J. Pesticide Sci. **21**, 430–433 (1996)

Original Article

Glutathione-Dependent *O*-Alkyl and *O*-Aryl Conjugations for Dicapthon and Fenitrothion in Several Insects*

Yasuhiko KONNO and Takashi SHISHIDO**

Division of Pesticides, National Institute of Agro-Environmental Sciences, Kannondai, Tsukuba 305, Japan
***Faculty of Agriculture, Tohoku University, Aoba-ku, Sendai 980, Japan*

(Received April 19, 1996; Accepted August 9, 1996)

Glutathione-dependent *O*-alkyl and *O*-aryl conjugations for ^{14}C-dicapthon (*O, O*-dimethyl *O*-2-chloro-4-nitrophenyl phosphorothionate) and ^{14}C-fenitrothion (*O, O*-dimethyl *O*-3-methyl-4-nitrophenyl phosphorothionate) were studied in the each cytosolic fraction prepared from the whole bodies of the azuki bean beetle, *Callosobruchus chinensis*, the chestnut weevil, *Curculio dentipes*, the green rice leafhopper, *Nephotettix cincticeps*, the almond moth, *Ephestia cautella*, the yellow peach moth, *Conogethes punctiferalis*, and the rice stem borer, *Chilo suppressalis*. Fenitrothion was degraded almost by *O*-alkyl conjugation, whereas relatively high ratio of *O*-aryl conjugation was observed for dicapthon degradation. Particularly in the azuki bean beetle, the green rice leafhopper and the almond moth, *O*-aryl conjugation was dominant (60–80%). This result is the first evidence that *O*-aryl conjugation is preferentially occurred for *O, O*-dimethyl *O*-aryl phosphorothionates in insects. Malpighian tubes, fat body and midgut prepared from the common cutworm, *Spodoptera litura*, were also assayed with ^{14}C-dicapthon. Interestingly, the ratios of *O*-alkyl and *O*-aryl conjugation were clearly different among the three tissues.

INTRODUCTION

It is well known that *O, O*-dialkyl *O*-aryl phosphorothionate insecticides are metabolized by two types of glutathione *S*-transferase (GST) reaction in insects, that is *O*-alkyl and *O*-aryl conjugations. The ratio of the two conjugations depends on the structure of the insecticides.[1] *O, O*-Dimethyl *O*-aryl phosphorothionates are known to be degraded almost by one reaction, *i.e.*, *O*-alkyl conjugation. The well known examples of the reaction are methyl parathion in the horn beetle, *Xylotrupes dichotomus*,[2] and the housefly, *Musca domestica*,[3] whereas it has been known that both reactions occur for *O, O*-diethyl *O*-aryl phosphorothionates, such as diazinon in the American cockroach, *Periplaneta americana*, and parathion in the housefly.[4–6]

The purpose of this study is to clarify whether the *O*-alkyl conjugation is really the principal GSH-dependent reaction for the degradation of *O, O*-dimethyl *O*-aryl phosphorothionates in insects or not. To check this, we measured the GST activities in various insects using ^{14}C-fenitrothion and ^{14}C-dicapthon as substrates. As a result, it was revealed that dicapthon was degradaded mainly by *O*-aryl conjugation in the azuki bean beetle, *Callosobruchus chinensis*, the green rice leafhopper, *Nephotettix cincticeps* and the almond moth, *Ephestia cautella*. We also report here the ratio of *O*-alkyl and *O*-aryl conjugation for dicapthon in three insect tissues, *i.e.*, Malpighian tubes, fat body and midgut prepared from the common cutworm, *Spodoptera litura*.

* This work was supported in a part by a Grand-in-Aid (Bio Media Program) from the Ministry of Agriculture, Forestry and Fisheries, Japan (BMP 96-V-1-5-5).

** Present address: 3-2-125-15 Narushima, Yonezawa 990, Japan.

MATERIALS AND METHODS

1. *Insects*

The chestnut weevil, *Curculio dentipes*, the green rice leafhopper, the yellow peach moth, *Conogethes punctiferalis*, and the common cutworm, were collected at Tsukuba city, Ibaraki prefecture in August 1992. The rice stem borer, *Chilo suppressalis*, was collected at Soja city, Okayama prefecture in August 1993. The azuki bean beetle, the almond moth and the American cockroach were reared in the laboratory for more than 25 years, but their origins were unknown. All of these insects were maintained in the laboratory at 25°C under a 15L-9D photoperiod.

2. *Chemicals*

[Methoxy-^{14}C]fenitrothion (*O, O*-dimethyl *O*-3-methyl-4-nitrophenyl phosphorothionate, 12,564 dpm/μg, >99% purity by TLC) was obtained from Sumitomo

Chemical Co. (Osaka, Japan). [Methoxy-^{14}C]dicapthon (*O, O*-dimethyl *O*-2-chloro-4-nitrophenyl phosphorothionate, 3227 dpm/μg, >99% purity by TLC) were prepared by the reaction of 2-chloro-4-nitrophenol with [methoxy-^{14}C]dimethyl phosphorochloridothionate in the presence of potassium carbonate at 70–75°C in methyl ethyl ketone as described by Nishizawa *et al.*[7] The intermediate [methoxy-^{14}C]dimethyl phosphorochloridothionate was prepared by the reaction of ^{14}C-methanol with phosphorus pentasulfide as described by Fletcher *et al.*[8] Desmethyl fenitrothion and desmethyl dicapthon were prepared by dealkylation of fenitrothion and dicapthon, respectively, with benzenethiol as described by Oppenoorth *et al.*[6] *S*-Methyl glutathione, *S*-methyl cysteine and dimethyl phosphorothioic acid were synthesized as described previously.[9] All other chemicals used were of the highest grade and commercially available.

3. *Enzyme Preparation*

All enzyme preparations were done at 4°C. Whole body was used as enzyme source. But the adults of the Ameriacn cockroach and 5th-instar larvae of the common cutworm were dissected beforehand carefully using micro-dissecting forceps and scissors (Sigma, U.S.A.). These tissues or whole body of insects were homogenized in 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA (ethylenediaminetetraacetic acid) using a glass homogenizer (Wheaton, U.S.A.). The homogenates were then centrifuged for 60 min at 100,000×*g*. The cytosolic fractions were used as the enzyme solution. Protein concentration was determined by the method of Bradford.[10]

4. *GST Assay with ^{14}C-Fenitrothion and ^{14}C-Dicapthon*

In a 10-ml stoppered tube, 1 ml of the reaction mixture was introduced consisting of 0.15 μmol ^{14}C-fenitrothion or ^{14}C-dicapthon dissolved in 15 μl of ethanol, 3 mM GSH, 45 μg Triton X-100 dissolved in 15 μl of ethanol, 0.05 M phosphate buffer (pH 7.4) containing 0.5 mM EDTA, and enzyme solution. The mixture was incubated for 60 min at 25°C. After removing the intact radioactive fenitrothion or dicapthon by extraction with chloroform, the amount of GSH conjugates of fenitrothion or dicapthon in the water-soluble fraction was determined by liquid scintillation counting (LSC).

Water-soluble metabolites were identified by thin-layer chromatography (TLC) analyses on silica gel plates (60-F_{254}, Merck, Germany) with authentic standards. Solvent system used was 2-propanol : ammonia water : water (75 : 10 : 15). In this solvent system, *Rf* values of desmethyl fenitrothion or desmethyl dicapthon, *O, O*-dimethyl phosphorothioic acid, *S*-methyl cysteine and *S*-methyl glutathione were 0.84, 0.68, 0.33 and 0.19, respectively. Radioactive metabolites were detected by autoradiography, and unlabeled standards were detected by UV visualization and/or spraying a $PdCl_2$, ninhydrin or Hanes-Isherwood's reagent as described previously.[9] Radioactive compounds scraped off from TLC plates were quantified by LSC. The reaction mixture without GSH was used as a control, and data shown in this paper were calculated by subtracting the control from the reaction with GSH. Data in the Tables show the average of triplicate experiments.

RESULTS AND DISCUSSION

1. *GST Activities towards ^{14}C-Fenitrothion and ^{14}C-Dicapthon in Various Insects*

The cytosolic fractions of the six insect species were assayed with ^{14}C-fenitrothion and ^{14}C-dicapthon (Tables 1, 2). The GST activities towards ^{14}C-fenitrothion were clearly lower than those towards ^{14}C-dicapthon. Among the six insects, the highest GST activity was found in the green rice leafhopper, *i.e.*, 134 pmol GSH conjugation/mg protein/min for fenitrothion and 460 pmol GSH conjugation/mg protein/min for dicapthon.

The ratio of *O*-alkyl and *O*-aryl conjugation for fenitrothion are shown in Table 1. Fenitrothion was degraded almost by *O*-alkyl conjugation in the six insects. That is, more than 94% of the total GSTreaction was by *O*-alkyl conjugation. This result agreed with our previous report showing that fenitrothion was degraded almost by *O*-alkyl conjugation in Malpighian

Table 1 Glutathione *S*-transferase activity for ^{14}C-fenitrothion in various insects.[a]

Insect	Life stage[b]	Protein content (mg/g tissue)	pmol of ^{14}C-fenitrothion conjugated (min^{-1} mg protein^{-1})	% of reaction	
				O-Alkyl conjugation	*O*-Aryl conjugation
Azuki bean beetle	A	45±3.6	111±24	94.0	6.0
Chestnut weevil	L	42±2.8	100±19	95.0	5.0
Green rice leafhopper	A	50±4.8	134±44	97.6	2.4
Almond moth	L	68±6.2	17±4.4	96.6	3.4
Yellow peach moth	L	65±5.8	87±26	98.5	1.5
Rice stem borer	L	58±4.5	13±2.1	97.3	2.7

[a] Values are mean±S.D. of triplicate runs.

[b] A : adult, L : larvae.

Table 2 Glutathione *S*-transferase activity for ^{14}C-dicapthon in various insects.[a]

Insect	Life stage[b]	Protein content (mg/g tissue)	pmol of ^{14}C-dicapthon conjugated (min^{-1} mg protein^{-1})	% of reaction	
				O-Alkyl conjugation	*O*-Aryl conjugation
Azuki bean beetle	A	45±3.6	289±69	22.1	77.9
Chestnut weevil	L	42±2.8	357±100	71.5	28.5
Green rice leafhopper	A	50±4.8	460±134	32.1	67.9
Almond moth	L	68±6.2	191±56	41.2	58.8
Yellow peach moth	L	65±5.8	184±43	76.1	23.9
Rice stem borer	L	58±4.5	105±31	67.1	32.9

[a] See Table 1. [b] A : adult, L : larvae.

tubes, fat body and midgut prepared from the American cockroach and the common cutworm.[11]

Compared with the fenitrothion degradation, relatively high ratio of *O*-aryl conjugation was observed for dicapthon degradation. As shown in Table 2, more than 23.9% of the total GST reaction was by *O*-aryl conjugation in the six insects. Particularly in the azuki bean beetle (77.9%), the green rice leafhopper (67.9%) and the almond moth (58.8%), *O*-aryl conjugation was dominant. This result is the first example that *O*-aryl conjugation preferentially occurs for the degradation of *O, O*-dimethyl *O*-aryl phosphorothionates in insects.

Why is dicapthon degraded preferentially by *O*-aryl conjugation in some insects? Figure 1 shows the structures of dicapthon and fenitrothion. When comparing the structure of dicapthon with that of fenitrothion, dicapthon has a chloro substituent adjacent to *P-O*-aryl ester bond (2-position of the phenyl ring). Because the chloro substituent has an electron-withdrawing property, we consider that the presence of the 2-chloro substituent on the phenyl ring may weaken the *P-O*-aryl ester bond resulting in an increase of reactivity at the ester linkage by GSH-dependent *O*-aryl conjugation.

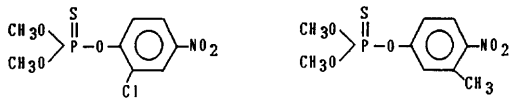

Fig. 1 Structures of dicapthon and fenitrothion.

2. *GST Activities towards ^{14}C-Dicapthon in Insect Tissues*

The cytosolic fractions of Malpighian tubes, fat body and midgut prepared from the American cockroach and the common cutworm were assayed with ^{14}C-dicapthon (Table 3). Among the three tissues, Malpighian tubes had the highest GST activities in the two insects. This result agreed with our previous report using CDNB, DCNB, DNPBS and fenitrothion as substrates in the same two insects.[11]

In the American cockroach tissues, ^{14}C-dicapthon was degraded mainly by *O*-aryl conjugation (73.4 to 80.1%). Whereas in the common cutworm tissues, the ratio of *O*-alkyl and *O*-aryl conjugation were cleary different among the three tissues. That is, although *O*-aryl conjugation was dominant in the fat body (67.1%), *O*-alkyl conjugation was dominant in the Malpighian tubes and midgut (74.1 and 88.5%, repsectively) (Table 3, Fig. 2). This result is the first example that the ratio of *O*-alkyl and *O*-aryl conjugations were clearly different among insect tissues, suggesting that the common cutworm has at least two GST isozymes which are distributed separately in different tissues.

Table 3 Glutathione *S*-transferase activity for ^{14}C-dicapthon in insect tissues.[a]

Insect tissue	Life stage[b]	Protein content (mg/g tissue)	pmol of ^{14}C-dicapthon conjugated (min^{-1} mg protein^{-1})	% of reaction	
				O-Alkyl conjugation	*O*-Aryl conjugation
American cockroach	A				
Malpighian tubes		45±4.2	978±198	22.9	77.1
Fat body		42±5.6	476±100	26.6	73.4
Midgut		130±12	200±52	19.9	80.1
Common cutworm	L				
Malpighian tubes		25±3.6	800±140	74.1	25.9
Fat body		30±4.2	633±113	32.9	67.1
Midgut		25±3.3	320±60	88.5	11.5

[a] See Table 1. [b] A : adult, L : larvae.

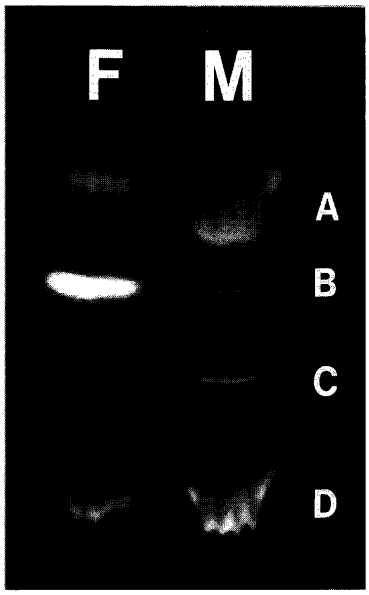

Fig. 2 Typical autoradiogram of ^{14}C-dicapthon metabolites produced in the fat body (F) and midgut (M) of *Spodoptera litura*.
Metabolites are desmethyl dicapthon (A), dimethyl phosphorothioic acid (B), *S*-methyl cysteine (C) and *S*-methyl glutathione (D).

The fact that each common cutworm tissue possesses the tissue-dependent GST isozymes has an important implication in considering the insect defense systems against xenobiotics or foreign compounds. That is, such GST diversity should be of great advantage for insects themselves to survive the chemically polluted environments, such as by insecticide application.

ACKNOWLEDGMENTS

We thank Dr. F. TANAKA (Okayama Prefectural Agricultural Experiment Station) for the supply of rice stem borers.

REFERENCES

1) J. Fukami : *Pharmacol. Ther.* **10**, 473 (1980)
2) J. Fukami & T. Shishido : *J. Econ. Entomol.* **59**, 1338 (1966)
3) N. Motoyama & W. C. Dauterman : *Insect Biochem.* **7**, 361 (1977)
4) T. Shishido, K. Usui, M. Sato & J. Fukami : *Pestic. Biochem. Physiol.* **2**, 51 (1972)
5) J. B. Lewis & R. M. Sawicki : *Pestic. Biochem. Physiol.* **1**, 275 (1971)
6) V. R. Oppenoorth, V. Rupes, S. ElBashir, N. W. H. Houx & S. Voerman : *Pestic. Biochem. Physiol.* **2**, 262 (1972)
7) Y. Nishizawa, M. Nakagawa, Y. Suzuki, H. Sakamoto & T. Mizutani : *Agric. Biol. Chem.* **25**, 597 (1961)
8) J. H. Fletcher, J. C. Hamilton, I. Hechenbleikner, E. I. Hoegberg, B. J. Sertl & J. T. Cassaday : *J. Am. Chem. Soc.* **72**, 2461 (1950)
9) Y. Konno & T. Shishido : *J. Pesticide Sci.* **12**, 469 (1987)
10) M. M. Bradford : *Anal. Biochem.* **72**, 248 (1976)
11) Y. Konno & T. Shishido : *Appl. Entomol. Zool.* **27**, 391 (1992)

要　　約

数種昆虫におけるダイキャプソンとフェニトロチオンのグルタチオン抱合

昆野安彦，宍戸　孝

グルタチオン *S*-トランスフェラーゼによる有機リン殺虫剤ダイキャプソンとフェニトロチオンの *O*-アルキル抱合と *O*-アリール抱合の反応割合を各種昆虫の上清（全虫体）を酵素源に用いて検討した．フェニトロチオンは供試した6種の昆虫（アズキゾウムシ，クリシギゾウムシ，ツマグロヨコバイ，スジマダラメイガ，モモノゴマダラノメイガ，ニカメイガ）すべてにおいて *O*-アルキル抱合が優先したが（90%以上），ダイキャプソンでは *O*-アリール抱合の割合が高く，とくにアズキゾウムシ，ツマグロヨコバイ，スジマダラメイガでは *O*-アリール抱合が優先した（60～80%）．またハスモンヨトウの各種臓器におけるダイキャプソンのグルタチオン抱合を調べた結果，マルピギー氏管，中腸では *O*-アルキル抱合が優先（74～89%）したのに対し，脂肪体では *O*-アリール抱合が優先（67%）するという，臓器間での際だった違いが認められた．