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GLUTATHIONE-*S*-TRANSFERASE FROM BOAR TESTIS: PROPERTIES OF THE CYTOSOLIC AND MICROSOMAL FORMS

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Glutathione-*S*-transferase (GST, EC 2.5.1.18) is the enzyme widely distributed among various animal species [1]. Differences in the content of GST isoenzymes in particular tissues as well as low substrate specificity of GST point to its strong involvement in the phase II of metabolism reactions of xenobiotics [2].

Glutathione-*S*-transferase, by catalysing conjugation with glutathione (GSH), participate in the processes of detoxication of numerous electrophilic exogenous compounds, such as drugs, pesticides, dyes and cancerogenic substances [3]. Participation of GST in protection of cells and tissues against excessive activity of some physiologically active endogenous compounds, is also of importance [4]. In addition to its catalytic function glutathione-*S*-transferase plays an essential role in intracellular binding and transport of some lipophilic compounds, both endogenous such as bilirubin, bile acids, steroid hormones and exogenous ones, e.g. drugs and other xenobiotics [5].

In our previous studies we have demonstrated that, in the cytosol of bull testis, two forms of glutathione-*S*-transferase can be separated on DEAE-cellulose and that they differ in substrate affinity, susceptibility to the –SH group and arginine residue blocking compounds, in thermostability as well as in susceptibility to some drugs used in veterinary medicine [5].

We have also demonstrated that glutathione-*S*-transferase forms occurring in the cytosol and microsomes of boar testis differ from each other [7].

In the present work we have continued our studies on glutathione-*S*-transferase from boar testis, concentrating on the mechanism of the GST-catalysed reaction and the effect of some drugs on particular forms of the enzyme.

The required tissues were obtained from the Animal Breeding Station. GST was extracted with 10 mM sodium phosphate buffer, pH 7.4, containing 0.25 M sucrose. The cytosolic fraction was obtained by centrifugation of the extract at 100 000 × *g* for 60 min. The microsomal fraction was obtained by two consecutive centrifugation at 30 000 × *g* for 20 min each, followed by suspending the sediment in 1% Triton-X-100 solution and recentrifugation at 30 000 × *g* for 20 min. The cytosolic and microsomal fractions were each purified by consecutive ion-exchange DEAE-cellulose chromatography and CM-Sephadex column chromatography.

The activity of glutathione-*S*-transferase was determined according to Habig *et al.* [8] and Fujita *et al.* [9], with 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 1 mM reduced glutathione (GSH), and it was 1.28 ± 0.06 µmol/mg per min, and 0.22 ± 0.04 µmol/mg per min in cytosolic and microsomal fraction, respectively.

In the cytosolic fraction of boar testis a single form of the enzyme ($GST_{C}I$) was present; it was not adsorbed on DEAE-cellulose but, when subjected to CM-Sephadex chromatography, separated into two forms. Chromatographic separation on DEAE-cellulose of the microsomal enzyme fraction from boar testis gave two forms: a non-adsorbed cationic form ($GST_{M}I$) and a form eluted by the KCl gradient ($GST_{M}II$). On CM-Sephadex chromatography these forms were not further separated and emerged as single activity peaks.

The effect of compounds interacting with the active groups of the enzyme was determined after 10 min preincubation at 37°C in the presence of 100 mM sodium phosphate buffer, pH 6.5, and the compound studied.

In the experiments on the effect of substrates on the activity of GST inactivated by *N*-ethylmaleimide (NEM) or phenylglyoxal (PG), the preincubation mixture contained 0.5 mM CDNB or 1 mM GSH, or both substrates together.

Thermostability of GST was studied by incubating the enzyme at 37°C for 5 - 30 min without any additions.

The effect of drugs was determined by adding the given compound directly to the incubation mixture containing the enzyme, buffer, 0.5 mM CDNB and 1 mM GSH.

The effect of testosterone was assayed after 10 min preincubation of the enzyme in the buffer containing the hormone at defined concentration.

It was found that cytosolic glutathione-*S*-transferases from boar testis (GST$_C$I and GST$_C$II) were susceptible to the –SH group blocking compounds, *N*-ethylmaleimide (NEM) and iodoacetamide (IAA). I_{50} for NEM was similar for both cytosolic forms: 1.8 and 2.2 mM for GST$_C$I and GST$_C$II, respectively, whereas I_{50} values for IAA were different: 10 mM for GST$_C$I and 1 mM for GST$_C$II.

Both enzyme forms were inhibited by phenylglyoxal, a compound reacting selectively with arginine residues (I_{50} for GST$_C$I 0.25 mM, for GST$_C$II 0.4 mM).

On preincubation of GST$_C$I with NEM or PG at a chosen concentration in the presence of one of the substrates (CDNB or GSH) or with both together, it was found that GSH protects the enzyme against the inhibition both by NEM and PG (Table 1), which indicates that –SH groups and arginine residues are required for binding of the substrate by the enzyme.

Table 1

The effect of substrate on the activity of glutathione-S-transferase inhibited by N-ethylmaleimide (NEM) or phenylglyoxal (PG)

GST$_C$I purified by DEAE-cellulose chromatography was preincubated for 10 min at 37°C either in the presence of NEM or PG, or in their absence. The activity measured after preincubation without any inhibitor was taken as 100. The results are mean values (± S.D.) from 4 determinations

Substrate	NEM (mM)		PG (mM)	
	0.5	1.0	0.25	0.5
	Activity (%)			
None	60.5 ± 2.5	41.1 ± 3.6	45.0 ± 3.3	25.8 ± 1.8
CDNB	53.5 ± 3.6	44.3 ± 2.8	38.7 ± 5.2	23.3 ± 2.4
GSH	86.0 ± 4.8	51.6 ± 4.5	76.0 ± 4.5	54.5 ± 3.0
CDNB + GSH	101.7 ± 2.3	74.1 ± 4.2	88.7 ± 5.5	53.0 ± 3.7

On preincubation of the enzyme forms at 37°C for 30 min it was observed that, in contrast to the analogous thermolabile GST forms from bull testis [6], the two enzyme forms from boar testis both from cytosol and microsoms (not shown) proved to be temperature resistant.

The effect *in vitro* of various drugs on GST activity was studied with both forms of cytosolic GST from boar testis and with the main microsomal fraction GST_MII (about 90% of the total activity). Hydrocortisone inhibited to a much greater extent the anionic forms, GST_CII and GST_MII, than the cationic form GST_CI. Pilocarpine (parasympaticomimetic) at concentrations up to 10 mM inhibited the two cytosol forms but did not affect the activity of the microsomal form; cholagogue, clanobutin (4-*p*-chloro-*N*-[*p*-methoxyphenylo]-benzamido butyric acid) inhibited strongly all the enzyme forms studied. An antiinflammatory drug, phenazoline, had but little effect on GST_CI, inhibited GST_CII whereas it strongly activated the microsomal GST_MII (Table 2).

The catecholamines: epinephrine and norepinephine which strongly inhibited cytosolic GST from bull testis [6] had no effect on the activity of all studied GST forms from boar testis (not shown).

Testosterone, the main masculine hormone, inhibirted *in vitro* both cytosolic forms of GST from boar testis, whereas it had no effect (at

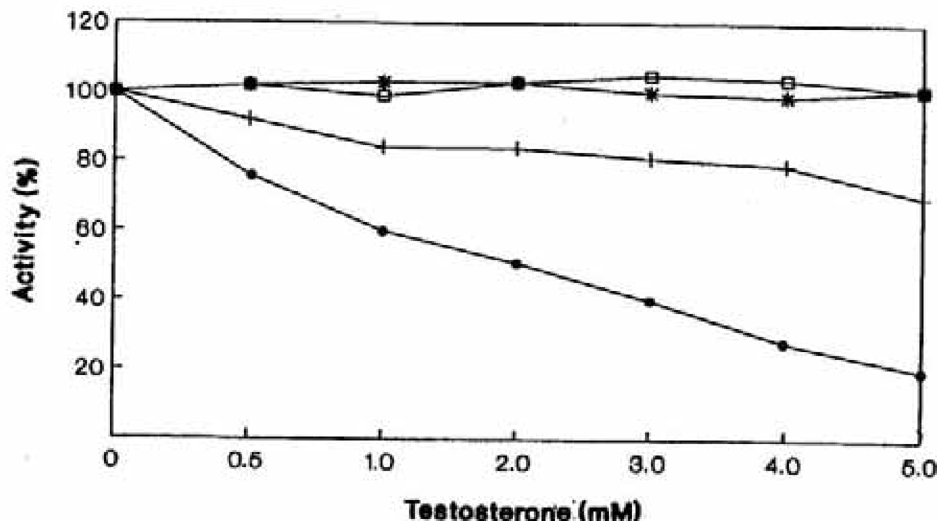

Fig. 1. The effect of testosterone on the activity of glutathione-*S*-transferase from boar testis. The activity of GST was determined after 15 min preincubation, in the presence of varying concentrations of testosterone. GST_MI, □ ; GST_MII, * ; GST_CI, ● ; GST_CII, + Each point is the mean of 3 determinations

Table 2

The effect of drugs on the activity of particular forms of glutathione-S-transferase from boar testis

The activity of GST was determined in the presence of a drug, 1 mM GSH and 0.5 mM CDNB. The results are mean values (± S.D.) from 3 determinations. The activity without the drug was taken as 100

Drug	GST_{CI}		GST_{CII}		GST_{MII}	
	Activity (%)					
Hydrocortisone	104.0 ± 3.8	67 ± 4.1	51 ± 3.3	33 ± 1.8	93 ± 2.0	52 ± 4.0
Pilocarpine	62.5 ± 1.5	32 ± 5.0	52 ± 2.8	18 ± 2.0	101 ± 3.1	100 ± 3.1
Clanobutin	61.5 ± 4.6	23 ± 1.9	53 ± 4.0	8 ± 0.5	11 ± 0.8	0
Phenazoline	87.5 ± 2.0	82 ± 2.0	82 ± 3.9	51 ± 1.6	183 ± 5.3	390 ± 8.0

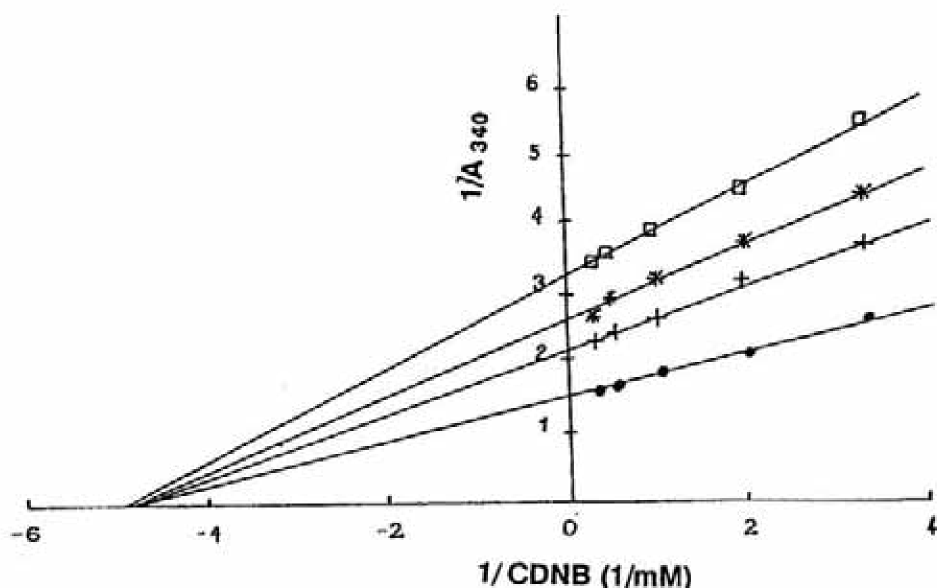

Fig. 2. Inhibition of glutathione-S-transferase (GSTcI) from boar testis by testosterone. GSTcI was preincubated with testosterone at the concentration indicated: ●, none; +, 0.63 mM; ✳, 1.2 mM and ◻, 2.5 mM

concentrations not exceeding 10 mM) on the enzyme forms isolated from microsomes. Cationic GSTcI was more susceptible to this hormone than was the anionic GSTcII (Fig. 1). In the presence of CDNB as the substrate, GSTcI was non-competitively inhibited by testosterone (Fig. 2).

On the basis of the results presented it can be concluded that, in boar testis, at least four different molecular forms of glutathione-*S*-transferase are present protecting this organ against the action of electrophilic compounds. Some veterinary drugs inhibit or induce the activity of these molecular forms.

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