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THE DISTRIBUTION OF MICROSOMAL GLUTATHIONE TRANSFERASE AMONG DIFFERENT ORGANELLES, DIFFERENT ORGANS, AND DIFFERENT ORGANISMS

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Abstract—1. In the present study we have used both enzyme assay with 1-chloro-2,4-dinitrobenzene as substrate and immunochemical quantitation to examine the distribution of microsomal glutathione transferase in different organelles, in different organs, and in different organisms.

2. This enzyme was found to constitute 3% and 5%, respectively, of the total protein recovered in the microsomal and outer mitochondrial membrane fractions from rat liver. Microsomal glutathione transferase present in other subcellular fractions can be accounted for by contamination by the endoplasmic reticulum.

3. In contrast to the situation with rat liver microsomes the glutathione transferase activities of microsomes from extrahepatic tissues of this same animal could not be activated by treatment with *N*-ethylmaleimide. Nonetheless, significant albeit low levels of a protein with the same molecular weight and immunochemical properties as the rat liver enzyme could be detected in microsomes from several extrahepatic tissues, notably the intestine, the adrenal, and the testis.

4. Of those mammals for which fresh liver could be obtained, all demonstrated *N*-ethylmaleimide-activatable glutathione transferase activity in their liver microsomes. On the other hand, representatives for fish, birds, and amphibia did not demonstrate such activatable transferase activity in their liver microsomes. Toad was the only species that had a notable (twofold) sex difference in their level of hepatic microsomal glutathione transferase activity.

The glutathione transferases are a large group of ubiquitous enzymes which catalyze the conjugation of various electrophiles with the tripeptide glutathione [1]. Among the substrates for these enzymes are a number of pharmacologically active, toxic, or genotoxic xenobiotics or their metabolites [2]. Thus, the glutathione transferases are involved, among other things, in detoxifying reactive intermediates of xenobiotic metabolism and in converting xenobiotics to water-soluble conjugates which can be excreted in the urine and bile [3].

Cytosolic glutathione transferases have been purified from a large number of different species and from various organs and many of these enzymes have been thoroughly characterized [4]. However, less is known at present about the distinct microsomal glutathione transferase, which we recently purified and have begun characterizing [5, 6]. This microsomal enzyme may have a special role to play in xenobiotic metabolism, since it is localized primarily in the same membrane [7] as the cytochrome P-450 system, which converts many xenobiotics to reactive electrophilic intermediates which are known or potential substrates for glutathione transferases [2].

In the present investigation we are concerned with the distribution of microsomal glutathione transferase in different organelles, different organs, and different organisms. The subcellular localization of this enzyme might reveal further functional aspects. The level of microsomal glutathione transferase in different tissues should be compared to the corresponding levels of other drug-metabolizing systems and might also give hints with regard to possible roles of this enzyme in other physiological processes. Finally, the distribution of microsomal glutathione transferase among various species provides a picture of its evolutionary development and may yield additional clues concerning its functional significance.

Microsomal glutathione transferase activity has been measured here by assaying the ability of different preparations to conjugate 1-chloro-2,4-dinitrobenzene with glutathione before and after treatment with *N*-ethylmaleimide. We have shown previously that this sulfhydryl reagent selectively activates the microsomal glutathione transferase [8]. In addition the amount of this enzyme present has been quantitated by immunoblotting. Some of the data reported here have been published in preliminary form [9, 10].

MATERIALS AND METHODS

Animals. Male white New Zealand rabbit (3 kg, State Bacteriological Laboratory, Stockholm, Sweden), Sprague–Dawley rats (180–200 g, Anticimex, Stockholm, Sweden), C57 black mice (18–

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20 g, Anticimex, Stockholm, Sweden), Syrian hamsters (70–80 g, State Bacteriological Laboratory), guinea pig (Duncin Hartley) (175 g, H.B. Sahlins Försöksdjursfarm, Malmö, Sweden), hens and roosters (2 years and 7 months, respectively), toads (*Xenopus laevus*, several years of age, a gift of Docent Peter Århem, Department of Neurophysiology, Karolinska Institute), pike (*Esox lucius*, 0.55–1.83 kg, captured in the brackish water of the archipelago outside of Stockholm), *Rhodospirillum rubrum* (log-phase culture was a gift from Dr. Stefan Nordlund of this department), and livers from bulls and cows (2.5 years, Eklunds Slakteri, Stockholm, Sweden) and from pigs (4 months (the males were castrated), Eklunds Slakteri, Stockholm, Sweden) were obtained from the sources indicated. All animals used were sexually mature except for the guinea pigs (10 days old). Human material in the form of post-mortem liver samples frozen in liquid nitrogen was obtained from Dr. Christer von Bahr at the Huddinge Hospital, Huddinge, Sweden.

Preparation of microsomes and other subcellular fractions. Liver microsomes from nearly all these species (except for the bacteria, which were simply sonicated before use) were prepared according to Ernster *et al.* [11] and washed one additional time with 0.15 M Tris–HCl, pH 8, in order to minimize cytosolic contamination [11]. Microsomes from pike liver were prepared according to [12]. Microsomes from extrahepatic organs of the rat were prepared in a similar manner with slight modifications [9]. Nuclei [13], whole mitochondria [14], inner [15] and outer [16] mitochondrial membranes, Golgi I (enriched in lipid filled Golgi vesicles) [17], Golgi II (mainly consisting of Golgi cisternae) [17], lysosomes [18], plasma membrane [19], and cytosol [11] were all prepared from rat liver using published procedures and the fractions obtained were characterized using marker enzymes to assure that the preparation had been successful.

Enzyme assay. Glutathione transferase activity was determined spectrophotometrically with 1-chloro-2,4-dinitrobenzene as substrate [20] both before and after treatment of samples with 1 mM *N*-ethylmaleimide for 30 sec at 0° [8]. These conditions were found to result in optimal activation of all the activatable fractions studied here. Except in the case of human liver, all activity measurements were performed on freshly isolated material.

Immunochemical quantitation. Conventional procedures were used to raise antibodies in rabbits against the purified, apparently homogeneous rat liver microsomal glutathione transferase. Serum from these rabbits was subsequently used to quantitate microsomal glutathione transferase by immunoblotting [21] after sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 15% gels according to Laemmli [22]. Purified rat liver enzyme was used to obtain a standard curve for each immunoblot. Quantitation was performed using ^{125}I-Protein A [23].

Miscellaneous. Protein was determined by the method of Peterson [24] using bovine serum albumin as standard. All chemicals used were of reagent grade and obtained from common commercial sources.

RESULTS

Subcellular distribution of the microsomal glutathione transferase in rat liver

Using a highly sensitive and specific immunochemical assay the levels of microsomal glutathione transferase in extensively purified subcellular fractions from rat liver have been examined. As can be seen in Table 1, about 3% of the total microsomal protein (of which 85–90% originates from the endoplasmic reticulum [25]) consists of the microsomal glutathione tranferase, a value in agreement with our earlier findings [5, 26]. As also shown, the low levels of microsomal glutathione transferase in all other organelles—with the exception of the mitochondrial outer membrane—can apparently be accounted for by contamination by the endoplasmic reticulum (judging from the distribution of NADPH-cytochrome *c* reductase, a marker for this organelle).

In the case of mitochondrial outer membranes approximately 5% of the total protein consists of the microsomal glutathione transferase.

Table 1. Subcellular distribution of the microsomal glutathione transferase in rat liver as quantitated by immunoblotting. Experimental details as described under Materials and Methods

Fraction	Microsomal glutathione transferase (μg/mg protein)	NADPH-cytochrome *c* reductase (μmol/min/mg)
Nuclei	4 ± 2* (13)	0.008 (10)
Mitochondria (whole)	7 ± 0.2 (23)	0.015 (18)
Mitochondria (inner membranes)	5 ± 1 (16)	0.005 (6)
Mitochondria (outer membranes)	48 ± 6 (150)	0.013 (16)
Golgi I	3 ± 1 (10)	0.020 (25)
Golgi II	3 ± 1 (10)	0.015 (18)
Lysosomes	1 ± 0 (3)	0.004 (5)
Microsomes	31 ± 6 (100%)	0.082 (100%)
Plasma membrane	<0.3† (1)	N.D.
Cytosol	<0.3 (1)	N.D.

* S.E.M. (N = 2–6). The numbers in parenthesis are percentages of the microsomal value.
† Detection limit.
N.D. = not determined.

Table 2. Activation of mitochondrial glutathione transferase activity by *N*-ethylmaleimide*

	Activity towards 1-chloro-2,4-dinitrobenzene (nmol/min-mg protein)		
	Control	Activated	Activated minus control
Observed for whole mitochondria	70 ± 8	340 ± 50	270
Calculated as due to microsomal glutathione transferase present†	14	210	196

* Mitochondria were isolated, glutathione transferase activity measured and activation performed as described in Materials and Methods.

† Calculated by multiplying the level of microsomal glutathione transferase present (7 μg/mg protein—see Table 1) by the specific activity of the purified enzyme (2 and 30 mole/min-mg protein for the unactivated and activated enzymes, respectively).

As shown in Table 2, the glutathione transferase activity of whole mitochondria towards 1-chloro-2,4-dinitrobenzene can be activated about fivefold by treatment with *N*-ethylmaleimide, further demonstrating the presence of the same enzyme in this organelle as in the endoplasmic reticulum. The increase in mitochondrial activity observed after *N*-ethylmaleimide treatment agrees fairly well with the increase expected on the basis of the level of microsomal glutathione transferase present as determined immunochemically (see Table 2, activated minus control).

Distribution of the microsomal glutathione transferase among different organs of the rat

In an earlier study we found that microsomes from extrahepatic organs of the rat do not contain significant levels of *N*-ethylmaleimide-activatable glutathione transferase activity towards 1-chloro-2,4-dinitrobenzene ([9]; see also Table 2). From this finding we concluded that microsomal glutathione transferase is localized primarily, if not exclusively in the liver of this animal.

However, when the microsomal glutathione transferase was quantitated immunochemically in microsomes from extrahepatic tissues of the rat, significant levels of an immunochemically cross-reacting protein with the same molecular weight as the liver enzyme were detected in a number of organs, most notably the intestine, adrenal, and testis (Table 3). If, as seems highly probable, this cross-reacting protein is the microsomal glutathione transferase, then the glutathione transferase activity of intestinal and adrenal microsomes, for instance, should have increased at least twofold upon treatment with *N*-ethylmaleimide. One possible explanation for this discrepancy is considered in the Discussion.

Phylogenetic and sexual distribution

The glutathione transferase activity of liver microsomes from a variety of species towards 1-chloro-2,4-dinitrobenzene has also been assayed before and

Table 3. Extrahepatic distribution of microsomal glutathione transferase. Experiments as described under Materials and Methods

Organ	Activity* (nmoles/mg protein/min) Microsomes	Activated microsomes	Activated microsomes × 100	Content† (μg/ml microsomal protein)
Liver	126 ± 6 (100)	700 ± 107	556.0	31 ± 6
Kidney	8.54 ± 3.98 (7)	8.44 ± 3.98	98.9	≤0.3‡
Lung	15.4 ± 4.0 (12)	17.8 ± 9.4	116.0	0.9 ± 0.2
Intestine	60.2 ± 24.7 (48)	37.4 ± 11.4	62.0	4.0 ± 2
Adrenal	51.9 ± 19.8 (41)	41.2 ± 24.3	79.3	3.2 ± 0.5
Testicle	129 ± 28 (102)	129 ± 16	100.0	2.5 ± 0.7
Spleen	9.01 ± 3.25 (7)	5.96 ± 2.99	66.1	0.5 ± 0.1
Brain	7.95 ± 0.49 (6)	0.58 ± 0.29	7.3	≤0.3
Heart	7.23 ± 2.00 (6)	0.37 ± 0.37	5.1	N.D.§
Thymus	4.37 ± 2.51 (4)	6.90 ± 6.91	158.0	1.1 ± 0.3

* Three rats were pooled for each preparation and three such preparations were performed. Values are the means ± S.D. of the means for these three independent determinations. Figures in parenthesis are percentage of liver value (from Ref. 9).

† Mean ± S.E.M. of two determinations (from Ref. 10).

‡ Detection limit.

§ Not detectable.

Table 4. Glutathione transferase activity before and after treatment with *N*-ethylmaleimide in liver microsomes prepared from males and females of various species and a subjective estimate of cross-reactivity with antisera against the rat enzyme (− = no cross-reactivity)

Species	Sex	N	Activity* (1-chloro-2,4-dinitrobenzene) (nmol/min/mg) Untreated	Treated	Cross-reactivity	Ref.†
Human	M	2	65 ± 6	81 ± 8	—	
	F	2	76 ± 14	76 ± 14	—	
Human		9	68 ± 20			33
Rhesus monkey		2–3 pools of 5	176			33
Chimpanzee		2–3 pools of 5	143			33
Bull	M	3	57 ± 11	194 ± 61 (340‡)	medium	
Cow	F	3	45 ± 4	157 ± 22 (349)	medium	
Pig	M	3	100 ± 28	196 ± 29 (196)	—	
	F	3	80 ± 21	200 ± 25 (250)	—	
Rabbit	M	1	156	222 (142)	—	
Rat	M	4	110 ± 9	540 ± 25 (491)	strong	
	F	4	109 ± 13	560 ± 28 (514)	strong	
Mouse C57B1	M	4	157 ± 19	500 ± 31 (318)	strong	
C57B1	F	4	186 ± 19	509 ± 40 (274)	strong	
NMRI	F	9	147 ± 35	689 ± 140 (469)		34
Syrian hamster	M	4	329 ± 43	957 ± 58 (291)	medium	
	F	4	374 ± 52	1031 ± 123 (276)	medium	
Guinea pig	M	4	180 ± 20	423 ± 58 (235)	weak	
	F	4	152 ± 19	409 ± 42 (269)	weak	
Rooster	M	3	240 ± 47	275 ± 53	—	
Hen	F	2	243 ± 77	297 ± 63	—	
Toad	M	2	1054 ± 16	0	—	
	F	2	2417 ± 226	0	—	
Pike	M	3	129 ± 4	101 ± 10	—	
Rhodospirillum rubrum		1	0	0	—	

* Mean (± S.E.M.).
† When other than present.
‡ % of control.

after treatment with *N*-ethylmaleimide (Table 4). The basal activities of the different mammalian microsomes varied between 45 nmol conjugate formed/min-mg protein for the cow and 374 nmol conjugate formed/min-mg protein for the female hamster, with most values falling between 100 and 200 nmol conjugate formed/min-mg protein. These basal activities could all be activated by *N*-ethylmaleimide and the degree of activation varied between 42% for the rabbit and 5–6-fold for rats and NMRI mice.

The one exception to this last statement is human liver microsomes. However, in this case the material we used had been stored frozen for months before we received it. In rat liver microsomes activation by *N*-ethylmaleimide is highest in freshly prepared material and is gradually and completely lost upon storage in the freezer [7]. Thus, studies with fresh human liver (to which we have not yet had access) must be performed in order to determine whether *Homo sapiens* have *N*-ethylmaleimide-activatable microsomal glutathione transferase in their livers.

Of the non-mammalian species, the chicken, toad and pike all demonstrated glutathione transferase activity in their liver microsomes which was not activated by *N*-ethylmaleimide treatment, whereas the bacterium *Rhodospirillum rubrum* demonstrated no activity at all. Of particular interest is the specific glutathione transferase activity in microsomes from toad liver, which is 2–4-fold higher than the corresponding cytosolic specific activity in the same tissue (569 ± 69 nmol/mg-min) and several-fold greater than the basal microsomal activities in the other species examined. The basal specific glutathione transferase activity of rat liver microsomes towards 1-chloro-2,4-dinitrobenzene is approximately 10 times lower than the corresponding cytosolic activity. The toad is the first example where the microsomal activity is higher.

Microsomes from the livers of cows and bulls, mice, Syrian hamsters, and guinea pigs contained a protein which has the same minimum molecular weight (i.e. 14,000) as the rat liver microsomal glutathione transferase and which cross-reacts with antibodies directed towards the rat enzyme. However, it was not possible to quantitate the enzyme immunochemically in these preparations, since the degree of cross-reaction is not known. None of the other liver microsomal preparations contained such a protein.

Finally, as can also be seen from Table 4, there are no marked differences in the glutathione transferase activities of liver microsomes from males and females of any of the species examined, with the exception of the toad.

DISCUSSION

Upon examination of the subcellular distribution of microsomal glutathione transferase in rat liver we were surprised to discover that this protein accounts for 5% of the total protein in the outer mitochondrial membrane. The question immediately arises as to what the functional significance of this localization is. We do not know, but speculate that the microsomal glutathione transferase localized on the outer mitochondrial membrane may be involved in the binding and transport of heme (the synthesis of which is completed in mitochondria) and of heme metabolites, as suggested for the cytosolic glutathione transferase B [27]. The localization of this protein in both the endoplasmic reticulum and the outer mitochondrial membrane also suggests a biogenetic relationship and/or topological continuity between these two membranes. Interestingly, a number of other enzymes known to be localized primarily on the endoplasmic reticulum—including cytochrome b_5, cytochrome b_5 reductase, and cytochrome P-450—have also been reported to be present on the outer mitochondrial membrane [28–30]. Since the endoplasmic reticulum contains 19% of the total cellular protein in rat hepatocytes and the corresponding value for the outer mitochondrial membrane is only 2% (calculated from Refs. 25 and 31), it can be calculated that 80% of the total microsomal glutathione transferase in rat liver is localized on the endoplasmic reticulum.

However, it is becoming apparent that many marker enzymes for cellular organelles are enriched in a particular organelle, but also found in lesser amounts in other cellular components [28–30]. For instance, the enzyme NADPH-cytochrome *c* reductase used here as a marker for the endoplasmic reticulum, is also present on the outer nuclear and outer mitochondrial membranes. Thus, if the outer nuclear membrane contains the same ratio of microsomal glutathione transferase/NADPH-cytochrome *c* reductase as does the endoplasmic reticulum, we would draw the incorrect conclusion that the microsomal glutathione transferase activity present in the nuclear fraction is due to contaminating microsomes. However, as seen in Table 1, even if all of the microsomal glutathione transferase present in the nuclear fraction is actually localized on the nuclei, the level of this enzyme in nuclei is still only 10% of the corresponding level in the endoplasmic reticulum.

The microsomal glutathione transferase has been found to demonstrate *N*-ethylmaleimide-activatable glutathione peroxidase activity towards cumene hydroperoxide [5]. This observation suggests that the enzyme may be involved in preventing lipid peroxidation and, indeed, it has been reported that rat liver microsomes contain a heat labile trypsin-sensitive factor, presumably a protein, which prevents lipid peroxidation in a glutathione-dependent manner [32]. Such a function might explain the bimodal distribution of microsomal glutathione transferase in the endoplasmic reticulum and the mitochondrion, since the high heme and flavin contents in these organelles might give rise to relatively high levels of oxygen species which can initiate lipid peroxidation.

As is the case for many drug-metabolizing systems, the levels of microsomal glutathione transferase in rat liver are considerably higher than in other tissues. This finding also indicates that if the microsomal glutathione transferase has another role to play in addition to its involvement in drug metabolism, this function must be one for which the liver is responsible. However, there are also significant levels of this protein in certain extrahepatic tissues, most notably the intestine, the adrenal, and the testis. The fact that these three tissues contain microsomal glutathione transferase, as indicated immunochemically, which cannot be further activated with *N*-ethylmaleimide suggests that this enzyme may already be at least partially activated *in vivo* in these tissues. Of course, this is speculative, but since one of our main interests at present is the possibility of such *in vivo* activation of the microsomal glutathione transferase, it might be of interest to isolate this enzyme from the intestine, adrenal, or testis and look for clues concerning such a process.

Along these same lines, we have also been interested in the glutathione transferase of toad liver microsomes. This activity cannot be activated by *N*-ethylmaleimide—indeed, within 3 min of treatment with 1 mM of this reagent, the toad liver microsomal enzyme is completely inactivated. In order to determine whether this enzyme is present in the microsomes in an already activated form involving a disulfide bond, the toad liver microsomes were incubated with 5 mM dithioerythritol for 5 min, but their glutathione transferase activity was not affected in this experiment.

Finally, the distribution of microsomal glutathione transferase activity in different species suggests that the property of activation by *N*-ethylmaleimide is one that has arisen late in evolution, since this property was demonstrated only by liver microsomes from the mammalian species examined (with the exception of human beings). In addition, the rat enzyme is apparently sufficiently different from those of the other species tested—with the exceptions of cows and bulls, mice, Syrian hamster and guinea pig—that it does not cross-react with them immunologically.

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