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PLACENTA AS A PROTECTING BARRIER – SOME PROPERTIES OF GLUTATHIONE-*S*-TRANSFERASE

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Pollution of the environment and the drugs taken by pregnant women can adversely affects both the developing embryo and the course of pregnancy. Placenta is the main barrier between mother and embryo, and its protecting role is, to a large extent, dependent on the activity of its detoxicating enzymes.

In the present work we have studied the properties of glutathione-*S*-transferase isolated from the amnion and chorion of human placenta.

Glutathione-*S*-transferase (GST, EC 2.5.1.18) is an enzyme which plays the key role in inactivation of a very large number of electrophilic compounds [1]. Multiplicity of the molecular forms of this enzyme [2] as well as their low substrate affinity [3] permit to suppose that GST can play a very important role in protection of the embryo against various exo- and endogenous compounds.

Placentae, obtained directly after the delivery, were washed with physiological saline until they were completely free of blood, the amnion and the chorion were separated and homogenized in 10 mM sodium phosphate buffer, pH 7.0 containing 0.25 M sucrose, and centrifuged at 12 000 × *g* for 15 min. Then the supernatants were recentrifuged at 100 000 × *g* for 60 min, the cytosols concentrated and subjected to ion-exchange chromatography on DEAE-cellulose. The active fractions were pooled,

concentrated using Aquacide, dialysed against 10 mM sodium phosphate buffer, pH 6.5, and used for further studies.

The activity of GST was determined according to Habig *et al.* [4]. Thermostability of the enzyme was determined after 15 min preincubation at varying temperature. The effect of chemical compounds on GST activity was assayed after 10 min preincubation at 37°C in the presence of the compound studied and 100 mM sodium phosphate buffer, pH 6.5.

The activity of glutathione-*S*-transferase in 100 000 × *g* supernatant of human placenta amnion and chlorin, determined with 1 mM 1-chloro-2,4--dinitrobenzene (CDNB) as the acceptor substrate, and 1 mM GSH was 0.017 ± 0.003 and 0.055 ± 0.004 μmol per mg protein per minute, respectively. The enzyme from both tissues were also active with other substrates (Table 1).

Table 1

Substrate specificity of glutathione-S-transferase from human placenta

Substrate	Amnionic GST	Chorionic GST
	Activity (%)	
1-Chloro-2,4-dinitrobenzene	100	100
1,2-Dichloro-4-nitrobenzene	8.3 ± 4.05	17.2 ± 2.5
p-Nitrobenzyl chloride	36.0 ± 1.5	4.3 ± 0.2
Bromosulfophtalein	7.0 ± 0.5	44.0 ± 5.0
Ethacrynic acid	24.0 ± 5.0	48.0 ± 3.0

Amnionic GST was not adsorbed on DEAE-cellulose (Fig. 1A) whereas chorionic GST was adsorbed and was eluted with a linear KCl concentration gradient (Fig. 1B). The amnionic enzyme showed much higher affinity towards CDNB (K_m 0.38 ± 0.04 mM) than the chorionic enzyme (K_m 2.0 ± 0.06 mM) whereas their affinity towards GSH was similar (K_m 0.4 ± 0.05 and 0.6 ± 0.08 mM, respectively). The two transferases differed in thermostability, amnionic GST being more heat resistant than was the chorionic enzyme.

After preincubation at 42°C the amnionic enzyme retained about 90% of its initial activity, and about 50% at 49°C, whereas the chorionic enzyme retained about 40% of activity at 42°C and no more than 10% at 49°C.

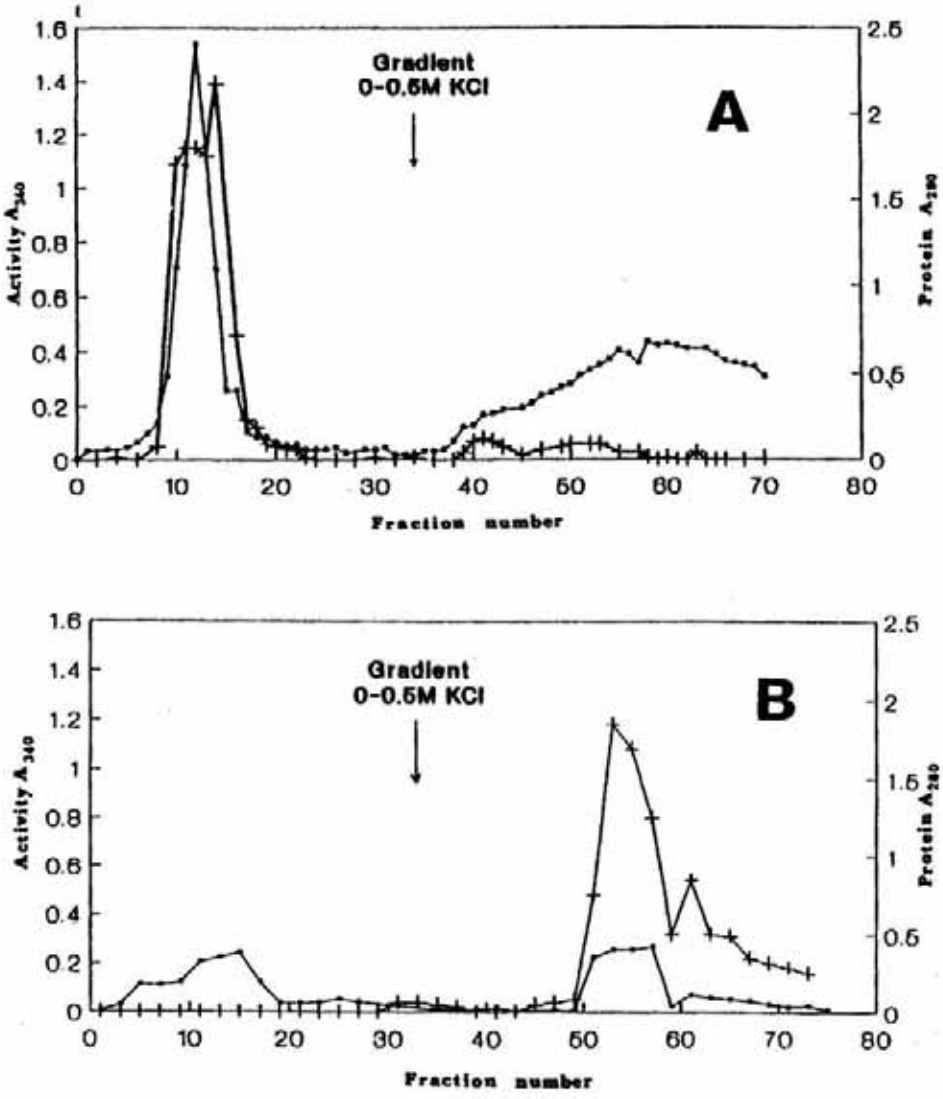

Fig. 1. DEAE-cellulose chromatography of glutathione-*S*-transferase from the amnion (A) and chorion (B) of human placenta. The 100 000 × *g* supernatant (about 100 mg protein) was applied to the DEAE-cellulose column (20 × 1 cm) equilibrated with 10 mM sodium phosphate buffer, pH 7.4. The amnion enzyme was eluted with the buffer and the chorion enzyme with a linear KCl concentration gradient. Fractions of 5 ml were collected and the activity (+) and protein content (●) were determined.

N-Ethylmaleimide (NEM) and phenylglyoxal (PG), the compounds blocking –SH groups and arginine residues, respectively, inhibited both the amnionic and chorionic GST. Reduced glutathione, when added to the NEM-inactivated chorionic enzyme, led to rapid complete restoration of its activity, whereas under the same conditions CDNB had no effect (Fig. 2). In the case of the PG-inactivated enzyme the addition of either GSH or CDNB resulted in its partial (about 60%) reactivation (Fig. 2). Similar results were obtained with the amnionic enzyme (not shown).

The results presented point to the involvement of –SH groups and arginine residues in binding of reduced glutathione, as well as the involvement of arginine residues in binding of CDNB.

The activity of the two transferases studied was strongly inhibited by catecholamines, the highest inhibition being observed with dopamine (Table 2). DOPA and methyl-DOPA, unlike catecholamines, did inhibit GST activity but slightly or not at all. Propranolol, the β-adrenergic antagonist, inhibited amnionic GST to a greater extent than the chorionic enzyme. The steroid hormones proved also to be effective inhibitors of the two enzymes studied (Table 2).

Table 2

The effect of catecholamines and steroid hormones on the activity of glutathione-S-transferase from human placenta

The activity after 10 min preincubation without the compound studied was taken as 100%

Compound studied (10 mM)	Amnionic GST	Chorionic GST
	Inhibition (%)	
Epinephrine	46 ± 1.2	52 ± 2.0
Norepinephrine	64 ± 1.5	55 ± 0.5
Dopamine	80 ± 2.1	76 ± 2.2
DOPA	12 ± 0.2	5 ± 0.4
Methyl-DOPA	0	20 ± 1.8
Propranolol	46 ± 1.3	20 ± 2.0
Testosterone	20 ± 2.3	23 ± 1.8
Hydrocortiosne	28 ± 2.0	50 ± 1.6
Corticosterone	57 ± 7.0	65 ± 3.0

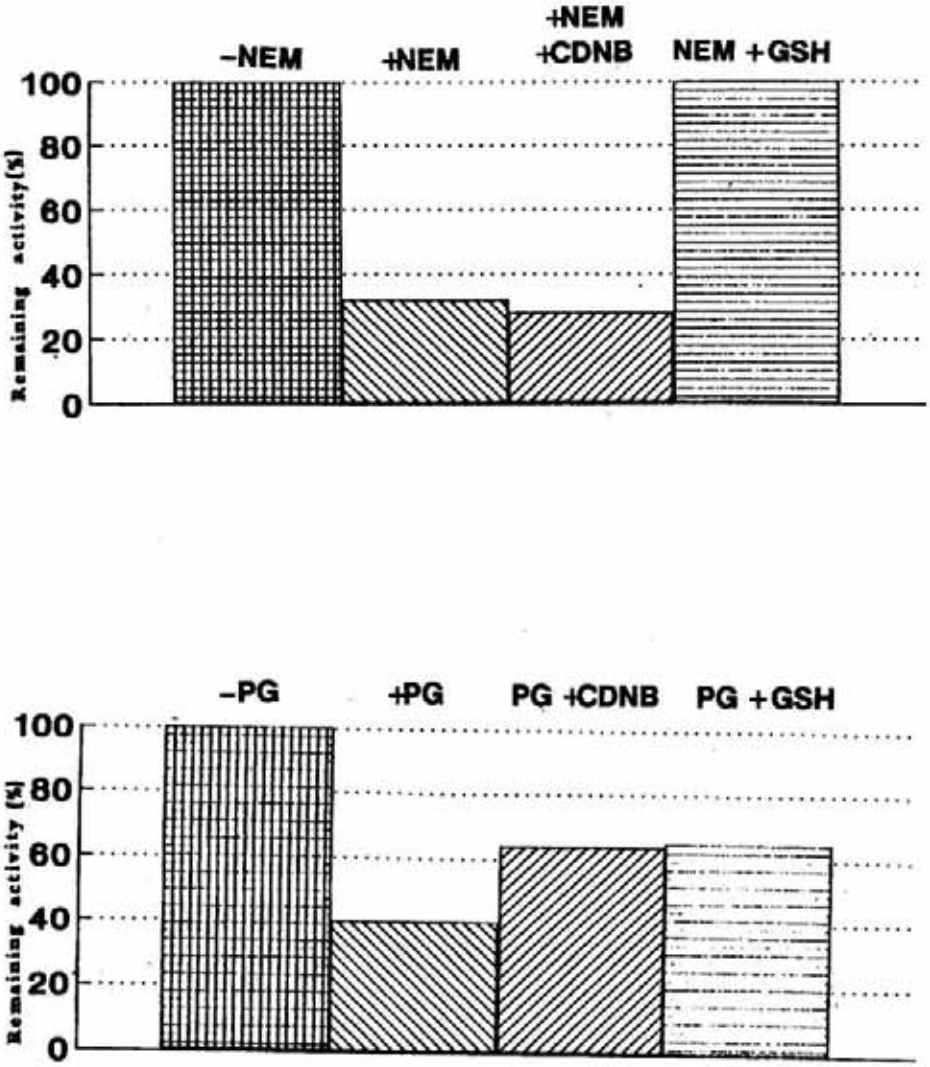

Fig. 2. The effect of *N*-ethylmalemide (NEM) and phenylglyoxal (PG) on the activity of chorionic glutathione-*S*-transferase and its restoration by reduced glutathione or CDNB. The enzyme obtained after DEAE-cellulose chromatography was preincubated with or without the inhibitor or with inhibitor and substrate at the concentration of 1 mM

On the basis of the results presented it can be concluded that either membrane of human placenta possesses an active glutathione-*S*-transferase. The form of the enzyme present in the amnion differs from the chorionic one. Both forms inactivate various exogenous compounds, and their activity is controlled by physiologically active endogenous compounds. Since the concentration of blood catecholamines is rather frequently elevated in pregnant women, inhibition of placental glutathione-*S*-transferase by these compounds could augment the exposure of the embryo to the action of electrophilic compounds.

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