グルタチオンとの抱合活性化によるサリゲニン環状リン酸エステル類のグルタチオントランスフェラーゼ阻害

誌名	日本農薬学会誌
ISSN	03851559
著者	塩月, 孝博 小磯, 彰宏 江藤, 守総
巻/号	14巻3号
掲載ページ	p. 337-344
発行年月	1989年8月

J. Pesticide Sci. **14**, 337–344 (1989)

Original Article

Glutathione Conjugation as a Mechanism for Glutathione Transferase Inhibition by Saligenin Cyclic Phosphates

Takahiro SHIOTSUKI, Akihiro KOISO and Morifusa ETO

Department of Agricultural Chemistry, Kyushu University, Higashi-ku, Fukuoka 812, Japan

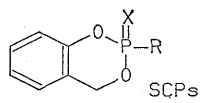
(Received January 11, 1989)

Saligenin cyclic phosphates (SCPs) such as salithion were tested for effects on glutathione transferase (GSH-*t*) from equine liver and fly abdomen. The reaction of SCPs with glutathione (GSH) resulted in the formation of a certain metabolite possessing inhibitory activity higher than SCPs *per se*. The inhibitive metabolite was identified as *S*-(2-hydroxybenzyl)glutathione by TLC analyses and fast atom bombardment mass spectrometry. This GSH conjugate inhibited two GSH-*t* systems: 1-Chloro-2,4-dinitrobenzene/GSH conjugation and GSH-dependent degradation of fenitrothion. The inhibition was competitive with GSH. The effectiveness of salithion against organophosphate-resistant insects and the synergistic effect of SCPs such as K-1 and K-2 on organophosphorus insecticides may be attributed, at least partially, to GSH-*t* inhibition after activation.

INTRODUCTION

Some reports have said that salithion, one of saligenin cyclic phosphates (SCPs, see Fig. 1), shows higher insecticidal activity against organophosphate (OP)-resistant insects[1,2] and bulb mite[3] than some other conventional OPs, *e.g.*, fenitrothion and malathion. In the resistance mechanisms of a Daisan Yumenoshima housefly strain (3-Y) to fenitrothion, the insensitivity of acetylcholinesterase with low affinity to fenitrooxon[1] and the high activity of glutathione (GSH)-dependent degradation of fenitrothion[4] play important roles. Salioxon analogs K-1 and K-2 are known to inhibit carboxylesterase to synergize with malathion against diazinon-resistant houseflies,[5] malathion-resistant green rice leafhoppers,[5] and OP-resistant citrus red mites.[6] It has been also reported that K-2 is synergistic with fenitrothion, fenitrooxon, and paraoxon because it inhibits hydrolytic degradation of OPs, against OP-resistant rice stem borers,[7,8] and with paraoxon against green peach aphides.[9] We have suggested that K-1 and K-2 inhibit glutathione *S*-transferase (GSH-*t*) to synergize with fenitrothion against a fenitrothion-selected 3-Y strain (3-Y_F).[10] Clarification of GSH-*t* inhibition mechanisms by SCPs will provide valuable information for effective control of insects. Effects of SCPs and the related compounds on the GSH conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) and the GSH-dependent degradation of ^{14}C-fenitrothion are discussed in this paper as indexes of the activity of GSH-*t*.

Abbreviations: OP, organophosphate; SCP, saligenin cyclic phosphate; GSH, reduced glutathione; GSH-*t*, glutathione transferases; K-1, 2-phenyl-4*H*-1, 3, 2-benzodioxaphosphorin 2-oxide; K-2, 2-phenoxy-4*H*-1, 3, 2-benzodioxaphosphorin 2-oxide; MTBO, 2-methylthio-4*H*-1, 3, 2-benzodioxaphosphorin 2-oxide; EDTA, disodium ethylenediaminetetraacetate, dihydrate; CDNB, 1-chloro-2, 4-dinitrobenzene; SRS, insecticide-susceptible standard registered housefly strain; 3-Y_F, fenitrothion-selected OP-resistant Daisan Yumenoshima housefly strain.



SCPs

	X	R
salithion	S	OMe
salioxon	O	OMe
K-1	O	Ph
K-2	O	OPh
MTBO	O	SMe

Fig. 1 Structures of saligenin cyclic phosphates (SCPs).

MATERIALS AND METHODS

1. *Chemicals*

Salithion, an insecticidal SCP, was supplied by Sumitomo Chemical Co., Ltd., and its oxon analog salioxon was synthesized by us according to the described method.[1] K-1 and K-2 were also prepared with the procedure reported in the previous paper,[11] and MTBO, a thiolo-isomer of salithion was a gift from Dr. M.-Y. Eto of Himeji College of Hyogo.

S-(2-Hydroxybenzyl)glutathione was non-enzymatically obtained from GSH and K-2 by applying Frankel's method for preparation of *S*-benzyl glutathione with some modifications.[12] To a solution of GSH (1 mmol) in the mixture of EtOH (1 ml) and 2 N NaOH (1.2 ml), K-2 (1 mmol) was added at room temperature. The mixture was weakly acidified after 2 hr, and submitted to gel-filtration (Sephadex G-10). Fractions that absorb UV light at both 220 and 280 nm were gathered, concentrated and recrystallized from water to obtain white crystals. The structure of the product was determined by TLC analyses and fast atom bombardment mass spectrometry (FAB MS).

All other chemicals were of available reagent grade.

2. *Analytical Methods*

TLC analyses were performed with precoated silica gel plates (60 F_{254}) supplied by E. Merck. A mixture of *n*-BuOH, AcOH, and H_2O (4:1:2) was used as a developing solvent system. Chromatograms were visualized with UV light and a ninhydrin reagent for amino acids, an acid molybdate reagent for phosphate, or a diazotized sulfanilic acid reagent for phenols. FAB MS was performed on a JMS-DX300 mass spectrometer (JEOL Ltd., Tokyo) equipped with a FAB ion source and interfaced to the data processing system JMA-3500 (JEOL Ltd.). The sample was suspended in a matrix of glycerol and DMSO.

3. *Housefly Strains*

A WHO's SRS strain represented susceptible houseflies and a 3-Y_F strain selected by fenitrothion OP-resistant houseflies. Both strains were supplied by Dr. T. Shono and reared successively in this laboratory. The characteristics of these strains have been previously reported.[10] Three- to four-day-old adult females were used in all experiments.

4. *Enzyme Preparations*

Purified equine liver GSH-*t* was purchased from Sigma Chemical Company. Housefly GSH-*t* was prepared as follows: The abdominal sections of flies were separated with tweezers after freezing in granular solid carbon dioxide, and homogenized with a teflon-glass Potter-Elvehjem tube in ice-cold 0.1 M phosphate buffer at pH 7.0 (10 abdomens per ml) containing 1 mM EDTA. The homogenate was centrifuged at $600\times g$ at 0°C for 10 min, and the supernatant was again centrifuged at $10{,}000\times g$ for 10 min. The supernatant obtained through further centrifugation at 40,000 $\times g$ for 60 min was used as fly abdominal GSH-*t*.

5. *Inhibition of GSH-t Activity*

The activity of GSH-*t* was measured with two substrates, 1-chloro-2, 4-dinitrobenzene (CDNB) and [ring-^{14}C]-fenitrothion. The spectrometric method described by Booth *et al.*[13] was used with slight modifications for CDNB/GSH conjugation: Enzyme solution (2.9 ml) containing 5 μg protein of equine liver GSH-*t* or 0.25 housefly abdomen, was prepared with 0.1 M phosphate buffer of pH 7.0 containing 1 mM EDTA. After preincubation at 25°C for 2 min, 0.1 ml of 75 mM GSH and 20 μl of 150 mM CDNB were added to the enzyme solution, and the change in absorbance at 340 nm was recorded as the production rate of 2,4-dinitrophenyl glutathione of a Shimadzu UV-200 spectrophotometer. In the inhibition test, inhibitor solution in acetone or DMSO was added to the enzyme solution during

preincubation.

To study time-dependent changes in inhibitory activity by mixing SCP and GSH (Figs. 3 and 7), further GSH (75 mM, 0.1 ml) was added at the enzyme assay: To 49.5 ml of GSH solution in 0.1 M phosphate buffer, pH 8.0, 0.5 ml of SCP solution in acetone was added. After an appropriate interval, housefly abdominal GSH-*t* (0.1 ml) and 10 mM GSH solution (1.0 ml) were incubated with an aliquot (1.9 ml) of SCP-GSH mixture at 25°C for 2 min. Then, 20 μl of 60 mM CDNB was added to the enzyme reaction mixture and changes in absorbance at 340 nm were recorded. A control contained neither GSH-*t* nor SCP.

The activity of an enzyme with fenitrothion as the substrate was measured according to the method described previously.[4] The enzyme reaction was performed with equine liver GSH-*t* at 37°C for 45 min or with fly abdominal GSH-*t* at 25°C for 120 min. In this paper, only the acid-soluble fraction, corresponding to the "aqueous" fraction in the previous papers,[4,10] containing demethyl products and *S*-(3-methyl-4-nitrophenyl)glutathione, is described.

6. *Time-dependent Decrease of the GSH Sulfhydryl Group*

The sulfhydryl group of GSH was determined by Ellman's method.[15] Reagent solution was prepared by dissolving 39.6 mg of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and 15 mg of sodium bicarbonate in 10 ml of 0.1 M phosphate buffer, pH 7.0. To a sample solution (3 ml), 0.1 ml of the DTNB reagent solution was added, and the absorbance at 412 nm was measured 2 min after the addition of the reagent. The temperature was maintained at 25°C. A standard curve was obtained by plotting absorbances versus concentrations of GSH in good correlation ($R=1.000$). From the theoretical values calculated from the molecular extinction coefficient and observed data, the used GSH preparation was estimated to contain 6.2% of oxidized form. Time-course decrease of the sulfhydryl group in GSH was measured by keeping at 25°C a solution containing 50 μM of GSH and an equivalent molar SCP in 0.1 M phosphate buffer, pH 8.0. GSH was spontaneously oxidized by 3.9% after 60 min under the applied conditions as in Fig. 2.

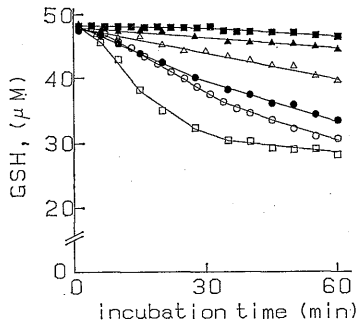


Fig. 2 Time course decrease of a GSH sulfhydryl group in the presence (50 μM) or absence of SCPs.

■: no SCPs, ▲: salithion, △: salioxon, ●: K-1, ○: K-2, □: MTBO, [GSH]=50 μM.

7. *Kinetic Study of CDNB/GSH Conjugation and Its Inhibition*

The Michaelis constant (*Km*) and the maximum velocity (V_{max}) were obtained from the rates of CDNB/GSH conjugation at six or more concentrations of each substrate. The kinetic constants were calculated based on nonlinear regression analysis.[14] The Lineweaver-Burk plot in the presence of the inhibitor was obtained first, and the data was replotted on the Dixon plot to obtain the K_i value from the intersection.

RESULTS AND DISCUSSION

1. *Effect of SCPs on the GSH-t Activity and GSH-Dependent Degradation of ^{14}C-Fenitrothion*

Since K-1 and K-2 inhibit the GSH-dependent degradation of ^{14}C-fenitrothion as reported in our previous paper,[10] the effect of SCPs on GSH-*t* activity to another substrate, CDNB, was examined. As presented in Table 1, however, no or only little effect was observed in all the enzymatic systems of houseflies. The activity of CDNB/GSH conjugation with equine liver GSH-*t* was hindered by all the SCPs tested (33–56%). On the other hand, the GSH-dependent degradation of fenitrothion by fly abdominal GSH-*t* was inhibited by salithion and MTBO to a larger extent than by K-2 (Table 2). Salioxon had lower inhibi-

Table 1 Effect of SCPs (0.1 mM) on CDNB/GSH conjugation by fly abdominal or equine liver GSH-*t*.[a)]

SCPs	% of control		
	Fly (SRS)	Fly (3-Y_F)	Equine
Salithion	103.7	108.2	74.7
Salioxon	98.5	108.2	59.2
MTBO	99.6	98.3	76.5
K-1	97.1	85.0	63.7
K-2	91.5	72.1	43.8

a) Control values were 324.4 and 546.3 nmol conjugation·min^{-1}·abdomen^{-1} by SRS and 3-Y_F housefly GSH-*t* and 29.6 nmol conjugation·min^{-1}·μg protein^{-1} by equine liver GSH-*t*.

Table 2 Effect of SCPs on GSH-dependent degradation of ^{14}C-fenitrothion by fly abdominal or equine liver GSH-*t*.[a)]

SCPs	% of control		
	Fly (SRS)	Fly (3-Y_F)	Equine
Salithion	12.2	6.7	41.9
Salioxon	90.2	61.0	45.2
MTBO	19.5	15.3	25.8
K-1	62.2	58.5	22.6
K-2	20.7	20.1	25.8

a) Control values were 20.5 and 88.5 nmol conjugation·min^{-1}·abdomen^{-1} by SRS and 3-Y_F GSH-*t*, and 8.26 nmol conjugation·min^{-1}·μg protein^{-1} by equine liver GSH-*t*.

tory activity than K-1. The fenitrothion degradation by equine liver GSH-*t* was inhibited by MTBO, K-1 and K-2 to a larger extent than by salithion and salioxon. In these enzymatic systems, the reaction time differed depending on the substrates used (120 min for fenitrothion degradation by fly abdominal GSH-*t*, 45 min by equine liver GSH-*t* and 2 min for CDNB/GSH conjugation). The inhibitors might have been activated by reacting with GSH while GSH-dependent fenitrothion degradation was inhibited for a long period.

2. *Time-Dependent Changes in the Amount of the GSH Sulfhydryl Group and in the Inhibitory Activity of SCPs*

In order to elucidate the activation of SCPs with GSH, time-course changes in the amount of the GSH sulfhydryl group were investigated in the absence of enzyme. In the phosphate buffer (pH 8.0), GSH changed slowly into an oxidized form (3.9% 60 min after dissolution), as shown in Fig. 2. The addition of SCPs (50 μM, equivalent to GSH) rapidly decreased the sulfhydryl group in GSH. The depleting ability was in decreasing order in MTBO, K-2, K-1, salioxon and salithion. This order is well correlated with their hydrolytic rates.[16] Since their partially hydrolyzed products, *i.e.*, 2-hydroxybenzyl phosphates, are known to be effective alkylating agents,[17,18] these facts suggest that SCPs react with the sulfhydryl group of GSH to produce GSH-*t* inhibitors.

Standing mixtures of GSH and SCPs in phosphate buffer were then tested for inhibitory activity against the CDNB/GSH conjugation. Figure 3 shows the effect of preincubation time on the inhibitory activity of K-1 and K-2 in the presence of GSH against 3-Y_F abdominal GSH-*t*. The similar time-dependent increase of inhibitory activity was also observed in the cases of SRS abdominal and equine liver GSH-*t*. Salithion, salioxon, and MTBO similarly increased inhibitory activity when preincubated with GSH, but there was no change in the absence of GSH. A possibility of GSH-*t* inhibition owing to GSH depletion by SCPs is not important, if any, because

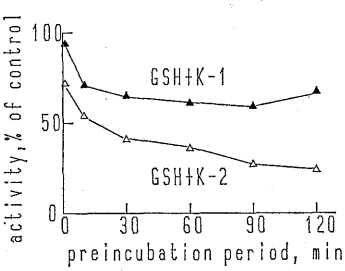


Fig. 3 Relationship between the activity of CDNB/GSH conjugation with 3-Y_F abdominal GSH-*t* and the nonenzymatic preincubation time of mixture of GSH (5 mM at a preincubation time and further 1 ml of 10 mM solution when CDNB added) and K-1 or K-2 (0.1 mM).

further addition of GSH after preincubation increased the inhibitory activity of SCPs as well.

3. *GSH-t Inhibitive Conjugate from SCPs and GSH*

A preincubated mixture containing GSH and SCPs without enzyme was subjected to TLC using *n*-BuOH/AcOH/H_2O (4/1/2) as a developing solvent. K-2 gave five products besides starting materials as shown in Fig. 4. The main product (Rf=0.60) absorbed UV light and was positive to ninhydrin and diazotized sulfanilic acid reagents, but negative to an acid molybdate reagent. The reaction of GSH and other SCPs were similar in chromatograms, and the main products from every SCP and GSH gave the same Rf value. Based on these results, the main products were presumed as *S*-(2-hydroxybenzyl)glutathione. Then the main product was purified by gel-filtration and recrystallization (see Materials and Methods for details). The negative FAB MS showed two main peaks other than those owing to the glycerol matrix: One 306 m/z, negative ion of GSH ($(GSH-1)^-$) and the other 412 m/z ($(M-1)^-$ in Fig. 5). These results support that the main conjugative product of SCPs with GSH is *S*-(2-hydroxybenzyl) glutathione (M_w=413.4). The formation scheme of the GSH-conjugate is proposed in Fig. 6.

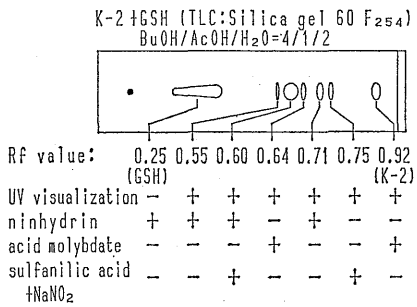


Fig. 4 TLC analyses of products from salithion and GSH.

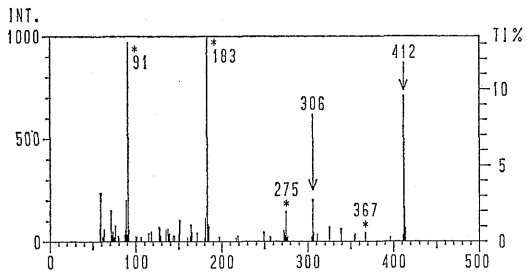


Fig. 5 Negative FAB MS of the major product isolated through from nonenzymatic reaction of K-2 and GSH.

The peaks with asterisk were attributable to the glycerol matrix.

The conjugate inhibited GSH-*t* (Table 3) and its inhibitory activity did not change, in contrast with SCPs, even after the preincubation with GSH prior to enzymatic CDNB/GSH conjugation by equine liver GSH-*t*, as shown in Fig. 7. This supports the suggestion that SCPs inhibit GSH-*t* after conjugating with GSH. *S*-(2-Hydroxybenzyl)glutathione inhibited CDNB/GSH conjugation with SRS and 3-Y_F fly abdominal and equine liver GSH-*t* by 11.6, 10.0 and 49.2% of control respectively. Table 3 also shows that the GSH-dependent degradation of fenitrothion was inhibited approximately 90% by the conjugates from all three GSH-*t* sources.

The inhibition of GSH-*t* by the glutathione conjugate must be attributed to the effective-

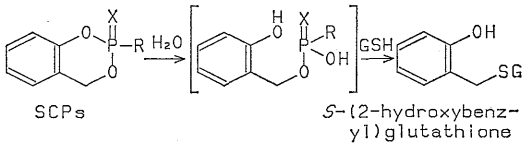


Fig. 6 Proposed formation scheme of the glutathione conjugate from SCPs.

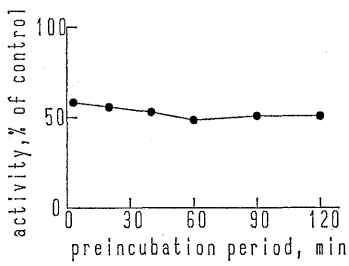


Fig. 7 Relationship between the activity of CDNB/GSH conjugation with equine GSH-*t* and the preincubation time of GSH and *S*-(2-hydroxybenzyl)glutathione under the same conditions as Fig. 3.

Table 3 Effect of *S*-(2-hydroxybenzyl)glutathione on CDNB/GSH conjugation GSH-dependent degradation of ^{14}C-fenitrothion by fly abdominal or equine liver GSH-*t*.[a)]

SCPs	% of control	
	CDNB/GSH conjugation	GSH-dependent degradation of fenitrothion
Housefly (SRS)	11.6	11.0
Housefly (3-Y_F)	10.0	8.8
Equine liver	49.2	12.9

a) See Tables 1 and 2 for enzyme activities of the control and inhibitory activity of SCPs.

ness of salithion against OP-resistant insects, and to the synergistic effect of K-1 or K-2 with OP-insecticides against resistant insects. Some glutathione derivatives or its conjugates, *e.g.*, *S*-(*n*-hexyl)glutathione,[19-23] *S*-(tridiphane)glutathione,[20,25] and *S*-((2-(4-phenylbenzoyl)-1-phenyl)ethyl)glutathione,[24] are known to inhibit GSH-*t*. GSH-*t* inhibition by *S*-benzyl glutathione[19,20] and its *p*-bromo derivative[19,21,22] have been reported.

4. *Kinetic Characteristics of CDNB/GSH Conjugation by GSH-t and Its Inhibition*

The affinity of GSH-*t* against two substrates, CDNB and GSH, was determined first in the absence of inhibitors. The reaction rates plotted against substrates with equine liver GSH-*t* are shown in Fig. 8, and kinetic parameters of CDNB/GSH conjugation by three kinds of GSH-*t* are summarized in Table 4. All of the enzymes had higher affinity to CDNB than to GSH. The *Km* values against GSH were not significantly different between two housefly strains, but the affinity ($1/Km$) of 3-Y_F GSH-*t* to CDNB was 13.6-fold higher than that of SRS GSH-*t*. The affinity ratios to CDNB and GSH (Km[GSH]/Km[CDNB]) were 12 and 192 with SRS and 3-Y_F GSH-*t*, respectively.

The Lineweaver-Burk plots of CDNB/GSH conjugation with equine liver GSH-*t* at various concentrations of *S*-(2-hydroxybenzyl)glutathione are shown in Fig. 9. At a fixed concentration of GSH (Fig. 9a), *S*-(2-hydroxybenzyl)glutathione was not competitive with

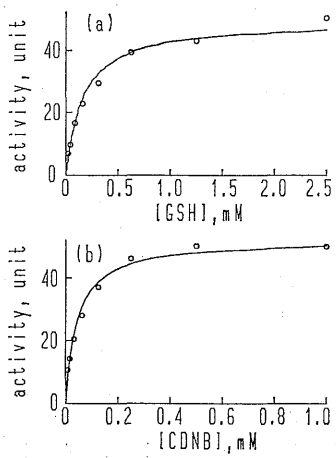


Fig. 8 Relationship between the activity of CDNB/GSH conjugation and the concentration of GSH (a) and CDNB (b).

Km[GSH]=162.3±21.0 μM, V_{max}[GSH]=49.49±2.38 unit,* Km[CDNB]=43.29±5.70 μM, and V_{max}[CDNB]=52.38±2.31 unit.* *One unit means 1 nmol conjugation·min^{-1}·mg protein^{-1}.

Table 4 Michaelis constants and maximum velocity of CDNB/GSH conjugation by fly abdominal or equine liver GSH-*t*.

Constant	Fly (SRS)	Fly (3-Y_F)	Equine
GSH			
Km, μM	615.3±178.1	720.6±267.9	162.3±21.0
V_{max}, unit[a)]	287.5± 40.0	732.9±104.6	49.5± 2.3
CDNB			
Km, μM	50.5± 12.4	3.7± 1.0	43.9± 5.6
V_{max}, unit[a)]	297.5± 25.0	437.7± 26.7	52.4± 2.3

a) One unit is 1 nmol conjugation·min^{-1}·abdomen^{-1} for housefly GSH-*t* and 1 nmol conjugation·min^{-1}·μg protein^{-1} for equine liver GSH-*t*.

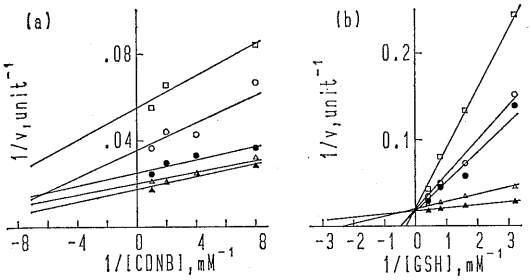


Fig. 9 Lineweaver-Burk plots of CDNB/GSH conjugation with equine liver GSH-*t* inhibited by *S*-(2-hydroxybenzyl)glutathione as function of CDNB (a, [GSH]=2.5 mM) and GSH (b, [CDNB] =1.0 mM). ▲: 0 μM, △: 6.25 μM, ●: 25 μM, ○: 50 μM and □: 100 μM. One unit means 1 nmol conjugation·min^{-1}·mg protein^{-1}.

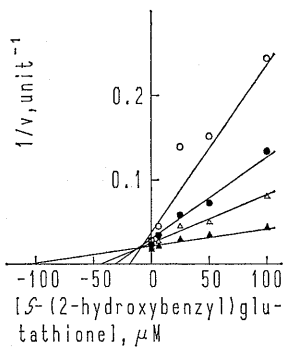


Fig. 10 Dixon plot of CDNB/GSH conjugation with equine liver GSH-*t* inhibited by *S*-(2-hydroxybenzyl)glutathione as a function of GSH concentration.

▲: 0.3125 mM, △: 0.625 mM, ●: 1.25 mM, and ○: 2.5 mM.

CDNB. As seen in Fig. 9b, however, the conjugate is a competitive inhibitor with GSH. The Dixon plot is presented in Fig. 10, where the data from Fig. 9b is horizontally replotted with the concentrations of the inhibitor. The intersection point in the Dixon plot gives a K_i value, whose reciprocal indexes the affinity of an inhibitor to an enzyme. In the present case, the K_i value of *S*-(2-hydroxybenzyl)-glutathione against equine liver GSH-*t* was 11.0 μM, indicating that the affinity of the conjugate to the enzyme is much higher than that of GSH and CDNB (see *Km* values in Table 4). The K_i values of *S*-(tridiphane) glutathione for CDNB/GSH conjugation against GSH-*t* from giant foxtails, corn,[20] and houseflies (susceptible 2168 strain)[25] have been reported to be 2, 8 and 0.2 μM, respectively. The inhibitory kinetics of the *S*-(2-hydroxybenzyl) glutathione with fly abdominal GSH-*t* could not be measured accurately because nonlinearity owing to certain components in the crude enzyme preparations interfered with enzymatic kinetics.

It has been known that SCPs inhibit several enzymes in some manners. Enzymes with a serine hydroxyl group as an active center, such as acetylcholinesterase, are attacked by phosphorus atom in SCPs, Pearson's hard acid, and enzymes with a cysteine sulfhydryl group such as papain and alcohol dehydrogenase are inactivated by reaction of the sulfhydryl group with the benzyl carbon, the soft acid.[16] In our study SCPs reacted with the sulfhydryl group in GSH, and competitive inhibition of GSH-*t* by the resulted glutathione conjugate was demonstrated. This conjugate is regarded as a kind of transition-state analogs, which may contribute to its high affinity to the enzyme.[26] SCPs are interesting chemicals from the viewpoints of pesticide biochemistry and physiology.

ACKNOWLEDGMENTS

The authors are indebted to Dr. M.-Y. Eto of Himeji College of Hyogo for the gift of MTBO, to Dr. T. Shono for supplying two strains of houseflies, and to Mr. R. Isobe in Faculty of Pharmaceutical Science of this university for FAB MS spectrometry.

REFERENCES

1) T. Shiotsuki & M. Eto: *J. Pesticide Sci.* **12**, 17 (1987)
2) A. Kudamatsu, T. Sato, A. Hayashi & R. Kano: *Jpn. J. Sanit. Zool.* **30**, 255 (1979) (in Japanese)
3) M. Kuwahara: *Jpn. J. Appl. Entomol. Zool.* **30**, 290 (1986) (in Japanese)
4) T. Shiotsuki, R. Takeya & M. Eto: *Agric. Biol. Chem.* **51**, 1851 (1987)
5) M. Eto, Y. Oshima, S. Kitakata, F. Tanaka & K. Kojima: *Botyu-Kagaku* **31**, 33 (1965)
6) Y. Takahashi, T. Saito, K. Iyatomi & M. Eto: *Botyu-Kagaku* **38**, 13 (1973)
7) Y. Konno & T. Shishido: *J. Pesticide Sci.* **10**, 285 (1985)
8) Y. Konno, T. Shishido & F. Tanaka: *J. Pesticide*

Sci. **11**, 393 (1986)
9) F. J. Oppenoorth & S. Voremann: *Pestic. Biochem. Physiol.* **5**, 431 (1975)
10) T. Shiotsuki, R. Takeya, M. Eto & T. Shono: *Agric. Biol. Chem.* **52**, 2191 (1988)
11) M. Eto and Y. Oshima: *Agric. Biol. Chem.* **26**, 452 (1962)
12) M. Frankel, D. Gartner, H. Jacobson & A. Zilkha: *J. Chem. Soc.* **1960**, 1390
13) J. Booth, E. Boyland & P. Sims: *Biochem. J.* **79**, 516 (1961)
14) K. Yamaoka, Y. Tanigawara, T. Nakagawa & T. Uno: *J. Pharmacobio-Dyn.* **4**, 879 (1981)
15) G. L. Ellman: *Arch. Biochem. Biophys.* **82**, 70 (1959)
16) M. Eto: *J. Environ. Sci. Health* **B18**, 119 (1983)
17) H. Ohkawa & M. Eto: *Agric. Biol. Chem.* **33**, 443 (1969)
18) H. Ohkawa, S. Maruo & M. Eto: *J. Fac. Agric. Kyushu Univ.* **17**, 13 (1972)
19) P. Askelof, C. Guthenberg, I. Jacobson & B. Mannervik: *Biochem. J.* **147**, 513 (1975)
20) G. L. Lamoureux & D. G. Rusness: *Pestic. Biochem. Physiol.* **26**, 323 (1986)
21) M. Warholm, C. Guthenberg & B. Mannervik: *Biochemistry* **22**, 3610 (1983)
22) M. K. Tahir, C. Guthenberg & B. Mannervik: *FEBS Lett.* **181**, 249 (1985)
23) L. K. Ong & A. G. Clark: *Biochem. Pharmacol.* **35**, 651 (1986)
24) T. Miyamoto, M. Silva & B. D. Hammock: *Arch. Biochem. Biophys.* **254**, 203 (1987)
25) G. L. Lamoureux & D. G. Rusness: *Pestic. Biochem. Physiol.* **27**, 318 (1987)
26) R. N. Lindquist: "Drug Design," ed. by E. J. Ariens, Academic Press, Inc., New York, pp. 23–80, 1975

要　　約

グルタチオンとの抱合活性化によるサリゲニン環状リン酸エステル類のグルタチオントランスフェラーゼ阻害

塩月孝博，小磯彰宏，江藤守総

サリゲニン環状リン酸エステル類 (SCPs) のグルタチオン *S*-トランスフェラーゼ (GSH-*t*) の阻害様式について検討した．SCPs それ自身ではそれほど高い阻害活性を有していないが，非酵素的にグルタチオン (GSH) と反応することにより GSH-*t* の阻害活性が増大した．この活性本体は，TLC ならびに FAB MS による分析の結果，*S*-(2-hydroxybenzyl) glutathione であることがわかった．GSH-*t* 阻害の速度論的解析から，この抱合体はグルタチオンと拮抗していることがわかった．サリチオンが有機リン殺虫剤抵抗性昆虫に有効であること，これらの系統に K-1，K-2 がマラチオン，フェニトロチオンの共力作用を示すことに，この GSH-*t* の阻害が寄与しているものと推測される．