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GLUTATHIONE S-TRANSFERASE ACTIVITY IN HUMAN PLACENTA

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Abstract—Glutathione S-transferase activity has been identified in human placenta cytosol. The soluble protein fraction subjected to isoelectric focusing was resolved into a single peak of activity towards 1-chloro-2,4-dinitrobenzene centred at pH 4.65. Gel filtration experiments indicated that the protein had a molecular weight of 60,000. There was no apparent binding of sulphobromophthalein to this protein. The placental glutathione S-transferase system was inhibited by some non-substrate anions but apparently not by bilirubin. Glutathione S-transferase activity was located in the cytosol of the chorial villi and to a lesser extent in the amnion; it appears in the early stages of pregnancy. This activity was not detected in the amniotic fluid.

The glutathione S-transferases (EC 2.5.1.18) are a family of soluble enzymes that play a key role in the biotransformation and detoxication of a wide number of exogenous compounds in liver, kidney and intestine [1–5]. Studies of the glutathione S-transferase obtained from human erythrocytes have been reported recently [6].

These enzymes have *inter altra* a dual physiological function: (i) they catalyse the first step in mercapturic acid formation by promoting attack by reduced glutathione (GSH) on a wide range of substrates which bear an electrophilic centre in the molecule [7]; and (ii) they are involved in the cellular transport of several non-substrate organic anions by binding some covalently and others non-covalently [8, 9].

Since it has been found that organs which are exposed to a primary contact with xenobiotics contain the glutathione S-transferase system, generally regarded to be detoxifying, we decided to study glutathione S-transferase activity in placental tissue [10, 11], where the processes of absorption and excretion are rather unique.

Our recent data suggest that this placental enzyme system can exert, as presumed for the analogous enzymes of liver, kidney and intestine, a detoxifying action against electrophilic compounds.

MATERIALS AND METHODS

Preparation of homogenate and cytosol. For all preparative procedures, only placentae obtained at term from healthy women were utilized. To obtain enzyme preparations, suitable amounts of tissue were repeatedly washed in ice-cold physiological saline immediately after delivery; weighed fragments were minced, homogenized in a Waring blender in 4 vol. 10 mM potassium phosphate buffer, pH 5.9, and 1 mM EDTA for 1 min and the resultant homogenate was centrifuged for 15 min at 9000 *g*. Cytosol fractions were harvested after centrifugation at 105,000 *g* for 60 min in a Beckman model Spinco L2-65 B ultracentrifuge. All the operations were carried out at 2–4° unless otherwise indicated. Specimens were stored at −20° for up to several weeks without significant loss of activity.

Enzyme assays. Glutathione S-transferase activities were determined spectrophotometrically at 340 nm, according to Habig *et al.* [7] in a 1 ml reaction mixture containing 0.1 M potassium phosphate buffer, pH 6.8, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 5 mM GSH, at 20°. Reactions were initiated by the addition of 5–20 μl of cytosol. All assays were linear functions of protein concentration and of time for at least 3 min. Spontaneous increases of absorbance due to non-enzymic reaction were subtracted from the reaction rates.

The conjugation of GSH with the other substrates was monitored by using techniques previously described [7]. The concentrations used were the following: 1 mM 1,2-dichloro-4-nitrobenzene, 0.5 mM *p*-nitrobenzylchloride, 0.2 mM ethacrynic acid, 0.5 mM *trans*-4-phenyl-3-buten-2-one, 0.5 mM 1,2-epoxy-3-(*p*-nitrophenoxy)propane. γ-Glutamyltranspeptidase was assayed with L- γ-glutamyl-*p*-nitroanilide as described by Orlowsky and Meister [12] with the addition of glycylglycine as an acceptor [13]. One unit (U) of enzyme activity is defined as the amount of enzyme required to catalyse the conjugation of 1 μmole of substrate per min. Specific activity is expressed as μmoles per min per mg of cytosol protein. Protein was determined by the method of Lowry *et al.* [14] using bovine serum albumin as standard.

The substrates for enzymic reactions were obtained from Aldrich Europe (Beerse, Belgium) and from Sigma (St. Louis, MO). Ethacrynic acid was a gift from Merck, Sharp & Dohme Research Laboratories, Rahway, NJ.

Gel filtration. Concentrated cytosol exposed to sulphobromophthalein was eluted at 4° from a column (39 × 2.5 cm) of Sephadex G-100 (Pharmacia, Uppsala, Sweden) equilibrated with 10 mM potassium phosphate buffer, pH 5.9, and 1 mM EDTA. The dye associated with the protein fractions was deter-

mined spectrophotometrically at 580 nm after alkalization. Estimates of molecular weight were performed by gel filtration of concentrated cytosol on a column (90 × 2.5 cm) of Sephadex G-100 standardized with a mixture of proteins of known molecular weight (Biochemia Protein Calibration Kit, Size II), including bovin serum albumin (68,000), hen egg albumin (45,000), bovine pancreas chymotrypsinogen A (25,000), and horse heart cytochrome *c* (12,500). Fractions of 2 ml were collected.

Electrofocusing. Cytosol prepared from fresh chorial villi or amniotic membrane was applied to a 110 ml LKB Uniphor column containing a sucrose gradient and Ampholines with a pH 3.5–9.5 range. Fractions of 1.6 ml were collected after 96 hr at 600 V.

Substrate and inhibitor kinetics. The kinetics of enzyme activity towards 1-chloro-2,4-dinitrobenzene and GSH in cytosol fractions were studied by the method of Lineweaver and Burk [15].

Inhibitor kinetics were investigated by using four substrate concentrations and at least three inhibitor concentrations. The method of Dixon was employed to determine the inhibitor constant K_i [16]. All the kinetic constants were calculated by applying the least-squares regression analysis procedure.

RESULTS

Homogenate and cytosol from placental tissue were initially tested with a range of substrates. The highest activity was recorded with CDNB (Table 1). About 100 per cent of the activity present in the homogenate was recovered in the 105,000 *g* supernatant fluid.

The low activities measured using other substrates did not provide sufficient accuracy for their further study and enzyme determinations in chromatographic and kinetic experiments were carried out in the standard assay only with CDNB.

Other thiols such as 2-mercaptoethanol, cysteine and dithiothreitol were unable to replace as the thiol substrate in undergoing enzyme-catalysed conjugation with CDNB.

Villus, chorion and amnion cells were found to have glutathione S-transferase activity of the following specific activities: 0.17, 0.13 and 0.07 μmoles/min/mg, respectively. When these data are expressed in enzyme units (U) per g tissue, the activity measured in villi seems to be unequivocally the highest: the values were 4.89, 1.81 and 0.24 U/g, respectively.

The presence of both γ-glutamyltranspeptidase and GSH S-transferase activities in placentae at early

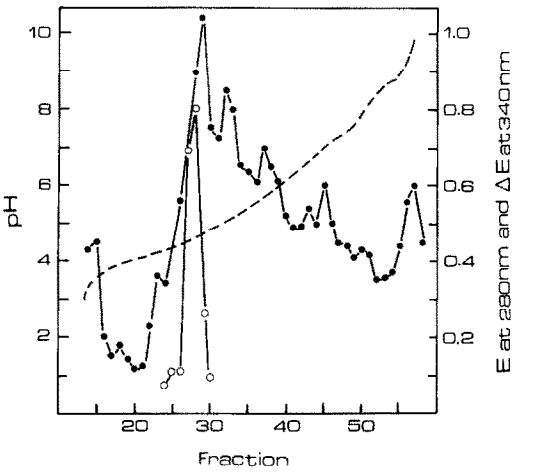

Fig. 1. Isoelectric focusing profile of placental cytosol GSH S-transferase activity. Fractions of 1.6 ml were collected. (●) Optical density at 280 nm; (○) transferase activity given as ΔE/min/0.2 ml at 340 nm under standard assay conditions.

stages of pregnancy was checked. In a crude homogenate prepared from three 8- to 9-week-old placentae, a specific activity of 0.11 μmoles/min/mg for γ-glutamyltranspeptidase was measured, while the specific activity calculated for GSH S-transferase in the cytosol of the above-mentioned preparation was 0.386 μmoles/min/mg, which closely approaches the value reported for human liver [17]. GSH S-transferase activity was not present in amniotic fluid obtained during pregnancy by amniocentesis or at the time of delivery.

Molecular filtration. Gel filtration experiments of human placenta cytosol gave an unexpected elution volume for the active protein fraction. The enzyme activity peak lays between the elution volume of bovine serum albumin (68,000) and that of hen egg albumin (45,000), corresponding to a molecular weight of 60,000 and assuming a globular shape resembling that of the protein standards.

When 40 mg of soluble protein, exposed to 1.6 μmoles of sulphobromophthalein, was eluted from a column (39 × 2.5 cm) of Sephadex G-100, there was no detectable binding of the drug to the fractions expressing glutathione S-transferase activity.

Isoelectric point. From the isoelectric focusing elution pattern, placental glutathione S-transferase appears to be an anionic protein and was resolved into a single peak of activity centred at pH 4.65 (Fig. 1). An identical pattern of activity was also achieved

Table 1. Substrate-specific activity of placental GHS S-transferase*

Substrate	Specific activity (μmoles product formed/min/mg cytosol protein)
1-Chloro-2,4-dinitrobenzene	0.170
1,2-Dichloro-4-nitrobenzene	Trace
p-Nitrobenzylchloride	0.002
Ethacrynic acid	0.025
trans-4-Phenyl-3-buten-2-one	Not detectable
1,2-Epoxy-3-(*p*-nitrophenoxy) propane	0.013

* All determinations were carried out in duplicate.

Table 2. Inhibition of GSH S-transferase by organic anions*

Inhibitor	K_i (mM)	Inhibition
Rose Bengal	0.002	Competitive
Cephalothin	2.2	Competitive
Cephoxitin	1.8	Competitive
Tobramycin sulphate	3.7	Non-competitive
p-Aminohippurate	9.6	Non-competitive

* See the text and caption to Fig. 2 for experimental details.

when the cytosol obtained from an 8-week-old placenta was subjected to isoelectric focusing.

Kinetic studies. In the standard assay conditions, enzyme activity exhibited a broad pH optimum around pH 7.0 (after corrections for nonenzymic reaction).

K_m values for GSH and 1-chloro-2,4-dinitrobenzene were 0.19 and 0.67 mM, respectively. Table 2 lists the inhibitor constants (K_i) and the type of inhibition observed for several non-substrate compounds. As typical examples of these inhibition studies, Fig. 2 shows the Dixon plots indicating competitive inhibition by cephoxitin (A) and noncompetitive inhibition by *p*-aminohippurate (B) of GSH S-transferase activity towards CDNB. The K_i constants correspond to the values on the abscissa

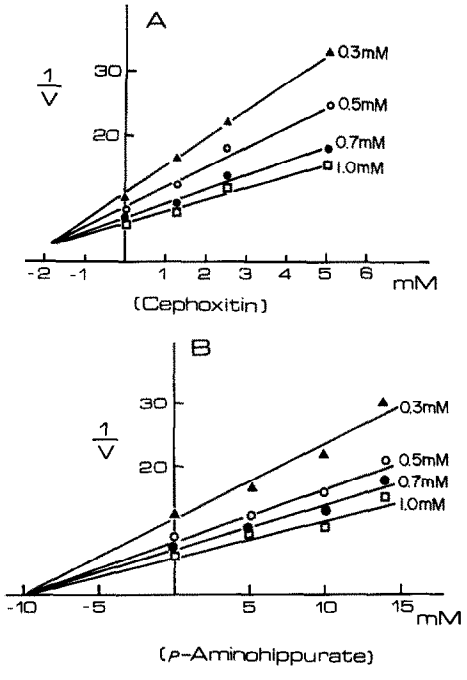

Fig. 2. Dixon plots showing competitive inhibition of placental GSH S-transferase activity by cephoxitin (panel A) and noncompetitive inhibition by *p*-aminohippurate (panel B). Reaction mixtures (1.0 ml) contained CDNB (1.0, 0.7, 0.5, 0.3 mM), GSH (5 mM) and inhibitor in 0.1 potassium phosphate buffer, pH 6.8, 1 mM EDTA, at 20°. The reaction was started by the addition of 5 μl of chorial villi cytosol. The reciprocal of the initial rate ($1/V$) is expressed as $(\mu\text{moles/min/mg})^{-1}$.

for the intersection of lines for four substrate concentrations.

Furosemide, a diuretic recognized to be teratogenic in animals [18], but used in pregnancy, was a strong inhibitor of placental glutathione S-transferase activity. The K_i value was not determined, but from the data presented in Table 3 it is possible to compare its inhibitory effect with that exerted by *p*-aminohippurate. About a 50-fold higher concentration of *p*-aminohippurate than of furosemide was required to produce approximately 50 per cent inhibition of the enzymic reaction.

No inhibitory effect was detected when bilirubin, was added to the standard assay mixture to a maximal concentration of 200 μM.

DISCUSSION

Human placental cytosol contains glutathione S-transferase activity, which has been shown to promote the first step of mercapturic acid formation by catalysing the conjugation of GSH with several electrophilic substances [19].

This enzymic activity in placenta was associated with an acidic protein fraction whose molecular dimensions were appreciably different from those attributed to glutathione S-transferases of human liver [17] and erythrocytes [6], which have molecular weights of 48,500 and 47,500, respectively. Thus the molecular weight of the placental enzyme, as determined by gel filtration, is 60,000.

However, some of its physicochemical properties appear to be similar to those recently reported for the purified glutathione S-transferase from human erythrocytes [6], e.g. the acidic isoelectric point, the optimum pH, the low interaction with bilirubin and its activity in catalysing the conjugation of GSH with ethacrynic acid.

As found for most GSH S-transferases from other sources, the placental enzyme system exhibited the highest activity towards 1-chloro-2,4-dinitrobenzene. The specific activity, calculated for this arylchloride, is approximately 50 per cent of that measured in rat liver and intestine [4, 7] and human liver [17] extracts, but is about two orders of magnitude (85-fold) higher than the activity recovered from human haemolysate [6].

Examination of the inhibition data (Table 2) shows that there was interaction of the placental enzyme

Table 3. Comparison of inhibition of placental glutathione S-transferase activity by diuretic agents*

Inhibitor	Concentration (mM)	Residual activity (%)
Furosemide	0.15	72
	0.24	51
	0.30	37
p-Aminohippurate	10.0	61
	15.0	43
	20.0	23

* The reaction mixture used was that described for Fig. 2 with 1.0 mM CDNB.

system with organic compounds which are not substrates. Rose Bengal, cephalothin and cephoxitin were competitive inhibitors. Thus, these studies demonstrate, at least qualitatively, that several types of anionic substances, such as cephalosporin and aminoglycoside antibiotics, diuretic agents and dyes, bind to the placental glutathione S-transferase system. In this respect, the GSH S-transferase system appears to possess the well-known properties of GSH S-transferases in other tissues, that of the dual role of catalytic and cytosol binding proteins.

Its conjugating activity towards a range of electrophilic compounds and its affinity for GSH (K_m 0.19 mM) suggests that placental tissue uses the metabolic process initiated by the GSH S-transferase system for detoxication. Moreover, GSH levels in placenta, estimated at about 0.6 mM [20], would be sufficient for this cellular function.

The presence of γ-glutamyltranspeptidase activity in placenta has also been detected. We have observed that this enzyme, which is believed to catalyse the second step in mercapturic acid biosynthesis metabolizing the GSH conjugates formed by glutathione S-transferase action, reaches a specific activity of 0.110 μmoles/min/mg in the placenta by the eighth week of pregnancy.

Finally, the observation of a lack of GSH S-transferase activity in the amniotic fluid could have significance if one thinks of the absence of a direct contact between this liquid and xenobiotics, while the relatively high activity found in early pregnancy would repond to the necessity of ensuring effective protection for the foetus, according to the pronounced organogenesis occurring at this phase of development.

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