Heterogeneity of the bovine brain glutathione-S-transferase

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Glutathione-S-transferase (GST[1], EC 2.5.1.18) catalyses conjugation of various electrophilic compounds with glutathione leading usually to formation of non-toxic and readily excretable products [1, 2]. Moreover, the enzyme binds and transports numerous hydrophobic compounds, such as bilirubin, steroids, neurotransmitters, pesticides or drugs [3, 4]. In most mammalian tissues [5, 6] it exists in multiple forms. GST forms (isoenzymes) differing in pI values have been characterized in human and rat brains [7, 8]. Despite numerous studies, the functional importance of glutathione-S-transferase and its role in brain detoxication is not clear.

In our previous studies we have demonstrated the presence of glutathione-S-transferase in primary cultures of monkey brain microvessel endothelium which forms the blood-brain barrier [9, 10] and characterized various GST forms from monkey brain cortex [11]. In the present work we have isolated and studied GST from bovine brain cortex.

The bovine brain was obtained from the slaughterhouse immediately after killing of the animal. The fresh grey matter was homogenized as soon as possible in five volumes of 10 mM sodium phosphate buffer, pH 7.6, containing 0.25 M sucrose. The homogenate was centrifuged at $15000 \times g$ for 15 min. The resulting supernatant was recentrifuged at $100000 \times g$ for 60 min yielding the cytosolic fraction. The cytosol was placed on a DEAE-cellulose column (20 × 2 cm) equilibrated with the same buffer. The non-adsorbed GST I, containing about 70% of the total activity was eluted with the buffer, and the adsorbed GST II (about 30% of the total activity), with a KCl linear gradient (Fig. 1). The activity of glutathione-S-transferase was determined according to Habig *et al.* [12] and Fujita *et al.* [13], with 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 1 mM reduced glutathione (GSH). The activity of the cytosolic fraction was 0.25 ± 0.08 µmol/mg protein per min.

Five different forms of GST were separated from the cytosol on the basis of isoelectric points, estimated by an agarose gel electrophoresis with ampholine in the pH range of 3.5 - 10.0: two basic (pI 8.55 and 7.35), one near-neutral (pI 6.7), and two acidic forms (pI 5.8 and 4.55). The pI values of the forms eluted with KCl from DEAE-cellulose were: 6.7 for GST I and 7.35 for GST II.

The relative molecular mass of GST I and GST II determined on a Sephadex G-100 calibrated column [14] was: 45000 and 40000, respectively.

Both GST I and GST II forms were active with CDNB and other exogenous electrophilic compounds (Table 1). Their specific activity with *p*-nitrobenzylchloride and ethacrynic acid (strong diuretic) was much higher than with 1,2-dichloro-4-nitrobenzene and bromosulfophthalein.

GST I had a lower than GST II affinity towards substrates, with K_m values being: 0.5 ± 0.02 mM and 0.29 ± 0.03 mM for CDNB, and 0.48 ± 0.04 mM and 0.15 ± 0.02 mM for GSH.

Both enzymes were losing their activity during 10 min preincubation with catecholamines and their derivatives at concentrations of

[1] Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; GSH, reduced glutathione; GST, glutathione-S-transferase; MOPEG, 3-methoxy-4-hydroxyphenylethylene glycol
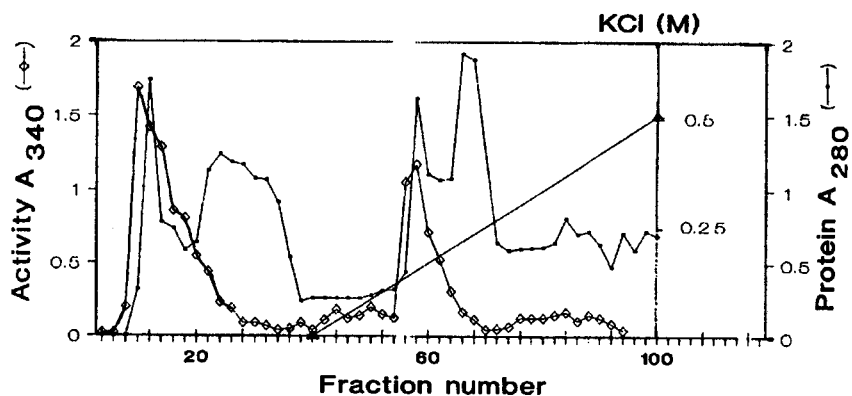
Fig. 1. DEAE-cellulose chromatography of glutathione-S-transferase from bovine brain cortex.
The cytosol (about 200 mg of protein) was applied on a column. Fractions of 5 ml were collected, and the activity and protein were determined
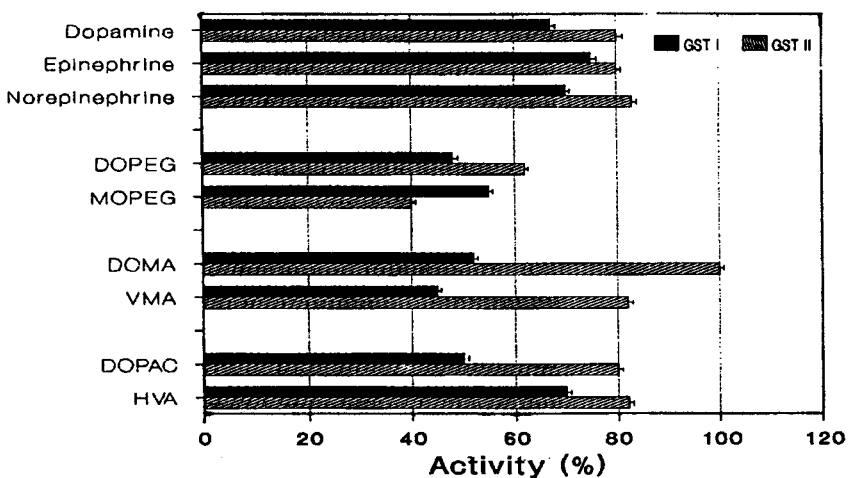

Fig. 2. The effect of catecholamines and their derivatives on the activity of glutathione-S-transferase from bovine brain cortex.
The activity was determined after 10 min preincubation of the enzyme with 2.5 mM compound in 100 mM sodium phosphate buffer, pH 6.5. The activity after preincubation with the buffer alone was taken as 100. Each value is the mean of three determinations. DOPEG, 3,4-dihydroxyphenylethylene glycol; MOPEG, 3-methoxy-4-hydroxyphenylethylene glycol; DOMA, 3,4-dihydroxymandelic acid; VMA, 3-methoxy-4-hydroxymandelic acid; DOPAC, 3,4- dihydroxyphenylacetic acid; HVA, 3-methoxy-4-hydroxyphenylacetic acid
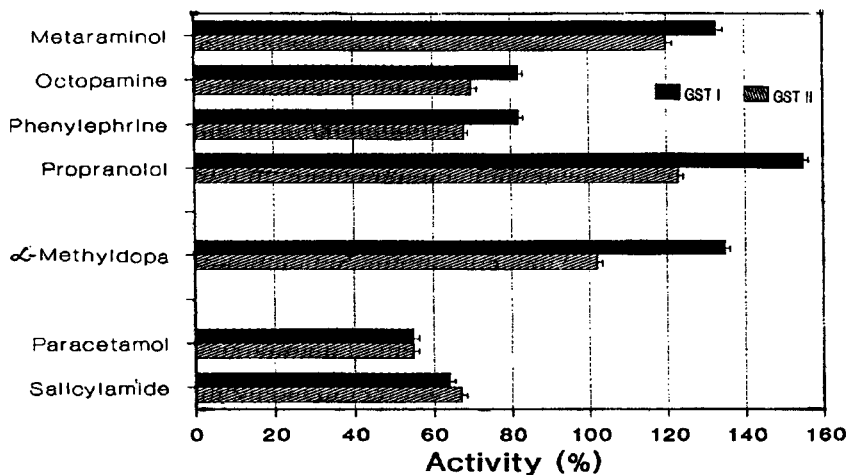

Fig. 3. The effect of some drugs on the activity of glutathione-S-transferase from bovine brain cortex.
The activity was determined as described in the legend to Fig. 2 and the same abbreviations were used. Each value is the mean of three determinations

Table 1

Substrate specificity of glutathione-S-transferase from bovine brain cortex.
Specific activity determined in the presence of 1 mM CDNB was: 0.87 and 0.63 μmol/mg per min for GST I and GST II, respectively

Substrate	GST I	GST II
	Specific activity (%)	
1-Chloro-2,4-dinitrobenzene	100.0	100.0
1,2-Dichloro-4-nitrobenzene	8.2	4.1
p-Nitrobenzylchloride	71.0	56.0
Ethacrynic acid	35.0	24.0
Bromosulfophthalein	7.7	5.7

2.5 mM (Fig. 2) and 10 mM (not shown). GST I was more sensitive to these compounds than GST II. Among the derivatives, 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG), the main metabolite of norepinephrine in brain, was the strongest inhibitor of GST II (Fig. 2). In the presence of GSH as the variable substrate GST II was non-competitively inhibited by MOPEG.

Among the adrenergics studied, octopamine and phenylephrine (α-adrenergics) inhibited either GST form, but metaraminol (α,β-adrenergic) and propranolol (β-adrenergic), activated them (Fig. 3). α-MethylDOPA, a methylated precursor of catecholamines widely used as an antihypertensive drug increased the activity of GST I, but had no effect on GST II (Fig. 3). Analgetics, paracetamol and salicylamide inhibited both GST forms.

Thus it can be concluded that many different molecular forms of glutathione-S-transferase are present in bovine brain cortex. The forms protect this organ against the action of electrophilic compounds and their activity can be regulated by catecholamines, their derivatives or precursors, as well as by drugs containing catechol or phenolic ring.

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