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PROPYLTHIOURACIL INDUCIBLE GLUTATHIONE TRANSFERASES

SELECTIVE INDUCTION OF LIGANDIN (GLUTATHIONE TRANSFERASE 1-1)

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Abstract—Repeated administration of propylthiouracil (PTU) resulted in an increase in gluthathione (GSH) transferases activity in rat liver cytosol toward various substrates except for epoxy(*p*-nitrophenoxy)propane. The enzyme from rat treated with PTU showed high activity with 1-chloro-2,4-dinitrobenzene (CDNB) and ethacrynic acid. GSH transferases were separated into five forms by CM-Sephadex C-50 column chromatography to detect which isozymes were induced by PTU treatment. Although the activity of the unretained fraction obtained from the column was slightly increased by PTU treatment, the treatment markedly elevated the activity of GSH transferase 1-1. On the other hand, the activities of GSH transferases 1-2, 3-3 and 2-2 were little changed. In addition, an analysis of GSH transferase 1-1 from CM-Sephadex C-50 using SDS–PAGE confirmed that it comprised the 1-1 homodimer. This fraction was then further purified by passing it through a hydroxylapatite column and the partially purified GSH transferase 1-1 from rats treated with PTU was found to have the same characteristics as the control, e.g. the same K_m values for GSH and CDNB and the same substrate spectrum. It was concluded that PTU specifically induced GSH transferase 1-1 among the cationic transferases.

The GSH† transferases (EC 2.5.1.18) are a group of multi-functional proteins serving several roles in detoxication [1]. In various organs and tissues, many kinds of the isozymes have been reported and termed by various researchers. Recently, the nomenclature of glutathione transferases has been arranged on the basis of the numbers given to their two constituate subunits, and six kinds of isozymes were recognized [2], namely, ligandin, GSH transferases B, AA, A and C are named as GSH transferases 1-1, 1-2, 2-2, 3-3 and 3-4, respectively.

Cationic GSH transferases which bind to CM-cellulose [3, 4] comprise GSH transferases 3-4, 1-1, 1-2, 3-3 and 2-2 [1]. Further, GSH transferases of rat liver cytosol may be induced by several xenobiotics [5–12] such as phenobarbital, 3-methylcholanthrene, benzo[*a*]pyrene and stylene oxide and the enzyme prepared from the hepatic cytosol of rats treated with these compounds showed differences in the substrate specificity. However, the isozyme(s) induced by these xenobiotics were not fully characterized.

Reyes *et al.* observed that hypophysectomy and thyroidectomy increased ligandin-like activity and thyroxin was able to prevent this effect [13]. Recently, it was found that GSH transferase 1-1, 3-3 and 1-2 were induced by phenobarbital [14] and Kitahara *et al.* reported that GSH transferase 3-3 was induced in rat preneoplastic legions [15].

Previously, it was found that repeated administration of PTU increased the activity of GSH transferase in rat liver cytosol but not particulate fraction [16]. Moreover, PTU-inducible enzyme activity was highest toward CDNB in comparison to the various substrates tested [16]. Therefore, it is possible that PTU treatment causes selective induction of GSH transferase isozyme(s) with high activity toward CDNB. In this report, the isozyme(s) induced by PTU treatment were determined.

MATERIALS AND METHODS

PTU, GSH, ethacrynic acid and 1,2-epoxy(*p*-nitrophenoxy)propane were purchased from Sigma Chemical Co., CDNB from Aldrich Chemical Co., DCNB from Tokyo Kasei Kogyo Co., *p*-nitrobenzyl chloride and *trans*-4-phenyl-3-buten-2-one from Wako Pure Chemical Industry. All other chemicals of highest grade were obtained from commercial sources. CM-Sephadex C-50 and hydroxylapatite were obtained from Pharmacia and Bio-Rad Laboratories, respectively.

PTU treatment was carried out as described previously [17]. PTU (1.5 mmol/kg) was intraperitoneally administered to Sprague–Dawley rats, weighing about 100 g body weight, for 2 weeks. Twenty-four hours after the last treatment the rats were sacrificed and their livers were removed immediately. The liver cytosol was prepared as described elsewhere [16].

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† Abbreviations used: PTU, propylthiouracil; GSH, reduced glutathione; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Each of the cationic isozymes was separated as described by Hayes *et al.* [14]. Rat liver cytosol was dialyzed against 100 vol. of 10 mM sodium phosphate buffer, pH 7.4 with two changes. The dialyzed cytosol (350 mg protein) was passed through CM-Sephadex C-50 column (1.4 × 30 cm) equilibrated with the same buffer. The column was washed with 90 ml of the same buffer and then eluted with 300 ml of 0–80 mM linear gradient of NaCl in the same buffer.

Further purification of GSH transferase 1-1 was carried out as followed: the active fraction of GSH transferase 1-1 from CM-Sephadex C-50 column was concentrated using Amicon PM-10 filter and was then dialyzed twice against 100 vol. of 10 mM potassium phosphate buffer, pH 6.5 containing 1.0 mM GSH and 30% (v/v) glycerol. The dialyzed sample was applied to hydroxylapatite column (1.2 × 8.0 cm) equilibrated with the same buffer. The column was washed with 45 ml of the same buffer and eluted with 100 ml of 10–200 mM linear gradient of potassium phosphate buffer, pH 6.5 containing 1.0 mM GSH and 30%(v/v) glycerol. The specific activities toward CDNB of these partially purified enzymes from control rats and rats treated with PTU were 12.3 and 16.9 μmol/mg protein/min, respectively. The purities of these enzymes, determined by SDS-PAGE, were more than 80%.

The enzyme activity was determined as described by Habig *et al.* [18]. SDS-PAGE was carried out according to Laemmli [19] using 12.5% acrylamide. The gel was stained with Coomassie brilliant blue, and the densitometry was performed with Dual-Wavelength TLC Scanner CS-910 (Shimadzu, Japan) at 570 nm.

Protein was assayed by the method of Lowry *et al.* [20].

RESULTS

It has been reported that PTU treatment causes a marked increase in GSH transferase activity of rat liver cytosol when CDNB was used as the substrate [16]. The effect of PTU treatment on the enzyme activity was further examined using several different substrates. Table 1 shows that activity to all substrates with the exception of epoxy(*p*-nitrophenoxy)-propane is increased by PTU treatment in a statistically significant manner even though this increase is small. In fact, when CDNB and ethacrynic acid were used as the substrates, the degree of increase in the activity was highest.

By use of CM-Sephadex C-50 ion exchange chromatography the isozymes induced by PTU treatment were determined. GSH transferases from control rats were separated into several forms, namely the unretained fraction, GSH transferases 1-1, 1-2, 3-3 and 2-2 in order of elution as shown in Fig. 1A. GSH transferases from rats treated with PTU were divided into the same fractions as depicted in Fig. 1B. When CDNB was used as the substrate, the activity of the unretained fraction was slightly increased by PTU treatment and the activity of GSH transferase 1-1 was elevated by twofold by PTU. In contrast, GSH transferases 1-2, 3-3 and 2-2 were little changed. On the other hand, the activity toward DCNB was observed in the unretained fraction and GSH transferase 3-3, since the other isozymes have a relative low activity toward this compound. PTU treatment could not affect the activity toward DCNB of the isozymes (Fig. 1).

The effect of PTU treatment on the subunit composition of GSH transferase 1-1 obtained from CM-Sephadex C-50 column chromatography was examined to confirm the selective induction of the subunit 1 by PTU. As shown in Fig. 2B, the GSH transferase 1-1 fraction was found to contain mainly subunit 1 in common with other proteins. Furthermore, PTU treatment increased subunit 1 by twofold (Fig. 2B), whereas the subunit composition of the fraction of GSH transferase 1-2, 3-3 and 2-2 was affected little by PTU treatment (Fig. 2C, D). These results indicate that only GSH transferase 1-1 among the cationic isozymes was induced by repeated administration of PTU. On the other hand, the unretained fraction, designated as the anionic transferases, contained proteins corresponding to the subunits 1, 2 and 3 (Fig. 2A).

PTU-inducible GSH transferase 1-1 was further purified on hydroxylapatite and then the enzyme was characterized. Table 2 showed the substrate specificity of GSH transferase 1-1 purified from rats treated with or without PTU. GSH transferase 1-1 had the highest activity toward CDNB with low activity toward the other substrates tested. In addition, the substrate spectrum of the enzyme from PTU-treated rat liver was identical to that from the control. Furthermore, the partially purified enzymes prepared from rats treated without or with PTU had

Table 1. Effect of PTU treatment on GSH transferase activity in rat liver cytosol

Substrates	Enzyme activity (nmol/mg protein/min)	
	Control	PTU
1-Chloro-2,4-dinitrobenzene	688.0 ± 56.0	1176.0 ± 64.0**(171)
1,2-Dichloro-4-nitrobenzene	41.2 ± 2.0	51.2 ± 1.8**(124)
p-Nitrobenzyl chloride	78.9 ± 2.1	90.8 ± 2.5**(115)
trans-4-Phenyl-3-buten-2-one	10.8 ± 0.4	12.8 ± 0.5**(119)
Ethacrynic acid	21.4 ± 2.3	38.4 ± 0.9**(179)
1,2-Epoxy-3-(*p*-nitrophenoxy)propane	21.5 ± 0.9	23.2 ± 1.3 (108)

Values are expressed as means ± S.E.M. of 5–7 experiments.
Numbers in parentheses are expressed as percent of control.
**$P < 0.01$, compared with control.

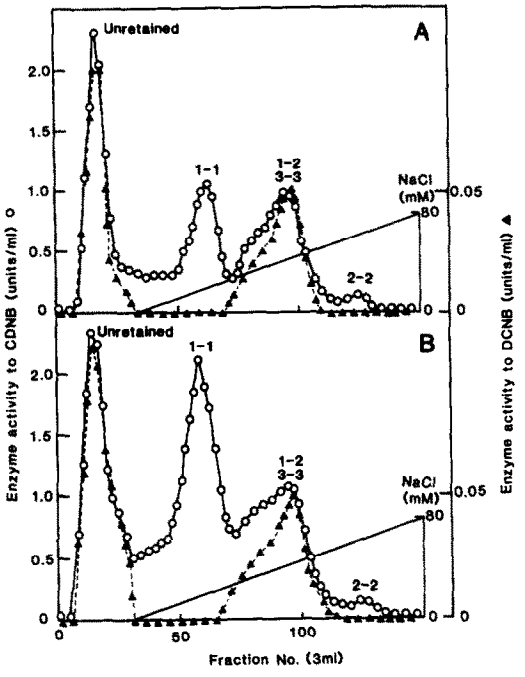

Fig. 1. CM-Sephadex C-50 column chromatogram of hepatic GSH transferases from rats treated with or without PTU. Hepatic cytosol (350 mg protein) from control or PTU-treated rats were dialyzed against 10 mM sodium phosphate buffer, pH 7.4, and then were applied to CM-Sephadex C-50 (1.4 × 30 cm) and eluted with a linear gradient of NaCl. The enzyme activity was measured with CDNB (○) or DCNB (▲) as the substrate. A, control; B, PTU-treated.

the same K_m values for GSH (control, 0.124 mM; PTU-treated, 0.117 mM) and CDNB (control, 0.091 mM; PTU-treated, 0.090 mM).

DISCUSSION

It is well known that xenobiotics such as phenobarbital and chemical carcinogens induce GSH transferases of rat liver cytosol [5–12]. Ligandin (GSH transferase 1-1), one of the molecular forms of GSH transferases, is also induced by the administration of these xenobiotics and hypophysectomy or thyroidectomy [13]. Nevertheless, these is no specific inducer of GSH transferase 1-1 because these compounds induce not only GSH transferase 1-1 but also other isozymes. Although 3-methylcholanthrene treatment increased the activity only of ligandin [14], it was not characterized as the 1-1 homodimer. The results presented here revealed that PTU-inducible GSH transferase consists of the 1-1 homodimer, indicating that PTU treatment causes selective induction of GSH transferase 1-1 among the cationic transferases in rat liver cytosol. In addition, the increase in GSH transferase activity of rats treated with PTU using various substrates was not necessarily to the same degree (Table 1). Although the activity of GSH transferase 1-1 toward CDNB is very high, the activity towards other substrates such as DCNB and *p*-nitrobenzyl chloride was relatively low (Table 2). It supports the evidence that PTU treatment resulted in the enhancement of GSH transferase activity toward CDNB in rat liver cytosol in comparison with other substrates tested as described previously [16]. GSH transferase 1-2 also shares the subunit 1, but this isozyme was induced little by PTU treatment. Therefore, it is emphasized that PTU treatment induces only the subunit 1 but not the subunit 2 and 3.

Characteristics such as the K_m values and the substrate specificity of GSH transferase 1-1 from rats treated with PTU were found to be the same as that from control, suggesting that GSH transferase 1-1 from control rats and rats treated with PTU are essentially identical. From this finding, it can be concluded that an increase in the enzyme activity of hepatic cytosol from rats treated with PTU using CDNB as the substrate is due to the induction of GSH transferase 1-1 with high activity toward the compound. However, the increase in the activity toward ethacrynic acid of the enzyme from rats treated with PTU is not due to the induction of GSH transferase 1-1, because this isozyme has a relatively low activity with ethacrynic acid as the substrate [21, 22]. Although the activity toward CDNB of the unretained fraction from CM-Sephadex C-50 chromatography was slightly increased by PTU treatment (Fig. 1), the treatment markedly enhanced the activity toward ethacrynic acid by 1.5-fold (control, 28; PTU-treated, 41 nmol/mg protein/min). Therefore, the induction of a molecular form in the unretained fraction may result in an increase in the

Table 2. Substrate specificity of GSH transferase 1-1 from control rats and rats treated with PTU

Substrates	Enzyme activity (μmol/mg protein/min)			
	Control		PTU	
1,2-Dichloro-4-nitrobenzene	0.0024	(1.0)	0.0034	(1.0)
1-Chloro-2,4-dinitrobenzene	12.3	(5120.0)	16.9	(4970.0)
p-Nitrobenzyl chloride	0.0098	(4.1)	0.0142	(4.2)
1,2-Epoxy-3-(*p*-nitrophenoxy)propane	0.015	(6.3)	0.022	(6.5)

GSH transferase 1-1 was partially purified as described in the text.
Numbers in parentheses represent the relative substrate efficacy with the conjugation of 1,2-dichloro-4-nitrobenzene set as 1.0.

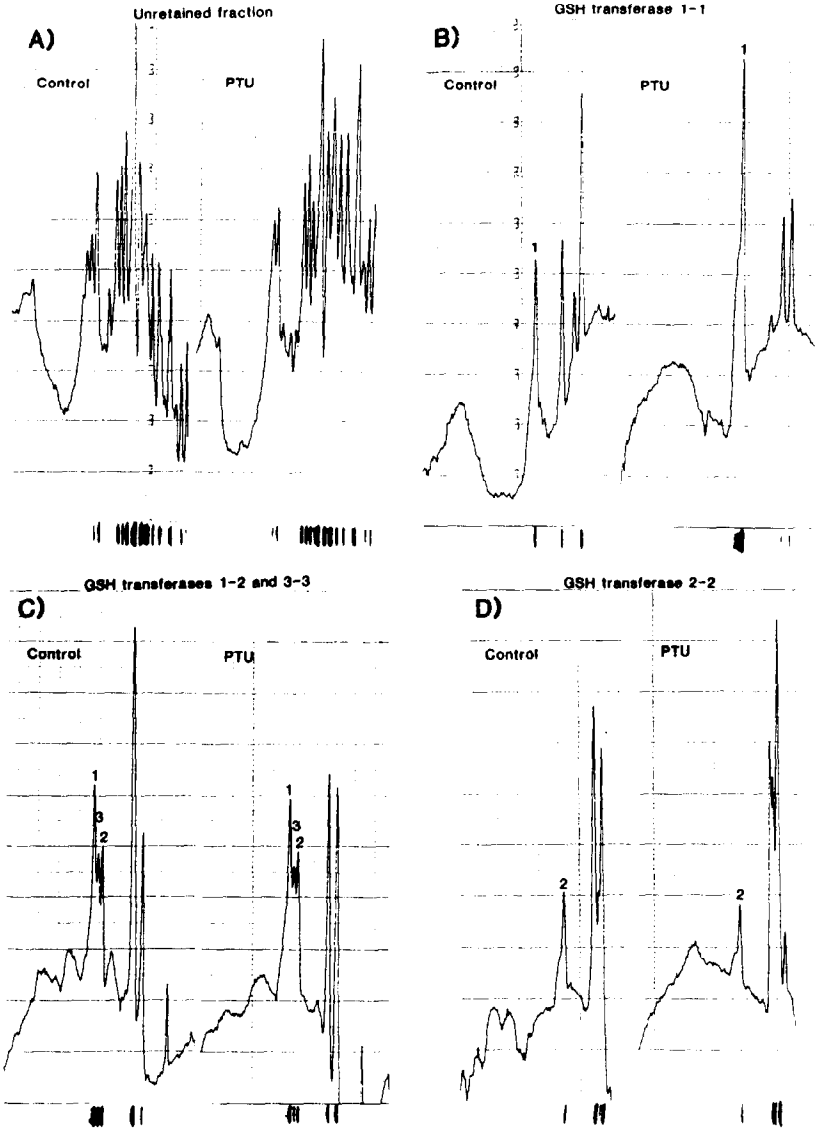

Fig. 2. SDS-PAGE of active fractions obtained from CM-Sephadex C-50 column chromatography of hepatic cytosol in rat treated with or without PTU. Fraction numbers of 9-27, 51-67, 81-103 and 121-130 in Fig. 1 were pooled as the unretained fraction, GSH transferases 1-1, 1-2.3-3 and 2-2, respectively. These isozymes were analyzed by SDS-PAGE with a 12.5% acrylamide. The standard proteins used for the estimation of the subunits of the enzymes were bovine serum albumin (68,000), ovalbumin (45,000), soy bean trypsin inhibitor (21,500) and cytochrome *c* (12,300). After the electrophoresis the gels were stained with Coomassie blue and densitometry performed at 570 nm. Their densities are shown in the panels A, B, C and D corresponding to the unretained fraction (50 μg protein), GSH transferases 1-1, 1-2·3-3 and 2-2 (20 μg protein), respectively.

activity toward ethacrynic acid of rat liver cytosol. In addition, the bands corresponding to the subunits 1 and 3 in this fraction were slightly increased by PTU treatment. However, it is unclear which molecular form(s) in the fraction is induced by PTU at this time. Since the unretained fraction contains not only GSH transferases 3-4 and 4-4 but also neutral/acidic transferases [23] which bind to DEAE-cellulose, named as anionic form, it is considered that the anionic isozyme(s) with high activity toward ethacrynic acid may also be induced by PTU treatment.

It is well known GSH transferase 1-1 plays a role in detoxication and binding of various hydrophobic compounds such as bilirubin [24–26]. However, the physiological role of this isozyme has not been understood sufficiently. In this experiment, it seems little doubt that PTU is a selective inducer of GSH transferase 1-1 among the cationic transferases. Therefore, the rat treated with PTU may be useful for the study of a role of GSH transferase 1-1 in the physiological state.

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