Purification and characterization of glutathione *S*-transferases of human kidney

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Several forms of glutathione *S*-transferase (GST) are present in human kidney, and the overall isoenzyme pattern of kidney differs significantly from those of other human tissues. All the three major classes of GST isoenzymes (α , μ and π) are present in significant amounts in kidney, indicating that GST1, GST2 and GST3 gene loci are expressed in this tissue. More than one form of GST is present in each of these classes of enzymes, and individual variations are observed for these classes. The structural, immunological and functional properties of GST isoenzymes of three classes differ significantly from each other, whereas the isoenzymes belonging to the same class have similar properties. All the cationic GST isoenzymes of human kidney except for GST 9.1 are heterodimers of 26 500- M_r and 24 500- M_r subunits. GST 9.1 is a dimer of 24 500- M_r subunits. All the cationic isoenzymes of kidney GST cross-react with antibodies raised against a mixture of GST α , β , γ , δ and ϵ isoenzymes of liver. GST 6.6 and GST 5.5 of kidney are dimers of 26 500- M_r subunits and are immunologically similar to GST ψ of liver. Unlike other human tissues, kidney has at least two isoenzymes (pI 4.7 and 4.9) associated with the GST3 locus. Both these isoenzymes are dimers of 22 500- M_r subunits and are immunologically similar to GST π of placenta. Some of the isoenzymes of kidney do not correspond to known GST isoenzymes from other human tissues and may be specific to this tissue.

INTRODUCTION

Glutathione *S*-transferases (EC 2.5.1.18) play an important role in the metabolism and detoxification of xenobiotics through several mechanisms (Booth *et al.*, 1961; Jakoby, 1978; Chasseaud, 1979). A number of glutathione *S*-transferase (GST) isoenzymes have been described in human liver (Kamisaka *et al.*, 1975; Awasthi *et al.*, 1980; Warholm *et al.*, 1983; Singh *et al.*, 1985a; Stockman *et al.*, 1985; Vander Jagt *et al.*, 1985; Soma *et al.*, 1986), lung (Koskelo *et al.*, 1981; Partridge *et al.*, 1984), erythrocytes (Marcus *et al.*, 1978; Awasthi & Singh, 1984), placenta (Awasthi & Dao, 1981; Guthenberg & Mannervik, 1981), lens (Singh *et al.*, 1985b), cornea (Singh *et al.*, 1985c), retina (Singh *et al.*, 1984a) and brain (Theodore *et al.*, 1985). Structural and kinetic data from various laboratories have led to the suggestion that the isoenzymes of GST can be classified in three separate classes designated as α , μ and π (Mannervik *et al.*, 1985). Genetic models, on the other hand, suggest that the multiple forms of human GST arise from at least three (Board 1981; Strange *et al.*, 1984, 1985) and possibly six (Laisney *et al.*, 1984) distinct gene loci. A molecular basis for the co-relation between these two models must exist, and it can be established only through detailed structural and immunological characterization of GST isoenzymes in various human tissues.

Kidney plays an important role in the detoxification and excretion of xenobiotics, and the presence of several cationic as well as anionic GST isoenzymes has been demonstrated in human kidney (Sherman *et al.*, 1983). The isoenzyme patterns of kidney GST do not

correspond to those of liver and other organs from the same individuals (Sherman *et al.*, 1983). This may be indicative of tissue-specific expression of human GST isoenzymes, similar to that observed for GST isoenzymes of rat (Tu *et al.*, 1983; Singh & Awasthi, 1984; Singh *et al.*, 1984b; Awasthi & Singh, 1985). To resolve the question of tissue-specific expression of GST isoenzymes in humans, a complete understanding of the inter-relationships among the isoenzymes of various tissues is necessary. The present studies were, therefore, designed to purify and study the structural, immunological and kinetic characteristics of various GST isoenzymes of human kidney and to investigate their inter-relationships with the known GST isoenzymes from other human tissues. These studies indicate that kidney tissue has all the three major classes of GST isoenzymes, and some of the isoenzymes of kidney may be specific to this tissue.

MATERIALS AND METHODS

Materials

Unless otherwise specified sources of the chemicals used in the present study were the same as those used in our previous studies (Singh *et al.*, 1985a).

Human kidney samples were obtained at autopsy either from Galveston County Hospital, Galveston, TX, U.S.A., or from the University of Texas Medical Branch, Galveston, TX, U.S.A., within 24 h of death. All the five kidney samples were from adult males of ages between 25 and 46 years, and were accident victims except for the subject for kidney sample I, who died of drug overdose. The tissue was stored at -20°C until used.

Abbreviation used: GST, glutathione *S*-transferase.

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Enzyme assay

GST activity towards different substrates was determined as described by Habig *et al.* (1974). GSH peroxidase II activity towards cumene hydroperoxide was determined according to the procedure of Awasthi *et al.* (1975). One unit of enzyme utilized 1 μ mol of substrate at 25 °C for GST and at 37 °C for GSH peroxidase II.

Protein was determined by the method of Bradford (1976), with bovine serum albumin as a standard.

Purification of GST

All the purification steps were performed at 4 °C. In a representative purification protocol, a 10% (w/v) homogenate of human kidney was prepared in 10 mM-potassium phosphate buffer, pH 7.0, containing 1.4 mM-2-mercaptoethanol (buffer A) by using a PT 10-35 Polytron (Kinematica, Littau, Switzerland). The homogenization was performed for 5 min. The homogenate was centrifuged at 14000 *g* for 40 min, and the supernatant was subjected to affinity chromatography on GSH linked to epoxy-activated Sepharose 6B (Simons & Vander Jagt, 1977). A column (0.75 cm $\times$ 10 cm) of GSH affinity resin was pre-equilibrated with 22 mM-potassium phosphate buffer, pH 7.0, containing 1.4 mM-2-mercaptoethanol (buffer B) at a flow rate of 10 ml/h, and this flow rate was maintained throughout the affinity chromatography. The 14000 *g* supernatant was applied to the column, the unbound proteins were thoroughly washed off with buffer B and the enzyme was eluted with 5 mM-GSH in 50 mM-Tris/HCl buffer, pH 9.6, containing 1.4 mM-2-mercaptoethanol. The fractions containing GST activity were pooled and dialysed against buffer A and subjected to isoelectric focusing in an LKB 8100-1 isoelectric-focusing column with Ampholines in the pH range 3.5–10, in a sucrose density gradient (0–50%, w/v). After electric focusing at 1600 V for 18 h, 0.8 ml fractions were collected and monitored for pH and GST activity with 1-chloro-2,4-dinitrobenzene as the substrate.

Electrophoresis

Urea/SDS/2-mercaptoethanol/polyacrylamide-slab-gel electrophoresis was performed with the buffer system described by Laemmli (1970). The concentration of urea in both stacking and resolving gels was 6 M. The stacking and resolving gels contained 5.9% (w/v) and 12.5% (w/v) polyacrylamide respectively. The concentrations of the cross-linker *NN'*-methylenebisacrylamide in the stacking and resolving gels were 0.15% (w/v) and 0.35% (w/v) respectively. Two-dimensional polyacrylamide-gel electrophoresis was performed according to the method of O'Farrell (1975).

Immunological studies

Antibodies raised against anionic GST π of human placenta (pI 4.5), anionic GST ψ (pI 5.5) of human liver and a mixture of the cationic GST (α , β , γ , δ and ϵ) of human liver were the same as those used in our previous studies (Dao *et al.*, 1984; Singh *et al.*, 1985a). Double immunodiffusion and immunotitrations were performed according to the procedures of Ouchterlony (1958) and Awasthi *et al.* (1980) respectively.

Peptide mapping after CNBr cleavage

Purified GST isoenzymes (approx. 100 μ g) were hydrolysed in 70% (v/v) formic acid with 50-fold molar

excess of CNBr over methionine residues essentially as described by Gross (1967). Resulting peptides were analysed by h.p.l.c. on an Ultrasphere octyl column (4.6 mm $\times$ 25 cm). The h.p.l.c. was performed on a Beckman 334 gradient liquid chromatograph connected with a model 165 variable-wavelength u.v. detector. The mobile phase consisted of 0.1% (v/v) trifluoroacetic acid in water (solvent C) and 0.1% (v/v) trifluoroacetic acid in aq. 50% (v/v) acetonitrile (solvent D). A 0.1 ml sample was injected into the column.

The column was washed with solvent C for 10 min, followed by a 0–100% (v/v) linear gradient of solvent D for 40 min, and solvent D was maintained for an additional 30 min. The flow rate was 1 ml/min and the chart speed was 15 cm/h. Eluents were monitored at 230 nm.

Inhibition studies

The inhibitory effects of haematin, bilirubin and bromosulphophthalein on GST isoenzymes of human kidney were tested by varying the concentrations of the inhibitor at 1 mM-GSH and 1 mM-1-chloro-2,4-dinitrobenzene. The nature of inhibition and K_i values were determined by plotting $1/v$ against $1/[S]$ (Lineweaver-Burk plot) in the presence and in the absence of the inhibitor.

RESULTS

Purification of GST isoenzymes of human kidney

In human kidney, GST activity is expressed towards a wide variety of substrates by a number of isoenzymes. In the five kidney samples, designated as I, II, III, IV and V in the present study, the total GST activity in the 14000 *g* supernatant towards 1-chloro-2,4-dinitrobenzene was found to be in the range 15–23 units/g wet wt. of tissue. The results on purification of GST isoenzymes from kidney II are presented in Table 1. The enzyme was purified to about 100-fold by affinity chromatography (Table 1) on GSH linked to epoxy-activated Sepharose 6B with a yield of approx. 50%. A substantial loss in enzyme activity was, however, observed during the final steps of isoelectric focusing and dialysis of individual isoenzyme fractions (Table 1). The major proportion (up to 50%) of enzyme inactivation was incurred during dialysis to remove the Ampholines and sucrose. This may account for the low specific activities of these isoenzymes towards 1-chloro-2,4-dinitrobenzene (Table 1) as compared with those given in Table 2, which were determined in each of the peak isoelectric-focusing fractions before dialysis.

When the affinity-purified enzymes from each of the five samples were subjected to column isoelectric focusing, significant differences were observed in their isoelectric-focusing profiles (Fig. 1). In all the five samples 60–80% of total GST activity as well as protein was accounted for by a number of the cationic isoenzymes. Remaining GST activity and protein was accounted for by the anionic isoenzymes (pI 4.7–4.9) and less-anionic or near-neutral isoenzymes (pI 6.6–5.5). Individual variations were observed in the isoenzyme patterns of all these three groups of isoenzymes. The isoelectric focusing of GST purified from each of the five samples was repeated several times over the period of study and reproducible results were obtained. Storage of

Table 1. Purification of GST isoenzymes of human kidney

The results presented in this Table were obtained from kidney II. One unit of enzyme utilized 1 μ mol of substrate/min at 25 °C. Experimental details are given in the text. The enzyme activity and the protein content of GST isoenzyme pools, after isoelectric focusing, were determined after dialysis for 24 h against buffer A (four changes, 2 litres each).

	GST activity		Total protein (mg)	Specific activity (units/mg of protein)	Yield (%)	Purification (fold)
	(units/ml)	(total units)				
14000 g supernatant	1.31	58.03	330	0.17	100	—
Affinity chromatography	1.04	30.39	1.7	17.8	52.3	104
Isoelectric focusing						
GST 9.1	0.35	5.38	0.55	9.7	28.8	—
GST 9.0	0.41	6.58	0.59	11.1		
GST 6.6	0.26	1.33	0.08	16.6		
GST 4.7	0.42	3.43	0.37	9.2		

Table 2. Specific activities of human kidney GST isoenzymes towards different substrates

One unit of enzyme utilized 1 μ mol of substrate/min at 25 °C. Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; E.Acid, ethacrynic acid; BSP, bromosulphophthalein; NPNO, 4-nitropyridine *N*-oxide; CUOH, cumene hydroperoxide; N.D., not detected. The specific activities presented in this Table were determined with the peak fraction of each GST isoenzyme without dialysis. The specific activity of each isoenzyme was determined at least twice and similar values were obtained.

GST isoenzymes	Specific activity (units/mg of protein)					
	CDNB	DCNB	E.Acid	BSP	NPNO	CUOH*
GST 9.1	13.1	0.03	0.03	0.02	0.05	2.4
GST 9.0–8.8	10.4–12.3	0.04–0.07	0.02–0.04	0.02–0.03	0.05–0.06	2.3–2.4
GST 8.7–8.2	4.2–5.5	0.03–0.05	0.016–0.023	0.002–0.008	0.028–0.050	1.4–2.8
GST 6.6	53.0	0.015	0.055	0.001	0.08	0.05
GST 5.5	40.2	0.02	0.04	N.D.	0.07	N.D.
GST 4.9–4.7	26.5–33.5	0.09–0.16	0.67–0.85	N.D.	0.013–0.014	N.D.

* Glutathione peroxidase II activity of GST towards cumene hydroperoxide at 37 °C.

kidney samples for a period of up to 6 months did not alter the isoelectric-focusing profiles or total GST activity of any of the kidney samples used in the present study. Therefore it seems unlikely that the differences seen in the isoenzyme profiles of kidney samples are due to the experimental variation and degradations during the storage of the tissue.

Cationic GST isoenzymes

The cationic GST of kidney I resolved into five peaks of enzyme activity corresponding to pI values of 9.1, 8.9, 8.8, 8.5 and 8.3 (Fig. 1). Kidney II had two peaks of enzyme activity in this region corresponding to pI values of 9.1 and 9.0 (Fig. 1), whereas kidney III had only one major cationic peak corresponding to a pI of 8.8 (Fig. 1). Kidneys IV and V had three and five cationic isoenzymes corresponding to pI values of 8.7, 8.4 and 8.2 and of 8.9, 8.8, 8.7, 8.5 and 8.1 respectively (Fig. 1). We have designated these isoenzymes as human kidney GST suffixed with their pI values. Even though the separation of these isoenzymes by isoelectric focusing was reproducible, it should be pointed out that the pI values of some of the isoenzymes are very close and they may not be

completely separated as homogeneous species by column isoelectric focusing. Therefore the possibility of cross-contamination of these isoenzymes cannot be ruled out.

Anionic and near-neutral GST isoenzymes

The anionic isoenzymes GST 4.7 and/or 4.9 constituted from 15% to 32% of the total GST activity (towards 1-chloro-2,4-dinitrobenzene) in the kidney samples analysed in this study. Kidney samples I and IV both had two peaks each of GST activity corresponding to pI values of 4.7 and 4.9 (Fig. 1). Kidneys II and V, on the other hand, had single sharp peaks in this region corresponding to pI values of 4.7 and 4.9 respectively (Fig. 1). Individual variations were also seen in the expression of the less-anionic or near-neutral groups of isoenzymes. GST 6.6 was present only in two of the five samples analysed, where it represented 5–8% of the total GST activity of kidney. GST 5.5 was present in only one of the five samples analysed in the present study.

Structural properties

Gel-filtration studies over a column of Sephadex G-100 indicate that the approximate M_r value of the mixture of

kidney GST isoenzymes obtained by affinity chromatography was about 50000 (results not shown). When the mixture of purified GST isoenzymes from kidney was subjected to urea/SDS/polyacrylamide-disc-gel electrophoresis, three polypeptide bands corresponding to M_r values of 26500, 24500 and 22500 were observed (results not shown). The presence of these three sizes of subunits among the kidney GST isoenzymes was confirmed by the results of studies on the subunit composition of individual isoenzymes. All the cationic GST isoenzymes of human kidney except for GST 9.1 were found to be dimers of 26500- M_r and 24500- M_r subunits, and representative electrophoretograms of GST 9.0 and GST 8.5 are presented in Fig. 2(a), lane 3, and Fig. 2(b), lane 2, respectively. In this respect, the cationic GST isoenzymes of kidney are similar to those of human liver (Awasthi *et al.*, 1980; Dao *et al.*, 1982; Singh *et al.*, 1985a). GST 9.1 appeared as a homodimer of 24000- M_r subunits (Fig. 2a, lane 2). In one-dimensional electrophoretic analysis both GST 5.5 (Fig. 2a, lane 4) and GST 6.6 (Fig. 2b, lane 3) appeared as homodimers of 26500- M_r subunits, whereas GST 4.9 and GST 4.7 both appeared as homodimers of 22500- M_r subunits (Fig. 2c, lanes 2 and 3).

Subunit composition of human kidney GST isoenzymes was also studied by using isoelectric focusing followed by urea/SDS/2-mercaptoethanol/polyacrylamide-gel electrophoresis in the system described by O'Farrell (1975). In this system the mixture of cationic isoenzymes isolated from kidney II revealed the presence of two charge isomers each corresponding to M_r values of 26500 and 24500 (results not shown), indicating that there may be at least four charge isomers among the subunits of the cationic GST isoenzymes of human kidney. In this respect cationic GST isoenzymes of human kidney are similar to those of human liver, because similar results have been reported (Singh *et al.*, 1985a) for the cationic GST isoenzymes of human liver. The near-neutral isoenzyme (pI 6.6) of human kidney II revealed the presence of two charge isomers of equal M_r values of 26500 on two-dimensional electrophoretograms (results not shown). This indicates that GST 6.6 of kidney has structural differences from the GST μ (Warholm *et al.*, 1983) of liver, which also has a pI value of 6.6. The anionic isoenzyme GST 4.7 of kidney II, which appeared as a homodimer of 22500- M_r subunits in one-dimensional polyacrylamide-gel electrophoresis, also showed the presence of two polypeptide spots corresponding to an M_r value of 22500 upon two-dimensional electrophoretic analysis (results not shown). Such charge heterogeneity among the subunits of anionic GST isoenzymes of human liver (Singh *et al.*, 1985a), lung, heart and erythrocytes (Singh *et al.*, 1986) has been documented previously.

Immunological properties

The immunological characterization of GST isoenzymes of human kidney was performed by double-

Fig. 1. Isoelectric-focusing profiles of affinity-purified GST from kidney I, kidney II, kidney III, kidney IV and kidney V

Experimental details are given in the text. ○, pH gradient; ●, GST activity with 1-chloro-2,4-dinitrobenzene as the substrate.

Fig. 2. Urea/SDS/2-mercaptoethanol/polyacrylamide-slab-gel electrophoresis of GST isoenzymes from human kidney

(a) Lane 1, standards; lane 2, GST 9.1; lane 3, GST 9.0; lane 4, GST 5.5. (b) Lane 1, standards; lane 2, GST 8.5; lane 3, GST 6.6. (c) Lane 1, standards; lane 2, GST 4.9; lane 3, GST 4.7. Experimental details are given in the text. Approx. 20 μ g of protein was applied in each lane except for lane 4 of (a), where only 5 μ g of protein was applied.

immunodiffusion studies (Ouchterlony, 1958) and immunotitrations (Awasthi *et al.*, 1980). The cationic isoenzymes of kidney cross-reacted with the antibodies raised against a mixture of cationic GST isoenzymes of liver (α , β , γ , δ and ϵ) and did not cross-react with the antibodies raised against either anionic enzymes of placenta (GST π , pI 4.5) or GST ψ (pI 5.5) of human liver (results not shown). GST 6.6 and GST 5.5 of human kidney cross-reacted only with the antibodies raised against GST ψ of human liver and did not cross-react with any other antibodies. GST 4.7 and GST 4.9 of kidney cross-reacted with the antibodies raised against GST π of placenta, but did not cross-react with the antibodies raised against either the cationic GST or GST ψ of human liver. These results, taken together with the other observations of the present study, suggest that, similar to human liver GST isoenzymes (Singh *et al.*, 1985a; Mannervik *et al.*, 1985), there are at least three groups of subunits among the GST isoenzymes of human kidney and all these three types of subunits are expressed in this tissue in sufficient amounts.

Peptide mapping of kidney GST

Peptide maps of human kidney GST isoenzymes were obtained by CNBr fragmentation followed by h.p.l.c. analysis (Fig. 3). The fragmentation patterns of the isoenzymes within a group were very similar to each other. However, remarkable differences in the peptide maps were observed among the isoenzymes belonging to different groups. As shown in Fig. 3, significant differences are observed in the fragmentation patterns of the cationic (pI 9.0), intermediate (pI 6.6) and anionic (pI 4.7), isoenzymes of GST isolated from kidney II.

Substrate specificities

The differences among the properties of GST isoenzymes of human kidney belonging to different classes and the similarities in the isoenzymes within the same class are also reflected in their substrate specificities (Table 2). GST 6.6 had the highest specific activity towards 1-chloro-2,4-dinitrobenzene, and the order of enzyme activity of the isoenzymes towards 1-chloro-2,4-dinitrobenzene was GST 6.6 > GST 5.5 > GST 4.9 or GST 4.7 > cationic isoenzymes. On the other

Fig. 3. H.p.l.c. of CNBr-treated GST 9.0 (a), GST 6.6 (b) and GST 4.7 (c) of human kidney

Experimental details are given in the text. The GST isoenzymes used for peptide mapping were purified from kidney II.

hand, GST 4.9 and GST 4.7 had much higher specific activities towards ethacrynic acid and 1,2-dichloro-4-nitrobenzene. Bromosulphophthalein was a better substrate for the cationic isoenzymes as compared with GST 6.6, GST 5.5, GST 4.9 and GST 4.7 (Table 2). 4-Nitropyridine *N*-oxide was found to be the preferred substrate for GST 6.6 and GST 5.5 (Table 2). Cationic isoenzymes had the highest GSH peroxidase II activity. A small amount of GSH peroxidase II activity was also associated with GST 6.6, but GST 5.5, GST 4.9 and GST 4.7 were completely devoid of this activity (Table 2).

Inhibition of human kidney GST by bilirubin, haematin and bromosulphophthalein

The inhibitory effects of bilirubin, haematin and bromosulphophthalein were determined on various GST isoenzymes isolated from human kidney II, and the results are summarized in Table 3. The K_i values and nature of inhibition of cationic isoenzymes of human kidney by these compounds were close or similar to those reported for cationic enzymes of human liver (Kamisaka *et al.*, 1975; Vander Jagt *et al.*, 1983, 1985). The inhibition constants of these compounds for GST 6.6 of human kidney were similar or close to that reported for GST μ of liver (Warholm *et al.*, 1983).

Studies by Vander Jagt *et al.* (1985) have shown time-dependent inhibition by bilirubin with most of the basic isoenzymes of human liver, and this loss of enzyme activity was suggested to be due to a rather slow conformational change (Vander Jagt *et al.*, 1983). Those authors also reported that the conversion of the bilirubin-GST complex into an inactive conformation can be prevented by the presence of low concentrations of a foreign protein such as haemoglobin. However, this phenomenon was not observed with GST μ of liver (Vander Jagt *et al.*, 1985). We examined at least one isoenzyme from each of the three groups of human kidney GST for this phenomenon. All the three isoenzymes, GST 9.0, GST 6.6 and GST 4.7, tested in this study were inhibited in a time-dependent manner following the addition of 5 μ M-bilirubin to the assay system. In this regard the GST 6.6 of kidney differs from GST μ of liver.

DISCUSSION

Human kidney has a relatively high amount of GST activity among the human tissues investigated so far, and this activity is expressed by all the three classes of GST α , μ and π (Mannervik *et al.*, 1985) described in human tissues. The overall isoenzyme pattern of kidney GST significantly differs from those of other tissues. In human liver the α (cationic) and μ (near-neutral) classes of GST are predominant and only a very small amount of anionic isoenzyme (GST ω) has been reported (Awasthi *et al.*, 1980). On the other hand, tissues such as placenta,

lung and erythrocytes have predominantly the anionic form of GST belonging to the π class, and the isoenzymes belonging to the α or μ class are either absent or present in very small amounts. In kidney all the three classes of the isoenzymes are expressed in significant amounts, emphasizing the important role of this tissue in detoxification of toxic xenobiotics. In reference to the proposed genetic models (Board, 1981; Strange *et al.*, 1984, 1985; Laisney *et al.*, 1984) for human GST alleles, this would mean that all the three loci GST1, GST2 and GST3 are expressed in kidney. Individual variations in the isoenzymes in kidney corresponding to all the three loci may indicate that all these loci may be polymorphic. Although genetic polymorphism has been suggested at GST2 locus (Board, 1981; Hussey *et al.*, 1986), investigators have suggested that individual variations observed in the electrophoretic patterns of cationic isoenzymes of liver may be due to post-synthetic modifications (Laisney *et al.*, 1984; Strange *et al.*, 1984, 1985). In the present study variations in the isoelectric-focusing profiles due to storage and processing of tissues were ruled out because during the 6-month period of this study reproducible isoelectric-focusing profiles could be obtained repeatedly for every sample.

While discussing the possibility of polymorphism at GST2 locus, the subunit structures of the cationic GST must be considered. Previous studies from this laboratory (Singh *et al.*, 1985a) have shown that at least two immunologically distinct subunits, A and B, are present in the cationic GST isoenzymes of liver. Studies by Stockman *et al.* (1985) also demonstrate the presence of two immunologically distinct subunits in cationic liver GST isoenzymes. Although in these studies there is some difference in the M_r values for subunits as determined in SDS/polyacrylamide-gel electrophoresis, these two studies nonetheless clearly indicate that the cationic GST isoenzymes are products of more than one gene. Since the cationic GST isoenzymes of kidney also indicate the presence of two types of subunits having M_r values and peptide fragmentation patterns, as well as immunological properties, similar to those of A-type and B-type subunits of liver GST isoenzymes (Singh *et al.*, 1985a), the possibility of the involvement of at least two gene loci, say GST2A and GST2B, can also be considered for the cationic enzymes. If both these loci are polymorphic, then much more diversity among the isoelectric-focusing

Table 3. Inhibition of GST isoenzymes of human kidney by bilirubin, haematin and bromosulphophthalein

The results presented in this Table were obtained with GST isoenzymes isolated from kidney II. The inhibition studies were performed as described in the Materials and methods section. The nature of inhibition was determined from double-reciprocal plots and the K_i values were determined by the replots of the double-reciprocal plots. Abbreviation: N.D., not determined.

GST isoenzyme	Nature of inhibition with respect to 1-chloro-2,4-dinitrobenzene		
	Bilirubin	Haematin	Bromosulphophthalein
GST 9.1	Non-competitive (K_i 28 μ M)	Non-competitive (K_i 8 μ M)	Competitive (K_i 100 μ M)
GST 9.0	Non-competitive (K_i 38 μ M)	Non-competitive (K_i 25 μ M)	Competitive (K_i 90 μ M)
GST 6.6	Non-competitive (K_i 8.7 μ M)	Non-competitive (K_i 5 μ M)	Non-competitive (K_i 3.8 μ M)
GST 4.7	N.D.	Competitive (K_i 6 μ M)	Competitive (K_i 18 μ M)

profiles of different individuals could be expected, as observed in the present study. The presence of more than one charge isomer in both A-type and B-type subunits seen in the two-dimensional gel electrophoresis may suggest that both GST2A and GST2B gene loci may be polymorphic.

Unlike the isoenzymes corresponding to the GST1 and GST2 gene loci, the presence of only one major isoenzyme corresponding to the GST3 locus has been reported in the human tissues examined so far (Marcus *et al.*, 1978; Guthenberg & Mannervik, 1981; Koskelo *et al.*, 1981; Polidoro *et al.*, 1981; Dao *et al.*, 1984; Laisney *et al.*, 1984; Awasthi & Singh, 1984; Singh *et al.*, 1985a,b,c; Strange *et al.*, 1984, 1985). In kidney at least two GST isoenzymes, pI 4.7 and 4.9, corresponding to the GST3 gene locus are present because both these isoenzymes cross-react with the antibodies raised against GST π of placenta. Individual variations in the expression of this group of enzymes suggest the possibility of a polymorphic nature of the GST3 gene locus. Studies involving a large number of kidney samples are needed to substantiate the polymorphic nature of the GST3 and GST2 gene loci, and definite conclusions cannot be drawn because only a small number of samples were used in the present study.

Similarly to the cationic isoenzymes of human liver GST (Singh *et al.*, 1985a), all the cationic isoenzymes of kidney (except for GST 9.1) are heterodimers of A-type and B-type subunits. Although the subunit structure of GST 9.1 of kidney (dimer of B-type subunits) resembles that of a cationic isoenzyme (pI 9.2) isolated from human lung (Partridge *et al.*, 1984), this kidney isoenzyme appears to be different from GST 9.2 of lung because of significant differences in the kinetic properties of these two isoenzymes. Except for 1-chloro-2,4-dinitrobenzene, no other substrate used in this study was utilized by GST 9.2 of lung (Partridge *et al.*, 1984), whereas GST 9.1 of kidney expressed activity towards all the substrates. Also, the specific activity of GST 9.1 of kidney towards 1-chloro-2,4-dinitrobenzene is about 6-fold higher as compared with that reported for GST 9.2 of lung. Among the near neutral or μ class of isoenzymes of kidney, GST 5.5 corresponds to GST ψ (Singh *et al.*, 1985a, 1987) of liver in its subunit composition, pI value and catalytic properties, and this enzyme may be the same as GST ψ . GST 6.6 of kidney corresponds to GST μ of liver in its pI value and catalytic properties. However, in two-dimensional electrophoretic analysis kidney GST 6.6 shows the presence of two charge isomers corresponding to M_r 26 500 whereas GST μ is a homodimer (Warholm *et al.*, 1983). These results indicate that GST 9.1 and GST 6.6 of kidney are distinct from GST isoenzymes characterized so far from human tissues.

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