The *trans*-stilbene oxide-active glutathione transferase in human mononuclear leucocytes is identical with the hepatic glutathione transferase μ

Janeric SEIDEGÅRD,*‡ Claes GUTHENBERG,† Ronald W. PERO* and Bengt MANNERVIK†

*Department of Molecular Ecogenetics, The Wallenberg Laboratory, University of Lund, Box 7031, S-220 07 Lund, Sweden, and †Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

A glutathione transferase from human mononuclear leucocytes with high activity towards *trans*-stilbene oxide (GT-tSBO) was purified. GT-tSBO is expressed in only about 50% of the individuals studied. As judged from activity measurements, immunological studies and the fact that only those individuals who express glutathione transferase μ have high activity towards *trans*-stilbene oxide, it is concluded that the hepatic transferase μ is identical with the glutathione transferase (GT-tSBO) in mononuclear leucocytes.

INTRODUCTION

The glutathione transferases are a group of multifunctional proteins that play an important role in the biotransformation of many different biologically active compounds [1,2]. On the basis of physical and immunological properties and by substrate specificities and protein structure, the human glutathione transferases have been divided into three distinct classes, named Alpha, Mu and Pi [3]. In addition to the class Alpha isoenzymes, some adult livers contain a class Mu isoenzyme (named glutathione transferase μ) [4]. Transferase μ is present in high concentration, but only in some individuals (about 60% in a small sample of $n = 23$). This enzyme has also been found in adult adrenal glands. The classification of the purified glutathione transferases [3] is consistent with a genetic model involving three autosomal gene loci [5,6]. Class Mu corresponds to locus GST1, which appears to exhibit multiple alleles. Approx. 50% of a large number (several hundreds) of individuals that have been analysed express the GST1 null phenotype. In contrast with the liver, most extrahepatic organs express class Pi transferases [2]. Thus there is a multitude of different glutathione transferases, and the occurrence of the various isoenzymes is organ-dependent. Recently it was found that an isoenzyme of glutathione transferase in mononuclear leucocytes (MNL) with high activity towards *trans*-stilbene oxide (tSBO) was expressed only in some individuals [7]. The inter-individual differences of activity showed a 100–200-fold variation, and this property is inherited in an autosomal dominant fashion [8]. A question has arisen whether this isoenzyme (GT-tSBO) is identical with, or closely related to, any other previously identified human glutathione transferase.

MATERIALS AND METHODS

Chemicals

1-Chloro-2,4-dinitrobenzene (CDNB) and *trans*-stilbene oxide were obtained from EGA-Chemie, Steinheim/Albruch, Germany. GSH was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. *trans*-[³H]stilbene

oxide (2 Ci/mmol) was synthesized in collaboration with Dr. Åke Pilotti, Department of Organic Chemistry, University of Stockholm, according to a published procedure [9].

Methods

Human liver tissue was obtained from kidney donors, judged to have normal livers (by morphological criteria) [10]. Liver biopsies were obtained from patients scheduled for gall-bladder surgery. Peripheral-blood samples were also obtained from the same patients. Glutathione transferases α - ϵ and μ were purified to homogeneity, as previously described [11]. Glutathione transferase π was purified from human placenta by the procedure of Mannervik & Guthenberg [12]. MNL were isolated from whole blood of apparently normal individuals by a standard procedure using Isopaque/Ficoll gradient centrifugation [13]. The cells were homogenized in 5 vol. of 0.25 M-sucrose and centrifuged at 10000 g for 15 min, and the resulting supernatant was re-centrifuged at 105000 g for 1 h. Supernatants from individuals showing high and low glutathione transferase activity with tSBO were pooled separately. The isoenzymes of glutathione transferase were purified from the pooled fractions by use of affinity chromatography on *S*-hexylglutathione coupled to epoxy-activated Sepharose 6B and chromatography on hydroxyapatite.

Antibodies to transferase μ , the hepatic basic transferases (class Alpha), and transferase π from human placenta were raised in rabbits. Immunoblotting analysis was performed essentially as described by Burnette [14].

Glutathione transferase activity towards CDNB was determined spectrophotometrically at 30 °C [15]. The assay procedure utilized for measuring glutathione transferase activity towards tSBO was essentially a modification [7] of that reported by Gill *et al.* [9].

RESULTS

A recent characterization of the glutathione transferase activity towards tSBO in peripheral resting MNL showed that a large difference in the activity between

Abbreviations used: MNL, mononuclear leucocytes; GT, glutathione transferase; tSBO, *trans*-stilbene oxide; CDNB, 1-chloro-2,4-dinitrobenzene.

‡ To whom correspondence should be addressed.

individuals exists [7,8]. This large inter-individual difference was not found when CDNB was used as the electrophilic substrate [7]. Furthermore, the high activity against tSBO was only detected in about 50% of a normal population of 248 individuals [8].

Glutathione transferases were purified from the cytosol fraction of MNL. The activity profiles after the final hydroxyapatite chromatography were determined with both CDNB and tSBO. MNL having high activity towards tSBO showed a peak in both activity profiles at 0.1 M-phosphate in the elution gradient, the position expected for glutathione transferase μ [11]. Hydroxyapatite chromatography of glutathione transferases from MNL showing low activity against tSBO gave no peak in that position. Glutathione transferase π does not bind to the hydroxyapatite, and class Alpha enzymes are eluted at a significantly higher phosphate concentration than transferase μ .

Fractions obtained after purification of transferases from MNL having high activity and low activity with tSBO were analysed by immunoblotting with antibodies raised against human class Alpha and class Pi transferases and against glutathione transferase μ . Representatives of classes Pi and Alpha could be detected in appropriate fractions of all MNL, whereas transferase μ was identified only in fractions obtained from MNL showing high activity towards tSBO. The reaction with antiserum

Table 1. Correlation between presence of glutathione transferase μ and GT-tSBO in liver supernatant from eight different individuals

The presence of transferase μ was determined by isoelectric focusing [16,17]. Activity less than 0.05 nmol/min per mg is marked '–'; activity greater than 30 nmol/min per mg is marked '+' [7].

Subject no.	Transferase μ	GT-tSBO activity
1	Present	+
2	Present	+
3	Lacking	–
4	Lacking	–
5	Present	+
6	Present	+
7	Lacking	–
8	Present	+

Table 3. Specific activities of purified human glutathione transferases with CDNB and tSBO as substrates

Transferase	Source	Substrate ...	Activity (μ mol/min)	
			CDNB	tSBO
α – ϵ	Liver		64	0.002
μ	Liver		187	5.2
π	Placenta		105	0.002

raised against transferase μ was weaker than that obtained with antiserum against transferase π , reflecting the lower concentration of transferase μ in MNL.

Table 1 shows that the presence of glutathione transferase μ is strictly correlated to expression of activity towards tSBO in liver samples from the eight individuals examined. The activity in livers containing transferase μ was at least 600-fold higher than the activity in livers lacking transferase μ .

In another experiment the glutathione transferase activities towards tSBO and CDNB were measured in both liver and blood from the same individuals (Table 2). The GT-tSBO phenotypes determined by analysis of MNL correspond to the homozygotes and heterozygotes for the described GT-tSBO activity, and to homozygotes for the lack of this enzyme activity. Table 2 shows that the activity in blood matches excellently the activity found in the liver from the same individual.

The activities of the purified glutathione transferases α – ϵ of class Alpha, transferase μ isolated from adult human liver, as well as of the class Pi enzyme isolated from human placenta were determined with both tSBO and CDNB as substrates (Table 3). Glutathione transferase μ displayed an about 2500-fold higher activity with tSBO as substrate than did the other isoenzymes, but the activities with CDNB were in the same range with all three classes of isoenzymes. On the basis of the results of the present investigation, we conclude that GT-tSBO is identical with the hepatic glutathione transferase μ .

DISCUSSION

In the purification of glutathione transferases from MNL having high activity towards tSBO, the activity

Table 2. Activities of glutathione transferase towards tSBO and CDNB in liver supernatant fractions and whole blood from different individuals phenotyped according to GT-tSBO

The phenotypes marked ++, + and – correspond to very high, high and low activity towards tSBO [8]. The activities in liver (means and ranges) are expressed as nmol/min per mg of protein, and the activities in blood (mean and ranges) are expressed as nmol/min per ml of whole blood.

Phenotype	n	Activity towards tSBO		Activity towards CDNB
		In liver	In blood	In liver
–	3	0.06 (0.05–0.07)	0.19 (0.12–0.26)	499 (387–611)
+	6	40.8 (36.5–45.0)	0.66 (0.52–0.80)	682 (526–837)
++	2	57.4 (51.9–62.8)	1.35 (1.18–1.53)	762 (757–767)

profiles determined with tSBO coincided with that expected for glutathione transferase μ .

The immunoblotting studies, with antibodies raised against different classes of glutathione transferase, revealed that the acidic class Pi transferase was the major isoenzyme in leucocytes having high activity against tSBO, as well as in leucocytes showing low activity with that substrate. According to the reaction with the different antisera, the transferases of class Alpha and class Mu (when present) were less abundant forms of enzyme.

The relatively high activity in the conjugation of tSBO is also characteristic of the purified glutathione transferase μ , which has a 2500-fold higher specific activity with this substrate than have isoenzymes of the other two classes of human glutathione transferase. Furthermore, liver samples from individuals expressing glutathione transferase μ show a 1000-fold higher cytosolic activity towards tSBO than do liver samples that lack this enzyme form.

The physiological consequences of the absence or presence of glutathione transferase μ are not fully understood. It has been suggested that the enzyme may provide protection against chemical mutagens and carcinogens [4]. Support for this proposal derives from a clinical study on the influence of GT-tSBO on the frequency of lung cancer in a smoking population. This investigation showed that GT-tSBO occurred in 35% of the smokers who developed lung cancer, whereas the enzyme was significantly more frequent (59%) in the smokers without symptoms of lung cancer [18]. Whether this isoenzyme may affect the susceptibility of smokers to forms of neoplasia other than lung cancer remains to be investigated.

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