Purification and characterization of hepatic glutathione *S*-transferases of rhesus monkeys

A family of enzymes similar to the human hepatic glutathione *S*-transferases

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Thirteen forms of glutathione *S*-transferase were purified from the livers of female rhesus monkeys (*Macaque mulatta*). Most (74.7%) of the activity in the hepatic cytosol adhered well to the GSH affinity column and could be eluted only with the addition of GSH to the eluting buffer. The predominant isoenzymes ($n = 5$) in this 'high-affinity' fraction had alkaline pI values (> 9.0) and contained a subunit with an M_r value of 24000. All of these isoenzymes had high organic peroxidase activity and, on the basis of amino acid analysis, substrate specificities and affinity for non-substrate ligands, appear to belong to the family of glutathione *S*-transferases that have been termed alpha [Mannervik, Ålin, Guthenberg, Jensson, Tahir, Warholm & Jörnvall (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 7202–7206]. Also within the high-affinity fraction was an isoenzyme with an acidic (5.8) pI value. This acidic isoenzyme was composed of a unique subunit (M_r 23000). The *N*-terminal sequence (ten residues) of this acidic enzyme was identical with that of a human form that is referred to as pi. The predominant form of enzyme in the 'low-affinity' (eluted from the GSH affinity column with an increase in buffer pH) fraction was a homodimer of a 26000- M_r subunit. It had an alkaline pI (> 9.0) but it lacked organic peroxidase activity. The *N*-terminal sequence (ten residues) of this enzyme was identical with that of a human enzyme referred to as mu. The substrate specificities and affinity for non-substrate ligands of this monkey enzyme also were similar to those of the human enzyme. In conclusion, the liver cytosol of rhesus monkeys contains a number of glutathione *S*-transferase isoenzymes that are very similar to the human hepatic enzymes.

INTRODUCTION

The glutathione *S*-transferases (EC 2.5.1.18) are a family of enzymes present in the soluble fraction of many tissues. They are dimeric proteins of M_r approx. 50000 that are formed by similar and dissimilar subunits. In rats, mice and humans several different subunits have been identified. Within each species these different subunits combine to form a variety of dimeric enzymes that have different but frequently overlapping substrate specificities (Boyer & Kenney, 1985; Mannervik, 1985).

The various isoenzymes within different species form a number of separate families, each of which has unique structural and functional features. For example, within the hepatic cytosol of the rat are six glutathione *S*-transferase isoenzymes that have basic pI values. These six isoenzymes are formed by combinations of four distinct subunits termed Ya (or 1), Yc (or 2), Yb₁ (or 3) or Yb₂ (or 4) (Jakoby *et al.*, 1984). On the basis of substrate specificities, cross-reactivity with antibodies, *N*-terminal sequence data and hybridization studies, the Ya- and Yc-containing enzymes form one family of enzymes and the Yb-containing enzymes a different family (Mannervik & Jensson, 1982; Boyer *et al.*, 1983; Jakoby *et al.*, 1984; Mannervik *et al.*, 1985). Similar studies of human forms of glutathione *S*-transferase also have found that the numerous isoenzymes can be grouped

into a number of different families that have unique structural and functional characteristics (Mannervik *et al.*, 1985; Singh *et al.*, 1985; Tahir *et al.*, 1985). Mannervik *et al.* (1985), using *N*-terminal sequence data, substrate specificities, sensitivities to inhibitors and cross-reactivity with antibodies, have separated the various isoenzymes obtained from rats, mice and humans into three large families termed alpha, mu and pi. Although the isoenzymes within these three families have many similarities, subtle differences may be present. For example, some forms of the rat glutathione *S*-transferases are able to function simultaneously as enzymes and binding proteins under conditions that are thought to be present in the cell (Boyer *et al.*, 1984). Although one can obtain human forms of glutathione *S*-transferase that are very similar to the above rat isoenzymes, they are not able to function simultaneously as enzymes and binding proteins under physiological conditions (Boyer & Vessey, 1987). Hence direct comparison of rodent with human enzymes may not be possible during investigation of certain aspects of the glutathione *S*-transferases.

It would seem that the glutathione *S*-transferases of non-human primates would most closely resemble the enzymes present in man. Asaoka and colleagues (Asaoka *et al.*, 1977; Asaoka & Takahashi, 1977) identified by CM-cellulose chromatography five forms of glutathione *S*-transferase in the liver cytosol of rhesus monkeys.

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They purified the predominant form obtained from the CM-cellulose column. As with enzymes purified from other animals, the monkey enzyme was a dimeric protein of two identical subunits. Its substrate specificities and amino acid composition were very similar to those of other glutathione *S*-transferases. Brazilian and Bolivian squirrel monkeys also have high levels of glutathione *S*-transferase activity in hepatic cytosol (Portman *et al.*, 1984), but the various isoenzymes have not been characterized. These studies suggest that the hepatic cytosol of monkeys contains a number of glutathione *S*-transferase isoenzymes; however, their similarity to the isoenzymes present in other species has not been systematically investigated.

In the current study we have purified and characterized the glutathione *S*-transferases from the hepatic cytosol of rhesus monkeys. We used a purification scheme that has been applied previously to the human hepatic glutathione *S*-transferases. We identified and purified 13 isoenzymes from the liver cytosol of female rhesus monkeys. They were similar, both structurally and functionally, to the human enzymes. Two forms were shown to have the same *N*-terminal sequence (ten residues) as two human isoenzymes. On the basis of the results of this current study it was possible to separate the glutathione *S*-transferases of the non-human primate liver into the same three classes that contain the major human and rodent enzymes.

MATERIALS AND METHODS

Materials

1-Chloro-2,4-dinitrobenzene, GSH, NADPH, glutathione reductase (EC 1.6.4.2), ethacrynic acid, *trans*-4-phenylbut-3-en-2-one, sulphobromophthalein and epoxy-activated Sepharose were purchased from Sigma Chemical Co. 1,2-Dichloro-4-nitrobenzene and *p*-nitrobenzyl chloride were obtained from Aldrich Chemical Co., and recrystallized from ethanol/water before use. Chromatofocusing resins PBE-94 and PBE-118, Polybuffers and Ampholines were from Pharmacia.

Animals

Female rhesus monkeys (*Macaque mulatta*), aged 13–21 years, from the California Primate Research Center at the University of California, Davis, were used for this study. The animals were maintained according to standard procedures employed at the Primate Center. Only healthy animals were selected for study and they had not received any drugs, either acutely or chronically. The animals were killed in order to control the size of the colony and not for the purposes of this study.

A laparotomy was performed under sodium pentobarbital anaesthesia. The animal was euthanized with an overdose of the same drug and the liver was removed immediately. After removal of the gall-bladder, the liver was rinsed with ice-cold 0.15 M-NaCl/10 mM-sodium phosphate buffer, pH 7.4, and transported on ice to the laboratory. The 100 000 *g* supernatant from each animal was prepared in 40 mM-sodium phosphate buffer, pH 7.4, containing 0.25 M-sucrose and was frozen separately at -20°C until used.

Enzyme assays

Glutathione *S*-transferase and glutathione peroxidase activities were determined spectrophotometrically as

described previously (Habig *et al.*, 1974; Lawrence & Burk, 1976). All determinations were performed in duplicate and corrected for non-enzyme rates, and the specific activities are expressed as units (μmol of product formed/min)/mg of protein. Protein was determined by the method of Schaffner & Weissmann (1973), with bovine serum albumin as the standard. Inhibition by non-substrate ligands was performed as described previously (Tahir *et al.*, 1985).

Preparation of enzymes

A portion of 100 000 *g* supernatant that contained approx. 1000 units of enzymic activity (assayed with 0.5 mM-1-chloro-2,4-dinitrobenzene and 1 mM-GSH) was taken from the material obtained from each of four different animals, pooled (total activity 4200 units, specific activity 1.2 units/mg of protein) and used for purification of the isoenzymes. This and all subsequent steps were performed at $0-4^{\circ}\text{C}$. The purification protocol was similar to that previously used to purify the human liver forms of glutathione *S*-transferase (Vander Jagt *et al.*, 1985). The 100 000 *g* supernatant was applied to a GSH affinity column as described previously (Simons & Vander Jagt, 1977). The column was washed with buffer (pH 7.0) to remove contaminating proteins. The pH of the eluting buffer was increased to 9.6 to elute the 'low-affinity' isoenzymes. GSH (10 mM) was added to the eluting buffer and the 'high-affinity' forms of the enzyme were recovered. These two forms of enzyme were handled separately throughout the remainder of the purification. Chromatofocusing was performed according to the manufacturer's instructions. The high-affinity enzymes were equilibrated with the eluting buffer (1:45 dilution of pH 10.5–8.0 Ampholines set at pH 8.0) for chromatofocusing and then applied to a PBE-118 column (1.5 cm $\times$ 30 cm) equilibrated with 25 mM-triethylamine, pH 11.0. A small amount of the eluting buffer was run into the column before the addition of the enzymes to minimize exposure of the proteins to extremes of pH. Fractions (2 ml) were collected and assayed for enzyme activity and pH (the latter at 4°C). After completion of the pH gradient the column was washed with 1 M-NaCl to remove the acidic forms of the enzyme. The high-affinity acidic fraction was dialysed into 10 mM-potassium phosphate buffer, pH 7.0, containing 30% (v/v) glycerol. The enzyme was applied to a PBE-94 column (1 cm $\times$ 15 cm) equilibrated in 25 mM-Tris/acetic acid buffer, pH 8.3. The column was eluted with a 1:10 dilution of a mixture of Polybuffers 96 and 74 (pH 5.0). Fractions (0.4 ml) were collected and assayed as described above. The low-affinity enzymes were treated similarly. The Ampholines were removed by re-applying the purified isoenzymes to a GSH affinity column, washing the column with 0.15 M-NaCl/10 mM-potassium phosphate buffer, pH 7.0, and eluting the enzymes with 10 mM-GSH. The purified enzymes were dialysed into 10 mM-potassium phosphate buffer, pH 7.0, containing 30% glycerol and stored at -80°C until used. The enzymes were stable under these conditions for at least several months.

SDS/polyacrylamide-gel electrophoresis

The enzymes were subjected to discontinuous polyacrylamide-gel electrophoresis as described by Maizel (1971). After electrophoresis the gels were fixed and stained with Coomassie Blue. The protein M_r standards

Fig. 1. Chromatofocusing column chromatography of high-affinity forms of monkey liver glutathione *S*-transferase

(a) The glutathione *S*-transferases that were eluted from the GSH affinity column by GSH were subjected to chromatofocusing as described in the Materials and methods section. This high-affinity fraction initially was focused on the PBE-118 column. (b) Isoenzymes that failed to be eluted with the pH gradient were recovered by washing the PBE-118 column with 1 M-NaCl (arrow in a) and then focused on a PBE-94 column. In both cases the fractions were assayed at pH 6.5 with 0.5 mM-1-chloro-2,4-dinitrobenzene and 1 mM-GSH. ○, Enzyme activity; ●, pH.

were bovine serum albumin (M_r 66000), ovalbumin (M_r 45000), glyceraldehyde-3-phosphate dehydrogenase (M_r 36000), carbonic anhydrase (M_r 29000), trypsinogen (M_r 24000), soya-bean trypsin inhibitor (M_r 20100) and α -lactalbumin (M_r 14200).

Amino acid analysis and *N*-terminal sequencing

Before amino acid analysis or sequencing the proteins were dialysed for several days against several changes of double-distilled water. The proteins for amino acid analysis were then freeze-dried and shipped to the Protein Structure Laboratory (University of California, Davis). Acid hydrolysis was performed in 6 M-HCl for 24 h. Performic acid oxidation was performed on a separate sample for measurement of thiol-containing amino acids. The amino acid composition was determined on a Beckman 6300 or Durrum D-500 automated amino acid analyser with a system integrator. *N*-Terminal sequencing of the purified enzymes was performed at the Biomolecular Resource Center at the University of California, San Francisco. The sequencing was performed by using an Applied Biosystems 470A automatic gas-phase Sequencer with an on-line 120 phenylthiohydantoin analyser (h.p.l.c.) as described previously (Hunkapiller *et al.*, 1983).

Preparation of antibody

A 100 μ g portion of purified enzyme was mixed with complete Freund's adjuvant and injected at multiple sites intradermally into male New Zealand White rabbits (Nitabel Rabbitry, Hayward, CA, U.S.A.). At 3 and 5 weeks later, the rabbits were injected intramuscularly with 50 μ g of the same protein again mixed with complete Freund's adjuvant. At 1 week after the second booster injection serum was collected and used for Ouchterlony immunodiffusion studies.

RESULTS

Enzyme preparation

Of the starting activity 15.5% and 74.2% were recovered from the GSH affinity column as the low-affinity and high-affinity fractions respectively. These two fractions were resolved further by chromatofocusing into a number of isoenzymes. At the completion of the purification 37.9% of the post-GSH-affinity-column enzymic activity was recovered as purified isoenzymes. Of the recovered activity 72.8% was accounted for by five high-affinity alkaline isoenzymes termed H1-H5,

Fig. 2. Chromatofocusing column chromatography of low-affinity forms of monkey liver glutathione *S*-transferase

(a) The enzymes that were eluted from the GSH affinity column following an increase in pH were focused on a PBE-118 column. (b) The activity recovered from the PBE-118 column by washing with 1 M-NaCl (arrow in a) was focused on the PBE-94 gel. Assays were performed as indicated in Fig. 1 legend. ○, Enzyme activity; ●, pH.

1.9% as three high-affinity acidic isoenzymes (H6–H8), 25.2% as three low-affinity alkaline isoenzymes (L1–L3) and 0.1% as two low-affinity acidic isoenzymes (L4 and L5). The elution profiles from the chromatofocusing columns are shown in Figs. 1 and 2. The amounts of the individual isoenzymes and their respective apparent *pI* values (pH at which enzymes were eluted from chromatofocusing column) are shown in Table 1. We were concerned that the heterogeneity found with the chromatofocusing columns may have reflected pH-induced formation of isoenzymes not normally present in the hepatic cytosol. The isoenzyme H1 was therefore rechromatofocused, and a single peak of enzymic activity was obtained at pH 9.6. This *pI* was identical with that obtained with the original chromatofocusing column (Table 1). The amount of isoenzyme L4 recovered was less than expected on the basis of the results of chromatofocusing (Fig. 2) and suggests that it may be somewhat unstable after purification.

Molecular characteristics

The high-affinity alkaline enzymes H1–H4 were homodimers of subunits with M_r values of 24000 (Fig. 3).

Isoenzyme H5 was a heterodimer of two subunits with M_r values of 24000 and 26000. Isoenzyme H6 was also a heterodimer of subunits with M_r values similar to those of isoenzyme H5, whereas isoenzyme H8 was composed of a single subunit with an M_r value of 23000. We lacked enough isoenzymes H7 and L5 to obtain any M_r values. Two of the three low-affinity alkaline isoenzymes (L1 and L2) were homodimers of subunits with M_r values of 24000, whereas isoenzymes L3 and L4 were predominantly homodimers of subunits with M_r values of 26000.

Substrate specificities and kinetic constants

The substrate specificities for the various isoenzymes are shown in Table 2. All forms had high levels of activity with 1-chloro-2,4-dinitrobenzene. High levels of organic peroxidase activity were limited to the isoenzymes with alkaline values of *pI* (both H and L forms). Low levels of peroxidase activity were present in isoenzymes L3 and H6, and acidic isoenzymes H7 and H8 lacked detectable activity. Activity with 1,2-dichloro-4-nitrobenzene was highest with isoenzymes H6 and H7.

The kinetic constants for the two predominant high-

Table 1. Apparent isoelectric points and relative amounts of monkey liver glutathione *S*-transferases

Apparent pI values are the pH values at which the various isoenzymes were eluted from the chromatofocusing columns in Figs. 1 and 2. A total enzymic activity of 3154 units was recovered from the GSH affinity column. At the end of the purification an enzymic activity of 1194 units was recovered as the various purified isoenzymes.

Isoenzyme	Apparent pI	Relative amount [% of total enzyme activity purified (% of initial activity)]
High-affinity forms		
H1	9.60	36.7 (13.9)
H2	9.51	18.7 (7.1)
H3	9.45	15.1 (5.7)
H4	9.40	1.1 (0.4)
H5	9.37	1.2 (0.4)
H6*	7.59	0.4 (0.1)
H7*	6.48	< 0.1 (< 0.1)
H8*	5.80	1.5 (0.6)
Low-affinity forms		
L1	9.70	2.0 (0.8)
L2	9.65	1.3 (0.5)
L3	9.50	21.9 (8.3)
L4*	7.48	< 0.1 (< 0.1)
L5*	6.85	< 0.1 (< 0.1)

* Enzymes eluted from the PBE-94 chromatofocusing column.

affinity and low-affinity forms, H1 and L3, were determined by bisubstrate kinetic analysis and are shown in Table 3. The binding of either 1-chloro-2,4-dinitrobenzene or GSH to isoenzyme H1 was affected little by the presence of the other substrate. In contrast, with isoenzyme L3 the presence of either 1-chloro-2,4-dinitrobenzene or GSH greatly decreased the affinity of the enzyme for the other substrate. This same isoenzyme's affinity for GSH was decreased by an increase in buffer pH (i.e. eluted from GSH affinity column with an increase in pH in the absence of GSH).

Inhibition by non-substrate ligands

The inhibitor sensitivities of the major high-affinity and low-affinity and acidic forms of monkey glutathione

S-transferase (H1, L3 and H8) for haematin are shown in Fig. 4. The I_{50} values for haematin with isoenzymes H1 and H2 (results for the latter not shown) were similar, 0.072 μ M and 0.058 μ M respectively, whereas the I_{50} value for isoenzyme L3 was 0.55 μ M and that for isoenzyme H8 was above 50 μ M. The I_{50} values for sulphobromophthalein with isoenzymes H1, H2 and H8 were similar, 10–22 μ M, whereas the I_{50} with isoenzyme L3 was only 1.7 μ M (results not shown).

Antibody studies

A multivalent antibody was raised against isoenzyme L3 as described in the Materials and methods section. This antibody reacted against isoenzyme L3, the two heterodimer isoenzymes H5 and H6 and isoenzyme L4. No reaction was noted with any of the other glutathione *S*-transferase isoenzymes purified from monkey liver.

Amino acid analysis and *N*-terminal sequence

The amino acid composition of the glutathione *S*-transferase isoenzyme H1 is shown in Table 4. The amino acid composition of this isoenzyme was quite similar to that reported previously for the basic human glutathione *S*-transferases purified from liver (Ålin *et al.*, 1985).

Two attempts were made to sequence the *N*-terminus of isoenzyme H1, and no amino acid residues were obtained. The *N*-termini of isoenzymes L3 and H8 were sequenced through ten residues (Table 5). The *N*-terminal sequence of isoenzyme L3 is identical with that obtained with a human glutathione *S*-transferase isoenzyme referred to as μ (Ålin *et al.*, 1985). Similarly the *N*-terminal sequence of isoenzyme H8 is identical with that of the human enzyme purified from placenta and termed π (Dao *et al.*, 1984; Ålin *et al.*, 1985).

DISCUSSION

Within the human liver are three classes of glutathione *S*-transferases, termed basic (or α), near neutral (or

Fig. 3. SDS/polyacrylamide-gel electrophoresis of monkey liver glutathione *S*-transferases

The side channels contained protein standards (S) with M_r values of 66000, 45000, 36000, 29000, 24000, 20100 and 14200. The different monkey isoenzymes are indicated by their affinity for the GSH affinity column, high (H) or low (L) and their numerical order of elution from the chromatofocusing columns (Figs. 1 and 2).

Table 2. Substrate specificities of monkey liver glutathione *S*-transferases

The abbreviations for the isoenzymes are described in Table 1. Conditions of assay were the same as those described previously (Boyer *et al.*, 1983). Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; CuOOH, cumene hydroperoxide; *p*-NBCl, *p*-nitrobenzyl chloride; BSP, sulphobromophthalein; Eacid, ethacrynic acid; N.D., no activity detected.

Substrate	Iso-enzyme ...	Glutathione <i>S</i> -transferase activity (units/mg)												
		H1	H2	H3	H4	H5	H6	H7	H8	L1	L2	L3	L4	L5
CDNB		59.6	26.4	93.8	57.1	52.7	110.8	71.7	73.8	18.7	67.1	38.0	47.4	20.8
DCNB		0.38	0.54	0.65	0.36	1.39	4.34	11.58	0.69	1.53	1.13	0.29	—†	—†
CuOOH		8.47	6.56	7.01	9.37	3.07	N.D.	N.D.*	N.D.	7.23	5.35	0.58	—†	—†
<i>p</i> -NBCl		1.27	0.76	0.87	0.48	1.21	—†	—†	—†	—†	—†	0.35	—†	—†
BSP		0.03	0.06	0.06	0.04	0.04	—†	—†	—†	—†	—†	0.02	—†	—†
Eacid		0.24	0.14	0.17	0.09	0.06	—†	—†	—†	—†	—†	0.01	—†	—†

* The small amount of material available limited the amount of protein in this assay to less than 0.5 μ g. The use of larger amounts of protein may have shown activity to be present.

† Too little material was available to perform these assays.

Table 3. Kinetic constants for monkey liver glutathione *S*-transferases

Enzyme assays were performed in duplicate with various concentrations of 1-chloro-2,4-dinitrobenzene (CDNB) (0.1–1.0 mM) and GSH (0.1–2 mM). K_m values were obtained at saturating concentrations of the second substrate. V_{max} was obtained at saturating concentrations of both substrates.

Isoenzyme	$K_{m(\text{CDNB})}$ (mM)	$K'_{m(\text{CDNB})}$ (mM)	$K_{m(\text{GSH})}$ (mM)	$K'_{m(\text{GSH})}$ (mM)	V_{max} (units/mg)	k_{cat} (min ⁻¹)
H1	0.46	0.37	0.75	0.48	155	7750
L3	1.10	6.60	0.57	9.26	310	15 500

Fig. 4. Inhibition profiles of three isoenzymes of monkey liver glutathione *S*-transferase as a function of concentration of haematin

The remaining enzymic activity of isoenzyme H1 (●), isoenzyme L3 (■) and isoenzyme H8 (○) was measured at pH 6.5 with 1 mM-1-chloro-2,4-dinitrobenzene and 1 mM-GSH at increasing concentrations of haematin.

mu) and acidic (or pi) (Mannervik *et al.*, 1985). The basic forms predominate and the isoenzymes within this class are dimers of subunits with M_r values between 26 500 and 24 500. They all have organic peroxidase activity and a high affinity for the non-substrate ligand haematin (Mannervik *et al.*, 1985; Stockman *et al.*, 1985; Singh

et al., 1985; Hussey *et al.*, 1986). The mu form is a homodimer of two subunits with M_r 26 300, has high activity with epoxides but little organic peroxidase activity and has a high affinity (relative to the other forms) for sulphobromophthalein (Warholm *et al.*, 1983; Tahir *et al.*, 1985). The acidic or pi form was initially

Table 4. Amino acid composition of monkey liver glutathione *S*-transferase isoenzyme H1

Amino acid	Composition (residues/1000 residues)
Asp	82
Thr	26
Ser	55
Glu	125
Pro	48
Gly	66
Ala	73
Cys*	24
Val	38
Met†	28
Ile	61
Leu	130
Try	42
Phe	38
His	13
Lys	97
Arg	53

* Measured as cysteic acid.

† Measured as methionine sulphone.

Table 5. *N*-Terminal sequences of monkey liver glutathione *S*-transferase isoenzymes L3 and H8

The analysis was performed in a gas-phase Sequenator as described in the Materials and methods section.

Edman cycle	Isoenzyme...	Residue (pmol)	
		L3	H8
1		Pro (300)	Pro (112.9)
2		Met (386)	Pro (108.1)
3		Ile (303)	Tyr (87)
4		Leu (287)	Thr (48)
5		Gly (260)	Val (96.1)
6		Tyr (280)	Val (115)
7		Trp (96)	Tyr (73.2)
8		Asp (260)	Phe (106.5)
9		Ile (226)	Pro (57.3)
10		Arg (210)	Val (43.6)

purified from placenta and was shown to be a dimer of a relatively small subunit, M_r 22500. It has an *N*-terminal sequence that is different from the mu form of the glutathione *S*-transferases, and has high activity with ethacrynic acid and relatively low affinity for non-substrate ligands (Dao *et al.*, 1984; Ålin *et al.*, 1985; Mannervik *et al.*, 1985; Tahir *et al.*, 1985). This form also has been found in the liver (Singh *et al.*, 1985) and it may increase selectively in hepatocellular carcinoma (Soma *et al.*, 1986).

The current study shows that there is a striking degree of similarity between human and non-human primate hepatic glutathione *S*-transferases. The predominant isoenzymes (71.6% of recovered activity) in the non-human primate liver also have basic pI values (> 9.0) and high organic peroxidase activity (Table 2). All of the isoenzymes that had organic peroxidase activity con-

tained at least one of the 24000- M_r subunits, with the highest peroxidase activity being present in the homodimers of this subunit. As with the human basic isoenzymes (Mannervik *et al.*, 1985), the forms of the monkey glutathione *S*-transferases possessing organic peroxidase activity were *N*-terminally blocked. The amino acid composition of one of the basic isoenzymes purified from the monkey liver (H1) (Table 4) was very similar to the basic human forms of this enzyme (Ålin *et al.*, 1985). Lastly, as is observed with the alpha class of human isoenzymes (Tahir *et al.*, 1985), the monkey basic glutathione *S*-transferase isoenzymes had a high affinity for haematin but not for sulphobromophthalein. On the basis of the above observations, it seems reasonable to include the H1-H5 isoenzymes and perhaps isoenzymes L1 and L2 (both have high organic peroxidase activity) purified from monkey liver in the alpha family of glutathione *S*-transferases.

The next most common isoenzyme (22% of recovered activity) in the rhesus monkey liver was L3. This isoenzyme had a low affinity for the GSH affinity column at a high value of pH and was composed of a subunit with an M_r of 26000. Isoenzymes H5, H6 and L4 also contained a 26000- M_r subunit, and an antibody raised against isoenzyme L3 cross-reacted with these other three isoenzymes. Thus all four isoenzymes (L3, H5, H6 and L4) appear to contain a similar subunit. Vander Jagt *et al.* (1985) identified in human liver three basic (pI ≥ 7.0) and two acidic forms of glutathione *S*-transferase that did not adhere to the glutathione affinity column at a high value of pH. These isoenzymes accounted for 10-43% of the glutathione *S*-transferase activity present in human liver. Other workers (Warholm *et al.*, 1983), using a different purification scheme, have isolated a form of glutathione *S*-transferase from human liver (termed mu) that has high activity with exopoxides but a relatively low pI value (6.6). Vander Jagt *et al.* (1985) also identified two mu forms, both with pI values of < 7.0 and one of which had a low affinity for the GSH affinity column. In the current study one of the low-affinity isoenzymes, L3, purified from monkey liver had an *N*-terminal sequence identical with that of the mu form purified from human liver (Ålin *et al.*, 1985). Also, isoenzyme L3 had a high affinity for sulphobromophthalein, which is characteristic of the human mu isoenzyme (Tahir *et al.*, 1985), and we believe that isoenzyme L3 can be included with the mu class of enzymes. However, the pI value of the human enzyme is much lower than that found with the monkey enzyme. This difference may represent either significant structural differences between the two species or the fact that the pH at which a protein is eluted from the chromatofocusing column may not be its true pI value. In general the pH of elution and pI value are similar, but one group noted that the mu isoenzyme purified from human liver had a higher pH of elution than its isoelectric point (Stockman *et al.*, 1985). It is important to note that, although isoenzyme L3 had a relatively low affinity for GSH at high values of pH or in the presence of saturating concentrations of the second substrate, at more physiological values of pH and in the absence of saturating concentrations of the second substrate (a situation much more likely to occur *in vivo*) the affinity of isoenzyme L3 for GSH was similar to that of isoenzyme H1 and other reported values for the glutathione *S*-transferases (Boyer & Kenney, 1985).

Of the monkey hepatic glutathione *S*-transferases 2% had pI values that were less than 7.0, and we could purify only a single form (isoenzyme H8) in sufficient quantity to perform any studies. This latter form was composed of a subunit with an M_r value (23000) lower than those of the other isoenzymes. The *N*-terminal sequence of isoenzyme H8 was identical with that of the acidic isoenzyme purified from placenta and human liver termed pi (Dao *et al.*, 1984; Ålin *et al.*, 1985). In addition, the relatively small size of the subunit that forms isoenzyme H8 and the latter's low affinity for the tested non-substrate ligands is characteristic of the pi class of glutathione *S*-transferases (Dao *et al.*, 1984; Tahir *et al.*, 1985).

In conclusion, the liver of non-human primates contains three classes of glutathione *S*-transferase that are formed by at least three distinct subunits. The human isoenzymes are formed by four distinct subunits, which actually may be mixtures of charge isomers (Singh *et al.*, 1985), and the same may be true of the monkey. These charge isomers then can join together to form the large number of isoenzymes present in monkey (Table 1) and human liver (Singh *et al.*, 1985). In the human, these distinct subunits are thought to be products of at least three separate genes (Board, 1981; Strange *et al.*, 1984). The differences between the subunits that form the alpha, mu and pi enzymes in the monkey liver also suggests that at least three separate genes are responsible for the production of these subunits. The unique characteristics of the various isoenzymes are determined by their subunit composition, and this feature provides the organism with an array of glutathione *S*-transferases that can protect it from a variety of toxic organic molecules. The conservation of these different families of enzymes among species suggests that the overlapping but unique functions of each enzyme family are important to the survival of the organism.

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