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Isoenzyme profile of glutathione S-transferases in human kidney

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Abstract Novel glutathione S-transferase (GST) isoenzymes, which do not bind to the glutathione (GSH) affinity column, were recently identified in dog kidney and dog renal cell lines. In humans, similar affinity flow-through GST has been previously found only in the urinary bladder. To ascertain whether these affinity flow-through GST isoenzymes also exist in the human kidney, we separated GST isoenzymes from five kidney samples on the basis of their affinity to GSH affinity resin. GSTs were further purified by anion exchange chromatography and chromatofocusing and characterized with specific substrates. Our results show that the human kidney has both affinity flow–through GST isoenzymes and those which bind tightly to the GSH affinity column. Purification of affinity-bound GST resulted in a rich profile of different isoenzymes with balanced expression of both anionic and cationic forms. Affinity flow-through GST was represented by one isoenzyme (pI-7.9) in all kidney samples tested, but one kidney specimen also contained another GST isoenzyme (pI-7.0). Our results for the first time show the presence of GST isoenzymes that do not bind to GSH-affinity resin in the human kidney. Although the assessment of similarity between the human kidney and urinary bladder affinity flow-through GST requires further elucidation, it can be speculated that these particular GSTs may play an important role in providing protection against the common carcinogens.

Key words Glutathione · S-transferase · GST · Humans · Kidney · Detoxification

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Introduction

Glutathione S-transferases (GSTs) are a super-family of enzymes divided according to their structural, physicochemical, enzymatic, and immunological properties into four classes: α, μ, π and θ [12, 13]. Different isoenzymes were observed within the GST classes. In certain tissues, isoenzymes belonging to classes α, μ, and π are identified as cationic, near neutral, and anionic forms, respectively. Although these designations may sometimes equate with α, μ and π classes, this equivalence can not be taken for granted [4].

GSTs play an important role in the detoxification of hydrophobic and electrophylic molecules. They have been associated with putative preneoplastic foci of rat hepatocarcinogenesis [4] and chemotherapy resistance of human cancers [6]. GSTs catalyse the conjugation of xenobiotics and carcinogens with glutathione (GSH). This reaction is a first step in the formation of mercapturic acids, a pathway resulting mainly in detoxification and the elimination of potentially genotoxic compounds by the kidney [9]. However, there are few exceptions, e.g., *t*-butilhydroquinone [14] and trichloroethene [3], for which conjugation with GSH produces more toxic compounds.

Differential glutathione S-transferase expression (e.g., the number and the activity of GST within each class) may significantly influence the anti-carcinogenic potential of tissues. GSTs are therefore currently being investigated as biomarkers of risk for various cancers [10, 16]. Conflicting data have been obtained, but GST μ deficiency seems to be associated with susceptibility to certain cancers [15, 19].

Since the potential nephrotoxicity of carcinogens depends on the biotransformation capacity of the kidney, it is of major importance to identify the GST isoenzymes in the human kidney. According to Di Ilio and coworkers [5], all the three major classes (α, μ and π) are present in significant amounts in the human kidney, while Tateoka et al. [20] found only GSTs belonging to classes α and π. In these studies, GST isoenzymes were separated from the

cytosol by affinity chromatography, followed only by further purification and characterization of isoenzymes tightly bound to GSH affinity resin. However, novel GST isoenzymes, which do not bind to the GSH affinity column were recently identified in dog kidney and Madin-Darby canine kidney (MDCK) cells (dog renal cell line) [1]. GST with similar characteristics with respect to binding for GSH-affinity resin in humans, was detected only in the urinary bladder [18]. We find it important to assess whether these affinity flow-through GST isoenzymes also exist in the human kidney, since both renal parenchymal cells and uroepithelium of the urinary bladder are exposed to a broad range of the same, potentially genotoxic, compounds. The present study was therefore designed to separate GSTs in the human kidney on the basis of their affinity to GSH affinity resin, as well as to purify and characterize them.

Materials and methods

Tissue samples

Human kidney tissue was obtained at autopsy from five individuals who died either from accidental causes or from a non-renal disease. All tissue samples were washed in cold saline, freezed in liquid nitrogen and stored at −80 °C until used.

Preparation of subcellular fractions

After thawing, the samples were homogenized with a Potter-Elvehjem glass-teflon homogenizer in approximately 4 vol. of 50 mM Tris-HCl buffer, pH 7.8, containing 200 mM NaCl and 0.5 mM dithiotreitol (TBS buffer). The homogenate was ultracentrifuged for 1 h at 100,000*g* and the clear supernatant was used as the cytosol fraction for GST activity determination and further enzyme purification. Protein concentration was determined using the Bradford method [2], with bovine serum albumin (BSA) as the standard.

Determination of the GST activity

GST activity towards different substrates was determined as described by Habig et al. [7]. One unit (U) of enzyme utilized 1 μmol of substrate per minute at 25 °C. Class Alpha GSTs are highly active with cumene hydroperoxide (CuOOH). The reaction represents the "nonselenium" glutathione peroxidase activity [21]. GST activity with CuOOH was determined according to the procedure of Singh [18]. The activity of GST towards cumene hydroperoxide was determined at 37 °C, and expressed as micromole per minute of NADPH (nicotinamide adenine dinucleotide phosphate, reduced) consumed. Specific enzyme activity is expressed as units per milligram of protein.

Separation of GST isoenzymes

Affinity chromatography

Separation of GSTs by affinity chromatography was performed by using Glutathione Sepharose 4B affinity column (1.6 × 12 cm). The Glutathione Sepharose 4B affinity column was equilibrated with TBS buffer, pH 7.8. After applying the cytosol, the column was eluted with TBS buffer until in the eluate no further absorbance was measured at 280 nm. The bound GSTs were eluted with 20 mM GSH in 200 mM Tris-HCl buffer, pH 9.2. Both the affinity-bound and the flow-through GST fractions were pooled and concentrated with Amicon YM30 ultrafiltration membrane.

Purification of affinity-bound GST fraction

Affinity-bound GST fractions were applied to a Polybuffer exchanger 94 column (PBE 94, 1 × 25 cm) equilibrated with the 25 mM imidazole-HCl buffer, pH 7.4. The column was eluted with Polybuffer 74, diluted (1:8), and adjusted to pH 4.0 with HCl. The pH values in the fractions were monitored by digital pH-meter. The cationic GST activity did not bind to the Polybuffer exchanger 94 equilibrated with 25 mM imidazole-HCl buffer, pH 7.4, and was recovered in flow-through GST fraction.

The flow-through GST fractions after anionic chromatofocusing were pooled, concentrated, and equilibrated with 25 mM ethanolamine-HCl, pH 9.4. These cationic GSTs were applied to a PBE 94 column (1 × 25 cm), equilibrated with 25 mM ethanolamine-HCl buffer, pH 9.4. Elution was carried out with Polybuffer 96, diluted (1:8), and adjusted to pH 7.0 with HCl.

Purification of affinity flow-through GST fraction

The affinity flow-through GST fractions were pooled, concentrated, equilibrated with 10 mM Tris-HCl buffer, pH 8.0, and then applied to a DE-52 column (2.6 × 40 cm) equilibrated with 10 mM Tris-HCl, pH 8.0. GST activity was eluted with 400 ml of a NaCl gradient (0–200 mM). Substantial amount of GST activity did not bind to anion exchanger column and was recovered in DE-52 flow-through GST fractions.

DE-52 bound GST fractions were collected, concentrated, and equilibrated with 25 mM imidazole-HCl buffer, pH 7.4 and applied to a Polybuffer exchanger 94 column (1.0 × 25 cm) equilibrated with 25 mM imidazole-HCl buffer, pH 7.4. GST activity was eluted with Polybuffer 74-HCl, diluted (1:8), and adjusted to pH 4.0 with HCl.

DE-52 flow-through GST fractions were collected, concentrated, and equilibrated with 25 mM ethanolamine-HCl buffer, pH 9.4, and applied to a Polybuffer exchanger 94 column (1 × 25 cm) equilibrated with 25 mM ethanolamine-HCl buffer, pH 9.4. Elution was carried out with Polybuffer 96, diluted (1:8), and adjusted to pH 7.0 with HCl.

Characterization of the GST isoenzymes

Kinetic properties

Kinetic properties were examined by measuring the initial velocities of GST at a fixed concentrations of one substrate GSH (or 1-chloro-2,4-dinitrobenzene, CDNB) and varying concentrations (0.25-1 mM) of the other substrate CDNB (or GSH). Kinetic properties were analysed by computer program SPSS.

Substrate specificity

Substrates used in this study were 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid, 1,2-dichloro-4-nitrobenzene (DCNB) and cumene hydroperoxide (CuOOH). Ethacrynic acid and DCNB are reported to be the best substrates for classes π and μ, respectively [7]. Cumene hydroperoxide was used for the identification of α class GSTs.

Results

Glutathione S-transferase activity

All specimens were subjected to purification procedure, but only representative profile of human kidney was selected for presentation, because of interindividual variation in the conjugation of CDNB (Table 1).

Table 1 Glutathione S-transferase activity in human kidney. One unit (U) of enzyme activity utilized 1 μmol of substrate 1-chloro-2,4-dinitrobenzene per minute at 25 °C

Case	Age (years)	Sex	GST activity (U/mg)
1	46	M	0.593
2	53	F	0.982
3	61	M	0.685
4	57	M	0.818
5	62	F	1.240
Median and confidence intervals	57		0.818
	(46–62)		(0.593–1.240)

Affinity chromatography

Affinity chromatography purification of GSTs from all human kidney samples studied, revealed the presence of two forms of enzyme (Fig. 1). The first, flow-through GST fraction, which corresponds to the peak I in Fig. 1, represents 25%–47% of the GST activity towards CDNB. The affinity-bound GST fraction of human kidney was eluted with 20 mM GSH (peak II, Fig. 1) and represents 53%–75% of the GST activity eluted.

Separation of affinity flow-through GSTs

The flow-through fraction of the affinity step was further subjected to anion exchange chromatography (DE 52) (Fig. 2A). The majority of affinity flow-through GST failed to bind to anion-exchanger column (Fig. 2A) in all five specimens. However, in one kidney sample a fraction of GST activity was bound. This GST activity was eluted with a NaCl concentration of 100 mM from a DE-52 column (Fig. 2A). Anionic chromatofocusing showed that this bound GST fraction contained a single isoenzyme with nearly neutral isoelectric point (pI-7.0, Fig. 2B). Another GST fraction, which failed to bind to anion exchanger, was identified by cationic chromatofocusing (Fig. 2C), as one basic GST isoenzyme (pI-7.9) in all kidney samples tested.

Separation of affinity-bound GSTs

Affinity-bound GST fraction was further separated by anionic and cationic chromatofocusing. Representative chromatofocusing profile of human kidney GSTs is reported in Fig. 3. A profile consisting of at least nine GST activity peaks, with balanced expression of anionic and cationic forms, was obtained (Fig. 3). The anionic and near-neutral GST isoenzymes resolved into five peaks of enzyme activity corresponding to pI values of 4.7, 4.9, 5.2, 5.5 and 6.8 (Fig. 3A). The anionic isoenzymes GST-4.7 and GST-4.9 constituted approximately 43% of the GST activity eluted. Less-anionic and near-neutral isoenzymes were also present in substantial amounts (13 and 17% of eluted GST activity, respectively). The cationic GSTs of human kidney resolved into at least four peaks of enzymatic activity corresponding to pI values of 7.4, 7.8, 8.1 and 8.3. The major cationic GST peak was GST pI 7.8, and represented 20% of GST activity eluted (Fig. 3B).

Physicochemical properties of glutathione S-transferases

The differences among the properties of GST isoenzymes of human kidney belonging to different classes are also reflected in their substrate specificities. Physicochemical characteristics of the human kidney GST isoenzymes are summarized in Table 2. All affinity-bound cationic GSTs and less-anionic GST-5.2 and GST-5.5 were active with the class α substrate CuOOH. Activity with the class μ substrate DCNB was also observed, although less general and much lower. The acidic isoenzymes GST-4.7

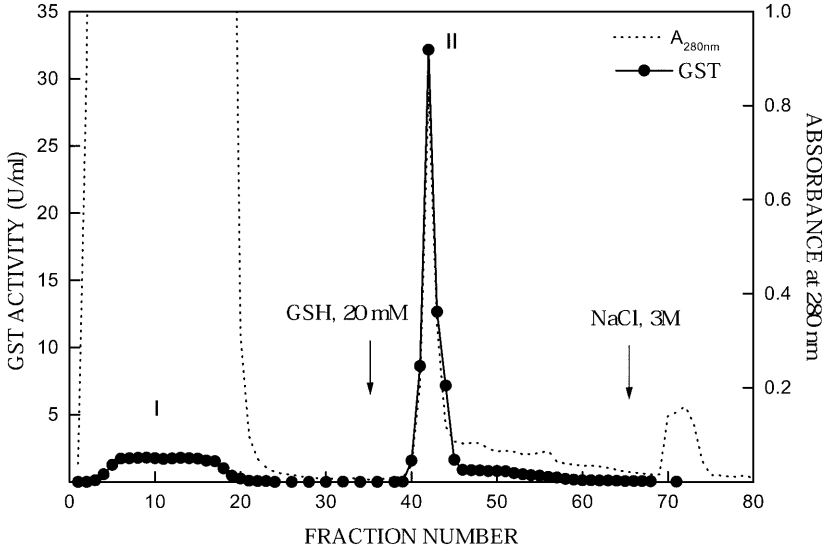

Fig. 1 Elution of human kidney GST activity from a Glutathione Sepharose 4B affinity column. The column was equilibrated with 50 mM TBS, pH 7.8. After application of the cytosol, the column was rinsed with the start buffer until no absorbance at 280 nm was detected in the eluate. The bound GST activity was eluted with 20 mM GSH in 20 mM Tris-HCl buffer, pH 9.2. Remaining proteins were removed with 3 M NaCl

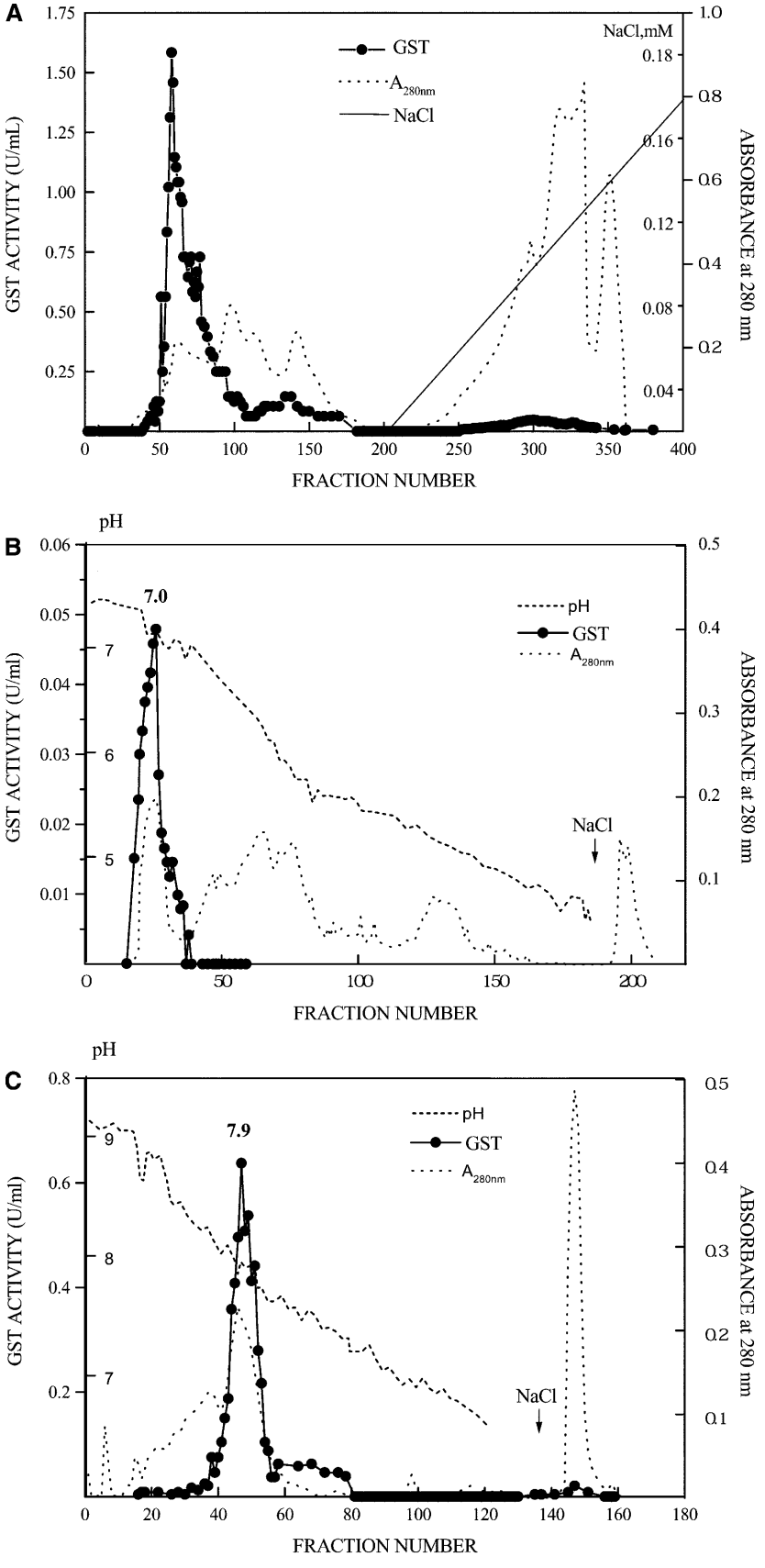

Fig. 2 Purification of the affinity flow-through human kidney GST. **A** Anion exchanger (DE 52) chromatography of human kidney affinity flow-through GST. The column was equilibrated with 10 mM Tris-HCl buffer, pH 8.0. After application of the affinity flow-through GST activity, the column was rinsed with the start buffer until no absorbance at 280 nm was detected in the eluate. The bound GST activity was eluted with 400 ml of a 0–200 mM NaCl gradient. Remaining proteins were removed with 3 M NaCl. **B** Anionic chromatofocusing of the affinity flow-through GST, which was bound to DE 52, on a Polybuffer exchanger 94 column. The column was equilibrated with 25 mM imidazole-HCl buffer, pH 7.4. GST activity was eluted with Polybuffer 74-HCl, diluted (1:8), and adjusted to pH 4.0 with HCl. **C** Cationic chromatofocusing of the affinity flow-through GST, which failed to bind to DE 52, on a Polybuffer exchanger 94 column. The column was equilibrated with 25 mM ethanolamine-HCl buffer, pH 9.4. GST activity was eluted with Polybuffer 96-HCl, diluted (1:8), and adjusted to pH 7.0 with HCl

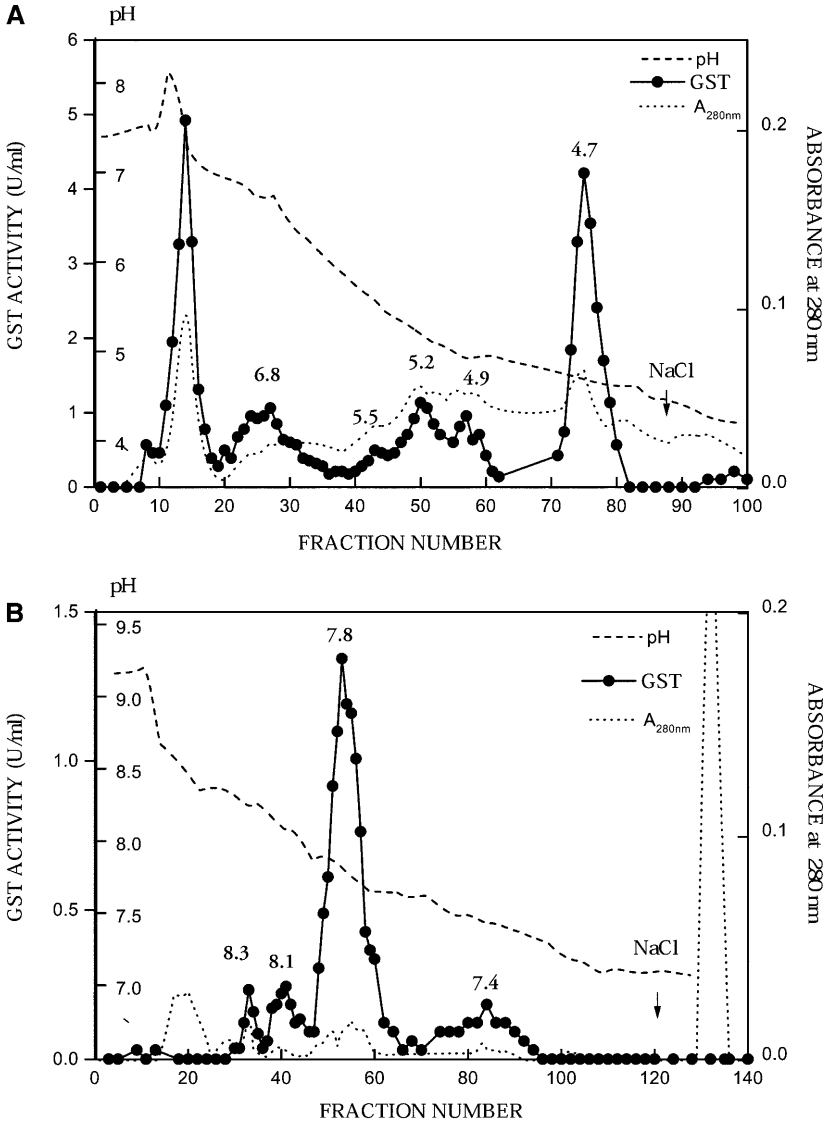

Fig. 3 Purification of the affinity-bound human kidney GST. **A** Anionic chromatofocusing of the affinity-bound human kidney GST on a Polybuffer exchanger 94 column. The column was equilibrated with 25 mM imidazole-HCl buffer, pH 7.4. GST activity was eluted with Polybuffer 74-HCl, diluted (1:8), and adjusted to pH 4.0 with HCl. **B** Cationic chromatofocusing of the affinity-bound human kidney GST on a Polybuffer exchanger 94 column. The column was equilibrated with 25 mM ethanolamine-HCl buffer, pH 9.4. GST activity was eluted with Polybuffer 96-HCl, diluted (1:8), and adjusted to pH 7.0 with HCl

Table 2 Physicochemical properties and substrate specificity of human kidney GST isoenzymes. One unit of enzyme activity utilized 1 μmol of substrate per minute at 25 °C*. The activity of GST towards cumene hydroperoxide was determined at 37 °C, and expressed as micromole per minute of NADPH consumed. The specific activities presented in this table were determined with peak fraction of each GST isoenzyme at least twice and similar results were obtained. For kinetic properties determination peak fractions were pooled, concentrated, and dialysed against 5 mM sodium phosphate buffer pH 7.0, containing 10% glycerol, 50 mM NaCl and 0.2 M dithiotreitol. (*ND* not detected)

Apparent isoelectric point (pI)	Km for GSH (mM)	Km for CDNB (mM)	Specific activity (U/mg)			
			CDNB	Ethacrynic acid	DCNB	CuOOH*
8.1	0.43	0.53	24.40	ND	0.05	5.67
7.9[a]	0.23	0.39	4.74	ND	0.02	2.73
7.8	0.32	0.79	35.26	ND	0.03	8.95
7.4	0.15	0.45	7.04	ND	0.017	5.63
6.8	0.13	0.27	16.60	0.11	ND	ND
5.5	0.37	1.06	10.5	ND	ND	2.00
5.2	0.34	0.68	9.29	ND	ND	3.32
4.9	0.29	0.71	47.00	0.6	ND	ND
4.7	0.55	0.65	31.89	2.12	ND	ND

[a] Affinity flow-through GST

and GST-4.9 were active with the ethacrynic acid, class π substrate. The isolated affinity flow-through GST-7.9 isoenzyme showed activity towards CuOOH, while the GST-7.0 could not be further identified due to the paucity of material available.

Discussion

Our study indicates that human kidney has both glutathione S-transferase isoenzymes, which are not retained on Glutathione Sepharose 4B affinity column and those which bind tightly to affinity column.

Purification of affinity-bound GSTs resulted in a rich profile of different GST isoenzymes with balanced expression of both anionic and cationic forms. Several renal GST isoenzymes isolated in our study have the same pI values as GST isoenzymes, which previously had been purified by other investigators [5, 20] The particular class of GST they belonged to was tested with specific substrates. It seems that the cationic, near neutral, or acidic pI of GST isoenzymes does not necessarily correspond to the classes α, μ and π, respectively. Namely, anionic GST 5.2, obtained in our study, has shown the activity towards cumene hydroperoxide, which has been considered characteristic substrate for class α. These data are consistent with those of Di Illio and coworkers [5], who also identified this isoenzyme as a member of class α with specific antibodies.

Although the majority of human kidney GSTs did bind to the Glutathione Sepharose 4B affinity column, a substantial amount of the GST activity towards CDNB was found in the flow-through fraction. To the best of our knowledge, our results for the first time show the presence of GST isoenzymes that do not bind to GSH-affinity resin in human kidney. These findings prompted us to purify this affinity flow-through GST fraction. In further purification, the enzyme fraction subjected to anion exchange chromatography (DE 52 column) and chromatofocusing, revealed two isoenzymes (GST-7.9 and GST-7.0). GST isoenzymes with similar characteristics with respect to binding to the GSH affinity column were also found in dog liver [8], dog kidney, and MDCK cells [1] and rat liver [11]. The affinity flow-through GST of dog liver, dog kidney, and MDCK cells, possesses high activity towards DCNB and CDNB and has near neutral pI [1]. Rat liver flow-through GST is characterized by a high peroxidase activity and extremely low activity with CDNB [11]. This enzyme was later identified as class θ enzyme [13]. The human kidney affinity flow-through GST-7.9, isolated in our study, has rather low activity towards both CDNB and CuOOH. The characterization of another kidney affinity flow-through GST (GST-7.0) could not be performed, due to the paucity of the material available. It is interesting to note that an enzyme with low affinity for GSH-affinity resin in humans was also detected in the urinary bladder [18]. This urinary bladder flow-through GST was identified as class α enzyme. Kidney affinity flow-through GST-7.9 isolated in our study is probably also a member of class α. This conclusion is based on its activity with CuOOH (a characteristic class α substrate). Kidney and urinary bladder GST α might be structurally different, if not completely, from this class of enzymes expressed in other human tissues, which do bind to GSH affinity resin [17]. However, further characterization of bladder and kidney flow-through GST would be needed to establish the similarity between these enzymes and to elucidate their physiological role. Besides, it would be important to determine (i) whether interindividual variations in the expression of renal affinity-flow through GSTs exist, since the particular form of enzyme may influence the ability to efficiently detoxify certain carcinogens and (ii) if they do exist, what are the consequences of the altered expression of renal affinity-flow through GST isoenzymes for the susceptibility to renal cell carcinoma.

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References

1. Bohets HH, Nouwen EJ, De Broe ME, Dierickx PJ (1996) The cytosolic glutathione S-transferase isoenzymes in the dog kidney cortex as compared with the corresponding MDCK renal cell line. Biochim Biophys Acta 1311: 93
2. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Anal Biochem 72: 248
3. Bruning T, Lammert M, Kempkes M, Thier R, Golka K, Bolt HM (1997) Influence of polymorphism of GSTM1 and GSTT1 for risk of renal cell cancer of workers with long-term high occupational exposure to trichloroethene. Arch Toxicol 71: 596
4. Coles B, Ketterer B (1990) The role of glutathione and glutathione transferases in chemical carcinogenesis. Clin Rev Biochem Mol Biol 25: 47
5. Di Ilio C, Aceto A, Zezza A, Ricci G, Federici G (1989) Electrophoretic and immunological analysis of glutathione transferase isoenzymes of human kidney carcinoma. Biochem Pharmacol 38: 1045
6. Gulick MA, Fahl WE (1995) Mammalian glutathione S-transferase: Regulation of an enzyme system to achieve chemotherapeutic efficacy. Pharmacol Ther 66: 237
7. Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases. The first enzymatic step in mercaturic acid formation. J Biol Chem 249: 7130
8. Igarashi T, Kohara A, Shikata Y, Sagami F, Sonoda J, Horie T, Satoh T (1991) The unique feature of dog liver cytosolic glutathione S-transferases. An isozyme not retained on the affinity column has the highest activity toward 1,2-dichloro-4-nitrobenzene. J Biol Chem 266: 21709
9. Ketterer B, Christodoulides LG (1994) Enzymology of cytosolic glutathione S-transferases. Adv Pharmacol 27: 37
10. Lafuente A, Pujol F, Carretero P, Villa JP, Cuchi A (1993) Human glutathione S-transferase μ (GST-μ) deficiency as a marker for the susceptibility to bladder and larynx cancer among smokers. Cancer Lett 68: 49
11. Mannervik B (1985) The isoenzymes of glutathione S-transferase. In: Meister A (ed) Advances in enzymology. J Wiley and Sons. New York, p 357–417
12. Mannervik B, Alin P, Guthenberg C, Jensson H, Tahir MK, Warholm M, Jornvall H (1985) Identification of three classes common to several mammalian species: correlation between structural data and enzymatic properties. Proc Natl Acad Sci USA 82: 7202

13. Meyer DJ, Coles B, Pemble SE, Gilmore KS, Fraser GM, Ketterer B (1991) Theta, a new class of GSH transferases purified from rat and man. Biochem J 274: 409
14. Peters MMCG, Rivera M, Jones TW, Monks TJ, Lau SS (1996) Glutathione conjugates of tert-butyl-hydroquinone, a metabolite of the urinary tract tumour promoter 3-tert-butyl-hydroxyanisole, are toxic to kidney and bladder. Cancer Res 56: 1006
15. Rebbeck TR (1997) Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomarkers Prev 6: 733
16. Seidegard J, Pero RW, Markowitz MM, Roush G, Miller DG, Beattie EJ (1990) Isoenzymes of gluthatione S-trasferase (class mu) as a marker for susceptibility to lung cancer: a follow up study. Carcinogenesis 37: 8
17. Simons PC, Vander Jagt DL (1977) Purification of glutathione S-transferases from human liver by glutathione-affinity chromatography. Anal Biochem 82: 334
18. Singh SV, Roberts B, Gudi VA, Ruiz P, Awasthi YC (1991) Immunohistochemical localisation, purification, and characterisation of human urinary bladder glutathione S-transferases. Biochim Biophys Acta 1074: 363
19. Strange RC, Lear JT, Fryer AA (1998) Glutathione S-transferase polymorphism: influence on susceptibility to cancer. Chem Biol Interact 111–112: 351
20. Tateoka N, Tshchida S, Soma Y, Sato K (1987) Purification and characterization of glutathione S-transferases in human kidney. Clin Chim Acta 166: 207
21. Wendel A (1980) Glutathione peroxidase. In: Jakobi WB (ed) Enzymatic basis of detoxication. Academic Press, New York, p 333