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Human intestinal glutathione *S*-transferases

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Cytosolic glutathione *S*-transferases were purified from the epithelial cells of human small and large intestine. These preparations were characterized with regard to specific activities, subunit and isoenzyme composition. Isoenzyme composition and specific activity showed little variation from proximal to distal small intestine. Specific activities of hepatic and intestinal enzymes from the same patient were comparable. Hepatic enzymes were mainly composed of 25 kDa subunits. Transferases from small intestine contained 24 and 25 kDa subunits, in variable amounts. Colon enzymes were composed of 24 kDa subunits. In most preparations, however, minor amounts of 27 and 27.5 kDa subunits were detectable. Separation into isoforms by isoelectric focusing revealed striking differences: glutathione *S*-transferases from liver were mainly basic or neutral, enzymes from small intestine were basic, neutral and acidic, whereas large intestine contained acidic isoforms only. The intestinal acidic transferase most probably was identical with glutathione *S*-transferase Pi, isolated from human placenta. In the hepatic preparation, this isoform was hardly detectable. The specific activity of glutathione *S*-transferase showed a sharp fall from small to large intestine. In proximal and distal colon, activity seemed to be about equal. In the ascending colon there might be a relationship between specific activity of glutathione *S*-transferases and age of the patient, activity decreasing with increasing age.

INTRODUCTION

The intestinal mucosa is involved in the digestion and absorption of nutrients and in the protection of the underlying tissue against toxic or carcinogenic compounds, ingested via the gastrointestinal tract. For this purpose a complex defence system is present. First, the epithelial cells are protected by a barrier of mucus; second, epithelial cells have a relatively short lifetime (several days); and third, the epithelial cells are provided by a complex system of enzymes, able to metabolize these harmful compounds in a way ultimately leading to the excretion of innocuous metabolites via bile, faeces or urine [1]. One of the most important classes of enzymes that fulfil this function are the glutathione *S*-transferases (EC 2.5.1.18). They are involved in binding, transport and detoxication of a wide variety of compounds. The enzymes are present in several tissues and species [2–5].

In man, the enzymes have been most intensively studied in the liver [3,4,6–15]. Recently, the purification and characterization of glutathione *S*-tranferases from human placenta [7], kidney [16], prostate [17], heart [18,19], lung [19], erythrocytes [7,19], leucocytes [20] and skin [21] was reported. However, little information on human intestinal glutathione *S*-tranferases is available yet [22–24]. We recently purified glutathione *S*-transferases from human small intestine, and compared some of its properties with those from the liver [25]. We now report in more detail on the glutathione *S*-transferases from small and large intestine.

MATERIALS AND METHODS

Tissue

Human intestinal tissue was obtained at autopsy (patient 1) or by surgical resections. Patient data are summarized in Table 1. Normal human placentas were obtained from the Department of Obstetrics and Gynaecology. All tissue used was macroscopically normal and was available within 30 min after resection. Subsequently the tissue was transported to the laboratory in ice-cold 0.9% NaCl. The mucosal scrapings from the colon of polyposis coli patients were slightly contaminated with polyp tissue. Tissue was cleaned by thoroughly washing with ice-cold 0.9% NaCl and was either used immediately, or otherwise frozen in liquid N_2 and stored at −80 °C. The 150000 *g* supernatants of liver tissue and intestinal mucosa were made as described previously [25].

The investigation was approved by the local ethical committee on human experimentation.

Purification of cytosolic glutathione *S*-transferases

Cytosol from liver and intestine (150000 *g* supernatant) was dialysed against 20 mM-Tris/HCl, pH 7.0, containing 1.4 mM-dithiothreitol (Sigma Chemical Co., St. Louis, MO, U.S.A.). GSH–agarose (Sigma) was equilibrated with the same buffer, and the 150000 *g* supernatant was loaded on a small column of bed volume approx. 3 ml. The column was washed with equilibration buffer until no A_{280} was detectable any more. Glutathione *S*-transferases were eluted with 50 mM-Tris/HCl, pH 9.5, containing 1.4 mM-dithiothreitol and 5 mM-GSH (Sigma).

Glutathione *S*-transferases were dialysed for 16 h against 10 mM-Tris/HCl, pH 7.4, containing 1.4 mM-dithiothreitol. The purified enzymes were rapidly frozen in liquid N_2 and stored at −80 °C. All treatments were done on ice.

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Miscellaneous

Assay for glutathione *S*-transferase activity with 1-chloro-2,4-dinitrobenzene (Sigma) as substrate was performed as described by Habig *et al.* [26]. Protein was determined by the method of Lowry *et al.* [27]. SDS/polyacrylamide-gel electrophoresis was done as described in ref. [28]. Isoelectric focusing was done with commercial gels (Phastgel; Pharmacia, Uppsala, Sweden) with a pH range of 3–9. Gels were run for 500 V·h (about 20 min) on the Pharmacia Phastsystem. Gels were scanned at 600 nm with a laser densitometer (LKB 2202 Ultrascan; LKB, Bromma, Sweden).

RESULTS

The small intestine of a kidney transplant donor (patient 1, Table 1) was divided in segments of 10 cm length at distances of 0, 100, 250 and 450 cm from the pylorus. Mucosal cells were isolated from such a segment. The cells were homogenized and subfractionated, and glutathione *S*-transferases were isolated from the 150000 *g* supernatant. Fig. 1 shows the purified glutathione *S*-transferases after SDS/polyacrylamide-gel electrophoresis (slots 3–6). From the same patient, hepatic glutathione *S*-transferases were also isolated (slots 2 and 7). Hepatic glutathione *S*-transferases are composed of two bands with molecular masses of 25 and 27 kDa, whereas small-intestinal glutathione *S*-transferases have subunit molecular masses of 24 and 25 kDa. From proximal to distal small intestine, there is little variation in subunit composition (Table 2).

The specific activities of purified glutathione *S*-transferases from liver and small intestine are also shown in Table 2. Hepatic activity is slightly higher than in the small intestine, where little variation in specific activity seems to be present. In Fig. 2 several GSH–agarose-purified glutathione *S*-transferase preparations from small and large intestine are shown. Purified glutathione *S*-transferases from small intestine are mainly composed of subunits with molecular masses of 24 and 25 kDa, whereas glutathione *S*-transferases from colon contain almost exclusively 24 kDa subunits. In both small- and large-intestinal preparations, small amounts of 27 and 27.5 kDa subunits are visible (Fig. 2, slots 2 and 10), especially when larger amounts of protein were loaded

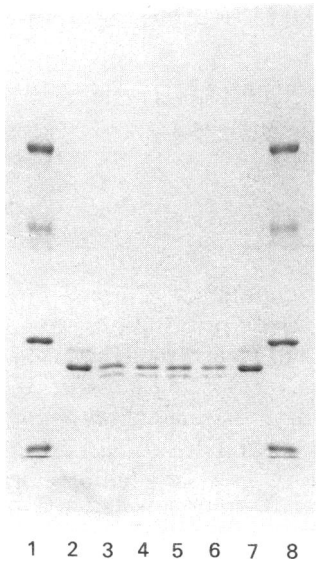

Fig. 1. SDS/polyacrylamide-gel electrophoresis of GSH–agarose-purified glutathione *S*-transferases from small intestine and liver

GSH–agarose-purified glutathione *S*-transferases were separated on an SDS/polyacrylamide gel [12.5% (w/v) acrylamide]. Slots 1 and 8 contain marker proteins with molecular masses (from top to bottom) of 68 kDa (bovine albumin), 45 kDa (egg albumin), 29 kDa (carbonic anhydrase) and 20 kDa (trypsin inhibitor). Purified glutathione *S*-transferases (0.7 μg each, patient 1) from liver (slots 2 and 7) and from small intestine at distances of 0, 100, 250 and 450 cm from the pylorus are shown in slots 3, 4, 5 and 6 respectively.

Table 1. Patient data

Patient no.	Tissue	Gender	Age (years)	Pathology
1	Liver	M	18	None*
1	Small intestine	M	18	None*
2	Jejunum	F	42	Colon carcinoma
2	Ascending colon	F	42	Colon carcinoma
3	Ileum	M	68	Caecum carcinoma
3	Ascending colon	M	68	Caecum carcinoma
4	Ileum	F	25	Crohn's disease
5	Ascending colon	F	64	Colon carcinoma
6	Ascending colon	F	34	Polyposis coli
7	Ascending colon	M	21	Polyposis coli
8	Ascending colon	M	55	Polyposis coli
9	Sigmoid	F	66	Rectum carcinoma
10	Sigmoid	M	49	Colon carcinoma
11	Ileum	F	21	Crohn's disease?
11	Ascending colon	F	21	Crohn's disease?
12	Ileum	–	–	Caecum carcinoma
12	Ascending colon	–	–	Caecum carcinoma
13	Sigmoid	–	–	Caecum carcinoma

* This kidney transplant donor died by cerebral damage after a traffic accident.

Table 2. Subunit composition and specific activities of purified glutathione *S*-transferases from liver and small intestine of patient 1

Specific activity was determined with 1-chloro-2,4-dinitrobenzene as substrate. Subunit composition was determined after separation of the glutathione *S*-transferase subunits on SDS/polyacrylamide-gel electrophoresis (Fig. 1) and subsequent scanning at 600 nm of the Coomassie-Brilliant-Blue-stained gels. Intestine segments of 10 cm length were analysed. Determinations were performed in triplicate, and values are given as means ± S.D.

Tissue	Subunit composition (% of total)			Specific activity (μmol/min per mg of protein)
	24 kDa	25 kDa	27 kDa	
Liver		95±1	5±1	77±3
Small intestine (distance from pylorus)				
0 cm	14±2	86±2		43±4
100 cm	14±1	86±1		45±1
250 cm	16±1	84±1		52±3
450 cm	19±1	82±1		35±2

on the gel (results not shown). These high-molecular-mass subunits could be identified as glutathione *S*-transferase μ, by incubation of Western blots with a monoclonal antibody against transferase μ (W. H. M. Peters, unpublished work).

The isoenzymes of the purified glutathione *S*-transferases were separated by their protein charge, by isoelectric focusing. Glutathione *S*-transferase isoenzymes from colon, small intestine and liver, originating from several patients are presented in Fig. 3. Hepatic glutathione *S*-transferases are mainly composed of basic and neutral isoforms, enzymes from small intestine contain both basic and acidic isoforms, and colon enzymes are exclusively acidic.

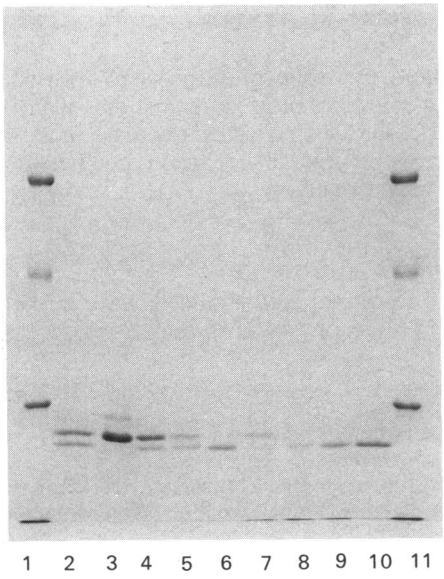

Fig. 2. SDS/polyacrylamide-gel electrophoresis of GSH–agarose-purified glutathione *S*-transferases from intestine and liver

GSH–agarose-purified glutathione *S*-transferases are separated on an SDS/polyacrylamide gel [11% (w/v) acrylamide]. Slots 1 and 11 contain marker proteins identical with those of Fig. 1. Purified glutathione *S*-transferases are shown in: slot 2, ileum (1.3 μg, patient 4); slot 3, liver (1.2 μg, patient 1); slot 4, jejunum (1.1 μg, patient 1); slot 5, ileum (0.8 μg, patient 11); slot 6, colon (0.7 μg, patient 11); slot 7, ileum (0.9 μg, patient 12); slot 8, colon (0.8 μg, patient 5); slot 9, colon (0.7 μg, patient 6); slot 10, colon (1.2 μg, patient 7).

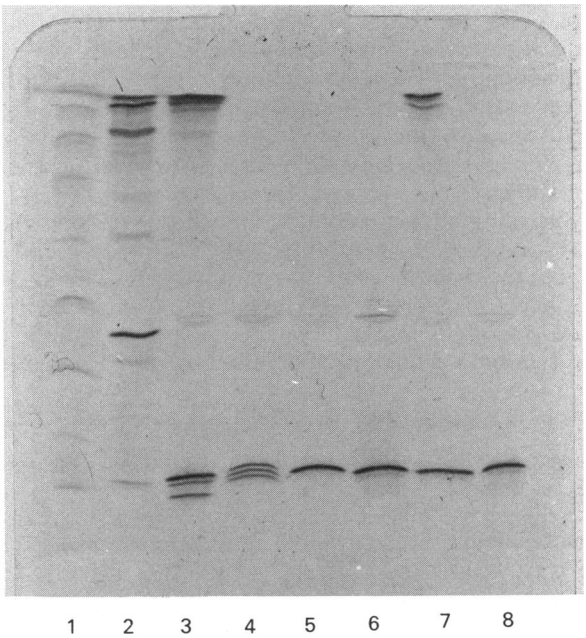

Fig. 3. Isoelectric focusing of GSH–agarose-purified glutathione *S*-transferases from intestine and liver

Isoelectric focusing was performed on Phastgel IEF 3–9. In slot 1, marker proteins with pI values (from top to bottom) 9.3 (doublet), 8.65, 8.45 (doublet), 8.15, 7.35 (weak), 6.85, 6.55, 5.85, 5.2, 4.55 and 3.75 (doublet) are shown. Glutathione *S*-transferases from liver are seen in slot 2 (12 μg, patient 1). Purified intestinal glutathione *S*-transferases are shown in: slot 3, jejunum (12 μg, patient 1); slot 4, colon (10 μg, patient 5); slot 5, colon (10 μg, patient 6); slot 6 colon (10 μg, patient 7); slot 7, ileum (10 μg, patient 11); and slot 8, colon (9 μg, patient 11).

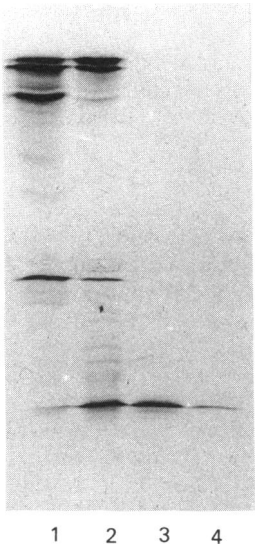

Fig. 4. Isoelectric focusing of GSH–agarose-purified glutathione *S*-transferases

Isoelectric focusing was performed on Phastgel IEF 3–9. Slot 1 is identical with slot 2 of Fig. 3. Purified glutathione *S*-transferases from ileum (10 μg; patient 3), placenta (10 μg) and colon (5 μg; patient 6) are shown in slots 2, 3 and 4 respectively.

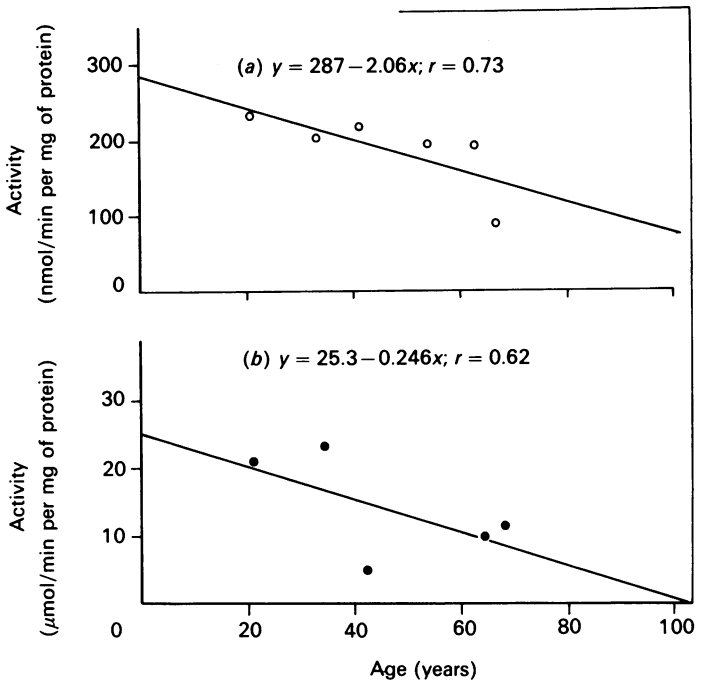

Fig. 5. Age-dependency of cytosolic glutathione *S*-transferases from human ascending colon

Specific activity of cytosolic glutathione *S*-transferases (*a*) and of GSH–agarose-purified glutathione *S*-transferases (*b*) from ascending colon is plotted against the age of the corresponding patients. Each point represents the mean value for three determinations.

Fig. 4 shows that the acidic isoform(s) from small and large intestine have pI values identical with that of human placental glutathione *S*-transferase Pi. On SDS/polyacrylamide-gel electrophoresis the acidic isoform from placenta gives a band of molecular mass 24 kDa, identical with the 24 kDa band of small- and large-intestinal glutathione *S*-transferase preparations (results not shown).

Specific activities were determined of cytosolic and of GSH–agarose-purified glutathione *S*-transferase. Table 3 gives the cytosolic activities. Going from small intestine to the colon, there is a sharp fall in enzyme activity. In the ascending colon, values are comparable with those of the sigmoid colon. Specific activities of GSH–agarose-purified and cytosolic glutathione *S*-transferases from ascending colon are plotted against age of the patients in Fig. 5. In both cases a gradual decrease in activity is seen with increasing age. Correlation coefficients are 0.62 and 0.73 respectively.

DISCUSSION

Human intestinal epithelial cells have a relatively high content of cytosolic glutathione *S*-transferase activity

Table 3. Specific activities of cytosolic glutathione *S*-transferases from small and large intestine

Activity is determined in the cytosol (150000 ***g*** supernatant) with 1-chloro-2,4-dinitrobenzene as substrate. Determinations were performed in triplicate, and *n* gives the number of specimens from different patients. Values are given as means ± S.D.

	Sp. activity (nmol/min per mg of protein)				
			Patient no.*		
		Range	2	3	12
Liver ($n = 1$)	1320 ± 120				
Jejunum ($n = 2$)	785 ± 295	(490, 1080)	490 ± 51		
Ileum ($n = 4$)	440 ± 86	(328–549)		328 ± 17	549 ± 8
Ascending colon ($n = 7$)	205 ± 57	(90–295)	220 ± 21	90 ± 5	295 ± 18
Sigmoid colon ($n = 3$)	225 ± 15	(209–245)			

* Individual data from three patients, from which both small- and large-intestinal tissue was available, are shown to indicate the differences in specific activity in both parts of the intestine. These data are also included in the left part of the Table.

(Tables 2 and 3; ref. [25]). Values are comparable with those found previously in liver [9,29], kidney [16,29], adrenal [29], and to a lesser extent spleen [29] and prostate [17].

The specific activity of GSH–agarose-purified enzyme from small intestine is about half the corresponding hepatic activity (Table 2). Cytosolic activities in both organs may be equal (W. H. M. Peters, unpublished work). From the cytosolic protein content in both organs it could be derived that the hepatic cytosolic content of glutathione S-transferases is about 1.5 times higher than in the small intestine.

In normal small-intestinal mucosa, by electrophoretic studies little variation in glutathione S-transferase composition could be detected, from proximal to distal small intestine. However, more detailed studies to quantify the longitudinal distribution of the different isoenzymes will be useful.

The subunit composition of glutathione S-transferases, as revealed by SDS/polyacrylamide-gel electrophoresis (Figs. 1 and 2), is partially different for enzymes from liver, small and large intestine. Hepatic preparations almost exclusively contain 25 kDa subunits, but small amounts of 27 kDa subunits are also present (Fig. 1). This is in agreement with earlier published data on hepatic enzymes [7–10]. Enzymes from small intestine are composed of 24 and 25 kDa subunits, whereas glutathione S-transferases from colon are mainly composed of 24 kDa subunits. Several other preparations were investigated, with very similar results. In most intestinal preparations minor amounts of 27 and 27.5 kDa subunits are also present. These minor forms are visible only when the gels are loaded with adequate amounts of protein. The subunit composition of small-intestinal glutathione S-transferases may be very similar to that of kidney glutathione S-transferases [16]. With regard to subunit composition, colon enzymes seem to be more similar to glutathione S-transferase Pi from placenta [7]. Investigation of the glutathione S-transferases by isoelectric focusing (Figs. 3 and 4) reveals that basic, neutral and small amounts of acidic isoforms are present in liver. Small intestine contains basic and acidic isoforms, and the colon enzyme is exclusively composed of acidic isoforms. Thus acidic isoforms are correlated with low-molecular-mass (24 kDa) subunits, and basic isoforms are correlated with intermediate-molecular-mass (25 kDa) subunits. Isoelectric focusing also shows that glutathione S-transferase in most colon specimens is a single isoform, of pI 4.6 (Fig. 3). This isoform, which is also present in small intestine (Figs. 3 and 4), has an identical subunit molecular mass (results not shown), and an exactly identical pI value as compared with human placental glutathione S-transferase Pi (Fig. 4). Also, on isoelectric focusing on a gel with pH range 4–6.5 the placental form co-migrates with the acidic forms from small and large intestine (results not shown). This strongly suggests that glutathione S-transferase Pi is present in large amounts in normal small- and large-intestinal mucosa from patients with and without intestinal pathology. This result would be in contrast with the findings of Kodate *et al.* [30], who showed that glutathione S-transferase Pi in human colon is present only in adenomas and carcinomas, and very little in normal mucosa. Analysis of glutathione S-transferase mRNA by Kano *et al.* [31] revealed the presence of considerable amounts of human glutathione S-transferase Pi mRNA in normal colon, as well as in colon carcinoma tissue. However, this mRNA may not be completely identical with the human placental glutathione S-transferase Pi mRNA, since the former mRNA could not be detected in tissue from placenta itself [31]. Specific activities of cytosolic glutathione S-transferases gradually decrease from proximal to distal small intestine in humans (W. H. M. Peters, unpublished work) and show a sharp fall in activity in the colon (Table 3). In the ascending and sigmoid colon, activity seems to be equal (Table 3). Similar results were obtained by Siegers *et al.* [24], although the colon activities reported in the present study are somewhat higher (Table 3).

In the ascending colon an age-dependency of cytosolic as well as GSH–agarose-purified glutathione S-transferase activity seems to be present (Fig. 5). The relatively low activity in the colon may thus be further decreased with age. This could be a factor of importance with regard to the age-related carcinogenesis, since the detoxication potential of epithelial cells from colon will be decreased in parallel with the glutathione S-transferase activity.

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