

Purification of Glutathione *S*-Transferase from Human Placenta*

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Glutathione *S*-transferases comprise a group of enzymes catalyzing the nucleophilic attack of the sulfhydryl group of glutathione on electrophilic centers in a large number of chemical compounds. These enzymes appear to play an important role in the detoxification of xenobiotics and also seem to function as intracellular binding proteins for various exogenous and endogenous compounds.¹ The occurrence of glutathione *S*-transferases in animal species is wide-spread^{1,2} and purification of the cytoplasmic enzymes in several organs has been carried out.¹ Glutathione *S*-transferases have been isolated from human liver³ and from human erythrocytes.⁴ This communication presents a purification of glutathione *S*-transferase from human placenta and some of the properties of the enzyme.

Table 1 summarizes the results of the purification of the enzyme. Glutathione and 1-chloro-2,4-dinitrobenzene were used as substrates.⁵ A purification factor of 280 and a recovery of 50 % of the activity present in a supernatant fraction of human placenta were obtained. This corresponds to 42 mg of enzyme recovered per kg of placenta. The purified enzyme appeared homogeneous when analyzed by disc gel electrophoresis, isoelectric focusing and column chromatography. Fig. 1 shows that even in the crude preparation only a single form of the enzyme

could be observed using isoelectric focusing. No evidence for the existence of multiple forms of glutathione *S*-transferase in placenta was obtained under any conditions. The isoelectric point was at pH 4.8. The molecular weight was estimated as about 45 000 by use of gel filtration.⁶ Sodium dodecyl sulfate polyacrylamide gel electrophoresis⁷ indicated that the enzyme was composed of two similar or identical subunits. The substrate specificity was tested with 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, *p*-nitrophenyl acetate, and sulfobromophthalein and the following specific activities were determined: 60, 0.06, 0.1, and < 0.002 $\mu\text{mol}/\text{min}$ per mg protein, respectively. The assay conditions are those given in Refs. 5, 8 and 9.

In the purification procedure, the choice of the ligand for affinity chromatography was based on the assumption that the human enzyme would bind to *S*-alkyl glutathione derivatives, which have been found to inhibit glutathione *S*-transferase from rat liver.¹⁰ Such a rationale was previously successful in the purification of glutathione *S*-transferases from rat lung tissue.¹¹

Experimental. Determination of enzymatic activities and protein concentrations were carried out by published procedures.^{5,12} Placentas were processed at 4 °C within 12 h after parturition; 1000 g of tissue was cleared of amniotic membranes and washed with 0.25 M cold sucrose. Small pieces of placenta were homogenized with a blender in 0.25 M sucrose to give a 35–40 % (w/v) suspension. The homogenate was centrifuged for 1 h at 30 000 *g*. The supernatant (fraction 1) was equilibrated with 10 mM Tris/HCl buffer (pH 7.8) by passage over a Sephadex G-25 column (12 cm \times 79 cm) and applied to a DEAE-cellulose column (9 cm \times 8 cm) equilibrated with the same buffer. The enzyme was eluted with a linear gradient (0–0.3 M NaCl) formed in the original buffer. The pooled effluent (fraction 2) was dialyzed against 10 mM sodium phosphate (pH 6.2) containing 1 mM EDTA and then applied to a CM-cellulose

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Table 1. Purification of glutathione *S*-transferase from human placenta.

Fraction	Volume (ml)	Total activity ^a ($\mu\text{mol}/\text{min}$)	Specific activity ($\mu\text{mol}/\text{min}$ per mg protein)	Yield (%)
1. Placenta supernatant	1680	5100	0.21	100
2. DEAE-cellulose	490	3100	0.65	61
3. CM-cellulose	960	2700	2.8	53
4. <i>S</i> -Hexylglutathione Sepharose 6B	53	3000	n.d.	59
5. Sephadex G-75	190	2500	60	49

^a Activity measured at 30 °C and pH 6.5 using 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM GSH as substrates.

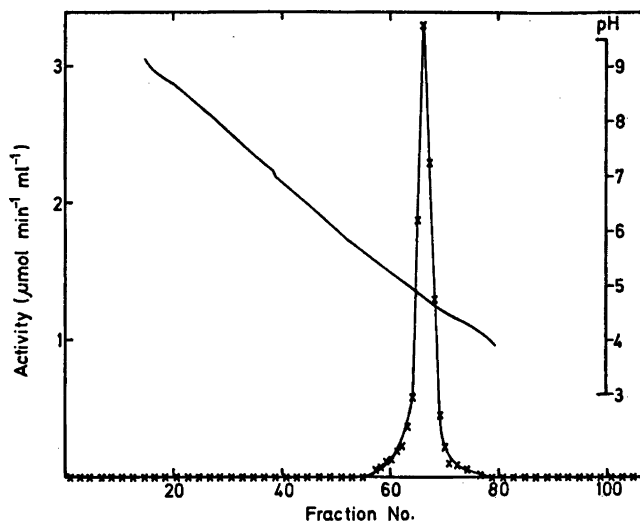


Fig. 1. Isoelectric focusing of glutathione *S*-transferase in a crude supernatant fraction of human placenta. A sample containing 75 mg protein of dialyzed supernatant (fraction 1 in the purification) was applied to a 110 ml column (LKB Produkter, Stockholm) and focused at 4°C (60 h) in a gradient formed by ampholytes covering the range pH 3.5–10. The contents of the column were collected in 1.5 ml fractions.

column (9 cm × 7 cm) equilibrated with the same buffer. The enzyme passed unadsorbed through the column and was collected. The collected enzyme (fraction 3) was adsorbed on an affinity column (2 cm × 15 cm) consisting of *S*-hexylglutathione immobilized on epoxy-activated Sepharose 6B¹³ which had been equilibrated with 10 mM Tris/HCl (pH 7.8)–1 mM EDTA. The column was washed with 150 ml 0.2 M NaCl in the same buffer and the enzyme was then eluted with 75 ml of 5 mM *S*-hexylglutathione in the buffer fortified with 0.2 M NaCl. The pooled enzyme activity (fraction 4) was chromatographed on a Sephadex G-75 column (4 cm × 140 cm) packed in 10 mM sodium phosphate (pH 6.7)–1 mM EDTA and the active fractions were combined (fraction 5).

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