyama and Dauterman, 1980; Clark and Shamaan, 1984). Our recent work also shows that glutathione transferases are involved in the metabolism of toxic allelochemicals in phytophagous insects (Wadleigh and Yu, 1987, 1988a, b)). The fact that the specialist insects possessed low glutathione transferase activities toward the allelochemicals as compared with the generalist insects suggests that these enzymes play an important role in host plant selections.

Glycosidases are another group of detoxication enzymes which catalyze the hydrolysis of glycosidic linkages in glycosides. A glycoside consists of a glycone (sugar moiety) and an aglycone (nonsugar moiety), the most common glycone being glucose in plants. Nearly all naturally occurring plant glycosides are β-linked *O*-glycosyl compounds and therefore β-glycosidases are very important in the metabolism of plant glycosides. β-Glycosidases are commonly measured with artificial substrates such as *p*-nitrophenyl β-D-glucoside and 4-methylumbelliferyl β-D-glucoside. Although insect β-glycosidases are capable of hydrolyzing glycosides to release carbohydrates, allelochemical aglycones are generally toxic to insects. Therefore, β-glycosidases in insects may work to their disadvantages when they ingest plant glycosides. Very little information is presently available regarding the hydrolysis of plant glycosides by β-glycosides in phytophagous insects.

This paper concerns the characterization of glutathione transferases and β-glucosidases in the fall armyworm, *Spodoptera frugiperda* (FAW), corn earworm, *Heliothis zea* (CEW), tobacco budworm, *Heliothis virescens* (TBW), cabbage looper, *Trichoplusia ni* (CL), and velvetbean caterpillar, *Anticarsia gemmatalis* (VBC).

GLUTATHIONE TRANSFERASES

Purification and characterization of glutathione transferases from insects

Glutathione transferases from several species of insects have been purified to apparent homogeneity. Earlier work involved conventional column chromatographic methods utilizing ion exchangers, gel filtration and hydroxyapatite. Using these methods, a glutathione transferase was partially purified from an insecticide resistant and a susceptible strain of house flies. The enzyme had a molecular weight of 50,000 with two equal subunits of 23,000. The optimum pH for enzyme activity toward 3,4-dichloronitrobenzene (DCNB) was 9.5 to 10. The same enzyme catalyzed the conjugation of methyl iodide, organophosphorus insecticides, Γ-BHC, but not DDT (Motoyama and Dauterman, 1977).

Yawetz and Koren (1984) also used these techniques to purified a glutathione transferase from the Mediterranean fruitfly (*Ceratitis capitata*) to apparent homogeneity. The transferase had an isoelectric point (pI) near pH 5.7 and a molecular weight of 43,000. The protein was composed of two similar subunits of 22,000 and 21,000 molecular weight. The enzyme catalyzed the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB), methyl iodide, styrene oxide and parathion.

Five glutathione transferase isozymes which catalyzed the conjugation of GSH with organophosphorus insecticides were also separated from American cockroaches. Two of these transferases were active on diazinon and three of these were active on methyl parathion. The molecular weights of these transferases were estimated to be 35,000-37,000 (Usi *et al.*, 1977).

However, the purification work was found to be tedious and ineffective until affinity column chromatography became available. In 1977, Clark *et al.* were first to use affinity chromatography on glutathione-sulphobromophthalein-agarose to purify the enzyme. Because of its selective ability to bind with GSH transferase, this enzyme was isolated from greater waxmoth larvae in substantially pure form. The transferase had a molecular weight of 41,000 with two identical subunits of 25,000 molecular weight. Its substrate specificity was found to resemble that of the glutathione transferase B from rat liver (Chang *et al.*, 1981).

Clark *et al.* (1984) examined the multiplicity of glutathione transferase in three house fly strains using affinity chromatography on GSH-sulphobromophthalein-agarose, followed by preparative isoelectrofocusing. Each strain was

found to possess at least three functionally different glutathione transferase isozymes with subunits of M_r 20,000, 22,000 and 23,000. These isozymes fell into two clearly defined groups. The first group, which had relatively low isoelectric point, showed activity toward CDNB but little toward DCNB, *p*-nitrobenzylchloride, or 1,2-epoxy-3-(*p*-nitrophenoxy) propane. The second group which had higher isoelectric points, showed higher activity toward all substrates tested.

DDT-dehydrochlorinase from house flies was purified by a procedure involving affinity chromatography, gel filtration and preparative isoelectrofocusing. The enzyme was found to be a glutathione transferase (Clark and Shamaan, 1984).

Using ammonium sulfate fractionation, affinity chromatography, anion exchange chromatography and chromatography on hydroxyapatite, Clark *et al.* (1985) purified two glutathione transferase isozymes having pI 5.9 and 8.3 from the New Zealand grass grub (*Costelytra zealandica*). They were homodimers with subunits of M_r 22,000 and 23,000, respectively.

Glutathione transferase from *Drosophila melanogaster* was purified to apparent homogeneity by means of affinity chromatography on a glutathione-agarose column. The transferase is a dimer with subunits of M_r 35,000 and 28,500. The enzyme possessed many of their multiple functions as found in mammalian ones including bilirubin and heme binding capacities and glutathione peroxidase activity (Cochrane *et al.*, 1987).

The *Tribolium castaneum* glutathione transferase was purified by a three-step procedure involving ammonium sulfate fractionation, gel filtration and affinity chromatography (Cohen, 1987). The K_m values for the conjugating activity of the transferase were 3.6×10^{-4} M for GSH and 2.76×10^{-5} M for CDNB. The enzyme had subunits of M_r 24,000 and 26,000.

More recently, Grant and Matsumura (1988) purified glutathione transferase from *Aedes aegypti* larvae using *S*-hexyl-glutathione affinity chromatography and anion exchange chromatography. According to these authors, the transferase is composed of two subunits, each with a molecular weight of 26,800, and has an isoelectric point of 5,0.

It is clear from the brief review above that insect glutathione transferase is active in numerous insect species and may exist in multiple forms. The enzyme is adimer with two subunits of M_r 20,000-35,000. However, no work has been done regarding the purification and characterization of glutathione transferases from lepidopterous insects. Since glutathione transferases are involved in the metabolism of plant allelochemicals (Wadleigh and Yu, 1987, 1988a, b), it would be very important to learn the biochemical characteristics of the transferases and whether the enzymes are different qualitatively among phytophagous insects with varying degrees of polyphagy and feeding speccialization.

Multiple glutathione transferases in phytophagous insects

Evidence accumulated so far suggests that glutathione transferase exists in multiple forms in lepidopterous insects. For example, selective induction of glutathione transferase activities toward α,β-unsaturated carbonyl compounds, isothiocyanates and thiocyanate in fall armyworm larvae by indole 3-acetonitrile clearly indicates the multiplicity of glutathione transferase (Wadleigh and Yu, 1987, 1988a, b). Furthermore, the ratio of activity toward DCNB and CDNB was different in the FAW, CEW, TBW and VBC, suggesting that glutathione transferases are different among these lepidopterous species (Yu, 1987).

We have recently purified and characterized glutathione transferases from five species of Lepidoptera with differing degrees of polyphagy. The FAW and CEW are highly polyphagous insects. The TBW and CL are also polyphagous insects but are more specialized. The former mainly feeds on tobacco and cotton, while the latter preferentially feeds on cabbage family. The VBC is a specialist insect feeding mainly on soybeans.

We have found that glutathione transferase can be purified from these

Table 1

Purification of glutathione transferases from five lepidopterous insects[b]

Species	Purification step	Yield (%)	Specific activity (μmol CDNB/min/mg protein)[a]	Purification factor
FAW	Cytosolic fraction	100	0.47±0.06	1.0
	Ammonium sulfate 45-75%	69	1.05±0.13	2.2
	GSH-agarose	12	10.37±1.49	22.1
CEW	Cytosolic fraction	100	1.30±0.04	1.0
	Ammonium sulfate	80	1.93±0.08	1.5
	GSH-agarose	8	58.35±2.29	44.9
TBW	Cytosolic fraction	100	0.47±0.10	1.0
	Ammonium sulfate	62	1.41±0.09	3.0
	GSH-agarose	10	17.50±3.72	37.2
CL	Cytosolic fraction	100	1.35±0.27	1.0
	Ammonium sulfate	69	2.73±0.47	2.0
	GSH-agarose	3	12.16±1.86	9.0
VBC	Cytosolic fraction	100	0.07±0.01	1.0
	Ammonium sulfate	54	0.11±0.07	1.6
	GSH-agarose	26	2.29±0.33	32.7

[a] Mean ± of four experiments, each with duplicate determinations.
[b] Data from Yu (1989a).

phytophagous insects to apparent homogeneity using a two-step procedure involving ammonium sulfate fractionation and affinity chromatography on a glutathione-agarose column (Yu, 1988a). Data in Table 1 show that the purifications were 22, 45, 37, 9 and 33-fold in the FAW, CEW, TBW, CL and VBC, respectively, as compared with the specific activity in the respective soluble fraction. The specific activity of the purified glutathione transferases ranged from 2.29 μmol/min/mg protein in the VBC to 58.35 μmol/min/mg protein in the CEW, a difference of 25-fold in enzyme activity.

The biochemical characteristics of purified glutathione transferases from the five lepidopterous species are shown in Table 2. Analysis of the affinity purified preparations by polyacrylamide gel electrophoresis (PAGE) under nondenaturing conditions revealed the presence of 6, 4, 1, 1, and 1 protein species in the FAW, CEW, TBW, CL and VBC, respectively. These protein species were regarded as glutathione transferase isozmes since each band exhibited glutathione transferase

Table 2
Biochemical characteristics of purified glutathione transferases from five lepidopterous insects[a]

Species	K_m (mM)	V_{max} (μmol/min/mg protein)	Inhibition by quercetin (% of control)[b] 10^{-4} M	10^{-5} M	Subunit M_r	Isozyme (R_f)	pI
FAW	1.33	17.2	23	71	30,000	0.34	6.0
					29,500	0.24	5.10
					29,000	0.46	5.00
					28,200	0.51	4.75
						0.55	4.55
						0.59	4.45
CEW	0.40	50.0	0	12	32,000	0.38	5.10
					29,000	0.42	5.05
					27,500	0.46	4.90
						0.54	4.75
TBW	0.40	50.0	0	0	32,000	0.47	5.45
					29,000		
					27,500		
CL	0.56	12.5	22	46	31,000	0.53	5.10
					29,000		
VBC	9.09	18.2	100	100	31,000	0.44	4.85
					29,000		

[a] Affinity purified preparation from each species was used as enzyme source toward CDNB. Data from Yu (1989a).
[b] Mean of 2-3 experiments.

transferase activity toward CDNB. When the preparations were analyzed by isoelectric focusing in polyacrylamide gels, the same number of protein bands as found in PAGE was also observed in each species. The pI values ranged from 4.45 to 6.00 among the five species, indicating that these transferases were all acidic isozymes. SDS-PAGE of the purified enzymes showed 4, 3, 3, 2 and 2 protein bands in the FAW, CEW, TBW, CL and VBC, respectively. The subunit molecular weights ranged from 27,500 to 32,000, with the subunit (M_r 29,000) being found in each species.

Kinetic studies also showed that the purified glutathione transferases were different qualitatively among these insects based on their K_m values with the exception of the CEW and TBW in which both had the same K_m value. That glutathione transferases are different qualitatively in the five species was also supported by the results obtained from the enzyme inhibition study. As shown in Table 2, the TBW glutathione transferase was most sensitive to inhibition by quercetin, followed by CEW, CL, FAW and VBC.

Effect of enzyme induction on isozyme composition

Plant and plant allelochemicals have been found to induce glutathione transferases in several phytophagous insects (Yu, 1986; Riskallah *et al.* 1986; Brattsten, 1987). It would be important to learn whether enzyme induction causes production of new isozyme(s) of glutathione transferase in these insects. Our results showed that dietary xanthotoxin at 1% caused increases of 20 and 2-fold in glutathione transferase activities toward DCNB and CDNB, respectively, in FAW larvae (Yu, 1989a). However, the induction resulted in increased levels of the existing isozymes but did not produced any new form of glutathione transferase isozymes. The results obtained from SDS-PAGE also showed that there was no difference in subunit composition of purified glutathione transferases from control and xanthotoxin-induced FAW larvae. Kinetic studies also revealed that these two groups of glutathione transferases did not differ qualitatively based on their K_m and V_{max} values. The results are consistent with our previous finding showing that cowpea-induced FAW larvae had the same K_m value for glutathione transferase compared with the soybean-fed larvae served as control (Yu, 1982). In agreement with those observations, dietary phenobarbital which induced glutathione transferase did not change apparent K_m value for glutathions transferase (DCNB) in house fiies (Hayaoka and Dauterman, 1982) and Australian sheep blow flies (Kotze and Rose, 1987). In contrast, Ottea and Plapp (1984) found significant changes in K_m value for glutathione transferase on induction with phenobarbital in house flies. Biochemical differences in house fly strains used may have attributed to the observed discrepancy.

Role of glutathione transferases in insect herbivory

Our recent work shows that glutathione transferases are involved in the metabolism of toxic allelochemicals in phytophagous insects (wadleigh and Yu, 1987, 1988a, b). The allelochemical *trans*-cinnamaldehyde, *trans*-2-hexenal, *trans*, *trans*-2, 4-decadienal and benzaldehyde, all of which contain the α,β-unsaturated carbonyl moiety, were metabolized by glutathione transferase from midgut homogenates of FAW. Isothiocyanates such as allyl, phenyl and benzyl isothiocyanates such as benzyl thiocyanate were also found to be metabolized by glutathione transferase from larvae of the FAW, CL and VBC. Furthermore, numerous allelochemicals induced glutathione transferase activity which is presumed to enhance the detoxication of allelochemicals. In addition, these transferase activities were lower in the specialist VBC than in the generalists, FAW and CL. These results seem to support the notion that glutathione transferases play an important role in the feeding strategies of phytophagous insects. The highly polyphagous insects may have evolved multiple glutathione transferases to detoxify the diverse toxic allelochemicals encountered in their host plants. On the other hand, specialized insects which feed on a narrow range of host plants and encountered more specific allelochemicals, have as few as one form of glutathione transferase. Therefore, the isozyme composition of glutathions transferase in phytophagous insects is related to host plant feeding. More work is needed to learn the substrate specificities of these isozymes before one can fully understand the molecular methanisms of glutathione-dependent detoxication in these phytophagous insects.

β-GLUCOSIDASES

Characteristics of β-glucosidases in phytophagous insects

We have recently characterized β-glucosidase in the fall armyworm using helicin as a model substrate and the midgut soluble fraction as enzyme source (Yu, 1989b). The pH optimum was determined with sodium phosphate buffer (0.1 M) over the pH range 5.7-8.0. The activity was highest at pH 6.9. Enzyme activity was linear with tissue level up to 1.6 mg protein/incubate; it increased with incubation time up to 75 min. Activity was inhibited by the known β-glucosidase inhibitors, castanosphermine and D-gluconic acid lactone, showing I_{50} values of 3.08×10^{-6} and 5.62×10^{-4} M, respectively. The β-glucosidase activity had the apparent K_m value of 0.63 mM and V_{max} value of 17.39 nmol/min/mg protein. Of the midgut subcellular fractions tested, the soluble fraction contained the highest β-glucosidase activity. Enzyme activity was found in various tissues and was mainly located in the midgut. No activity was found in the fat body. It was also observed that

using the midgut crude homogenates as enzyme source, the β-glucosidase activity in the fourth and fifth instars was only one half and two thirds in the sixth instar, respectively, showing a gradual increase in enzyme activity as larvae matured.

The stability of helicin β-glucosidase was investigated by periodic assays of the soluble fraction stored at −10°C. It was found that enzyme activity declined gradually during the first four weeks and then remained approximately the same, showing about 80% of the initial activity by the 56th day.

Data in Table 3 show that the fall armyworm β-glucosidase system also hydrolyzed a variety of glucosides including toxic plant allelochemicals and artificial substrates. The activity toward these glucosides in this insects varied considerably ranging from 0.43 to 7.70 nmol/min/mg protein. Among those glucosides studied, helicin, D-amygdalin and D(+)-cellobiose were the preferred

Table 3
β-Glucosidase activities toward various glucosides in four species of Lepidoptera[c]

Substrate (3.5 mM)	β-Glucosidase (nmol glucose/min/mg protein)[a]			
	VBC	CL	FAW	CEW
Salicin	3.79±0.29	2.52±0.01	1.53±0.05	2.68±0.24
Helicin	5.09±0.26	12.93±0.32	7.70±0.29	4.10±0.24
Phloridzin	0.41±0.05	1.12±0.15	0.43±0.02	5.90±0.08
Arbutin[b]	2.59±0.33	3.73±0.13	2.02±0.14	1.89±0.09
D-Amygdalin	9.62±0.12	3.03±0.22	5.48±0.16	10.60±0.58
Prunasin	4.54±0.08	4.36±0.07	1.48±0.04	2.29±0.02
Linamarin	0.65±0.04	1.40±0.03	1.89±0.09	1.29±0.01
α-Solanine	0.49±0.00	1.63±0.03	0.63±0.04	2.63±0.20
Tomatine	0.61±0.09	1.99±0.01	1.74±0.36	2.09±0.50
Sinigrin	1.78±0.26	1.54±0.04	0.48±0.02	2.02±0.33
D(+)-cellobiose	6.67±0.60	3.82±0.33	6.95±0.27	3.43±0.49
Indoxyl β-D-glucoside	4.68±0.09	5.60±0.35	3.78±0.15	3.09±0.25
p-Nitrophenyl β-D-glucoside[b]	11.22±0.18	4.87±0.08	3.30±0.02	3.87±0.03
4-methylumbelliferyl β-D-glucoside	4.64±0.08	8.27±0.39	4.34±0.44	5.20±0.39
Methyl β-D-glucoside	1.33±0.26	2.12±0.27	1.35±0.23	2.08±0.21

[a] Midgut crude homogenates prepared from 2-day-odl final instar larvae were used as enzyme source. Mean±SE of two experiments, each with duplicate determinations.
[b] nmol aglycone/min/mg protein.
[c] Data from Yu (1989b).

substrates, whereas phloridzin, sinigrin and α-solanine were poor substrates for the enzyme. The results also show that β-glucosidase from the VBC, CL and CEW was active toward various glucosides. However, the total activity appeared to be higher in the CL and VBC than in the FAW and CEW, indicating that there was no correlation between the degree of herbivore polyphagy and β-glucosidase activity among these four species. Moreover, the substrate specificities toward

Table 4

Effect of allelochemicals in larval diet on development of fall aryworm[b]

Treatment[a]		Concn in	% of control	
Glucoside	Aglycone	diet (%)	Larval weight after 12 days	Adult emergence
Arbutin		0.5	48	100
		1.0	42	100
	Hydroquinone	0.5	5	60
		1.0	0	0
Salicin		0.5	82	100
		1.0	75	100
	Saligenin	0.5	70	100
		1.0	33	50
Rutin		0.5	16	72
		1.0	8	50
	Quercetin	0.5	2	0
		1.0	1	0
Esculin		0.5	85	80
		1.0	79	90
	Esculetin	0.5	60	100
		1.0	6	10
Sinigrin		0.01	66	100
		0.05	100	100
	Allyl isothiocynate	0.01	30	70
		0.05	0	0
Amygdalin		0.5	90	90
		1.0	93	90
	Mandelonitrile	0.5	0	0
		1.0	0	0
Tomatine		0.05	48	100
		0.1	2	0
	Tomatidine	0.05	100	100
		0.1	74	100

[a] First-instar larvae were fed artificial diets containing the allelochemicals.

[b] Data from Yu (1989b).

these glucosides were different among these insects.

Table 4 summarizes the results of the bioassays of various glucosides and their aglycones with fall armyworm. With the exception of tomatine/tomatidine, all the glucosides were less toxic than their corresponding aglycones to the fall armyworm when first-instar larvae were fed artificial diets containing the compounds. Among those studied, the aglycone allyl isothiocyanate appeared to be most toxic to the insects.

Role of insect β-glucosidases in host plant resistance

The study of β-glucosidase in phytophagous insects is important not only in understanding the biochemistry of the insects but also in developing new pest management strategies. Plants appear to produce a wide variety of allelochemicals as defensive weapons. These include alkaloids, cyanogenic and triterpenoid glycosides, nonprotein amino acids, phenols and flavonoids. Among these allomones, glycosides seem to play a very important role in host plant resistance to insects. For example, tomatine, an alkaloid glycoside and rutin (quercetin 3-rutinoside) have been shown to be involved in the resistance of tomato to the tomato fruitworm (*Heliothis zea*) by acting as feeding deterrents (Elliger *et al.*, 1981; Sinden *et al.*, 1978). Tomatine was also related to the resistance to the Colarado potato beetle (*Leptinotarsa decemineata*) (Juvik and Stevens, 1982). Another well-known case of host plant resistance is the involvement of the glycoside in the resistance of corn to the European corn borer (*Ostrinia nubilalis*). It was found that DIMBOA (2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one) glucoside was a precusor of the active resistance factor, DIMBOA, in young corn tissue (Klun *et al.*, 1967). Maysin, a flavone glycoside isolated from corn silk, was found to possess antibiotic activity toward the corn earworm (*H. zea*) and has been implicated in the resistance of corn to this insect (Waiss *el al.*, 1979). Recently, Reilly *et al.* (1987) showed that the cyanogenic glucoside prunasin was involved in the selection of peach tree by the peachtree borer (*Synanthedon exitiosa*) and the lesser peachtree borer (*S. pictipes*). The ability of these insects to metabolize prunasin and its toxic metabolite HCN plays a major role in the host plant selection.

In addition, numerous plant glycosides have been found to possess antifeedant activity toward phytophagous insects. For example, the phenolic glucoside phloridzin was found to be a deterrent to non-apple feeding aphids, *Myzus persicae* and *Amphorophora agathomica* (Montgomery and Arn, 1974). The alkaloid glucosides solanine and chaconine inhibited feeding of Colorado potato beetle (Sturchow and Low, 1961). Sinigrin, a mustard oil glucoside (glucosinolate), was found to act as a chemical barrier to the black swallowtail butterfly (*Papilio polysenes*) by

reducing larval survival and the number of fertile eggs laid by surviving adults (Erickson and Feeny, 1974).

The toxic action of these glycosides mentioned above could have been due to their corresponding aglycones liberated by the action of β-glucosidase. As shown in Table 4, with the exception of tomatine/tomatidine, all the aglycones were more toxic than their corresponding glucosides to fall armyworms. Therefore, high β-glucosidase activity is obviously detrimental to phytophagous insects when a plant glycoside is ingested.

If we know the substrate specificity of β-glucosidase and the consequences of the hydrolysis in a phytophagous insect, we can utilize this knowledge to develop plant varieties with more plant defensive systems. This could be achieved, for example, through traditional breeding programs to select for plant varieties possessing higher degrees of resistance factors (i.e. toxic glycosides that are not easily degraded or glycosides that are rapidly activated). Alternatively, genetic engineering techniques could be used to transfer selectively the plant toxin-producing genes into a plant to make it more insect resistant. In addition, knowledge gained from the present study can be used to explain species variations in response to resistant plants in phytophagous insects and hence the mechanisms of host plant resistance.

CONCLUSION

Glutathione transferasee were characterized in five lepidopterous species. The highly polyphagous insects, fall armyworm and corn carworm, possess multiple glutathione transferases containing 6 and 4 isozymes, respectively. The more specialized insects, tobacco budworm, cabbage looper and velvetbean caterpillar, had a single form of the enzyme. These isozymes consisted of 2-4 subunits with molecular weights of 27,000 to 32,000, depending on the species. Qualitative differences in glutathione transferase isozymes were observed among these species based on their Michaelis constant, isoelectric point and relative mobility (electrophoresis). Induction of glutathione transferase in fall armyworm larvae by xanthotoxin increased levels of the existing isozymes but did not result in production of any new isozyme.

β-Glucosidases in larvae of the fall armyworm was studied using helicin as the substrate. Enzyme activity was found in various tissues with the midgut exhibiting the highest activity. Among the midgut subcellular fractions, the soluble fraction was the most active. The apparent K_m value for the enzyme was 0.63 mM. The enzyme was fairly stable for 8 weeks when stored at −10°C. β-Glucosidases isolated from midgut homogenates of the fall armyworm, corn

earworm, cabbage looper and velvetbean caterpillar hydrolyzed a variety of glucosides including numerous toxic plant allelochemicals. Substrate specificity of β-glucosidase was different among these species. In general, D-amygdalin, helicin, D(+)-cellobiose, *p*-nitrophenyl β-D-glucoside and 4-methylumbelliferyl β-D-glucoside were the preferred substrates, whereas sinigrin, phloridzin, α-solanine, tomatine and linamarin were poor substrates for the enzyme in these insects. Toxicity tests reveal that, in most instances, the glucosides were less toxic than their corresponding aglycones to the fall armyworm, indicating that β-glucosidase is an activation enzyme.

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