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Identification of a glutathione S-transferase without affinity for glutathione sepharose in human kidney

Short Communication

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Summary. To identify kidney glutathione S-transferase (GST) isoenzyme, which does not bind to glutathione affinity column, biochemical characterization was performed by using an array of substrates and by measuring sensitivity to inhibitors. Immunological characterization was done by immunoblotting. Affinity flow-through GST exhibited activity towards 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole and cumene hydroperoxide, typical class α substrates and high sensitivity towards hematin, an α class inhibitor. It cross-reacted with antibodies against α class GST. Affinity flow-through GST in human kidney is an α class member.

Keywords: Glutathione S-transferase – Alpha class GST – Human kidney

Introduction

The glutathione S-transferases (GSTs) are encoded by two distinct multigene families, microsomal GSTs and the soluble GSTs (Jakobsson et al., 1999). Soluble enzymes function as dimmers and catalyze the conjugation of glutathione (GSH) with electrophylic compounds (Hayes and Pulford, 1995). Besides GSH-conjugating activity, GSTs can also serve as peroxidases, isomerases and thiol transferases and some isoenzymes have non-catalytic functions. Classes α, μ, π, θ and ζ are polymorphic and consist of several isoenzymes, showing different substrate specificities (Hayes and Strange, 2000). The distribution of GSTs is organ-dependent and this feature, together with polymorphic expression, significantly influences biotransformation capacity of different tissues, (Mimic-Oka et al., 1992; Strange et al., 2001).

The highest GST activity in human and rat organs has been observed in the liver followed by the kidney (Baars et al., 1981; Sherman et al., 1983). In kidney tissue, four major cytosolic GST classes (α, μ, π and θ), have been described (Tateoka et al, 1987; Di Ilio et al., 1987; Oberley et al., 1994; Delbanco et al., 2001). Besides, isoform of GST that has no affinity for immobilized GSH was also isolated in human kidney (Simic et al., 2001). GST isoenzymes without affinity for GSH resin have been first identified in dog kidney and named affinity flow-through GST (Bohets et al., 1996). In addition to kidney, in humans, affinity flow-through GST isoenzyme has been found only in urinary bladder, (Singh et al., 1991). This bladder affinity flow-through GST was identified as class α enzyme. Corresponding kidney GST isoenzyme was only partially characterized, as a cationic GST with low peroxidase and 1-chloro-2,4-dinitrobenzene (CDNB) conjugating activity (Simic et al., 2001). However, identification of kidney affinity flow-through GST based on determination of activities with specific substrates and inhibitors, subunit composition and immunological properties, has not been performed as yet. Such characterization is of particular interest, since a potential role for affinity flow-through GST in the process of kidney cancerisation has been recently suggested. Namely, in normal human kidney, a substantial amount of the GST was found in the affinity flow-through fraction (Simic et al., 2001). However, in kidney tissue of patients with renal cell carcinoma this isoenzyme was significantly down-regulated. Besides, affinity flow-through was not expressed by renal cell carcinoma (Simic et al., 2003). The present study describes the identification of affinity flow-through GST in human kidney.

Materials and methods

Isolation of affinity flow-through GST from human kidney

Isolation of affinity flow-through GST from five human kidney specimens was performed according to the procedure that has recently been described (Simic et al., 2001). In brief, tissue was obtained at autopsy from individuals who died either from accidental causes or from a non-renal disease. Cytosolic fraction was used for enzyme isolation. GST in fractions was identified by measuring the overall enzyme activity towards CDNB. GSTs were first separated on the basis of their affinity to Glutathione Sepharose 4B affinity column, followed by ion exchange chromatography and cationic chromatofocusing. Affinity flow-through GST was isolated from peak fractions after chromatofocusing at pH-7.9. Activities towards specific substrates were determined in each sample at least three times. For inhibition studies, electrophoresis and immunoblotting peak fractions were pooled.

Biochemical characterization

Overall GST activity was determined by using GSH and CDNB in a concentration of 1 mM at 340 nm (pH 6.5) (Habig et al., 1974). Specific substrates included in the study were: 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), cumene hydroperoxide (CuOOH), 1,2-dichloro-4-nitrobenzene (DCNB), vinyl pyridine (VP) and 1,2-epoxy-3-(p-nitrophenoxy)-propane (EPNP) (Habig and Jakoby, 1981; Ricci et al., 1994; Thier et al., 2002). Enzyme activities were determined at the following wavelengths and pH conditions: NBD-Cl, 419 nm and pH 6.5; CuOOH, 340 nm and pH 7.0; DCNB 345 nm and pH 7.5; VP, 248 nm and pH 6.5; EPNP 360 nm and pH 6.5. The activity of GST towards CuOOH was determined at 37 °C and units (U) of activity are expressed as μmol per minute of NADPH consumed. GST activities towards other substrates are presented as units (U) corresponding to the amount of enzyme that utilized 1 μmol of substrate per minute at 25 °C. Specific activity is expressed in U/mg protein, the proteins being measured by the Bradford method (1976) with bovine serum albumin as the standard.

Inhibitors included in the study were hematin, cibacron blue and ethacrynic acid (Habig and Jakoby, 1981). The inhibition was quantified in the I_{50} value. This is the concentration of inhibitor resulting in 50% inhibition of the overall enzyme activity with CDNB and GSH as substrates.

SDS-polyacrylamide gel electrophoresis and immunoblotting

Vertical electrophoresis was performed as described by Laemmli (1970) using 12.5% polyacrylamide gels. For subunit composition and molecular mass determination proteins were visualised by Coomassie brilliant blue staining. For immunoblotting proteins were transferred to a nitrocellulose membrane. The membranes were incubated with primary rabbit polyclonal antibodies against classes α, μ and π (1:500), washed and incubated with secondary goat anti-rabbit antibody (1:2000) conjugated with peroxidase. Binding of immobilized proteins was detected by enhanced chemiluminiscence.

Results

The physico-chemical properties of the affinity flow-through GST from human kidney are summarized in Table 1. Affinity flow-through GSTs isolated from all samples had the same cationic isoelectric point (pI 7.9). SDS-PAGE showed that affinity flow-through was a homodimer with a molecular mass of 25 000 Da.

Affinity flow-through GST was tested for the activity with specific substrates (Table 1). The enzyme was active with NBD-Cl and CuOOH, characteristic class α substrates. Affinity flow-through GST did not exhibit any activity with substrates (DCNB, VP, and EPNP), that are specific for μ, π, and θ GST classes, respectively (Table 1).

Table 1. Physicochemical characteristics, substrate specificities and inhibition parameters of affinity flow-through GST in human kidney

	Affinity flow-through GST
Physicochemical characteristics	
pI	7.9
Overall activity towards CDNB (U/mg protein)	4.92 ± 1.13
Subunit (Da)	25000
Specific substrates (U/mg protein)	
NBD-Cl (class α)	1.12 ± 0.27
CuOOH (class α)	3.67 ± 0.99
DCNB (class μ)	nd
VP (class pi)	nd
EPNP (class θ)	nd
Specific inhibitors (I_{50} in μM)	
Hematin	0.15 ± 0.04
Cibacron blue	2.01 ± 0.49
Ethacrynic acid	5.51 ± 1.41

pI Isoelectric point; *CDNB* 1-chloro-2,4-dinitrobenzene; *GSH* glutathione; *NBD-Cl* 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; *CuOOH* cumene hydroperoxide; *DCNB* 1,2-dichloro-4-dinitrobenzene; *VP* vinyl pyridine; *EPNP* 1,2-epoxy-3-(p-nitrophenoxy)propane; *nd* not detected; *I_{50}* Concentration (μM) of inhibitor resulting in 50% inhibition of the GST activity, measured with 1 mM GSH and CDNB as substrates

Different GSTs can be also distinguished according to their sensitivity towards different inhibitors (Habig and Jakoby, 1981). Class α is noted for a low I_{50} value for hematin and a high value for cibacron blue. Class μ has a high I_{50} value for hematin and a low value for cibacron blue and ethacrynic acid. Class π has a high I_{50} value for

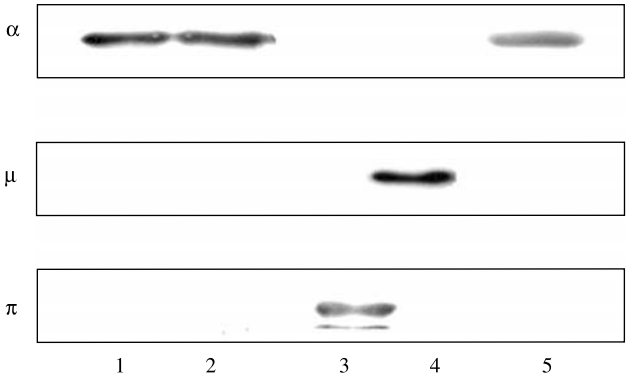

Fig. 1. Immunoblot analysis of affinity flow-through GST fraction in human kidney. Proteins were resolved by SDS/PAGE and analysed with antisera against class α, μ and π. *1*, *2* Affinity flow-through GSTs from human kidney; *3* human placental GST (class π); *4* rat liver GST (class μ), *5* bovine liver GST (class α)

hematin and ethacrynic acid and a low value for cibacron blue. The inhibition parameters for affinity flow-through GST with different inhibitors are given in Table 1. Affinity flow-through GST showed a high sensitivity towards hematin and a low sensitivity towards cibacron blue and ethacrynic acid, that is typical of α class GST.

Immunoblot analysis was performed for all isolated affinity flow-through GST isoenzymes and representative immunoblot is presented in Fig. 1. The affinity flow-through GST from human kidney showed the presence of class α antigen. The enzyme did not cross react with antibodies against classes μ and π (Fig. 1).

Discussion

We performed biochemical and immunological characterization of affinity flow-through GST in human kidney. It has been shown that affinity flow-through GST possesses NBD-Cl conjugating and peroxidase activity towards CuOOH, typical class α substrates. Inhibition studies have shown high sensitivity of this isoenzyme towards hematin, which is characteristic of α class. Finally, immunoblotting confirmed that affinity flow-through GST in human kidney belongs to α class.

Up to date, affinity flow through GST in human kidney has not been identified at class level. Cationic isoelectric point (pI – 7.9), suggested that affinity flow-through could be an α class member (Simic et al., 2001). Still, identification of kidney affinity flow-through GST had to be performed by comprehensive biochemical characterisation and immunoblotting. Our data on the activity of affinity flow-through GST with NBD-Cl are in agreement with the study of Delbanco et al. (2001), who showed the presence of NBD-Cl conjugating activity in renal tissue of patients with renal cell carcinoma. Peroxidase activity with CuOOH has also been reported in human kidney (Pacifici et al., 1989). Furthermore, NBD-Cl conjugating and CuOOH peroxidase activity in the kidney are reported to represent a subclass A2 of GSTs (Hayes and Strange, 2000). Regarding substrate specificity, affinity flow-through GST isoenzymes were characterised only in tissues of other species. Thus, affinity flow-through GST of dog liver and kidney possesses high activity towards DCNB, a class μ substrate (Igarashi et al., 1991; Bohets et al., 1996). Rat liver enzyme possesses peroxidase activity and extremely low activity with CDNB (Mannervik et al., 1985). It was later identified as class θ enzyme (Meyer et al., 1991). Since inhibitor sensitivities can also be used to distinguish different enzymes (Habig and Jakoby, 1981), we measured the activity of affinity flow-through GST in the presence of specific inhibitors. Our data that human kidney affinity flow-through GST is hematin sensitive, are in contrast to data on corresponding dog renal isoenzyme, which was similarly sensitive towards both cibacron blue and hematin (Bohets et al., 1996). Substrate specificities and inhibitor sensitivities suggest that human kidney affinity flow-through GST is functionally distinct from corresponding renal and non-renal affinity flow-through GST isoenzymes in other species.

The presence of α class in human kidney has already been documented at protein level by immunohistochemistry, and immunoblotting of cytosolic, and affinity bound renal GSTs (Di Ilio et al., 1987; Klone et al., 1990; Oberley et al., 1994; Rodilla et al., 1998). However, affinity flow-through GST, as quantitatively important part of GST in human kidney, was identified as a class α member for the first time in our study. Since bladder enzyme has also been identified as α class member, it is tempting to speculate that same GST enzymes provide protection against common carcinogenic compounds. On the other hand, overall down-regulation of GST α class in renal cell carcinoma has been well documented by several investigators (Klone et al., 1990; Oberley et al., 1994; Rodilla et al., 1998). Our recent study has shown that affinity flow-through GST is also down-regulated in patients with the renal cell carcinoma (Simic et al., 2003). However, the question of mechanism by which down-regulation of α class GST, contributes to malignant transformation remains unanswered and requires further investigations.

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References

Baars AJ, Mukhtar H, Zoetemelk CE, Jansen M, Breimer DD (1981) Glutathione S-transferase activity in rat and human tissues and organs. Comp Biochem Physiol 70: 285–288

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: 93–101

Bradford MM (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Delbanco EH, Bolt HM, Huber WW, Beken S, Geller F, Philippou S, Brands FH, Bruning T, Thier R (2001) Glutathione transferase activities in renal carcinomas and adjacent normal renal tissues: factors influencing renal carcinogenesis induced by xenobiotics. Arch Toxicol 74: 688–694

Di Ilio C, Del Boccio G, Aceto A, Federici G (1987) Alteration of glutathione transferase isoenzyme concentrations in human renal carcinoma. Carcinogenesis 8: 861–864

Habig WH, Jakoby WB (1981) Assays for differentiation of glutathione S-transferases. Methods Enzymol 77: 398–405

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferase-the first enzymatic step in mercapturic acid formation. J Biol Chem 249: 7130–7139

Hayes JD, Pulford DJ (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 30: 445–600

Hayes JD, Strange RC (2000) Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology 61: 154–166

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: 21709–21717

Jakobsson PJ, Morgenstern R, Mancini J, Ford-Hutchinson A, Persson B (1999) Common structural features of MAPEG – a widespread superfamily of membrane associated proteins with highly divergent functions in eicosanoid and glutathione metabolism. Protein Sci 8: 689–692

Klone A, Weidner U, Hussnatter R, Harris J, Meyer D, Peter S, Ketterer B, Sies H (1990) Decreased expression of the glutathione S-transferases alpha and pi genes in human renal cell carcinoma. Carcinogenesis 11: 2179–2183

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685

Mannervik B, Alin P, Guthenberg C, Jensson H, Tahir MK, Warholm M, Jornvall H (1985) Identification of three classes of cytosolic glutathione transferase common to several mammalian species: correlation between structural data and enzymatic properties. Proc Natl Acad Sci USA 82: 7202–7206

Meyer DJ, Coles B, Pemble SE, Gilmore KS, Fraser GM, Ketterer B (1991) Theta, a new class of glutathione transferases purified from rat and man. Biochem J 274: 409–414

Mimic-Oka J, Simic T, Djukanovic LJ, Stefanovski J, Ramic Z (1992) Glutathione and its associated enzymes in peripheral blood cells in different stages of chronic renal insufficiency. Amino Acids 2: 215–224

Oberley TD, Sempf JM, Oberley MJ, McCormick ML, Muse KE, Oberley LW (1994) Immunogold analysis of antioxidant enzymes in human renal cell carcinoma. Virchows Arch 424: 155–164

Pacifici GM, Viani A, Franchi M, Gervasi PG, Longo V, Di Simplicio P, Temellini A, Romiti P, Santerini S, Vannucci L (1989) Profile of drug-metabolizing enzymes in the cortex and medulla of the human kidney. Pharmacology 39: 299–308

Ricci G, Caccuri AM, Lo Bello M, Pastore A, Piemonte F, Federici G (1994) Colorimetric and fluorometric assays of glutathione transferase based on 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole. Anal Biochem 218: 463–465

Rodilla V, Benzie AA, Veitch JM, Murray GI, Rowe JD, Hawksworth GM (1998) Glutathione S-transferases in human renal cortex and neoplastic tissue: enzymatic activity, isoenzyme profile and immunohistochemical localization. Xenobiotica 28: 443–456

Sherman M, Titmuss S, Kirsch RE (1983) Glutathione S-transferase in human organs. Biochem Int 6: 109–118

Simic T, Mimic-Oka J, Ille K, Dragicevic D, Savic-Radojevic A (2003) Glutathione S-transferase isoenzyme profile in non-tumor and tumor human kidney tissue. World J Urol 20: 385–391

Simic T, Mimic-Oka J, Ille K, Savic-Radojevic A, Reljic Z (2001) Isoenzyme profile of glutathione S-transferases in human kidney. Urol Res 29: 38–44

Singh SV, Roberts B, Gudi VA, Ruiz P, Awasthi YC (1991) Immunohistochemical localization, purification, and characterization of human urinary bladder glutathione S-transferases. Biochim Biophys Acta 1074: 363–370

Strange RC, Spiteri MA, Ramachandran S, Fryer AA (2001) Glutathione-S-transferase family of enzymes. Mutat Res 482: 21–26

Tateoka N, Tsuchida S, Soma Y, Sato K (1987) Purification and characterization of glutathione S-transferases in human kidney. Clin Chim Acta 166: 207–218

Thier R, Golka K, Bruning T, Ko Y, Bolt HM (2002) Genetic susceptibility to environmental toxicants: the interface between human and experimental studies in the development of new toxicological concepts. Toxicol Lett 127: 321–327

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