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Human liver microsomal glutathione transferase

Substrate specificity and important protein sites

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Human liver microsomal glutathione transferase displays the following glutathione peroxidase/transferase activities: dilinoleoylphosphatidylcholine hydroperoxide (0.03 and 0.17 μmol/min · mg, unactivated and *N*-ethylmaleimide-activated enzyme, respectively), linoleic acid hydroperoxide (0.09 and 0.15 μmol/min · mg), cumene hydroperoxide (0.04 and 3 μmol/min · mg), methyl linoleate ozonide (0.02 and 1.2 μmol/min · mg) and 1-chloro-2,4-dinitrobenzene (1.9 and 24 μmol/min · mg). The activation of glutathione peroxidase activities are much higher than previously observed. The activity towards a phospholipid hydroperoxide is noteworthy since protection against lipid peroxidation has been implied. Methyl linoleate ozonide has not previously been characterised as substrate for any microsomal glutathione transferase. Human liver microsomal glutathione transferase displays an isoelectric point of 9.4 and a structure in agreement with that deduced from the cDNA sequence. Gel electrophoretic analysis shows that proteolytic activation of the human enzyme corresponds to cleavage at Lys-41, thus defining the critical activation site.

Microsomal glutathione transferase (human); Glutathione peroxidase activity; Proteolytic activation

1. INTRODUCTION

Microsomal glutathione transferase* [1] is the membrane-bound member of the glutathione transferase group of enzymes [2] that catalyse the conjugation of glutathione to hydrophobic electrophiles [3] thus serving in detoxication. Cytosolic glutathione transferases have been purified from many sources and exist as families of related proteins with M_r values in the 24–28 kDa region [4]. Rat liver microsomal glutathione transferase has a molecular mass of 17.3 kDa, with an amino acid sequence analysed both at the protein and cDNA level [5,6], and appears to have no closely related isozyme(s). Microsomal glutathione transferase is present predominantly in the liver and has been purified from rat [7,8], mouse [9] and human [10]. These species express closely related enzymes [6,9,10].

The existence of human liver microsomal glutathione transferase activity has been noted [7] but no activation with *N*-ethylmaleimide (NEM) was observed (a characteristic feature of rat liver microsomes and purified microsomal glutathione transferase [12]). Purification of the human microsomal glutathione transferase by McLellan et al. [10] showed that human liver contains an immunologically related enzyme which can be activated by NEM. The cDNA encoding the human microsomal glutathione transferase has been published [6]. Genomic analysis revealed the presence of a single gene on chromosome 12 in humans [11].

The present study characterises the glutathione peroxidase activity of the enzyme towards (phospho-)lipid hydroperoxides, activation by trypsin, physicochemical properties and definition of the N terminus of the protein.

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*Glutathione transferase, EC 2.5.1.18.

Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; NEM, *N*-ethylmaleimide.

2. MATERIALS AND METHODS

2.1. *Materials*

Methyl linoleate ozonide was a gift from Ria M. Vos and P.J. van Bladeren, TNO-CIVO, Toxicology and Nutrition Institute, Dept. of Biochemical Toxicology, Zeist, The Netherlands. Trypsin, trypsin inhibitor, linoleic acid, linoleic acid ethyl ester and glutathione reductase (Baker's yeast) were from Sigma Chemical Co., St. Louis, MO. Dilinoleoylphosphatidylcholine was from Lipid Products (Surrey, UK). Rainbow marker molecular weight standards were from the Radiochemical Centre (Amersham, UK). Lipid hydroperoxides were prepared as described [13]. Isoelectric point marker proteins were from Pharmacia (Uppsala, Sweden).

2.2. *Enzyme purification*

Human liver samples were obtained from post-mortem livers and

immediately placed in ice-cold 0.25 M sucrose containing 1 mM GSH, 0.1 mM EDTA, 10 mM potassium phosphate, pH 7. The liver sample (100–200 g) was diced and homogenised in a Polytron tissue homogeniser (2 × 10 s) after which preparation of microsomes and purification of enzyme was performed by a modification of the procedure described for the rat enzyme [8]. The hydroxyapatite column chromatography step was replaced by a batch procedure where solubilized microsomes were adsorbed for 10 min to hydroxyapatite (20 g) equilibrated with 10 mM potassium phosphate, pH 7, 1 mM GSH, 0.1 mM EDTA, 1% Triton X-100, 20% glycerol (buffer A). Low speed centrifugation (500 × *g*, 3 min) was used to pellet the hydroxyapatite. The hydroxyapatite was washed twice in the same buffer (2 × 100 ml) and subsequently once with buffer A containing 50 mM potassium phosphate (100 ml) after this the enzyme was desorbed with buffer A containing 200 mM potassium phosphate (2 × 100 ml). Sephadex G-25 desalting (in buffer A) and CM-Sepharose chromatography were performed as described [8]. To increase purity, the enzyme pool was diluted 10-fold in buffer A that had been adjusted to pH 8 (with 10 M KOH), and the CM-Sepharose step was repeated in this buffer. Further purification by sodium dodecylsulphate-polyacrylamide gel electrophoresis followed by electroelution/electroblotting [14] was performed for determination of the amino acid composition and N-terminal amino acid sequence.

2.3. *Enzyme assays*

Microsomal glutathione transferase activity was measured as described [8] using 0.5 mM 1-chloro-2,4-dinitrobenzene (CDNB) as the second substrate. Glutathione peroxidase activity was measured in a coupled assay system, with the inclusion of 0.1% Triton X-100 at pH 6.5 [12].

2.4. *Physicochemical characterisation*

Sodium dodecylsulphate-polyacrylamide gel electrophoresis was performed according to Laemmli [15] in 15% polyacrylamide gels.

Purified human microsomal glutathione transferase (0.3 mg/ml) was treated with trypsin (1 mg/ml) at room temperature for the times indicated and analysed by sodium dodecylsulphate-polyacrylamide gel electrophoresis after inclusion of trypsin inhibitor (2-fold excess). Protein was visualised by silver staining [16] and determined by the method of Peterson [17] using bovine serum albumin as a standard. Determination of the isoelectric point was performed as described [12], using marker proteins, rat microsomal glutathione transferase and cytochrome *c* as standards. Amino acid analysis after acid hydrolysis (6 M HCl containing 0.5% (w/v) phenol for 24 h at 110°C in evacuated tubes) performed directly on protein containing gel slices or with electroeluted samples [14] was carried out with an LKB 4151 Alpha Plus amino acid analyser. N-terminal amino acid sequence analysis of electroblotted protein was performed as described [14].

3. RESULTS AND DISCUSSION

Human microsomal glutathione transferase displays peroxidase activity towards a range of hydroperoxides (Table I). Especially noteworthy is the activity towards a phospholipid hydroperoxide, a feature unique to the enzyme compared to cytosolic glutathione transferases. The glutathione peroxidase activity of human microsomal glutathione transferase is similar to, or lower than that of the rat enzyme [20]. Human microsomal glutathione transferase also displays activity with methyl linoleate ozonide, particularly in the activated state. The glutathione peroxidase activity of human microsomal glutathione transferase could be involved in glutathione-dependent protection of membranes against lipid peroxidation [20,21] and thus protect cells from oxidative stress.

Table I

Glutathione transferase and glutathione peroxidase activity of human liver microsomal glutathione transferase

Substrate	Concentration (μM)	Activity (μmol/min · mg)	Activity of NEM-treated enzyme (μmol/min · mg)
1-Chloro-2,4-dinitrobenzene	500	1.9	24
Dilinoleoyl-phosphatidylcholine hydroperoxide	200	0.03	0.17
Linoleic acid hydroperoxide	100	0.09	0.15
Cumene hydroperoxide	500	0.04	3.0
Methyl linoleate ozonide	400	0.02	1.2

Assay and activation conditions as given in section 2.

We observed a CDNB conjugation activity of the purified human microsomal glutathione transferase (Table I) which is comparable to that of the rat, mouse and human enzymes [9,10,12]. Surprisingly, activation by NEM does not occur in human microsomes [10,18]. Perhaps endogenous inhibitory compounds [19] are lost during purification since activation is restored already after the hydroxyapatite step. Furthermore, detergents do increase the human microsomal CDNB activity up to 3-fold.

The isoelectric point of the human enzyme was determined to be 9.4 ± 0.1 (n = 3), which is lower than that

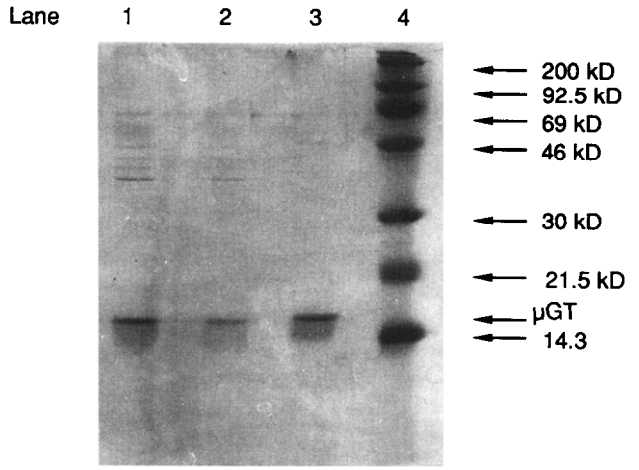

Fig. 1. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of purified human liver microsomal glutathione transferase. Lanes 1 and 2, the human enzyme (2 and 1 μg respectively); lane 3, the rat liver enzyme (2 μg); lane 4, molecular mass markers.

Table II

Amino acid composition of human liver microsomal glutathione transferase compared to the composition deduced from the cDNA sequence [6]

	Protein (mol/mol)	
	From hydrolysis	From cDNA
Asx	15.7	14
Thr	10.2	10
Ser	9.5	9
Glx	8.9	6
Pro	7.2	7
Ala	14.7	14
Val	9.0	10
Ile	4.5*	8
Leu	21.7	21
Tyr	6.8	9
Phe	8.0	10
His	3.0	3
Lys	8.5	7

* Low because of slow hydrolytic release.

of the rat enzyme (pI = 10.1 [12]). The band is not well focused which might depend on the quality of liver samples or on a post-translational modification. Nevertheless, the human enzyme is strongly basic which facilitates purification by the same method that was developed for the rat enzyme [8].

The amino acid composition of human microsomal glutathione transferase was determined by acid hydrolysis of protein containing gel slices from sodium dodecyl sulphate-polyacrylamide gel electrophoresis or after electroelution, since contamination by higher molecular mass proteins (Fig. 1) prevented analysis without the electrophoretic step. After hydrolysis of gels, some amino acids (Cys, Met, Arg, Gly) are not reliably quantified because of destruction or masking by liberated components, but the content of remaining amino acids (Table II) is in reasonable agreement with that expected from the cDNA [6]. Furthermore direct N-terminal sequence analysis of electroblotted protein was carried out for 16 cycles and the results obtained agreed with those deduced from the cDNA [6]. This fact, together with the composition and an M_r of approximately 17 kDa (Fig. 1), indicates that the human enzyme is expressed as the full-length 154-residue protein.

The rat liver microsomal glutathione transferase is activated by trypsin with cleavages at Lys-4 and Lys-41 [22], but which of the two (or both) cleavage sites that correlates with activation was previously unknown. Since the human enzyme lacks Lys-4 (replaced by Thr) it is now highly significant that the absence of this cleavage site does not influence the possibility to activate the enzyme (10-fold increase in activity), suggesting that Lys-41 is the important cleavage site for activation with trypsin. Gel electrophoretic analysis of trypsin-treated human microsomal glutathione transferase reveals the generation of a fragment corresponding to a cleavage at this site (Fig. 2). Previous studies on the rat enzyme [22] showed that NEM induces a conformational change that makes the enzyme more susceptible to tryptic cleavage at Lys-41. In fact, the NEM treated rat enzyme could retain maximal activity when fully cleaved at Lys-41 [22], which supports the conclusions above.

In view of the overall similarity between the human and rat microsomal glutathione transferases it is reasonable to assume that the same type of regulation, by reactive metabolites attacking Cys-49 [23] or other endogenous mechanisms [24], pertains to the human enzyme. It may play a role in protection against oxidative

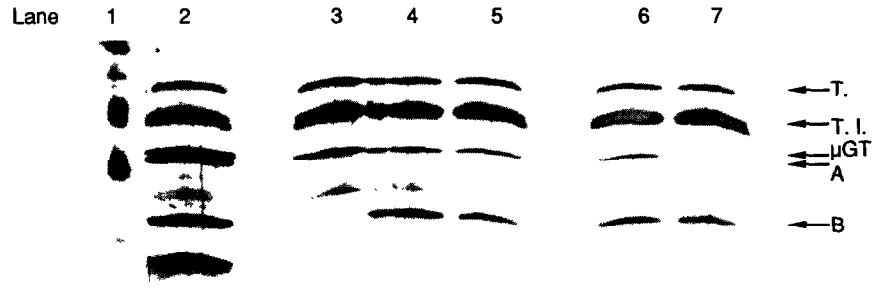

Fig. 2. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of purified human liver microsomal glutathione transferase activated by trypsin. Lane 1, molecular mass markers (21.5 and 14.3 kDa); lane 2, rat liver microsomal glutathione transferase treated with trypsin; lanes 3–7, human microsomal glutathione transferase (0.5 μg) treated with trypsin for 0, 35, 45, 60 and 480 min as described in section 2. The positions of trypsin (T), trypsin inhibitor (T.I.), microsomal transferase (μGT), fragments resulting from cleavage at Lys-4 (A) and Lys-41 (B) are indicated.

stress through its glutathione peroxidase activity and against electrophiles through its glutathione conjugation capacity.

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