Biochemical Pharmacology, Vol. 38, No. 2, pp. 353–359, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

PROPERTIES OF THE MICROSOMAL AND CYTOSOLIC GLUTATHIONE TRANSFERASES INVOLVED IN HEXACHLORO-1:3-BUTADIENE CONJUGATION

FRANZ OESCH* and C. ROLAND WOLF†‡

*Institute of Toxicology, University of Mainz, Obere Zahlbacher Strasse 67, D-6500 Mainz, Federal Republic of Germany; and †Imperial Cancer Research Fund, Laboratory of Molecular Pharmacology and Drug Metabolism, University Department of Biochemistry, Hugh Robson Building, George Square, Edinburgh, Scotland, U.K.

(*Received* 4 *May* 1988; *accepted* 12 *September* 1988)

Abstract—Hexachloro-1,3-butadiene (HCBD) is a substrate for the hepatic microsomal glutathione transferases and is metabolised at higher rates by these enzymes than their cytosolic counterparts. Conjugation reactions catalysed by the microsomal and cytosolic transferases have been studied and characterized using this substrate and 1-chloro-2,4-dinitrobenzene (CDNB). In rat liver microsomes the K_m values for HCBD and CDNB were 0.91 and 0.012 mM and in cytosol 0.51 and 0.10 mM respectively. V_{max} values for HCBD were 1.39 and 0.35 nmol conjugate formed/min/mg protein for microsomes and cytosol respectively. In microsomal systems HCBD was a potent competitive inhibitor of the metabolism of CDNB with a K_i value of approximately 10 μM. However, CDNB did not inhibit HCBD metabolism significantly. These data suggest that more than one microsomal enzyme is involved in HCBD metabolism. The microsomal membrane could be solubilized without significant inhibition of HCBD activity; however, some detergents did inhibit the conjugation reaction. Activity was also lost on treatment of microsomal membranes with trypsin indicating the enzyme is localized on the cytoplasmic surface of the endoplasmic reticulum. Pretreatment of the rats with Aroclor 1254, 3-methylcholanthrene or phenobarbital did not change the microsomal conjugation of HCBD or CDNB with glutathione. Of seven species investigated, a human liver sample showed the highest ratio of microsomal to cytosolic glutathione transferase activity for HCBD (in microsomes 40-fold higher specific activity than in cytosol). Glutathione conjugation appears to play a critical role in the toxicity and carcinogenicity of some halogenated hydrocarbons. These data substantiate the potentially important role for the microsomal glutathione transferase in catalysing these reactions.

Initial studies on the microsomal glutathione transferase (GST) indicated that this activity was due to the presence of cytosolic enzymes [1–4]. However, subsequent isolation of a membrane bound protein with this activity clearly showed that the microsomal membrane contained a distinct GST [5, 6]. In early studies of this enzyme system the specific activity of membrane bound GST was always much lower than in cytosol and therefore the likely substrates, and indeed the biological importance of the microsomal conjugation of foreign compounds was uncertain. It is now clear, however, that certain polyhalogenated hydrocarbons are good substrates for the microsomal enzymes [8–10]. One of the first to be identified was the nephrotoxic and carcinogenic compound hexachloro-1,3-butadiene (HCBC) [9, 10]. This substrate is metabolised by rat liver microsomes more rapidly than by the cytosolic GST enzymes. The finding that glutathione conjugation of HCBD and a variety of other halogenated compounds is important in their toxic and carcinogenic effects suggests that the microsomal GST will play an important role in these reactions. In this study the microsomal and cytosolic GST involved in HCBD and 1-chloro-2,4-dinitrobenzene metabolism has been compared and significant differences in the properties of these enzymes towards both substrates are described. In addition, a large species difference in the relative activity of microsomal versus cytosolic fractions in HCBD metabolism were observed. Data is also presented which indicates that more than one microsomal GST enzyme is involved in the conjugation of HCBD.

‡ Author to whom correspondence should be addressed.

MATERIALS AND METHODS

Hepatic microsomes and cytosol from male Sprague–Dawley rats (200 g), ZEI mice (25 g), New Zealand White rabbits (2.5 kg, inbred strain), Nikisch guinea-pigs (300 g), Syrian golden hamsters (100 g), Chinese hamsters (40 g), Green monkey and human liver were prepared using conventional procedures [4]. 1.15% KCl buffered with 10 mM phosphate buffer pH 7.4 was used throughout the procedure. The microsomal pellet was washed twice with this buffer and either used fresh or stored at −70° as a suspension in 0.25 M sucrose in 10 mM phosphate buffer, pH 7.4 (protein concentration approx. 20 mg/ml). In some cases the rats were treated before use with phenobarbital, 3-methylcholanthrene or Aroclor 1254 as reported previously [12]. Glutathione transferases; Yb_1Yb_1, Yb_1Yb_2, Yb_2Yb_2, YaYc, were purified according to published procedures [13].

HCBD conjugation was measured in microsomal or cytosolic samples in 1 ml 0.1 M phosphate buffer pH 7.4, containing 1 mM EDTA (microsomal or cytosolic protein, 0.5 to 4 mg/ml or 1–10 mg/ml

Table 1. Kinetic parameters for microsomal and cytosolic glutathione transferase activity with hexachloro-1,3-butadiene (HCBD) and 1-chloro-2,4-dinitrobenzene (CDNB)

	Microsomes		Cytosol					
			K_m (mM)			V_{max} (nmol/min/mg)		
	K_m (mM)	V_{max} (nmol/min/mg)	a	b	T	a	b	T
HCBD								
HCBD-dependency	0.91	1.39	0.50	0.51	0.56	0.09	0.21	0.35
GSH-dependency	0.80	1.05		ND			ND	
CDNB								
CDNB-dependency	0.012	187	—	—	0.10	—	833	833
GSH-dependency	0.62	142	—	—	ND	—	ND	ND

For the determination of the rate dependency on the concentration of HCBD or CDNB the glutathione concentration was 4 mM. For the determination of the rate dependency on the concentration of glutathione the HCBD and CDNB concentrations were 4 mM and 1 mM respectively. Other incubation conditions are given in Materials and Methods.

K_m, apparent K_m value; a, HCBD metabolite R_f 0.12; b, HCBD metabolite R_f 0.33; T, total cytosolic metabolites; ND, not determined.

respectively) and glutathione (0.1–4 mM). The reaction was started by the addition of 0.4 μCi (^{14}C)hexachloro-1,3-butadiene as a solution in ethanol in concentrations ranging from 0.1 to 4 mM. Incubations were carried out for periods up to 60 min at 37°. Following incubation microsomal samples were sedimented by centrifugation at 37,000 g for 10 min. Metabolites in this supernatant or in cytosolic fractions were separated from unchanged substrate by thin layer chromatography (TLC) [9] on Whatman GF Silica plates using n-butanol : acetic acid : water (12:3:5) (v/v) as running solvent. The plates were scraped in 1 cm strips into scintillation vials and 1 ml water added followed 10 min later by 5 ml of Unisolve scintillation liquid. Radioactivity was determined using a Packard 460 liquid scintillation counter. In an alternative more rapid assay procedure the clear supernatant fraction obtained following centrifugation of the incubation medium was extracted twice with 5 ml n-hexane. The radioactivity present in the remaining aqueous phase was then determined. As an alternative to using (^{14}C)HCBD as substrate in some experiments (^{3}H)glutathione was used.

Other enzyme activities determined were cytochrome P-450 reductase [14], CDNB conjugation with glutathione [15] and microsomal epoxide hydrolase, using (^{3}H)styrene 7,8-oxide as substrate [16] with the described modifications [17], specifically in absence of detergent. Protein was determined by the method of Lowry *et al.* [18].

(^{14}C)HCBD (10 μCi/μmol) was a generous gift from Dr. E. A. Lock at Imperial Chemical Industries PLC. (^{3}H)Styrene 7,8-oxide was synthesized as reported previously [16]. All other chemicals were of reagent grade and of the highest purity commercially available.

RESULTS

Incubation of (^{14}C)HCBD with hepatic microsomes and glutathione resulted in the formation of a conjugate which had an R_f value on TLC of 0.33 [9]. In the present study an assay procedure by extraction was developed (see Materials and Methods) which proved to be much simpler than the quantitation of the conjugate after separation by TLC. The two methods gave similar values (deviations from the mean less than 10%). When ^{14}C-labelled HCBD was not available the microsomal activity could also be determined using (^{3}H)glutathione. However, in this case only the TLC procedure could be used. Using (^{3}H)glutathione TLC analysis showed the radioactivity to be at exactly the same location as the (^{14}C)HCBD. The stoichiometry measured in parallel experiments showed that one molecule of glutathione was incorporated per molecule of HCBD. Under the assay conditions described in Materials and Methods HCBD glutathione conjugate formation by the microsomal reaction was linear with time and amount of protein for at least 30 min when up to 4 mg microsomal protein per ml were used.

A kinetic analysis of the activity of the microsomal and cytosolic enzymes in HCBD and CDNB metabolism is shown in Table 1. The apparent K_m and V_{max} values were similar for the xenobiotic substrates HCBD and for the endogenous substrate glutathione. In the cytosol two metabolites of HCBD can be detected with R_f values of 0.12 and 0.33, a and b respectively (Table 1). When (^{14}C)HCBD and (^{3}H)glutathione were used, incorporation of ^{14}C and ^{3}H in both products a and b was observed. As indicated above, the microsomal glutathione transferase(s) led to the formation of only one product with an R_f value of 0.33 (product b). The apparent K_m values for the formation of these two products by the cytosolic fraction are very similar and slightly lower than the microsomal K_m value. Two to three times more product b was formed than a. This difference was observed in the majority of cases; however, an overall variation in all experiments in

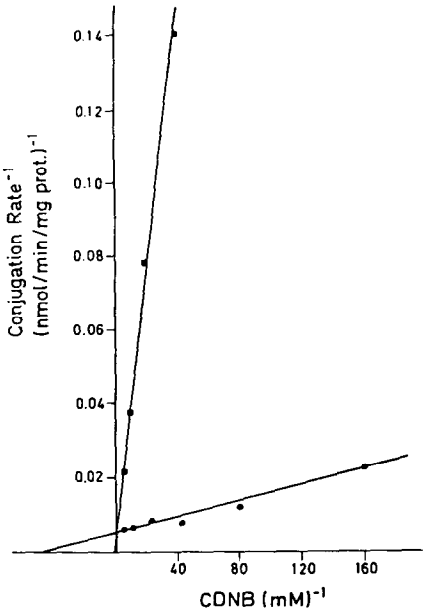

Fig. 1. Kinetic plot of CDNB conjugation by hepatic microsomal fractions (●) and inhibition by HCBD (■). CDNB conjugation was measured at 25° in 1 ml 0.1 mM phosphate buffer pH 6.8, by monitoring the absorption peak at 340 nm [13]. Glutathione concentration was 1 mM, microsomal protein concentration 0.01 mg/ml. HCBD (1 mM) was added in 5 μl ethanol.

was confirmed in experiments where substrate concentration was kept constant and inhibitor concentrations were varied (Dixon plot). In this case a K_i value of 10 μM was obtained (data not shown). In view of these findings it was interesting that HCBD metabolism at a substrate concentration of 1 mM was not significantly (less than 10%) inhibited by CDNB (1 mM). In the case of cytosolic enzymes a competitive inhibition of CDNB metabolism by HCBD was measured with a K_i value of 0.27 mM. This was similar to the K_m value for HCBD of 0.56 mM (Table 1).

The effect of solubilization of the microsomal membrane with ionic or non-ionic detergents on HCBD and CDNB metabolism is shown in Table 2. In the majority of cases a reduction in the metabolism of both substrates was observed. Solubilization with Triton X 100 reduced HCBD conjugation but had no effect on CDNB metabolism. When an equivalent amount of sodium cholate as that used to solubilize the microsomal membrane was added to the cytosol a very marked inhibition of HCBD conjugation was measured (90%, Table 3). The formation of both cytosolic metabolites was almost completely inhibited. At one quarter of this concentration 80% inhibition of the cytosolic activity was still observed, whilst this concentration had only a slight effect on the microsomal rate (Table 2). Microsomal CDNB conjugation was partially inhibited with sodium cholate (Tables 2 and 4). Other inhibitors of the cytosolic

Table 2. Effect of solubilization of the microsomal membrane with various detergents on microsomal glutathione transferase activities

Detergent	Concentration (mg/ml)	Conjugation rate (nmol/min/mg protein)	
		HCBD	CDNB
None (control)	—	0.54 (100)	223 (100)
Emulgen 911	0.8	0.38 (70)	178 (80)
Triton X100	1.6	0.34 (63)	220 (99)
Sodium cholate	0.8	0.49 (92)	ND
	3.2	0.28 (52)	135 (61)

Microsomal samples (4 mg/ml) were mixed with detergent on a rotary mixer for 5 min and then assayed for HCBD or CDNB conjugation as described in the Materials and Methods section. In the case of HCBD incubations were for 30 min at 37° in the presence of 4 mM glutathione. Values in parentheses are percentages of control values.
ND, not determined.

the ratio of these products from 1:1 to 1:2 "b" to "a" was measured. The rate of HCBD glutathione conjugation (nmol per min per mg protein) in the microsomes was 4 times higher than the total rate in the cytosol.

In the case of microsomal conjugation with CDNB the apparent K_m value for glutathione was similar to that observed with HCBD and the K_m for CDNB was 12 μM. The effect of HCBD on CDNB metabolism and vice versa showed HCBD to be a very effective competitive inhibitor of microsomal CDNB conjugation with glutathione (Fig. 1), and a K_i value of 7 μM was measured. This was much lower than the K_m value of HCBD of 0.91 mM. The K_i value

glutathione transferase, bilirubin, and dibromosulphophthalein caused a partial inhibition of HCBD metabolism by the microsomal fraction (Table 4). It is interesting to note a concentration of dibromosulphophthalein that partially inhibited microsomal HCBD metabolism (46%) completely inhibited CDNB conjugation, whilst, solubilization of the microsomal membrane with Triton X100, which reduced microsomal conjugation of HCBD did not affect that of CDNB (see above and Table 2). The inhibition of microsomal metabolism of CDNB by sodium cholate and dibromosulphophthalein gave double reciprocal plots of initial velocity against substrate concentration with an intercept consistently

Table 3. Effect of sodium cholate on the cytosolic conjugation of hexachloro-1,3-butadiene

Detergent	Conjugation rate (nmol/min/mg protein)		
	a	b	Total
None (control)	0.099 ± 0.10	0.073 ± 0.022	0.172 ± 0.038
Sodium cholate	0.005 ± 0.002 (5)	0.012 ± 0.005 (16)	0.017 ± 0.02 (10)

Sodium cholate concentration was 3.2 mg/ml. Assay conditions are described in the Materials and Methods section.

a, metabolite R_f 0.12; b, metabolite R_f 0.33; Values in parentheses represent per cent control values.

Table 4. Inhibition of microsomal glutathione transferase activity by sodium cholate, bilirubin and dibromosulphophthalein

Substrate	Conjugation rate (nmol/min/mg protein)					
	Sodium cholate		Bilirubin		Dibromosulphophthalein	
	0.1 mM	1.0 mM	0.08 mM	0.5 mM	0.2 mM	1.0 mM
Hexachloro-1,3-butadiene	1.68 (99)	1.51 (88)	1.56 (73)	1.40 (65)	1.34 (63)	1.15 (54)
1-Chloro-2,4-dinitrobenzene	188 (88)	142 (67)	—	—	66 (31)	0 (0)

Incubation conditions are described in the Materials and Methods section. Inhibitors were added as solutions in water immediately prior to HCBD or immediately prior to the addition of the microsomes in the case of CDNB. Values in parentheses are percentages of control values.

Table 5. Activity of purified cytosolic glutathione transferases in HCBD conjugation

Glutathione transferase form	Conjugation rate (nmol/min/mg)			
	a	b	Total	Ratio b:a
Yb_1Yb_1	1.61	3.92	5.52	2.4
Yb_1Yb_2	1.11	2.22	3.33	2.0
Yb_2Yb_2	1.40	1.68	3.08	1.2
YaYc	2.11	4.38	6.50	2.1

The assay procedure by TLC was as described in the Materials and Methods section with the exception that purified cytosolic glutathione transferases (1000 units/ml) were substituted for the microsomal protein. Incubations were for 60 min at 37°.

a, metabolite R_f 0.12; b, metabolite R_f 0.33; Total, total metabolite.

slightly below the abscissa, excluding competitive inhibition and indicating a mixed-type inhibition with a predominance of non-competitive inhibition and a slight kinetic contribution of uncompetitive inhibition.

The rates of conjugation of HCBD and CDNB with glutathione in microsomes were not affected by treatment of the animals with Aroclor 1254, 3-methylcholanthrene or phenobarbital, whilst cytochrome P-450 was increased 2–3-fold.

Four purified cytosolic glutathione transferases (YaYa, YaYc, Yb_1Yb_1, and Yb_2Yb_2) were tested for their activity towards HCBD. All of these enzymes were active and had similar turnover numbers. Forms containing, Yb_1Yb_1, YaYc, and Yb_1Yb_2 subunits gave two metabolic products, with R_f values of 0.12 (a) and 0.33 (b) in the TLC system described in Materials and Methods, in a ratio of approx. 2:1, b to a, however with form Yb_2Yb_2 the ratio of the two products was approximately 1:1 (Table 5).

N-Ethylmaleimide has been shown to activate microsomal glutathione transferase activity [19]. In agreement with literature findings preincubation of microsomes with this compound caused a 4–5-fold increase in the rate of CDNB metabolism. Several attempts to find an equivalent effect using HCBD were unsuccessful.

The effect of trypsin digestion on microsomal glutathione transferase activity and various other microsomal enzyme activities is shown in Fig. 2. The cytochrome P-450 reductase activity was rapidly released into the soluble fraction and was almost completely solubilized after 3 min. Epoxide hydrolase activity remained associated with the membrane fraction and remained unchanged (data not shown). Microsomal glutathione transferase activity measured with CDNB showed an initial increase in activity in the membrane fraction (activation) followed by a loss of activity with time. No concomitant increase in the activity in the soluble fraction was observed indicating that the enzyme was destroyed

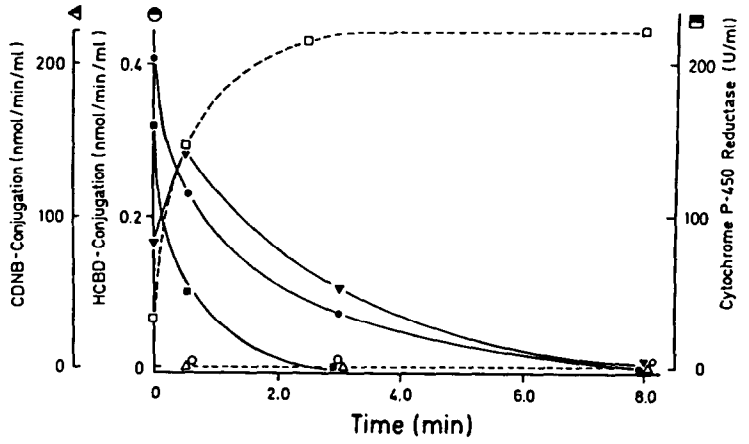

Fig. 2. Effect of trypsin on the conjugation of HCDB and CDNB and on cytochrome P-450 reductase in hepatic microsomes. Fifty milligrams of microsomal protein in 5 ml 0.1 mM phosphate buffer pH 7.4 were incubated with 5 mg trypsin at 15°. One millilitre aliquots were removed at the time points shown. One milligram of trypsin inhibitor was then added immediately and the samples spun at 35,000 *g* for 10 min. The supernatant fraction and the pellet were separated and aliquots taken for the various enzyme assays (see Materials and Methods). - - - -, open symbols represent assays with the supernatant fraction; ——, closed symbols represent assays with the pellet; ○●, conjugation of HCBD with glutathione; △ ▲, conjugation of CDNB with glutathione; □ ■, cytochrome P-450 reductase activity.

by the protease. Similar data were obtained when HCBD was used as substrate; however, no initial activation was observed. Using lower trypsin concentrations (0.1 mg/ml) cytochrome P-450 reductase was removed from the membrane at a much slower rate, all the activity was measured in the soluble fraction after 3 hr. As in the previous experiment a loss of HCBD-conjugating activity parallelled the release of reductase activity.

Table 6 gives rates of metabolism of HCBD and CDNB in samples from various animal species and compares them with those of microsomal epoxide hydrolase with styrene 7,8-oxide as substrate, an enzyme which, similar to the glutathione transferases, is involved in the deactivation of many reactive epoxides [20]. In nearly all species the specific activity of microsomal glutathione transferase for HCBD as substrate was much higher than that of the cytosolic fraction. For CDNB the reverse was the case. A large variation in the rate of microsomal conjugation of HCBD with glutathione was observed between species ranging from 0.03 in the guinea-pig to 1.4 nmol/min per mg protein in the rat and primate. In addition, a very large difference in the activity of the microsomes relative to the cytosol was observed. In the human sample studied the microsomal glutathione transferase was 37-fold more active in HCBD conjugation than the cytosolic enzymes.

DISCUSSION

It has recently become apparent that many haloalkanes and alkenes can be conjugated with glutathione [21–26] to give cytotoxic and mutagenic products [22, 27]. In the majority of cases a role for the microsomal glutathione transferases remains to be established. However, in the case of HCBD conjugation with glutathione in the liver and kidney catalyzed in part by the microsomal GST enzymes appears to be the initial event in these reactions [8, 9, 26]. The major product of HCBD conjugation with glutathione is *S*-(1,2,3,4,4-pentachloro-1,3-butadienyl)glutathione which results from a direct substitution reaction without prior oxidation by the cytochrome P-450 system [8, 9]. In the rat, the microsomal rate of conjugation is 3–4-fold higher than that measured in cytosol. Among other species a considerable variation in this difference is observed ranging from approximately equivalent rates in the guinea-pig to a microsomal rate of 40-fold higher in the human liver microsomal sample over cytosol. This finding is substantiated by the finding that GSTs from the human cytosolic alpha and mu classes do not appear to have HCBD conjugating activity [7]. This initial observation requires further study but may demonstrate an important role for the microsomal GST in the metabolism of various halogenated compounds in man.

The requirement of the microsomal enzyme for glutathione suggests that the catalytic site of the protein is localized on the cytoplasmic surface of the endoplasmic reticulum. This was substantiated by the loss of activity when the microsomes were treated with trypsin under conditions which released other microsomal enzymes from the membrane. In this case HCBD-conjugation was lost; however, the maintenance of activity on detergent solubilization demonstrates that an intact microsomal membrane is not critical for the function of this enzyme.

Both microsomes and cytosol were active in HCBD metabolism and all the purified rat cytosolic glutathione transferases had equivalent activity towards this substrate. The cytosolic conjugating activity could therefore be accounted for by the presence of identified cytosolic glutathione

Table 6. Glutathione transferase and epoxide hydrolase activities in liver microsomes and cytosol from various species

	Glutathione transferase activity (nmol/min/mg protein)						Epoxide hydrolase activity (nmol/min/mg protein)
	Substrate: Hexachloro-1,3-butadiene			Substrate: 1-Chloro-2,4-dinitrobenzene			
	Microsomes	Cytosol	Ratio microsomes : cytosol	Microsomes	Cytosol	Ratio microsomes : cytosol	Substrate: Styrene 7,8-oxide
Human	1.17	0.031	37.7	55	1666	0.033	59.3
Green monkey	1.39	0.074	18.8	268	2249	0.119	16.1
New Zealand rabbit	0.14	0.042	3.3	81	4091	0.020	10.1
Sprague–Dawley rat	1.39	0.350	3.9	107	1543	0.069	5.5
Syrian golden hamster	0.25	0.011	22.7	167	3922	0.043	25.9
ZE 1 mouse	0.16	0.109	1.5	54	617	0.088	1.4
Nikisch guinea-pig	0.03	0.028	1.1	152	4316	0.035	12.1

Incubation conditions are described in Materials and Methods.

transferases. The absence of any induction of the microsomal HCBD metabolism by compounds known to induce the cytosolic proteins and the finding that two metabolic products are formed in cytosol and only one in microsomes clearly distinguishes the microsomal and cytosolic enzymes. The different sensitivity of HCBD conjugation to sodium cholate in these systems substantiates this observation. The formation of two metabolites in the cytosolic fraction [9] has been shown to be due to the formation of mono and diconjugates of HCBD, the latter containing two glutathione molecules [28]. The microsomal GST do not appear to have the capacity to carry out the second conjugation step. K_m values for the microsomal and cytosolic conjugation of HCBD were approximately similar. This was not the case for CDNB where a 10-fold difference in K_m was observed, and raises interesting questions as to a role for microsomal GST *in vivo* at low substrate concentrations.

Unlike CDNB, microsomal HCBD conjugation could not be activated with *N*-ethylmaleimide. Another intriguing difference in the properties of these two substrates in microsomes was the finding that HCBD was a potent competitive inhibitor of CDNB metabolism whereas CDNB, with a 100-fold lower K_m value, did not significantly affect the metabolism of HCBD. Although care must be taken on the interpretation of kinetic parameters measured in microsomal preparations one explanation for this observation would be the involvement of more than one microsomal enzyme in HCBD metabolism. The very low turnover number of purified microsomal GST for HCBD would tend to substantiate this conclusion [6, 7]. However, this possibility requires further study.

Acknowledgements—The authors thank M. Kampe-Kurze for excellent technical assistance, Dr. T. Friedberg for the purified glutathione transferases, and the Deutsche Forschungsgemeinschaft for financial support.

REFERENCES

1. Booth J, Boyland E and Sims P, An enzyme from rat liver catalyzing conjugation with glutathione. *Biochem J* **79**: 516–524, 1961.
2. Nemoto N and Gelboin H, Assay and properties of glutathione *S*-benzo(a)pyrene-4,5-oxide transferase. *Arch Biochem Biophys* **170**: 739–742, 1975.
3. Glatt HR and Oesch F, Inactivation of electrophilic metabolites by glutathione *S*-transferase and limitation of the system due to subcellular localization. *Arch Toxicol* **39**: 87–96, 1977.
4. Friedberg T, Bentley P, Stasiecki P, Glatt HR, Raphael D and Oesch F, Identification, solubilization and characterization of microsome associated glutathione *S*-transferases. *J Biol Chem* **254**: 12028–12033, 1980.
5. Morgenstern R, Guthenberg C and DePierre JW, Microsomal glutathione *S*-transferase. *Eur J Biochem* **128**: 243–248, 1982.
6. Morgenstern R and DePierre JW, Microsomal glutathione transferase. Purification in unactivated form and further characterization of the activation process, substrate specificity and amino acid composition. *Eur J Biochem* **134**: 591–597, 1983.

7. McLellan LI, Wolf CR and Hayes JD, Human microsomal glutathione *S*-transferase; its involvement in the conjugation of hexachloro-1,3-butadiene with glutathione. *Biochem J* in press.
8. Wolf CR, Berry PN, Nash JA, Green T and Lock EA, The role of rat liver microsomal and cytosolic glutathione *S*-transferases in hexachlorol:3-butadiene mediated glutathione depletion. *Naunyn-Schmiedeberg's Arch Pharmakol* **319**: R17, 1982.
9. Wolf CR, Berry PN, Nash JA, Green T and Lock EA, Role of microsomal and cytosolic glutathione *S*-transferases in the conjugation of hexachloro-1,3-butadiene and its possible relevance to toxicity. *J Pharmacol Exp Ther* **228**: 202–208, 1984.
10. Lock EA and Ishmael J, The acute toxic effects of hexachlorobutadiene on the rat kidney. *Arch Toxicol* **43**: 47–57, 1979.
11. Kociba RJ, Keyes DG, Jersey GC, Ballard JJ, Dittenber DA, Quast JF, Wade CE, Humiston CG and Schwetz BA, Results of a two year chronic toxicity study with hexachlorobutadiene in rats. *Am Ind Hyg Assoc J* **38**: 589–602, 1977.
12. Wolf CR and Oesch F, Isolation of a high spin form of cytochrome P-450 induced in rat liver by 3-methylcholanthrene. *Biochem Biophys Res Commun* **III**: 504–511, 1983.
13. Friedberg T, Milbert U, Bentley P, Guenthner TM and Oesch F, Purification and characterization of a new cytosolic glutathione *S*-transferase (glutathione *S*-transferase X) from rat liver. *Biochem J* **215**: 617–625, 1983.
14. Yasukochi Y and Masters BSS, Some properties of a detergent-solubilized NADPH-cytochrome c (cytochrome P-450) reductase purified by biospecific affinity chromatography. *J Biol Chem* **251**: 5337–5344, 1976.
15. Habig WH, Pabst MJ and Jakoby WB, Glutathione *S*-transferases: the first enzymatic step in mercapturic acid formation. *J Biol Chem* **24**: 7130–7139, 1974.
16. Oesch F and Daly JW, Solubilization, purification and properties of a hepatic epoxide hydrase. *Biochim Biophys Acta* **227**: 685–692, 1971.
17. Oesch F, Purification and specificity of a human microsomal epoxide hydratase. *Biochem J* **139**: 77–88, 1974.
18. Lowry OH, Rosebrough NJ, Farr AL and Randall RT, Protein measurements with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
19. Morgenstern R, DePierre JW and Ernster L, Activation of microsomal glutathione *S*-transferase activity by sulfhydryl reagents. *Biochem Biophys Res Commun* **87**: 657–663, 1979.
20. Oesch F, Microsomal epoxide hydrolase. In: *Enzymatic Basis of Detoxication*, Vol. 2 (Ed. Jakoby, WB), pp. 277–289. Academic Press, London, 1980.
23. Dohn DR and Anders MW, The enzymatic reaction of chlorotrifluoroethylene with glutathione. *Biochem Biophys Res Commun* **109**: 1339–1345, 1982.
24. Morgenstern R, Meijer J, DePierre JW and Ernster L, Characterization of rat liver microsomal glutathione *S*-transferase activity. *Eur J Biochem* **104**: 167–174, 1980.
25. Martin JL, George JW and Pohl LR, Glutathione-dependent dechlorination of chloramphenicol by cytosol of rat liver. *Drug Metab Dispos* **8**: 93–97, 1980.
26. Green T and Oldham J, Structure activity studies of the nephrotoxic and mutagenic action of cysteine conjugates of chloro and fluoro alkenes. *Chem Biol Interact* **54**: 15–31, 1985.
27. Gandolfi AJ, Nagle RB, Soltis JJ and Plescia FH, Nephrotoxicity of halogenated vinyl cysteine compounds. *Res Commun Chem Pathol Pharmacol* **33**: 249–259, 1981.
28. Jones TW, Gerdes RG, Ormstad K and Orrenius S, The formation of both a mono- and bi-substituted glutathione conjugate of hexachlorobutadiene by isolated hepatocytes and following *in vivo* administration to the rat. *Chem Biol Interact* **56**: 251–267, 1985.