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PURIFICATION AND PROPERTIES OF A GLUTATHIONE-S-TRANSFERASE FROM CORN WHICH CONJUGATES s-TRIAZINE HERBICIDES

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Key Word Index—*Zea mays*; Gramineae; corn; glutathione-S-transferase; s-triazine herbicides; conjugation.

Abstract—A glutathione-S-transferase involved in atrazine conjugation was purified 43-fold from corn with a total yield of 36%. The purified enzyme has a MW of 45000 as determined by gel filtration. The estimated activation energy of the enzyme is 6.4 kcal/mol and the optimum pH for activity between 8 and 8.5. Substrate specificity studies with s-triazines indicated that atrazine was the best substrate followed by simazine and propazine. The —Cl group at the 2-position was essential for enzyme activity, and replacement by a $-SCH_3$ group resulted in a total loss of activity. The absence of an alkyl group resulted in a reduction of conjugation and 2-chloro-4,6-*bis*-amino-s-triazine was the poorest substrate. With insecticidal substrates (organophosphates), conjugating activity was observed only with diazinon and little or no activity was observed with ethyl parathion, malathion and etrimfos. No activity was found using methyl iodide as a substrate. The purified enzyme has properties similar to those of an aryl-S-transferase. Quinones were inhibitors of this enzyme.

INTRODUCTION

Chloro-s-triazines are a group of herbicides which are used world-wide for weed control. The *in vivo* metabolism of this group of compounds has been investigated in plants as well as in mammals [1–10]. The selectivity of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is believed to be associated with the ability of certain resistant plant species to conjugate the herbicide with glutathione (GSH). Glutathione-S-transferases are a group of enzymes which mediate the initial reaction in mercapturic acid biosynthesis. A wide variety of foreign compounds are conjugated with GSH by this group of enzymes. Glutathione conjugation plays an important role in resistance of insects to certain insecticides [11] as well as selectivity of certain herbicides [2]. The formation of a conjugate, S-(4-ethylamino-6-isopropylamino-2-s-triazinyl) glutathione, and its isolation in the presence of a soluble enzyme preparation from plants has been reported by Lamoureux *et al.* [4, 5]. Frear and Swanson [12] partially purified a GSH-S-transferase (7.6-fold) from corn. The same investigators purified a GSH-S-transferase from peas which is involved in the cleavage of the diphenyl ether linkage of fluorodifen [13]. There are many reports on the purification of GSH-S-transferase from mammals and insects [14–21].

The present investigation was undertaken to purify and characterize a plant GSH-S-transferase which is involved in the conjugation of atrazine.

RESULTS AND DISCUSSION

Purification procedure

To avoid endogenous inhibition by quinones and other compounds, 1% bovine serum albumin (BSA) was added to the homogenizing medium. Similar use of BSA to protect against endogenous inhibition of the house fly glutathione-S-transferase has been reported by Motoyama *et al.* [22].

All the steps carried out during purification of the enzyme are summarized in Table 1. The enzyme was

Table 1. Purification of glutathione-S-transferase from corn

Procedure	Volume (ml)	Units/ml	Total units	Protein (mg/ml)	Units/mg protein	Yield (%)	Purification
1 100000 *g* supernatant	100	0.23	22.9	10.0	0.023	100	1.0
2 Ammonium sulfate precipitate	10	2.21	22.1	63.0	0.035	96.5	1.5
3 Dialysis	15.2	1.53	23.2	23.25	0.069	101.3	3.0
4 DEAE-cellulose	230	0.08	17.5	0.58	0.222	76.3	9.6
5 Ultrafiltration	14.3	1.12	16.0	4.66	0.24	69.7	10.4
6 Sephacril	85	0.14	12.2	0.22	0.654	53.3	28.4
7 Ultrafilteration	9.5	1.25	11.9	1.65	0.76	51.0	31.9
8 Hydroxylapatite	80	0.1	8.0	0.1	1.0	36.4	43.4

Unit of activity is defined as 1 nmol atrazine conjugated per/hr at 37°.

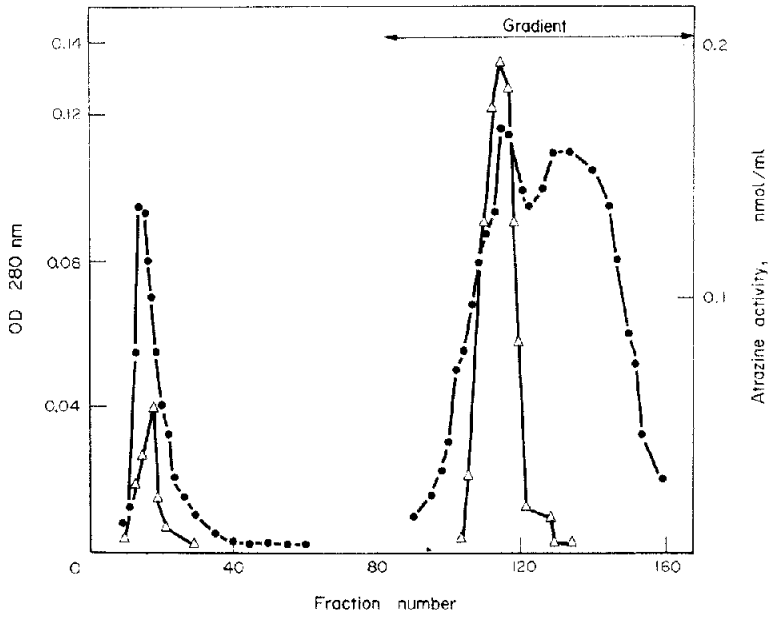

Fig. 1. DEAE-cellulose chromatography of purified glutathione-S-transferase. Linear gradient between 150 ml of 0.1 M potassium phosphate buffer, pH 6.5 and 150 ml of 0.5 M KCl in the same buffer. Atrazine activity (△) and protein at 280 nm (●—●).

purified 43-fold with a final yield of 36%. Three different types of column chromatography were utilized during the purification and the patterns of elution are shown in Figs. 1–3. Protein was monitored at 280 nm. Fig. 1 indicated that the major portion of the enzyme in the solution was bound to the DEAE-cellulose column and was eluted when a KCl linear gradient was applied. The eluted peak (peak II) from the DEAE-cellulose column was purified on a Sephacril column as shown in Fig. 2 and the elution pattern from a hydroxyl apatite column is shown in Fig. 3. Frear and Swanson [12] obtained a 7.6-fold purification of the enzyme using ammonium sulfate fractionation and Sephadex G-25 column chromatography. The same authors reported a 32-fold purification of a GSH-S-transferase present in peas which conjugated fluorodifen [13]. They suggested that a different GSH-S-transferase was responsible for fluorodifen conjugation than for atrazine conjugation.

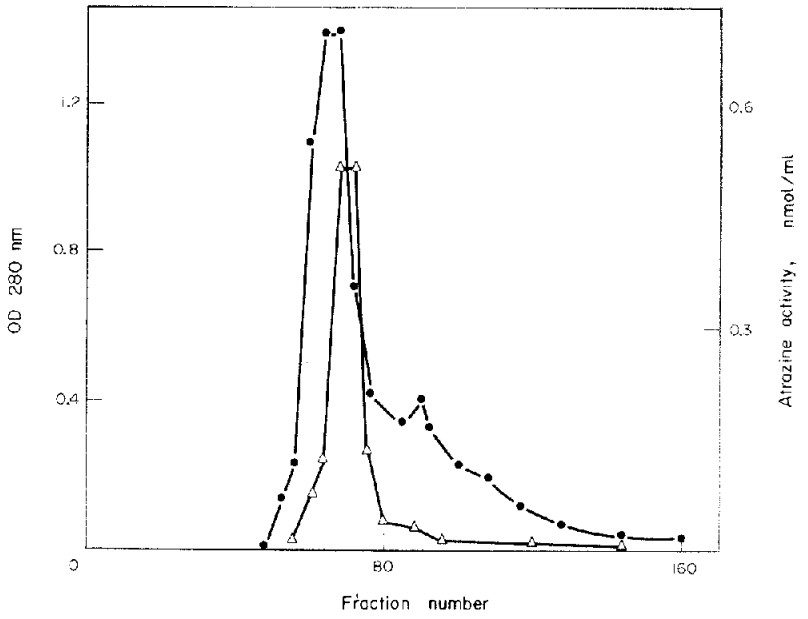

Fig. 2. Sephacril column chromatography of purified glutathione-S-transferase. Atrazine activity (△) and protein at 280 nm (●—●).

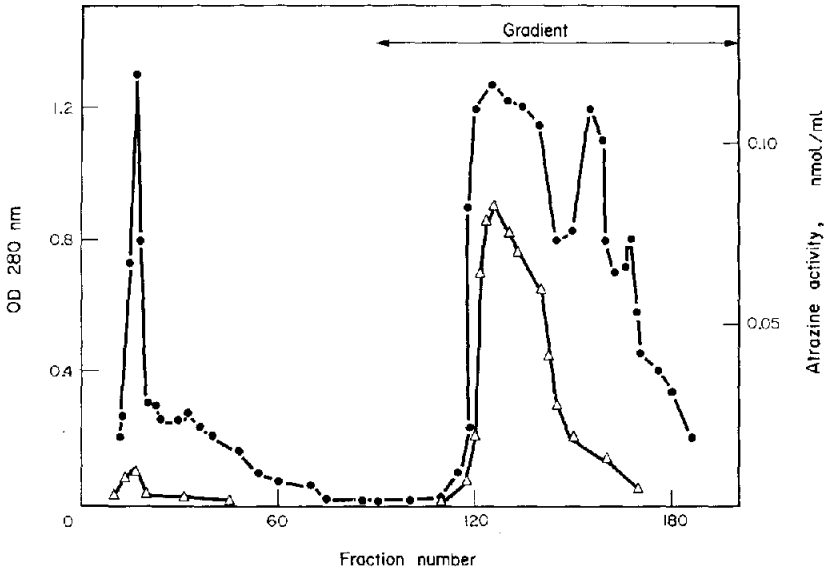

Fig. 3. Hydroxylapatite chromatography of purified glutathione-S-transferase. Linear gradient between 200 ml of 0.1 M potassium phosphate buffer, pH 6.8, containing 30% glycerol + 2 mM GSH + 0.1 mM EDTA (enzyme stabilizers) and 200 ml of 0.5 M potassium phosphate buffer with same stabilizers. Atrazine activity (△) and protein at 280 nm (● ●).

Identification of the reaction products

The aqueous phase from the enzyme reaction was concentrated and 0.1 ml samples were used for TLC with known standard conjugates [10]. Based on TLC, the reaction products were identified as glutathione conjugates.

Electrophoresis

The purity of the final preparation was determined using polyacrylamide gel electrophoresis and the results are shown in Fig. 4. To locate the active GSH-S-transferase bands on the gel, the gels were sliced (5 mm) and the activity extracted with 0.1 M phosphate buffer, pH 7.4 and assayed. Protein bands were located using Coomassie blue. Duplicate gels were run. The bands showing enzyme activity are indicated by (+) in Fig. 4.

MW determination

The MW of the purified GSH-S-transferase is 45000 as determined using Sephadex G-100 gel filtration. Based on the available literature, the MW of different transferases from mammals and insects ranges from 37000 to 80000. Pabst *et al.* [14] and Habig *et al.* [15] reported that GSH-S-transferases from rat liver had MWs of *ca* 46000. Clark *et al.* [23] reported the MW of a GSH-S-transferase from sheep liver and grass grub organism to be 80000. Usui *et al.* [20] reported the MW of a rat liver GSH-S-transferase to be 43000, while Askelöf *et al.* [19] estimated the MW of rat liver GSH-S-transferase to be 50000. Motoyama and Dauterman [21] studying a house fly GSH-S-transferase reported

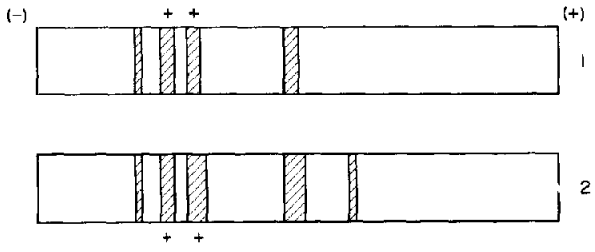

Fig. 4. Polyacrylamide gel electrophoresis of a purified glutathione-S-transferase from corn. (1) 50 µl and (2) 100 µl of enzymes were applied. After running electrophoresis, the gel without dye was sliced at 5 mm intervals and the enzyme was extracted with 0.5 ml of buffer for 10 hr and assayed. The (+) indicates the location of the active band.

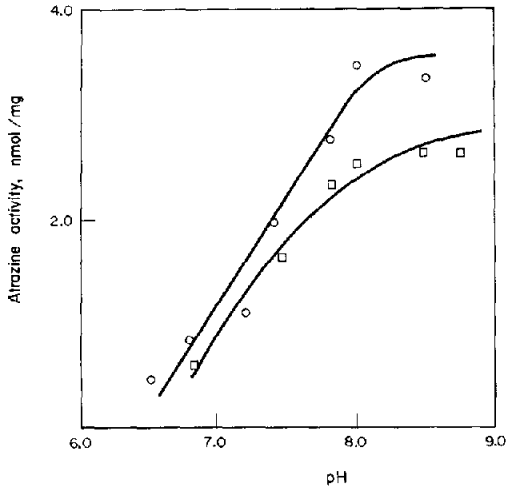

Fig. 5. Effect of pH on the activity of glutathione-S-transferase. Buffers used were: potassium phosphate buffer (○) and Tris–HCl buffer (□).

a MW of 50000. Guddewar and Dauterman [24] reported the MW of a mouse liver GSH-S-transferase involved in atrazine conjugation to be 47000. This is the first report on the MW of a plant GSH-S-transferase. The calculated value of 45000 appears to be within the range of enzymes purified from animal sources.

Effect of pH on enzyme activity

The effect of pH on the activity of purified GSH-S-transferase was studied with two different buffer systems (Fig. 5). The pH optimum is broad and between 8 and 8.5. This value is higher than that reported by Frear and Swanson [12] who found an optimum pH between 6.6 and 6.8 for the GSH-S-transferase from corn which is involved in atrazine conjugation. However, in another study on GSH-S-transferase from peas, they reported that the optimum pH was between 9.3 and 9.5 [13]. This variation may be due to the degree of purity of the enzyme or the source of enzyme. According to the literature, the optimum pH for GSH-S-transferase activity is usually between pH 7.5 and 9. Difference in pH optimum may depend upon the type of enzyme as well as the substrate. Habig *et al.* [16] reported a pH optimum of 7.4 for transferase B and E while a pH of 7.8 was demonstrated for transferace C and 8.5 for transferase A. Boyland and Chasseaud [25] reported pH 7.6 as the optimum for alkene-S-transferase. Clark *et al.* [23] reported an optimum pH of 8.3 for GSH-S-transferase from sheep liver and grass grub. Guddewar and Dauterman [24] reported an optimum pH of 8.5–9 for a GSH-S-transferase isolated from mouse liver which is involved in atrazine conjugation. Motoyama and Dauterman [21] reported an optimum pH between 9 and 9.5 for house fly glutathione-S-transferase. Thus, it appears that the optimum pH varies with the source of the enzyme, the purity of the enzyme and the type of buffer. In the present investigation, phosphate buffer resulted in higher activity than Tris–HCl buffer with a similar pH optimum.

Effect of temperature

The effect of temperature on enzyme activity was investigated between 25 and 40° as with the mouse liver GSH-S-transferase [24]. When the data were plotted, a straight line was obtained and an activation energy of 6.4 kcal/mol was calculated. Motoyama and Dauterman [21] and Boyland and Chasseaud [26] reported non-linearity with a break at 35° and activation energies over two different temperature ranges. Motoyama and Dauterman [27], in a later study, reported that the native transferase from the house fly gave a straight line whereas one of the purified forms separated by electrophoresis resulted in a discontinuity at 35°. It would appear that, depending on the form of the enzyme, the effect of temperature may vary. Although different forms of glutathione-S-transferases have been reported in the literature, little information is available on the effect of temperature on insect, mammalian, or plant enzymes.

Reaction of purified GSH-S-transferase with insecticides and methyl iodide

Parathion, diazinon, etrimfos, malathion, and methyl iodide were used as substrates for the purified enzyme (Table 2). Conjugation with glutathione was observed only with ring-labeled diazinon. This finding suggests that the purified enzyme has only aryltransferase activity and alkyltransferase activity is absent. Frear and Swanson [13] suggested that plants, like animals, may possess many transferases.

Table 2. Activity of purified glutathione-S-transferase towards certain insecticides and methyl iodide

Compound	Concentration (μmol)	% Conjugation
Ethyl parathion	0.254	
Diazinon	0.200	3.5
Etrimfos	0.200	0.02
Malathion	0.250	0.04
Methyl iodide	0.200	—

Substrate specificity studies

The K_m, V_{max} and enzymatic half-life $[(t_{0.5})e = 0.695 K_m/V_{max}]$ values were calculated for the s-triazines and data are presented in Table 3. The $-Cl$ group at the 2-position was required for enzyme activity and if this was replaced by $-SCH_3$, no activity was observed. Of the s-triazines with $-Cl$ groups at the 2-position, atrazine, simazine and propazine had higher activity than the dealkylated analogs. Atrazine, simazine and propazine are active herbicides while the dealkylated-s-triazines are metabolites. Comparison of the enzymatic half-lives showed that atrazine was conjugated faster than either simazine or propazine. The *bis*-amino compound (compound 4) lacking alkyl groups at both the 4- and 6-positions, was conjugated at the slowest rate of all the

Table 3. Activity of purified glutathione-S-transferase towards s-triazines

Compound	$K_m \times 10^{-2}$ mM	V_{max} μmol/mg/min	$0.695 \frac{K_m}{V_{max}}$
1 Atrazine 2-chloro-4-ethyl-amino-6-isopropyl-amino-s-triazine	18.0	25.0	0.50
2 2-Chloro-4 amino-6-iso-propylamino-s-triazine	14.3	5.5	1.81
3 2-Chloro-4 amino-6-ethyl-amino-s-triazine	11.8	9.0	1.31
4 2-Chloro-4,6-*bis*(amino)-s-triazine	11.0	5.0	2.80
5 Ametryn 2 methylthio-4-ethylamino-6-isopropylamino-s-triazine	—	—	—
6 Propazine 2-chloro-4,6-*bis*-(isopropylamino)-s-triazine	13.0	16.7	0.78
7 Simazine 2-chloro-4,6-*bis*(ethylamino)-s-triazine	15.5	17.0	0.63

Table 4. Inhibition of corn glutathione-S-transferase

	Inhibitor	Concentration (NM)	% Inhibition
1	Sulfobromophthalein	1.0	88.0
2	Dithiothreitol	1.0	13.3
3	1-Fluoro-2,4-dinitrobenzene	0.1	52.7
4	*N*-(*p*-dimethylamino-phenyl)-1,4-naptho-quinone	0.5	97.8
5	Tetrahydroxy-1,4-quinone dihydrate	0.5	93.8
6	2,5-Dimethyl-*p*-benzo-quinone	0.5	62.0

.triazines evaluated. Thus, alkyl groups at the 4- and 6-positions are required for maximum enzyme activity. These findings are similar to those of Frear and Swanson [12] who suggested that the alkyl side chains at the 4- and 6-positions were required for enzyme activity. With the mouse enzyme, Guddewar and Dauterman [24] obtained similar findings.

Inhibition studies

Studies were carried out with different potential inhibitors of glutathione-S-transferase such as quinones, sulfobromophthalein and other thio compounds. The results are presented in Table 4. Quinones inhibited the enzyme as well as sulfobromophthalein and 1-fluoro-2,4-dinitrobenzene. Dithiothreitol was the least effective inhibitor among the groups evaluated. Motoyama *et al.* [22] also reported inhibition of house fly glutathione-S-transferase by quinones.

EXPERIMENTAL

Chemicals. Atrazine-[^{14}C] and a number of other s-triazine analogs were kindly supplied by CIBA-Geigy Corp., Greensboro, N.C. The chemical names of the compounds used in the study are given in Table 5 with their sp. act. Prior to use, the purity of each compound was verified by TLC on 5 × 20 cm Polygram Silica Gel UV254 (0.25 mm) precoated plates. TLC plates were developed in C_6H_6–EtOH (9:1). If impurities were observed, the compounds were purified using a C_6H_6–EtOH TLC system. The radio-labeled compounds used as substrates had purities greater than 99%.

Table 5. s-Triazine substrates

	Compound	Specific activity (μCi/mg)
1	Atrazine 2-chloro-4-ethylamino-6-isopropylamino-s-triazine	24.9
2	2-Chloro-4-amino-6-isopropyl-amino-s-triazine	21.3
3	2-Chloro-4-amino-6-ethylamino-s-triazine	11.6
4	2-Chloro-4,6-*bis*(amino)-s-triazine	15.2
5	Ametryn 2-methylthio-4-ethylamino-6-isopropylamino-s-triazine	35.3
6	Propazine 2-chloro-4,6-*bis*(isopropylamino)-s-triazine	4.4
7	Simazine 2-chloro-4,6-*bis*(ethylamino)-s-triazine	26.8

Parathion [ethyl-1-^{14}C] [*O,O*-diethyl-*O*-*p*-nitrophenyl phosphorothioate] (5 mCi/mmol) and ^{14}C-malathion [*O,O*-dimethyl-S-(1,2-dicarbethoxy) [1,2-^{14}C] ethyl phosphorodithioate] (32.2 mCi/mmol) were purchased from Amersham Scarle Corp., Arlington Heights, Ill. [^{14}C]-diazinon [*O,O*-diethyl, *O*-(2-isopropyl-4-methylpyrimidin-2-^{14}C-6-yl)phosphorothioate] (1.1 mCi/mmol) was a gift from CIBA-Geigy Corp, Greensboro, N.C. while [^{14}C]-etrimfos [*O,O*-dimethyl-*O*-(2-ethyl-4-ethoxy-pyrimidin-4-^{14}C-6-yl)phosphorothioate] (3.2 mCi/mmol) was kindly supplied by Sandoz Inc., East Hanover, N.J.

Tissue preparation. Seedlings (15 days old) of corn (*Zea mays* L.) planted in vermiculite were cut into 2 to 3-inch pieces and homogenized in a Waring Blender for 2 min at a concn 1 g of plant material per 1.2 ml 0.1 M K-Pi buffer, pH 6.5, containing 1% BSA. The crude homogenate was filtered through cheesecloth and then centrifuged at 5000 *g* for 10 min. The supernatant was centrifuged at 22000g for 30 min, the pellet discarded and the liquid fraction recentrifuged at 100000 *g* for 1 hr. The 100000 *g* supernatant was used as a starting material for the purification of the enzyme.

Total protein was determined using Coomassie blue 250 as described in ref. [28]. BSA was used as standard.

Enzyme assay. The standard incubation mixture consisted of 5 mM GSH, 0.436 mM atrazine-[^{14}C] and 0.1 M K-Pi buffer, pH 7.4 in a final vol. of 2 ml. The reaction was incubated for 1.5 hr at 37° and the reaction terminated by the addition of 2 ml $CHCl_3$. The 2 phases were thoroughly mixed, centrifuged at 4000 *g* for 10 min and then separated. The radioactivity in 0.5 ml aliquots from each phase was quantiated in a liquid scintillation counter using Triton X-100 cocktail [29]. The efficiencies were corrected using *n*-hexadecane-[^{14}C] as an internal standard. All reactions were corrected for non-enzymatic conjugation.

Identification of conjugated products. The conjugate was separated from the parent compound by TLC on 5 × 20 cm Polygram Silica Gel UV254 (0.25 mm) precoated plates using methylethylketone–MeOH–H_2O (72:25:10) as the developing system. The radioactive products were co-chromatographed with known standards [10].

Purification of enzyme. Throughout the purification of the enzyme, the activity was monitored using atrazine-[^{14}C]. The temp. was maintained between 0 and 4°.

Step 1. 100000 *g* supernatant as described above was used as a starting material. The total vol. in this step was 100 ml.

Step 2. An appropriate amount of $(NH_4)_2SO_4$ was added slowly to the 100000 *g* supernatant to 90% satn and the pH was maintained at 6.5 by the addition of dil. base. After the addition of the $(NH_4)_2SO_4$, the enzyme soln was kept in the cold room for 30 min and then centrifuged at 10000 *g* for 10 min. The supernatant was discarded and the ppt. dissolved in a small vol. of 0.1 M K-Pi buffer, pH 6.5. The vol. was 10 ml.

Step 3. The soln from step 2 was dialysed against 0.1 M KPi buffer, pH 6.5 (4 l.) with 3 buffer changes during 24 hr. The vol. increased to 15.2 ml.

Step 4. A DEAE-cellulose column was prepared with 50 g of DEAE in 0.1 M K-Pi buffer, pH 6.5 and equilibrated. The enzyme soln was applied to the column, washed with 0.5 l. of buffer, and eluted with a gradient from 150 ml of 0.1 M K-Pi buffer, pH 6.5 to 0.5 M KCl in 150 ml of the same buffer.

Fractions (4 ml) were collected and assayed. Fractions showing enzyme activity were pooled (230 ml).

Step 5. The vol. of the peak from the DEAE-cellulose column was decreased to 14.3 ml by ultrafiltration using Diaflo membrane UM10 (Amicon Corp., Lexington, Mass., U.S.A.).

Step 6. A Sephacril column was prepared using 0.1 M K-Pi buffer, pH 6.5 and 14 ml of the concd enzyme from step 5 was added. Then the column was eluted with 250 ml buffer. Fractions (4 ml) were collected. The fractions were assayed for protein and enzyme activity and the fractions containing enzyme activity were combined (85 ml).

Step 7. The vol. of the enzyme fraction was reduced by ultrafiltration and 0.1 M K-Pi buffer with 30% glycerol + 2 mM GSH + 0.1 M EDTA (buffer B) was added. The final vol. was 9.5 ml and was used for further purification.

Step 8. The enzyme from step 7 was applied onto a hydroxylapatite column prepared in buffer B. The enzyme was washed into the column with 300 ml of buffer B and eluted with a gradient from 200 ml of buffer B to 200 ml of 0.5 M K-Pi buffer containing 30% glycerol + 2 mM GSH + 0.1 M EDTA. Fractions (4 ml) were collected. Fractions having enzyme activity were combined. The final vol. was 80 ml. For storage purpose the vol. of the enzyme soln was reduced by ultrafiltration and then frozen.

Electrophoresis. Polyacrylamide gel electrophoresis was carried out according to the method of ref. [30] and protein was stained with Coomassie blue. In certain expts, the tracking dye, bromophenol blue was utilized. Bromophenol blue was not utilized when the gels were to be analysed for enzyme activity.

Properties of enzyme. MW determination. The MW of the purified enzyme was determined using Sephadex G-100 filtration [31]. The column (2.7 × 80 cm) was prepared with 0.1 M K-Pi buffer pH 7.4. Hemoglobin (MW 64000), ovalbumin (MW 47000), β-lactoglobulin (MW 36500), trypsin (MW 23000) and cytochrome *c* (MW 12000) were used as standard proteins.

Effect of pH. Using two buffers, K-Pi buffer and Tris HCl buffer, a range of pH 6–8.5 was investigated to determine the effect on activity.

The effect of temp. was studied by carrying out the conjugation reaction with the purified enzyme and atrazine at 25–40°. The data obtained were used to calculate the activation energy.

Substrate specificity study. Atrazine-[^{14}C] and several s-triazines listed in Table 1 were used as substrates. K_m and V_{max} values were determined for each of these compounds. The enzymatic half-life values were calculated from the K_m and V_{max} values [24].

Studies on conjugation with insecticidal substrates. ^{14}C-ring labeled etrimfos, diazinon, ^{14}C-ethoxy-labeled parathion, and ^{14}C-succinate-labeled malathion were utilized as substrates to study the specificity of conjugation by the purified enzyme. Also, ^{14}C-methyl iodide was used to determine the alkyltransferase activity of the purified enzyme.

Inhibition study. Inhibition studies were carried out by incubating various inhibitors at the concn presented in Table 5 with the enzyme and buffer for 10 min. The reaction was then started by the addition of GSH and atrazine-[^{14}C] as described in the assay system. The % inhibition of each reaction was calculated.

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REFERENCES

1. Shimabukuro, R. H. and Swanson, H. R. (1969) *J. Agric. Food Chem.* **17**, 199.
2. Shimabukuro, R. H., Frear, D. S., Swanson, H. R. and Walsh, W. C. (1971) *Plant Physiol.* **47**, 10.
3. Shimabukuro, R. H., Walsh, W. C., Lamoureux, G. L. and Stafford, L. E. (1973) *J. Agric. Food Chem.* **21**, 1031.
4. Lamoureux, G. L., Shimabukuro, R. H., Swanson, H. R. and Frear, D. S. (1969) *Abstr.* 16, 157th *Meet. Am. Chem. Soc.* Minneapolis, Minnesota.
5. Lamoureux, G. L., Shimabukuro, R. H., Swanson, H. R. and Frear, D. S. (1970) *J. Agric. Food Chem.* **18**, 81.
6. Bakke, J. E., Robbins, J. D. and Feil, V. J. (1967) *J. Agric. Food Chem.* **15**, 628.
7. Bakke, J. E., Larson, J. D. and Price, C. E. (1972) *J. Agric. Food Chem.* **20**, 609.
8. Crayford, J. V. and Hutson, D. H. (1972) *Pestic. Biochem. Physiol.* **2**, 295.
9. Robbins, J. D., Bakke, J. E. and Feil, V. J. (1968) *J. Agric. Food. Chem.* **16**, 698.
10. Dauterman, W. C. and Muecke, W. (1974) *Pestic. Biochem. Physiol.* **4**, 212.
11. Motoyama, N. and Dauterman, W. C. (1974) *J. Agric. Food Chem.* **22**, 350.
12. Frear, D. S. and Swanson, H. R. (1970) *Phytochemistry* **9**, 2123.
13. Frear, D. S. and Swanson, H. R. (1973) *Pestic. Biochem. Physiol.* **3**, 473.
14. Pabst, M. J., Habig, W. H. and Jakoby, W. B. (1973) *Biochem. Biophys. Res. Commun.* **52**, 1125.
15. Pabst, M. J., Habig, W. H. and Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7140.
16. Habig, W. H., Pabst, M. J. and Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7130.
17. Habig, W. H., Pabst, M. J., Fleischner, G., Gatmaitin, Z., Arias, I. H. and Jakoby, W. B. (1974b) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3879.
18. Jakoby, W. B., Habig, W. H., Keen, J. H., Ketley, J. N. and Pabst, M. J. (1976) in *Glutathione: Metabolism and Function* (Arias, I. M. and Jakoby, W. B., eds.) p. 182. Raven Press, New York.
19. Askelöf, P., Guthenberg, C., Jakobson, I. and Mannervick, B. (1975) *Biochem. J.* **147**, 513.
20. Usui, K., Shishido, T. and Fukami, J. (1977) *Agric. Biol. Chem.* **41**, 2491.
21. Motoyama, N. and Dauterman, W. C. (1977) *Insect Biochem.* **7**, 361.
22. Motoyama, N., Kulkarni, A. P., Hodgson, E. and Dauterman, W. C. (1978) *Pestic. Biochem. Physiol* **9**, 225.
23. Clark, A. G., Darley, F. J. and Smith, J. N. (1969) *Biochem. J.* **103**, 49.
24. Guddewar, M. B. and Dauterman, W. C. (1978) *Pestic. Biochem. Physiol.* (submitted).
25. Boyland, E. and Chasseaud, L. F. (1969) *Adv. Enzymol.* **32**, 173.
26. Boyland, E. and Chasseaud, L. F. (1968) *Biochem. J.* **109**, 651.
27. Motoyama, N. and Dauterman, W. C. (1978) *J. Agric. Food Chem.* **26**, 1296.
28. Sedmak, J. J. and Grossberg, S. E. (1977) *Analyt. Biochem.* **79**, 544.
29. Patterson, M. J. and Green, R. C. (1965) *Analyt. Chem.* **37**, 854.
30. Davis, J. (1964) *Ann. N. Y. Acad. Sci.* **121**, 404.
31. Andrews, P. (1964) *Biochem. J.* **91**, 222.