Biochemical Pharmacology, Vol. 38, No. 18, pp. 2977–2983, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00
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GLUTATHIONE CONJUGATION OF NITRO COMPOUNDS BY MONKEY GLUTATHIONE *S*-TRANSFERASES

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(*Received* 12 *September* 1988; *accepted* 12 *January* 1989)

Abstract—The distribution in Japanese monkey tissues of glutathione *S*-transferase activity toward some aromatic nitro compounds was examined by measuring the release of the nitro group as nitrite ion. The activity was especially high in liver, kidney and small intestine when compounds such as 4-nitroquinoline *N*-oxide, 5-nitrofurfural diacetal and *o*-dinitrobenzene were used as substrates. The nitrite-releasing activity of the major enzyme purified from rhesus monkey liver was also tested on fifty-two nitro compounds including nineteen nitrofuran derivatives. Among the thirty-three nitro compounds other than the nitrofuran derivatives tested as substrates, the purified enzyme showed activity only toward *o*-dinitrobenzene, 4-nitroquinoline *N*-oxide, 3,4-dinitrobenzoic acid, *p*-dinitrobenzene, 2,5-dinitrobenzoic acid, 2,5-dinitrophenol, *tetra*-chloronitrobenzene and 2,4-dinitrobenzoic acid. The crude supernatant fraction of rhesus monkey liver showed activity in substrate specificity roughly similar to that of the purified enzyme. On the other hand, among at least ten carcinogenic 2-substituted 5-nitrofran derivatives tested, 4,6-diamino-2-(5-nitro-2-furyl)-*s*-triazine, 5-nitro-2-furaldehyde semicarbazone, *N*-[[3-(5-nitro-2-furyl)-1,2,4-oxadiazol-5-yl]methyl] acetamide, and *N*-[5-(5-nitro-2-furyl)-1-3,4-thiadiazol-2-yl)acetamide were shown to be enzymatically conjugated with reduced glutathione. Among the other nine 2-substituted 5-nitrofuran derivatives tested, six compounds could be the substrates of the enzyme, and 5-nitrofurfural and 5-nitrofurfural diacetal were especially good substrates. There was, however, little apparent correlation between their carcinogenicity and susceptibility to glutathione *S*-transferase. The bulky substituents at position 2 appeared to decrease the susceptibility of these nitrofuran derivatives to the enzyme. Both V_{max} and K_m values of the purified enzyme varied greatly among the substrates, and the optimum pH fell between 7.5 and 9.0 in most cases.

Nitro compounds are mostly cytotoxic. Some 4-nitroquinoline *N*-oxide derivatives are known to be carcinogenic [1]. It was further suggested that the 4-nitro group as well as the *N*-oxide group was necessary for this activity and that the carcinogenic action was due to the reaction of the nitro group with sulfhydryl compounds [2]. Some of the nitro compounds are used as antimicrobial drugs, and their effectiveness depends largely on the presence of the nitro group [3]. Many nitrofuran derivatives are used as drugs and as additives to human and animal foods [4, 5]. Several nitrofuran derivatives, however, have been found to be carcinogenic, mutagenic and/or DNA-damaging [6]. They are thought to be detoxicated *in vivo* by several detoxication mechanisms. Among them, reduced glutathione (GSH) conjugation seems to be one of the most important reactions, in which the nitro group is predominantly liberated as nitrite ion by the action of glutathione *S*-transferase. Previously, Al-Kassab *et al.* [7] investigated the glutathione conjugation of some nitro compounds using rat liver supernatant fraction and suggested that 4-nitroquinoline *N*-oxide and 4-nitropyridine *N*-oxide were conjugated with GSH by glutathione *S*-transferase. Further, Boyland and Speyer [8] investigated the reaction between some 2-substituted 5-nitrofuran derivatives and GSH, mainly using rat liver supernatant fraction. However, not much as yet has been discovered about the action spectrum of this enzyme toward various nitro compounds. Studies along this line are necessary to evaluate the cytotoxicity of these compounds, as well as to elucidate the characteristics of their detoxication *in vivo*.

In this study, we investigated (a) the distribution of glutathione *S*-transferase activity toward various nitro compounds in monkey tissues, and (b) the action of a purified monkey liver glutathione *S*-transferase toward various nitro compounds including carcinogenic substances.

MATERIALS AND METHODS

Preparation of crude and purified samples of monkey glutathione S-*transferase.* Adult Japanese monkeys (*Macaca fuscata*) and rhesus monkeys (*Macaca mulatta*) were obtained from the Monkey Care Laboratory at the Primate Research Institute, Kyoto University. Monkeys were killed by exsanguination after anesthetization with a minimal dose of Ketalar® (Sankyo Co.), and tissues were removed immediately and stored frozen at −80° until used.

All further procedures were performed at 0–4°. Cytosol fractions of various tissues were prepared as follows. Each monkey tissue was homogenized with 4 vol. (v/w) of 0.01 M sodium phosphate buffer, pH 7.4, containing 0.25 M sucrose in a Potter–Elvehjem type teflon homogenizer. After centrifugation of the homogenate for 1 hr at 105,000 *g*,

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the supernatant fraction was used as a cytosol enzyme fraction. The major isozyme (Fraction IV) of monkey glutathione *S*-transferases was purified to homogeneity from rhesus monkey liver as described previously [9, 10]. This isozyme appears to most closely resemble glutathione *S*-transferase δ, the major isozyme in human liver [11], in substrate specificity and some other properties. The crude liver supernatant fraction used for comparison (see Table 2) was obtained by homogenizing the liver with 3 vol. (v/w) of 0.1 M sodium phosphate buffer, pH 7.5, followed by centrifugation at 30,000 *g*.

Nitro compounds. Most of the nitrofuran derivatives used and 4-nitroquinoline *N*-oxide were supplied by Dr Takashi Sugimura (National Cancer Center, Tokyo). Fenitrothion (Sumithion®) was a gift from the Sumitomo Chemical Co. Ltd (Osaka, Japan). The following reagents were purchased from the sources indicated: GSH and chloramphenicol from Boehringer Mannheim (Mannheim, F.R.G.); 1,2-dichloro-4-nitro-benzene, 1-chloro-2,4-dinitrobenzene, 3,4-dinitrobenzoic acid, 4-chloro-3,5-dinitrobenzoic acid, *tetra*-chloronitrobenzene, 4-nitropyridine-1-oxide, 5-nitrofurfural diacetal and 5-nitrofurfural from the Tokyo Kasei Kogyo Co. (Tokyo, Japan); 1-nitropropane, 2-nitropropane, 2,4,6-trinitrophenol and flavianic acid from Nakarai Chemicals (Tokyo, Japan); *N*-(1-naphthyl)ethylenediamine dihydrochloride, sulfanilamide and the other nitro compounds from Wako Pure Chemicals Ind. (Tokyo, Japan). Other reagents were of the highest grade available and were used without further purification.

Measurement of enzyme activity. Enzyme activity was determined with each nitro compound and GSH as substrates by measuring the release of the nitro group as inorganic nitrite, as described previously [7] with slight modifications [12]. To determine kinetic parameters for various substrates, the substrate concentration was varied from 0.05 to 1 mM. The enzyme assay was performed at 25° and pH 7.0 at 5 mM GSH according to the standard assay, unless otherwise specified. The protein concentration was determined by the method of Lowry *et al.* [13], using bovine serum albumin as a standard.

RESULTS

When glutathione *S*-transferase activity in the cytosolic fractions of Japanese monkey tissues was measured using various nitro compounds as substrates, the results shown in Table 1 were obtained. Among the tissues tested, the soluble fractions of liver, kidney and small intestine markedly catalyzed the conjugation of the nitro compounds with GSH, and had especially high activity toward 4-nitroquinoline *N*-oxide. In most cases, the activities toward 4-nitroquinoline *N*-oxide, 5-nitrofurfural diacetal, and *o*-dinitrobenzene were highest in liver. No marked sexual difference in the transferase activity was observed for the tissues examined or for the substrates tested. Therefore, only the average values are shown in Table 1. The activities toward the four nitrofuran derivatives (Table 1), i.e. 4,6-diamino-2-(5-nitro-2-furyl)-*s*-triazine, furylfuramide, 4-(5-nitro-2-furyl)thiazole, and 5-nitro-2-furaldehyde semicarbazone were generally low and rather similar among the tissues tested.

The nitrite-releasing activities toward various nitro compounds of the purified enzyme and the supernatant fraction of a crude extract of rheusus monkey liver are compared in Table 2. The purified enzyme showed activity toward only eight compounds of the thirty-one compounds tested. The liver supernatant fraction showed activity in substrate specificity roughly similar to that of the purified enzyme. The activity of the purified enzyme toward *tetra*-chloronitrobenzene, however, was markedly lower than that of the supernatant fraction. In contrast with the purified enzyme, the liver supernatant fraction showed activities toward 4-nitropyridine *N*-oxide, 4-chloro-3,5-dinitrobenzoic acid and nitroethane. In the absence of GSH, the supernatant fraction also catalyzed, to some extent, the nitrite liberation from *o*-dinitrobenzene, 3,4-dinitrobenzoic acid, *tetra*-chloronitrobenzene and nitroethane. On the other hand, neither the purified enzyme nor the liver supernatant fraction showed nitrite-releasing activity toward 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene and 4-chloro-3,5-dinitrobenzoic acid although the enzymatic conjugation of these compounds was detected in a spectrophotometric assay [9]. The purified monkey enzyme had no activity toward nitroalkyl compounds.

We also examined the activity of the purified liver enzyme toward various nitrofuran derivatives (Table 3). Among the nineteen 2-substituted 5-nitrofuran derivatives tested, compounds 3, 4, 8, 10–14, and 16 are known to be carcinogenic *in vivo* [6]. Among these, compounds 3, 4, 8 and 10 were shown to be enzymatically conjugated with GSH. However, the enzyme was not active toward the other five carcinogenic compounds tested. Among the other ten nitrofuran derivatives tested, six compounds could be the substrates for the enzyme and four compounds were not. 5-Nitrofurfural and 5-nitrofurfural diacetal, in particular, were good substrates.

Table 4 shows K_m and V_{max} values of the purified enzyme toward some nitro compounds. Among the compounds tested, the K_m values varied about 160-fold, whereas the V_{max} values varied about 83,000-fold. Among the K_m values, that for 5-nitrofurfural diacetal was notably high. Although the above experiments were performed at pH 7.0, where the substrates are stable, the optimum pH of the enzyme varied somewhat. In most cases it fell between pH 7.5 and 9.0 (Fig. 1). 2,5-Dinitrophenol and *tetra*-chloronitrobenzene, however, had exceptionally low pH optima at about 6.0. The pH dependence of the non-enzymatic reaction in the presence of GSH is also shown in Fig. 1. The reactivity was almost zero at pH 7.0 and increased markedly as the pH was increased.

DISCUSSION

As described in the preceding section, among monkey tissues, the activity of GSH conjugation of nitro compounds was high in liver, kidney, and small intestine and, in most cases, the activities toward 4-nitroquinoline *N*-oxide, 5-nitrofurfural diacetal, and *o*-dinitrobenzene were highest in liver. These distri-

Table 1. GSH conjugation activity of cytosolic fractions of various tissues of Japanese monkeys on some nitro compounds

Substrate	GSH conjugation activity*				
	Liver	Kidney	Small intestine†	Stomach†	Lung
4-Nitroquinoline *N*-oxide	33,000 ± 7100 (470 ± 89)	28,000 ± 2700 (590 ± 160)	16,000 ± 4700 (490 ± 63)	12,000 ± 5800 (350 ± 190)	7700 ± 1800 (150 ± 32)
5-Nitrofurfural diacetal	6800 ± 910 (99 ± 9.1)	1300 ± 250 (26 ± 2.7)	2800 ± 1400 (71 ± 24)	590 ± 290 (16 ± 6.8)	130 ± 31 (2.6 ± 0.8)
o-Dinitrobenzene	7000 ± 330 (100 ± 5.6)	760 ± 130 (16 ± 2.2)	1200 ± 580 (34 ± 14)	240 ± 110 (6.7 ± 2.5)	670 ± 160 (14 ± 3.3)
4,6-Diamino-2-(5-nitro-2-furyl)-*s*-triazine	5.5 ± 1.8 (0.08 ± 0.03)	7.7 ± 1.2 (0.20 ± 0.07)	3.4 ± 1.9 (0.12 ± 0.06)	7.2 ± 3.1 (0.22 ± 0.12)	5.4 ± 2.1 (0.12 ± 0.06)
Furylfuramide (AF-2)	5.1 ± 0.6 (0.08 ± 0.01)	10 ± 2.2 (0.21 ± 0.06)	7.6 ± 0.6 (0.25 ± 0.10)	5.8 ± 3.0 (0.15 ± 0.06)	6.7 ± 2.0 (0.16 ± 0.04)
4-(5-Nitro-2-furyl)thiazole	3.9 ± 0.8 (0.06 ± 0.01)	3.7 ± 1.7 (0.08 ± 0.05)	2.4 ± 1.0 (0.08 ± 0.06)	2.6 ± 1.4 (0.07 ± 0.03)	2.7 ± 1.2 (0.06 ± 0.03)
5-Nitro-2-furaldehyde semicarbazone (Nitrofurazone)	6.5 ± 1.7 (0.10 ± 0.02)	7.3 ± 1.1 (0.16 ± 0.05)	11 ± 0.7 (0.36 ± 0.14)	6.6 ± 2.9 (0.19 ± 0.06)	14 ± 1.2 (0.30 ± 0.05)

* Means ± SD of six separate assays for three males and three females are shown. Activity is expressed as nmol per min per g of wet tissue weight (and in parentheses as nmol per min per mg of protein).

† The mucosa was used.

Table 2. GSH conjugation activity of purified glutathione *S*-transferase and crude supernatant fraction of rhesus monkey liver on some nitro compounds

Compound*	Purified enzyme†‡ (nmol/min/mg protein)	Supernatant fraction†§ (nmol/min/mg protein)	Non-enzymatic reaction§ (nmol/min)
o-Dinitrobenzene	1300	68 (0.02)	0.07 (0.001)
4-Nitroquinoline *N*-oxide	630	ND‖ (ND)	4.6 (0.001)
3,4-Dinitrobenzoic acid	550	120 (0.04)	2.7 (0)
p-Dinitrobenzene	95	2.8 (0)	0.001 (0.001)
2,5-Dinitrobenzoic acid	13	0.2 (0)	0.01 (0.001)
2,5-Dinitrophenol	9.9	0.6 (0)	0.003 (0.001)
tetra-Chloronitrobenzene	6.2	59 (0.02)	0.007 (0)
2,4-Dinitrobenzoic acid	2.5	0.1 (0)	0.003 (0)
4-Nitropyridine *N*-oxide	0	0.09 (0)	0.05 (0.003)
4-Chloro-3,5-dinitrobenzoic acid	0	0.08 (0)	0.001 (0.001)
Nitroethane	0	0.05 (0.06)	0.001 (0.001)

* No significant liberation of nitrite ion was detected from the following compounds either enzymatically or non-enzymatically: chloramphenicol, 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, 2,4-dichlorophenyl-4-nitrophenyl ether, *m*-dinitrobenzene, 2,2′-dinitrodiphenyl, 4,4′-dinitrodiphenyl, 2,4-dinitronaphthol, 2,4-dinitrophenol, *O*,*O*-dimethyl-*O*-(3-methyl-4-nitrophenyl)thiophosphate (fenitrothion, Sumithion®), flavianic acid, *p*-nitroaniline, nitrobenzene, *p*-nitrobenzoic acid, nitromethane, α-nitronaphthalene, *o*-nitrophenol, *p*-nitrophenol, 1-nitropropane, 2-nitropropane, 2-nitro-*p*-xylene, and 2,4,6-trinitrophenol.

† Values were corrected for the non-enzymatic liberation of nitrite ion from each compound in the presence or absence of GSH.

‡ No significant activity was detected in the absence of GSH.

§ Values in parentheses are those obtained in the absence of GSH.

‖ ND, not determined.

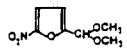
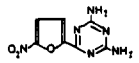
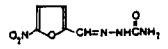
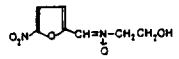
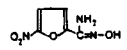
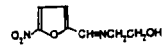
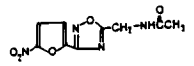
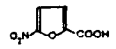
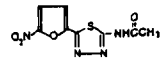
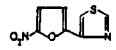
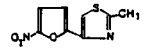
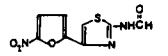
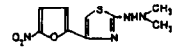
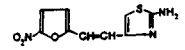
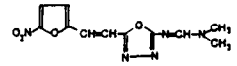
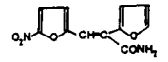
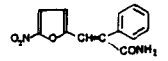
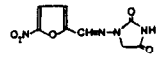
bution spectra roughly resemble those of the activities toward 1,2-dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene as observed previously [9, 14]. However, the activity per protein weight of monkey lung toward benz[*a*]anthracene-5,6-oxide has been reported to be higher than that of liver and kidney [15]. This difference may reflect the different composition of the glutathione *S*-transferase isozymes in each tissue.

The liver supernatant fraction showed activity in substrate specificity roughly similar to the purified enzyme. The activity toward *tetra*-chloronitrobenzene of the purified enzyme, however, was apparently markedly lower than that of the supernatant fraction. This may indicate the presence in the monkey liver supernatant of another isozyme(s) that is active toward this compound to liberate nitrite ions. The liver supernatant fraction showed activity toward 4-nitropyridine *N*-oxide, 4-chloro-3,5-dinitrobenzoic acid and nitroethane, whereas the purified enzyme did not. Further, in the absence of GSH, the supernatant fraction also catalyzed to some extent the nitrite liberation from *o*-dinitrobenzene, 3,4-dinitrobenzoic acid, *tetra*-chloronitrobenzene and nitroethane. A small amount of some other isozyme(s) may be present in the supernatant fraction that catalyzes the release of nitrite ions from these compounds.

On the other hand, neither the liver supernatant fraction nor the purified enzyme showed nitrite-releasing activity toward 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene and 4-chloro-3,5-dinitrobenzoic acid which were shown to be enzymatically conjugated with GSH by a spectrophotometric assay [9]. This indicates that the enzyme catalyzes the nucleophilic displacement of the halogeno group of these compounds rather than that of the nitro group with GSH. The lack of activity toward nitroalkyl compounds appears to indicate that the purified enzyme resembles the rat enzyme E more than the rat enzymes A, B and C [16].

The activity of the purified enzyme toward each compound is thought to reflect the intrinsic reactivity of the carbon atom to which a nitro group is bound as well as the degree of the effective interaction for catalysis of the enzyme with the compound. Thus, the degrees of susceptibility to the enzyme of the nitro compounds tested (Table 2) are roughly compatible with those expected from their structures and, hence, reactivities. For instance, among the dinitro compounds tested, *o*-dinitrobenzene and 3,4-dinitrobenzoic acid, which show marked non-enzymatic reactivities with GSH, were especially good substrates. The non-enzymatic reactivities of these compounds are thought to be attributable to their destabilization due to steric repulsion between the two adjacent nitro groups. The higher non-enzymatic reactivity of 3,4-dinitrobenzoic acid compared to *o*-dinitrobenzene may be due partly to the presence of the electron-drawing carboxyl group at position 1, which would further stabilize the reaction intermediate and thus facilitate the reaction. However, the enzymatic activity toward 3,4-dinitrobenzoic acid was lower than that toward *o*-dinitrobenzene. This was due presumably to the difference in the degree of effective interaction of the enzyme with these compounds. On the other hand, the hydroxyl group at position 1, such as in 2,5-dinitrophenol, is expected to rather reduce the non-enzymatic reactivity due to its electron-donating property. The lack in enzymatic

Table 3. GSH conjugation activity of purified rhesus monkey liver glutathione *S*-transferase toward some nitrofuran derivatives

	Compound	Structure	Purified enzyme (nmol/min/mg of protein)	Non-enzymatic reaction (nmol/min) GSH +	GSH −	Carcinogenicity [6]*
1	5-Nitrofurfural		14000	11	0.29	ND
2	5-Nitrofurfural diacetal		1600	2.7	0.087	ND
3	4,6-Diamino-2-(5-nitro-2-furyl)-s-triazine		170	0.059	0.058	+
4	5-Nitro-2-furaldehyde semicarbazone (Nitrofurazone)		92	0.016	0.005	+
5	2-(5-Nitro-2-furfurylidene) amino ethanol N-oxide		79	0.019	0.009	ND
6	5-Nitro-2-furamidoxime		41	0.013	0.006	−
7	2-(5-Nitro-2-furfurylidene) amino ethanol		32	0.011	0.009	ND
8	N-{[3-(5-Nitro-2-furyl)-1,2,4-oxadiazol-5-yl]methyl}acetamide		31	0.45	0.34	+
9	5-Nitro-2-furoic acid		23	0.27	0.27	ND
10	N-[5-(5-Nitro-2-furyl)-1,3,4-thiadiazol-2-yl]acetamide		20	0.002	0.0002	+
11	4-(5-Nitro-2-furyl)thiazole		0	ND	ND	+
12	2-Methyl-4-(5-nitro-2-furyl)thiazole		0	ND	ND	+
13	N-[4-(5-Nitro-2-furyl)-2-thiazolyl]-formamide		0	ND	ND	+
14	2-(2,2-Dimethylhydrazino)-4-(5-nitro-2-furyl)thiazole		0	ND	ND	+
15	2-Amino-4-[2-(5-nitro-2-furyl)vinyl]-1,3-thiazole		0	ND	ND	ND
16	2-[(Dimethylamino)methylimino]-5-[2-(5-nitro-2-furyl)vinyl]-1,3,4-oxadiazole		0	ND	ND	+
17	2-(2-Furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2, Furylfuramide)		0	ND	ND	−
18	2-(2-Phenyl)-3-(5-nitro-2-furyl)-acrylamide		0	ND	ND	ND
19	1-[(5-Nitrofurfurylidene)amino]-hydantoin (Furadantin®, Nitrofurantoin)		0	ND	ND	−

*Mutagenicity tests with *Escherichia coli* were all positive except for Compounds 1 and 2 (not tested) and 9 (negative), and those with *Salmonella typhimurium*, all negative except for Compounds 1 and 2 (not tested); repair tests with *E. coli* and *S. typhimurium* were all positive except for Compounds 1 and 2 (not tested), and 7 and 9 (negative) [6].

reactivity of most other compounds tested is generally reasonable as judged from the low reactivity of their carbon atoms to which a nitro group is bound toward nucleophilic reagents such as GSH. The lack of susceptibility of 4-nitropyridine *N*-oxide was somewhat unexpected, since it has significant non-enzymatic reactivity with GSH. The enzyme may be unable to accommodate this compound as a substrate unlike 4-nitroquinoline *N*-oxide which has very high non-enzymatic reactivity.

Table 4. Kinetic parameters of rhesus monkey liver glutathione *S*-transferase toward various nitro compounds

Substrate	V_{max} (mol/min/mol enzyme)	K_m (mM)	V_{max}/K_m (/mM/min)
5-Nitrofurfural	400	4.0	100
o-Dinitrobenzene	230	2.0	120
5-Nitrofurfural diacetal	220	28	7.9
3,4-Dinitrobenzoic acid	51	1.1	46
p-Dinitrobenzene	16	2.3	7.0
2,5-Dinitrobenzoic acid	1.8	1.9	0.95
2,4-Dinitrobenzoic acid	0.17	0.65	0.26
tetra-Chloronitrobenzene	0.14	0.18	0.78
2,5-Dinitrophenol	0.0048	1.3	0.0037

The enzyme assay was performed at 25° in 0.1 M sodium phosphate buffer, pH 7.0, containing 5 mM GSH.

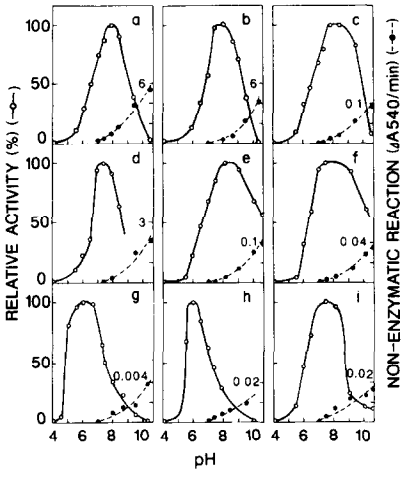


Fig. 1. Effect of pH on the activity of glutathione *S*-transferase toward various nitro compounds. Key: (a) 5-nitrofurfural; (b) 5-nitrofurfural diacetal; (c) *o*-dinitrobenzene; (d) 3,4-dinitrobenzoic acid; (e) *p*-dinitrobenzene; (f) 2,5-dinitrobenzoic acid; (g) 2,5-dinitrophenol; (h) *tetra*-chloronitrobenzene; and (i) 2,4-dinitrobenzoic acid. The activity was measured by the standard assay method at 1 mM substrate and 5 mM GSH except for a, b, and c (1 mM). The activity (—○—) is expressed as relative activity corrected for the reagent blank (maximal activity was taken as 100%). The reagent blank (--●--) (i.e. non-enzymatic reaction in the presence of GSH) is expressed in ΔA_{540}/min. The reagent blank was almost zero at pH 7.0.

The results (Table 3) obtained with various nitro furan derivatives using the purified enzyme also show that the degrees of susceptibility to the enzyme of compounds 1–14 roughly correlated with those expected from the structures and reactivities of these compounds. The enzymatic reactivities of compounds 1–10 are thought to be rather reasonable, as judged from their structures and non-enzymatic reactivities. The substituent groups at position 5 of compounds 1, 2, 5, 8, 9 and 10 have strong or relatively strong electron-drawing property whereas those of compounds 4 and 7 have weak electron-drawing property, and those of compounds 3 and 6, little electron-drawing property. The relatively high enzymatic reactivity of compound 3 may be due, in part, to the protonation of its amino group(s) upon binding to the enzyme. On the other hand, the lack of susceptibility to the enzyme of compounds 11–14 may be explained partly by the poor electron-drawing property of the thiazole ring. Further, the bulky substituent groups at position 2, especially in compounds 15–19 as well as compounds 8 and 10, appear to decrease the susceptibility of these nitrofuran derivatives to the enzyme. This is thought to be due mainly to the loss of effective interaction with the enzyme active site through steric hindrance.

The results also show that there is no apparent correlation between carcinogenicity and susceptibility to glutathione *S*-transferase. In the case of several 4-nitroquinoline *N*-oxide derivatives, their carcinogenicity was reported to parallel the extent of reaction with GSH [17]. In the case of these 2-substituted 5-nitrofuran derivatives, however, not only the reactivity of the carbon atom at position 5, which may be still potentially reactive, but also the nature of the substituent groups at position 2 may be important for carcinogenicity. It was reported [8] that rat liver supernatant fraction had little or no GSH-conjugating activity toward compounds 4, 13 and 19 in Table 3, whereas the activity was detected toward compounds 1, 2 and 9. Thus, the activity toward compound 4 (Nitrofurazone) is apparently different between monkey and rat. This difference may be due to species and/or isozyme differences.

The kinetic parameters in Table 4 show that the difference in the conjugation activity toward the nitro compounds used is due mainly to the difference in V_{max} values, which is thought to refect the difference in the electrophilicity of the carbon atom to which is attached the nitro group to be released by the enzyme. The high K_m value for 5-nitrofurfural diacetal is notable. This is probably due to the steric hindrance of the diacetal group which may hinder the effective binding of the compound to the enzyme. A rather broad range of pH optimum was also detected in rat [8, 16] and monkey [9]. However, in the case of 2,5-dinitrophenol and *tetra*-chlorodinitrobenzene, the optimum pH was markedly

lower (Fig. 1, g and h). The activity curve for 2,5-dinitrophenol seems to reflect the acid dissociation of this compound.

Acknowledgements—This study was supported, in part, by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan. We express our sincere thanks to Dr Takashi Sugimura of the National Cancer Center, Tsukiji, Tokyo, for the supply of nitrofuran derivatives and 4-nitroquinoline *N*-oxide. We also thank Dr Masayuki Yoshida of the University of Library and Information Science, Ibaraki, for helpful discussion, and Miss Keiko Uematsu for her expert technical assistance.

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