The conjugation of 4-nitroquinoline 1-oxide, a potent carcinogen, by mammalian glutathione transferases

4-Nitroquinoline 1-oxide conjugation by human, rat and mouse liver cytosols, extrahepatic organs of mice and purified mouse glutathione transferase isoenzymes

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The conjugation of 4-nitroquinoline 1-oxide with GSH by human, rat and mouse liver cytosols, by purified mouse GSH transferases and by extrahepatic organ cytosols of male and female mice was investigated. 4-Nitroquinoline 1-oxide was as effectively conjugated by human liver cytosol as was 1-chloro-2,4-dinitrobenzene, at a substrate concentration of 0.1 mM. Mouse isoenzymes composed of Yb₁ and Yf subunits exhibited high activity towards 4-nitroquinoline 1-oxide. Human, rat and mouse hepatic activities towards this substrate correlated with the hepatic isoenzyme compositions.

INTRODUCTION

4NQO is tumorigenic to the lung, oesophagus, fore-stomach, glandular stomach, skin and other organs, and is a co-carcinogen in the liver [1]. The metabolic capability for activation of 4NQO is widely distributed, being present in prokaryotes and eukaryotes and in most mammalian organs examined [2,3]. Whereas the mechanisms of metabolic activation and carcinogenesis of 4NQO have been subjects of rather intense investigation [2–6], relatively little attention has been focused on the biochemical mechanisms of inactivation or detoxication of this carcinogen.

Like many other carcinogenic and toxic compounds, 4NQO is a reactive electrophile that is subject to attack by nucleophiles such as GSH [7,8]. Such reactions may proceed spontaneously or be catalysed by GSH transferases [8–11]. Al-Kassab *et al.* [7] examined the reaction of 4NQO with GSH at pH 8, where the thiol group of GSH is appreciably ionized. They showed that rat liver cytosol catalysed the GSH-dependent liberation of nitrite from 4NQO, although the reaction proceeded spontaneously at an extremely high rate [7].

We recently developed a continuous spectrophotometric assay for the reaction of 4NQO with GSH and examined the pH-profiles of the rates of the spontaneous reaction and of the reaction catalysed by mouse liver cytosol [12]. The rate of spontaneous reaction was measurable at pH 6.7 and rose with pH, increasing most markedly above pH 7.7. Optimal catalysis by mouse liver cytosol occurred between pH 6.5 and pH 7.5. In the most prevalent physiological pH range the reaction of 4NQO with GSH was highly dependent upon enzymic catalysis [12].

In the present investigation we examined the cytosolic GSH transferase activities towards 4NQO of human liver, rat liver and livers from female and male mice.

Although the liver is a major site of xenobiotic metabolism, other organs also have the potential to detoxify reactive electrophiles via GSH conjugation [13,14]. Therefore we examined the effectiveness of cytosols from several extrahepatic mouse organs in promoting the conjugation of 4NQO with GSH. Previous studies showed that 4NQO was an effective substrate for mouse liver GSH transferase GT-8.8b [12]. We now report the activities of several mouse GSH transferase isoenzymes in catalysing the conjugation of 4NQO.

EXPERIMENTAL

Chemicals

4NQO was purchased from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Enzyme-grade sucrose was from Schwarz/Mann Biotech, Cambridge, MA, U.S.A. The reagent for the dye-binding protein assay was from Bio-Rad Laboratories, Richmond, CA, U.S.A. GSH, CDNB and bovine albumin were from Sigma Chemical Co., St. Louis, MO, U.S.A.

Measurement of enzyme activities

GSH transferase activity towards 4NQO was measured spectrophotometrically at 25 °C [12]. The assay system (1 ml; pH 6.5) contained 0.1 M-potassium phosphate buffer, 1 mM-GSH, an appropriate amount of enzyme, and 0.1 mM-4NQO, which was added in 10 µl of ethanol to initiate the reaction. Initial velocities were determined from the change in absorbance at 350 nm ($\Delta\epsilon = 7.20 \text{ mM}^{-1} \cdot \text{cm}^{-1}$). Product formation was linear with time and with enzyme concentration. Corrections were made for non-enzymic reaction of 4NQO with GSH ($\Delta A < 0.001/\text{min}$). GSH transferase activity towards CDNB was measured as described by Habig *et al.* [15]. Specific activities were based on protein concentrations

Abbreviations used: 4NQO, 4-nitroquinoline 1-oxide; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene.

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measured in cytosols by the dye-binding assay method of Bradford [16] in preparations of purified enzymes or by the method of Lowry *et al.* [17], with bovine plasma albumin as a standard.

Preparation of human liver cytosol

Liver was obtained upon autopsy of a 74-year-old man within 7 h after death from congestive heart failure. During the preceding 2 days the following medications had been administered: heparin, dilantin, dopamine, basjel and diazepam. After storage overnight at -80°C , liver (326 mg) was homogenized at 0°C in $978\ \mu\text{l}$ of 0.25 M-sucrose and a cytosol fraction was prepared [18].

Treatment of rodents and organs

CD-1 mice were purchased from Charles River Breeding Laboratories, Kingston, NY, U.S.A. A/HeJ mice were from Jackson Laboratories, Bar Harbor, ME, U.S.A. CD rats were obtained from the National Center for Toxicological Research, Jefferson, AR, U.S.A. Mice and rats were housed in stainless-steel cages with hardwood-chip bedding in an environmentally controlled room on a 12 h-light/12 h-dark cycle. They received Purina laboratory chow and tap water *ad libitum*. Rodents were 2–5 months old when killed. For reasons unrelated to this study, the A/HeJ mice received six intraperitoneal injections of 0.2 ml of sesame oil during the 9 days before they were killed. Rats and mice were killed by CO_2 asphyxiation and cervical dislocation respectively. Livers were perfused with cold 0.15 mM-KCl in 2 mM-EDTA, pH 7.0. All tissues were frozen in liquid N_2 and stored at -80°C . Tissues were homogenized in 0.25 M-sucrose at 0°C (3 ml/g of tissue) and cytosol fractions were prepared as described previously [18].

RESULTS

Conjugation of 4NQO by human liver cytosol

The human liver cytosol examined in these studies catalysed the conjugation of 4NQO with GSH with a specific activity of 42 nmol/min per mg of cytosol protein under the standard assay conditions described above. With 1 mM-CDNB as substrate [15] the specific activity (216 nmol/min per mg) was comparable with that reported by Kamisaka *et al.* [19] for human liver cytosol. At 0.1 mM-CDNB a specific activity of 41 nmol/min per mg was observed. Thus, when compared at the same concentration (0.1 mM) in the assay system, 4NQO and CDNB were similarly effective as substrates for conjugation by GSH transferases in the human liver cytosol preparation.

Conjugation of 4NQO by rodent liver cytosols

Rat and mouse liver cytosols (Table 1) exhibited substantially higher GSH transferase activities towards 4NQO than did the human liver cytosol. Among the rodents examined, the male mice had the highest hepatic 4NQO-conjugating activity, with liver cytosol for male rats being only 20% as active towards this substrate. Also, 2.8-fold higher hepatic 4NQO-conjugating activity was observed for the CD-1 male mice than for female mice of the same type.

Table 1. Conjugation of 4NQO with GSH by rodent liver cytosols and by purified GSH transferases

Assay conditions were as described in the Experimental section. Values for rodent liver cytosols represent the means $\pm$ S.E.M. for at least three animals except for female CD-1 mice, from which pooled tissue from four livers was used.

Enzyme preparation	Specific activity ($\mu\text{mol}/\text{min}$ per mg of protein)
Rodent liver cytosols	
Male CD rats	0.76 ± 0.08
Male CD-1 mice	3.72 ± 0.31
Female A/HeJ mice	0.79 ± 0.02
Female CD-1 mice	1.31
Mouse liver GSH transferases	
GT-8.7	44
GT-8.8a	82
GT-8.8b	85
GT-9.0	27
GT-9.3	3

Table 2. Conjugation of 4NQO by cytosols from extrahepatic organs of female A/HeJ mice and male CD-1 mice

Assay conditions were as described in the Experimental section. Values for A/HeJ mice represent the means $\pm$ S.E.M. for organs from six mice and values for CD-1 mice are for pooled tissue from three mice.

Organ	Specific activity ($\mu\text{mol}/\text{min}$ per mg of cytosol protein)	
	Female A/HeJ mice	Male CD-1 mice
Lung	0.21 ± 0.01	0.34
Kidney	0.30 ± 0.01	0.57
Oesophagus	0.18 ± 0.02	0.38
Fore-stomach	0.50 ± 0.03	0.54
Glandular stomach	0.28 ± 0.02	0.48
Upper small intestine	0.20 ± 0.01	0.63
Lower small intestine	0.10 ± 0.00	0.23
Colon	0.31 ± 0.04	0.62
Urinary bladder	—	4.94
Testis	—	1.72
Spleen	—	0.08
Heart	—	0.10

Specificities of mouse GSH transferases in the conjugation of 4NQO

Some but not all of the GSH transferases purified from livers of CD-1 mice ([20–22], and unpublished work by A. M. Benson, M. J. Humkeler & J. L. York) effectively catalysed the conjugation of 4NQO with GSH (Table 1). GT-8.8a, GT-8.8b and GT-8.7, which has been designated [23] a class Mu isoenzyme, exhibited the highest activities towards 4NQO. These three chromatographically distinct forms of GSH transferase were indistinguishable with respect to polypeptide M_r , several structural and immunological characteristics and substrate preferences [20,21]. High activity towards DCNB

(2–4 $\mu\text{mol}/\text{min}$ per mg) distinguished GT-8.7, GT-8.8a and GT-8.8b from the other hepatic transferases in Table 1. The conjugation of 4NQO was also actively catalysed by GT-9.0, a YfYf isoenzyme that was present in the liver and in the small-intestine mucosa of female CD-1 mice [21,22] and that had high activity towards ethacrynic acid, a characteristic substrate [23] for class Pi isoenzymes. GT-9.3, an isoenzyme that is structurally related to GT-8.7 [20] and that has also been designated a class Mu isoenzyme [23], had relatively low activity towards 4NQO, whereas more basic transferases, with Ya subunits and GSH peroxidase II activity of the class Alpha [23] GSH transferases, had no detectable activity towards 4NQO.

Extrahepatic GSH transferase activity towards 4NQO

GSH transferase activity towards 4NQO was widely distributed in mouse organs (Table 2). The highest extrahepatic 4NQO-conjugating activities were found in the urinary bladder and testis. In male CD-1 mice the cytosolic specific activity of the urinary bladder exceeded that of the liver and was more than 60 times greater than that of the spleen. However, appreciable GSH transferase activity towards 4NQO was observed in all of the extrahepatic organs examined.

DISCUSSION

The results of this investigation showed that 4NQO is a substrate for human, rat and mouse GSH transferases. Al-Kassab *et al.* [7] deduced that the spontaneous reaction of 4NQO with GSH at elevated pH produced a conjugate in which GSH replaced the nitro group of 4NQO. By use of m.s. and n.m.r. we have proven the structure of the conjugate, 4-glutathion-*S*-ylquinoline 1-oxide, formed during the GSH transferase-catalysed reaction (J. S. Stanley, J. O. Lay, Jr., D. W. Miller & D. C. DeLuca, unpublished work):

The observation that 4NQO was conjugated by human liver cytosol as efficiently as was DCNB when these two substrates were compared at the same concentration (0.1 mM) in the assay indicates that 4NQO is an excellent substrate for one or more of the human liver GSH transferase isoenzymes. The rodent liver cytosols examined in this study exhibited 18–89-fold higher activities towards 4NQO than did the human liver cytosol. The somewhat greater abundance of GSH transferases in rodent livers than in human liver [24] is insufficient to account for this large difference in 4NQO-conjugating activity, which appears to be due to species differences in GSH transferase isoenzyme composition. Comparative studies on the subunit compositions of human and rodent liver GSH transferases have shown Ya subunits to be predominant in human liver, whereas Yb subunits were more abundant in rodent livers, and hepatic expression of Yf subunits was observed only in mice,

particularly in male mice [25–27]. Mouse liver GSH transferases that were active towards 4NQO were composed of Yb and Yf subunits, and not of Ya subunits.

The same pattern of functional heterogeneity of Yb polypeptides was observed with 4NQO as with DCNB as substrate. GT-9.3 had relatively low activity towards 4NQO, whereas much higher activities were associated with the DCNB-conjugating Yb subunits of class Mu transferases GT-8.7, GT-8.8a and GT-8.8b and with a YfYf class Pi isoenzyme, GT-9.0. In studies on rat liver GSH transferases the homodimeric YbYb transferase with high activity towards DCNB was designated Yb₁Yb₁, in contrast with the structurally distinct Yb₂Yb₂ isoenzyme, which had high activity toward *trans*-4-phenylbut-3-en-2-one [28,29]. It has been concluded [27,30] that a Yb₁Yb₁ DCNB-conjugating isoenzyme purified from the livers of male mice corresponds to GSH transferase isoenzyme GT-8.7 [20] from female CD-1 mice.

The catalysis of 4NQO conjugation by both Yb₁- and Yf-containing isoenzymes suggests that hepatic conjugation of 4NQO is due primarily to different isoenzymes in male mice and in female mice. The major hepatic GSH transferase isoenzyme in male mice consists of Yf subunits, whereas Yb₁ polypeptides predominate in the livers of female mice [20,25–27,30]. The severalfold higher cytosolic 4NQO-conjugating activity in livers from male mice than in livers from the other rodents examined is in accord with the greater hepatic concentration of GSH transferases in male mice than in female mice or male rats [26,27] and with the absence of a YfYf isoenzyme from rat liver [25].

The conjugation of 4NQO by human and rodent liver GSH transferases, the catalysis of this reaction by different classes of isoenzymes, the wide distribution of activity towards 4NQO among extrahepatic organs and the low rate of spontaneous reaction of 4NQO with GSH in the most prevalent physiological pH range [12] indicate

that conjugation by GSH transferases may have major significance in the detoxication of 4NQO.

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