Two distinct forms of glutathione transferase from human foetal liver

Purification and comparison with isoenzymes isolated from adult liver and placenta

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Isoelectric focusing of a cytosol fraction from human foetal liver revealed the existence of an acidic and a basic isoenzyme of GSH transferase. The acidic and basic forms of GSH transferase were purified in good yield by use of ion-exchange chromatography on DEAE-cellulose followed by affinity chromatography on *S*-hexyl-GSH coupled to epoxy-activated Sephrose 6B. The content of the acidic and the basic isoenzymes of GSH transferase together was calculated to constitute 1–2% of the soluble proteins in the hepatic cytoplasm. Physical, catalytic and immunological analyses of the acidic and the basic isoenzymes from foetal liver demonstrated unambiguously that the two forms are different structures with distinct properties. On the other hand, the results show clearly extensive similarities between the foetal acidic transferase and transferase π from human placenta as well as between the foetal basic form and the basic isoenzymes isolated from adult liver. An exception is that both foetal enzymes seem to be considerably more efficient in catalysing the conjugation of GSH with styrene 7,8-epoxide than the the corresponding adult forms of GSH transferase.

INTRODUCTION

GSH transferases represent a family of enzymes (EC 2.5.1.18) that catalyse the conjugation of GSH with a large number of electrophilic substances, including chemical carcinogens and mutagens (Chasseaud, 1979). The occurrence of GSH transferases in animal species is widespread. In human tissues, as in tissues from other species, different isoenzymes of GSH transferase are present (Mannervik, 1985). The multiple forms in human tissues can be divided on the basis of their isoelectric points into three types of enzyme: acidic, near-neutral and basic GSH transferases (Warholm *et al.*, 1983). These three major types of GSH transferase are distinguished by physical and immunological properties and by substrate specificities (Warholm *et al.*, 1983), sensitivities to inhibitors (Tahir *et al.*, 1985) and protein structure (Ålin *et al.*, 1985). In adult liver, the number of basic forms of GSH transferase has been found to depend on the liver analysed. Five very similar proteins with basic isoelectric points, referred to as transferases α, β, γ, δ and ϵ, have been purified from a single liver (Kamisaka *et al.*, 1975). In addition to the basic isoenzymes, some livers (60% of the livers studied) contain the near-neutral transferase μ (Warholm *et al.*, 1980, 1983). The acidic type of GSH transferase is a major protein constituent in placenta (Guthenberg *et al.*, 1979; Polidoro *et al.*, 1980), erythrocytes (Marcus *et al.*, 1978), lung (Koskelo *et al.*, 1981; Koskelo, 1983; Mannervik *et al.*, 1983), brain (Olsson *et al.*, 1983) and spleen (Koskelo, 1983). Small amounts of acidic forms have also been detected in adult liver (Awasthi *et al.*, 1980; Koskelo & Valmet, 1980; Warholm *et al.*, 1980). The acidic type of GSH transferase occurs in all human foetal organs investigated. It has been shown that foetal lung, kidney, brain and intestine contain only an acidic form of GSH transferase (Polidoro *et al.*, 1982; Pacifici *et al.*, 1986). In contrast, foetal adrenal gland and foetal liver exhibit a major basic isoenzyme in addition to an acidic isoenzyme (Warholm *et al.*, 1981*a*; Pacifici *et al.*, 1986). GSH transferase μ has not been found in any of the human foetal organs tested. The inter-relationship between the acidic forms of GSH transferase from human tissues is not definitively established. However, chemical and immunological analyses show extensive similarities between acidic GSH transferases from various human tissues, suggesting that they are the same enzyme. Likewise, the basic isoenzymes isolated from different organs appear to be similar. The present paper describes the purification and characterization of two distinct forms of GSH transferase from human foetal liver, and a comparison of their properties with those of the isoenzymes purified from adult human liver and placenta.

MATERIALS AND METHODS

Materials

Human foetal livers were obtained at legal abortions performed on socio-medical indications. The livers were frozen at −80 °C within 30 min after abortion. DEAE-cellulose was purchased from Whatman Biochemicals, Maidstone, Kent, U.K. All other chromatography materials were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. *S*-Hexyl-GSH was synthesized and coupled to epoxy-activated Sepharose 6B as previously described (Mannervik & Guthenberg, 1981).

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Assays

GSH transferase activities with various electrophilic substrates were determined at 30 °C essentially according to the procedures described by Habig *et al.* (1974), Benson & Talalay (1976), DePierre & Moron (1979), Lawrence & Burk (1976), and Mukhtar & Bend (1977). One unit of enzyme activity is defined as the amount of enzyme that catalyses the formation of 1 μmol of product/min at 30 °C. Determinations of protein concentrations were based on absorbance at 260 and 280 nm (Kalckar, 1947) during the purification procedure. For solutions of purified enzyme, the protein concentration was determined as described by Lowry *et al.* (1951), with bovine serum albumin as standard. SDS/polyacrylamide-slab-gel electrophoresis was carried out essentially as described by Laemmli (1970). Isoelectric focusing was performed according to instructions by the manufacturer (LKB Produkter, Stockholm, Sweden), with Ampholine (pH 3.5–10) as ampholytes.

Purification of GSH transferases from human foetal liver

One frozen liver from a foetus of 25 weeks gestational age was thawed and homogenized in ice-cold 0.25 M-sucrose with a Potter-Elvehjem homogenizer. All data reported in the present paper (except the isoelectric-focusing profile in Fig. 1) were obtained with enzymes purified from this liver. The homogenate was diluted to 20% (w/v) with 0.25 M-sucrose and centrifuged at 18000 *g* for 20 min. The centrifugation and all subsequent operations were carried out at 4 °C. A microsome-free supernatant fraction was obtained after further centrifugation at 105000 *g* for 60 min. The 105000 *g* supernatant (35 ml) was chromatographed on a Sephadex G-25 column (4 cm × 17 cm) packed in 10 mM-Tris/HCl buffer, pH 7.8 (buffer A). The protein-containing effluent (66 ml) was collected and applied to a DEAE-cellulose column (4 cm × 7 cm) equilibrated with buffer A. The column was rinsed with the starting buffer until GSH transferase ceased to appear in the effluent. The active fractions were pooled (88 ml) (basic GSH transferase). About 46% of the activity was retained on the column. This activity was eluted with a 1400 ml linear gradient of NaCl (0–0.2 M) in buffer A. Fractions showing GSH transferase activity were pooled (73 ml) (acidic GSH transferase). The two pools from the chromatography on DEAE-cellulose were applied on separate affinity columns (2 cm × 4 cm) consisting of *S*-hexyl-GSH linked to epoxy-activated Sepharose 6B (Mannervik & Guthenberg, 1981). Non-specifically adsorbed protein was eluted with 75 ml of 0.2 M-NaCl in buffer A. GSH transferase was eluted with 5 mM-*S*-hexyl-GSH dissolved in the NaCl-fortified Tris buffer. The active pools from the two affinity-chromatography separations were desalted on a Sephadex G-25 column (4 cm × 11 cm) packed in buffer A containing 0.2 mM-dithioerythritol. The enzyme-containing fractions were pooled and concentrated by ultrafiltration on an Amicon PM 10 filter.

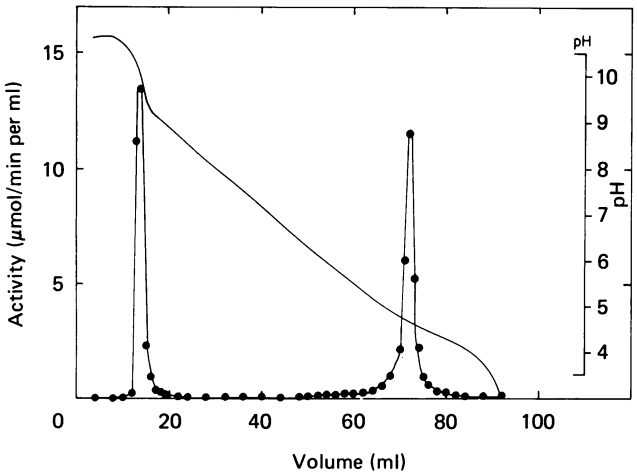

Fig. 1. Isoelectric focusing of the basic and the acidic forms of GSH transferase in the cytosol fraction of human foetal liver

The separation was performed by standard procedures in a 110 ml column (LKB Produkter) with Ampholine pH 3.5–10 (——) in a sucrose density gradient. The sample applied was 7 ml of a cytosol fraction dialysed against 10 mM-sodium phosphate buffer, pH 7.0. GSH transferase activity (●) was measured with 1-chloro-2,4-dinitrobenzene as electrophilic substrate. The liver sample was from a foetus of 23 weeks gestational age.

RESULTS

A typical GSH transferase activity profile after isoelectric focusing of a cytosol fraction from human foetal liver is shown in Fig. 1. The activity was measured with 1-chloro-2,4-dinitrobenzene as the electrophilic substrate. Two major peaks of activity were found. One peak focused at a high pH value (pH > 9) and the other peak focused at about pH 4.8.

The purification of these two forms of GSH transferase from a single liver is summarized in Table 1. About 46% of the activity applied was bound to the DEAE-cellulose and an additional 40% of the activity passed unadsorbed through the column. These two peaks of activity represent acidic and basic forms of GSH transferase respectively. The affinity chromatography on *S*-hexyl-GSH–Sepharose 6B resulted in an 18-fold purification of the acidic form of enzyme and in an 8-fold purification of the basic form. The total recovery of GSH transferase was high. Approx. 74% of the GSH transferase in the cytosol was recovered as acidic or basic forms of enzyme after DEAE-cellulose and affinity chromatography. On the basis of the results of the purification, it can be calculated that 1–2% of the soluble extractable protein in human foetal liver consists of GSH transferases.

Physical, catalytic and immunological properties of the purified acidic and basic isoenzymes from foetal liver were studied. The results are summarized in Tables 2–4. The data presented are from the purification of GSH transferases from a single liver. The basic and the acidic isoenzyme have also been purified from three other foetal livers. (The livers were from foetuses of 19–24 weeks of gestational age). Although the relative amounts of basic and acidic isoenzymes varied among individuals, the properties of isoenzymes isolated from different livers were found to be identical. For comparison, the properties of GSH transferase π obtained from human placenta and of the basic isoenzymes (α–ϵ) from adult human liver are included. When analysed by SDS/polyacrylamide-slab-gel electrophoresis, the purified foetal acidic GSH transferase gave one band with an apparent M_r of 23000, whereas the subunits of the basic isoenzyme displayed an apparent M_r of 25000 (Table 2).

Table 1. Purification of GSH transferases from human foetal liver

For full experimental details see the text.

Fraction	Volume (ml)	Total activity (μmol/min)	Specific activity (μmol/min per mg)	Yield (%)
Liver supernatant	35	206	0.56	100
DEAE-cellulose				
Peak 1 (basic GSH transferase)	88	82	4.6	40
Peak 2 (acidic GSH transferase)	73	94	4.8	46
Basic GSH transferase				
S-Hexyl-GSH–Sepharose 6B + Sephadex G-25 + concentration on PM 10 filter	4.5	88	35	43
Acidic GSH transferase				
S-Hexyl-GSH–Sepharose 6B + Sephadex G-25 + concentration on PM 10 filter	2.0	65	87	32

Table 2. Physical and immunochemical properties of human GSH transferases

Properties	Foetal basic transferase	Adult basic transferase (α–ε)	Foetal acidic transferase	Adult acidic transferase (π)*
Apparent subunit M_r	25000	25000	23000	23000
Isoelectric point	> 8	> 8	4.8	4.8
Precipitin reaction with antiserum against:				
GSH transferase α–ε	+	+	−	−
GSH transferase μ	−	−	−	−
GSH transferase π	−	−	+	+

* Data reported by Guthenberg & Mannervik (1981).

Antibodies have been raised in rabbits to the three major types of human GSH transferase found in adults. Each antiserum gave a precipitin line with its corresponding antigen, but not with the other GSH transferases (Warholm *et al.*, 1983). When the placental enzyme and the acidic isoenzyme from foetal liver were tested in parallel against anti-(GSH transferase π) antibodies, the enzymes gave precipitin lines that merged without spurs, suggesting immunological identity between the two transferases. The immunological reactivity with basic isoenzymes from foetal and adult liver were tested against antibodies directed against the adult basic isoenzymes. The continuous precipitin line obtained indicates the presence in the foetal sample of a polypeptide(s) immunologically identical with one (or more) of the polypeptide(s) present in the basic adult transferases (Table 2 and Fig. 2).

The substrate specificities of the foetal GSH transferases were examined, and the results were compared with those for the placental enzyme and the adult basic isoenzymes (Table 3). In general, the specific activities with different substrates determined for the acidic foetal enzyme are very similar to those of GSH transferase π, even though the activity with styrene 7,8-epoxide suggested a difference between the two enzymes. The similarity in substrate specificities between the foetal and the adult basic forms of GSH transferase is also obvious, with the exception of a remarkable difference obtained with

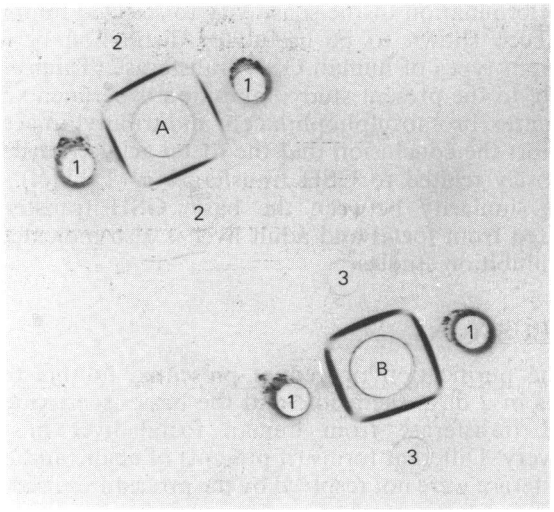

Fig. 2. Ouchterlony double immunodiffusion of GSH transferase

A, Antiserum raised against GSH transferase π from human placenta was placed in the centre well and enzyme from foetal liver (1) and placenta (2) in the peripheral wells. B, Antiserum raised against the adult basic form of human GSH transferase was placed in the centre well and enzyme from foetal liver (1) and adult liver (3) in the peripheral wells.

Table 3. Specific activities with selected substrates of human GSH transferases

Abbreviation: N.D., not determined.

Substrate	Specific activity (μmol/min per mg)			
	Foetal basic transferase	Adult basic transferase (α–ε)*	Foetal acidic transferase	Adult acidic transferase (π)†
1-Chloro-2,4-dinitrobenzene	34	64	87	105
Ethacrynic acid	N.D.	N.D.	1.0	0.86
Androst-5-ene-3,17-dione	5.7	8.0	N.D.	0.01
Cumene hydroperoxide	9.7	10.6	0.03	0.03
Benzo[*a*]pyrene 4,5-epoxide	0.044	0.047	0.13	0.13
Styrene 7,8-epoxide	0.86	0.02	0.93	0.14

* Data reported by Warholm *et al.* (1983).
† Data reported by Guthenberg & Mannervik (1981).

Table 4. Sensitivities to selected inhibitors of human GSH transferases

The I_{50} value is the concentration of inhibitor giving 50% inhibition of enzyme activity assayed at pH 6.5 and 30 °C with 1 mM-1-chloro-2,4-dinitrobenzene and 1 mM-GSH as substrates.

Inhibitor	I_{50} (μM)			
	Foetal basic transferase	Adult basic transferase (α–ε)	Foetal acidic transferase	Adult acidic transferase (π)
Haematin	0.5	0.5	10	10
Tributyltin acetate	0.04	0.2	6	4
Bromosulphophthalein	70	75	100	100

styrene 7,8-epoxide. The activity with the foetal basic transferase is approx. 40-fold higher than that of the adult basic isoenzyme.

Determination of the sensitivity to selected inhibitors has been shown to be useful for distinction between different types of human GSH transferase (Tahir *et al.*, 1985). In the present study, inhibition experiments with haematin, bromosulphophthalein and tributyltin acetate support the conclusion that the foetal acidic transferase is closely related to GSH transferase π (Table 4). The close similarity between the basic GSH transferases isolated from foetal and adult liver is also indicated by the inhibition studies.

DISCUSSION

The purification procedure presented in this paper yields in 2 days the acidic and the basic isoenzymes of GSH transferase from human foetal liver in high recovery. Different forms (if present) of acidic and basic transferase were not resolved by the procedures used, but acidic and basic types of enzyme were clearly separated. The acidic and basic isoenzymes of GSH transferase together constitute 1–2% of the total cytosolic protein in human foetal liver. This value is similar to the value of 2–3% found for GSH transferases in adult human liver [calculated from data presented by Kamisaka *et al.* (1975) and Warholm *et al.* (1981*b*)]. In spite of the similarity in total amounts there are marked differences in occurrence of the multiple forms of GSH transferase present in foetal and adult human liver (Warholm *et al.*, 1980, 1981*a*). Foetal liver contains only one major basic GSH transferase, even though traces of additional basic isoenzymes have occasionally been observed. In contrast, adult liver exhibits one or several major basic isoenzymes depending on the liver analysed (see, e.g., Warholm *et al.*, 1980). Another difference is that foetal liver contains an acidic form of enzyme as a major constituent, whereas adult liver contains only relatively small amounts of acidic forms (Awasthi *et al.*, 1980; Warholm *et al.*, 1980). In some foetal livers two very similar acidic GSH transferases have been resolved by isoelectric focusing (results not shown), but the cause of the appearance of an additional form has not been elucidated. The near-neutral GSH transferase μ has not been found in any of the 12 livers examined in foetuses of 19–25 weeks gestational age.

The results presented in this study show clearly extensive similarities between the foetal acidic isoenzyme and GSH transferase from human placenta as well as between the foetal basic form and the basic isoenzymes isolated from adult liver. The comparison includes physical, catalytic and immunological properties (Tables 2–4). The present paper does not answer the question whether the foetal GSH transferases described represent new forms of enzyme or forms identical with those expressed in adult tissues. Nevertheless, a notable difference was found in the sensitivity of adult and foetal basic GSH transferases to the inhibitor tributyltin acetate. An even more significant exception to the similarities between the foetal and adult isoenzymes was demonstrated in the activity with the substrate styrene

7,8-epoxide. Both foetal isoenzymes seem to be considerably more efficient in catalysing the conjugation of GSH with this substrate than are the corresponding adult forms of GSH transferase. The most remarkable difference was that the foetal basic GSH transferase gives about 40-fold higher activity with styrene 7,8-epoxide than the corresponding adult transferase. The interpretation of these results is not obvious, but it may be significant that the adult basic GSH transferase is represented by a mixture of isoenzymes. Stockman *et al.* (1985) have shown that the adult basic isoenzymes consist of immunologically distinct subunits. However, so far no clear-cut differences in substrate specificities (Kamisaka *et al.*, 1975) or in bilirubin-binding properties (Vander Jagt *et al.*, 1983) of different adult basic GSH transferases have been reported. Nevertheless it has been found that different forms of adult basic GSH transferases show differences in the affinity for haematin (Vander Jagt *et al.*, 1985). By analogy it cannot be excluded that they also exhibit differences in the activity with styrene 7,8-epoxide. If so, the foetal isoenzyme could be identical with an isoenzyme that exhibits high activity with styrene 7,8-epoxide. Another interpretation is that the foetal basic GSH transferase is a separate, but very similar, form of enzyme, which is not present in adult human liver. It is attractive to speculate that the high activity of the foetal basic GSH transferase with styrene 7,8-epoxide indicates a physiological role for this isoenzyme in the foetus by catalysing the conjugation of endogenous epoxides to GSH.

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