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GLUTATHIONE S-TRANSFERASE FROM BOVINE TISSUES: RELATIONSHIP BETWEEN MULTIPLE FORMS, DISTRIBUTION AND CATALYTIC ACTIVITY

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Cytosolic functions obtained from various bovine tissues was individually subjected to column isoelectric focusing in order to resolve the glutathione S-transferase isoenzymes. The results showed a large variability in the isoenzyme pattern. All the tissues were found to have neutral-acidic forms of the enzyme, whilst liver, adrenal gland, testicle, lung and kydney contained a conspicuous amount of activity associated with the cationic forms of the enzyme. In spite of these differences, by comparison of the conjugating activity of transferases, we did not find essential inter-organ variations. Conversely, when the same tissue samples were tested for selenium independent glutathione peroxidase activity, using cumene hydroperoxide as second substrate, we observed a higher activity in the organs having the cationic form of glutathione S-transferase.

Key words: Bovine tissues, glutathione S-transferase, Activity, Isoelectric focussing.
Abbreviations: GSH, reduced glutathione; DCNB, 1, 2-dichloro-4-n-nitrobenzene; p-NBCl, p-nitrobenzyl chloride; EA, ethacrynic acid; EPNPP, 1, 2-epoxy-3-(p-nitrophenoxy)-propane; TPNO, trans-4-Phenyl-3-butene-2-one; BSP, bromo-sulfophthalein; p-NPA, p-nitrophenyl acetate; H_2O_2, hydrogen peroxide; t-BHP, t-butyl hydroperoxide; CHP, cumene hydroperoxide; DTT, dithiothreitol.

INTRODUCTION

The glutathione S-transferases (EC 2.5.1.18) represent a complex family of proteins present in all mammalian tissues so far examined. It has been proposed that they can exert multifunctional roles in the cellular metabolism: (i) a detoxifying effect by this ability to catalyse the conjugation of a wide variety of compounds, carrying an electrophilic centre, with reduced glutathione, thus initiating the removal of many toxic and/or carcinogenic agents[1]; (ii) a binding action with respect to physiological compounds of low solubility such as haemin, bilirubin and steroids thus facilitating

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the redistribution of these substances in the internal milieu of the cell[2] and (iii) an antiperoxidative function by reducing organic hydroperoxides[3].

Glutathione S-transferase activity has been identified in different subcellular components of rat liver such as cytosolic, microsomal and mitochondrial fractions[4-6]. Rat liver soluble glutathione S-transferases are, in turn, separable in different groups of proteins, each one constituted by a large variety of distinct isoenzymatic forms[7,8].

A very complex pattern of multiple forms has also been found in several other hepatic tissues from different animals[9,10] and even in extrahepatic tissues of many species[11], including man[12].

Despite the increasing interest and recent knowledge about the existence of the polymorphic pattern of glutathione S-transferase, the biological role of its several isoenzymes and subforms remains essentially uncertain. However it has been supposed that multiple forms of such an enzyme could accomodate different types of potentially toxic agents as substrates or transfer ligands between differents locations in the cell[8,13]. We have examined the multiple form distribution and the catalytic properties of glutathione S-transferase in different beef tissues in an attempt to evaluate a possible correlation between the isoenzyme content and the metabolic activity of tissues investigated.

MATERIALS AND METHODS

Chemicals The following chemicals were purchased from Aldrich Chemical Co. (U.S.A): 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene, p-nitrobenzyl-chloride, 1,2-epoxy-3-(p-nitrophenoxy)-propane, trans-4-phenyl-3-butene-2-one. Ethacrynic acid and bromosulfophthalein were obtained from Fluka (Switzerland). GSH and NADPH were purchased from Biochemia-Boheringer (Germany). CM-Sephadex-C-50 and Epoxy-Sepharose-6-B were products of Pharmacia (Sweden). Ampholytes for column isoelectric focusing were obtained from LKB (Sweden). All other chemical materials were obtained from Carlo Erba (Italy) or Merck (Germany).

Enzyme assays Glutathione S-transferase activity was determined as described by Habig *et al.*[4] at 20°C. One milliunit of activity is defined as the amount of enzyme catalysing the formation of 1 nmol of product/min/mg under the specific assay conditions. Protein concentrations were measured by the method of Bradford[14] using bovine serum albumin as standard. Glutathione peroxidase activity was measured by the method of Little *et al.*[15] using 0.1 mM-cumene hydroperoxide or 0.1 mM ter-butyl-hydroperoxide or 0.1 mM hydrogen peroxide as the substrate.

Preparation of GSH-Epoxy-Sepharose GSH was coupled by Epoxy-activated Sepharose using the procedure of Simons and Vander Jagt[16].

Preparation of tissue extracts All tissues from 9 to 12 month-old male cows were obtained from a local slaughter house and immediately processed. The tissues were washed with cold saline solution, minced and homogenized in a Waring Blendor at low speed for 90 sec with 2 vol of 100 mM-potassium phosphate buffer containing 1 mM-EDTA and 1 mM-DTT at pH 7.2. The homogenate was centrifuged at 50,000 g for 60 min and the supernatant was filtered through a glass wool plug.

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Purification of glutathione S-transferase The tissue extracts (10–15 ml), prepared as above described, were applied to a GSH-epoxy-Sepharose column (15 cm × 1.5 cm) equilibrated with 50 mM potassium phosphate buffer, pH 7.2. Most of proteins were eluted with this buffer. The column was washed with 200 ml of the same buffer containing 500 mM-KCl and 1 mM-EDTA to remove protein bound aspecifically. Transferase activity was eluted with 50 mM-Tris/Cl containing 500 mM-KCl and 5 mM-GSH. Active fractions were pooled and concentrated by ultrafiltration in an Amicon apparatus provided with an UM-10 membrane, to a final volume of 2–3 ml, dialysed in the same apparatus against 300 ml of potassium phosphate buffer, pH 6.0, containing 1 mM-EDTA and 1 mM-DTT and stored at −20°C up to 3 months without appreciable loss of activity.

Isoelectric focusing Isoelectric focusing was performed on a LKB column (110 ml) using 1% mixed ampholine solution generating a pH gradient of 3.5 to 9.5 at 700 V for 70 h. Fractions of 0.9 ml were collected for determination of enzyme activity measurement.

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RESULTS

The elution profiles of glutathione S-transferase activity from preparative isoelectric focusing of the cytosolic fractions of different bovine tissues are reported in Fig. 1. In most tissues, the glutathione S-transferase activity is distributed in different peaks. However, whereas all tissues always possess a set of neutral-acidic forms, some of them (liver, kidney, testicle, lung) contain additional basic components. The relative amount of the subforms does drastically vary among the different tissues. Nevertheless, within the neutral-acidic group, the neutral form (pI around 7.0) is always prevalent with respect to the others. Particularly, in cardiac tissue it constitutes more than 95% of total transferase activity. Similarly, within the cationic form, the most basic isoenzyme is always present in a larger amount with respect to other basic components.

The glutathione S-transferase activity of the different tissues was also examined using several potential substrates. Since the glutathione S-transferase activity in the cytosolic fractions could be detected only with 1-chloro-2,4-dinitrobenzene as substrate, all further kinetic measurements were performed after partial purification of the enzymes by affinity chromatography.

More than 85–90% of initial transferase activity was recovered with a high degree of purity, as indicated by elevated increase of specific activity (results not shown). The active fraction was concentrated and dialysed and used to establish the spectrum of effective substrates.

As reported in Table I, in spite of a variable pattern of isoenzyme forms occuring in several tissues, no relevant differences could be found, in the catalytic conjugation of GSH with different substrates. In fact, the relative conjugating activities with respect to the best substrate (1-chloro-2,4-dinitrobenzene) are substantially identical in all the tissues examined. Conversely, the level of peroxidase activity, measured with cumene hydroperoxide, significantly differs in many tissues. It can be noted that where the basic forms greatly prevails (i.e. liver) the peroxidase activity is higher, suggesting a possible specialization of the cationic found in enzyme for this catalytic function.

In order to verify such a possibility, neutral-acidic and basic subforms (obtained

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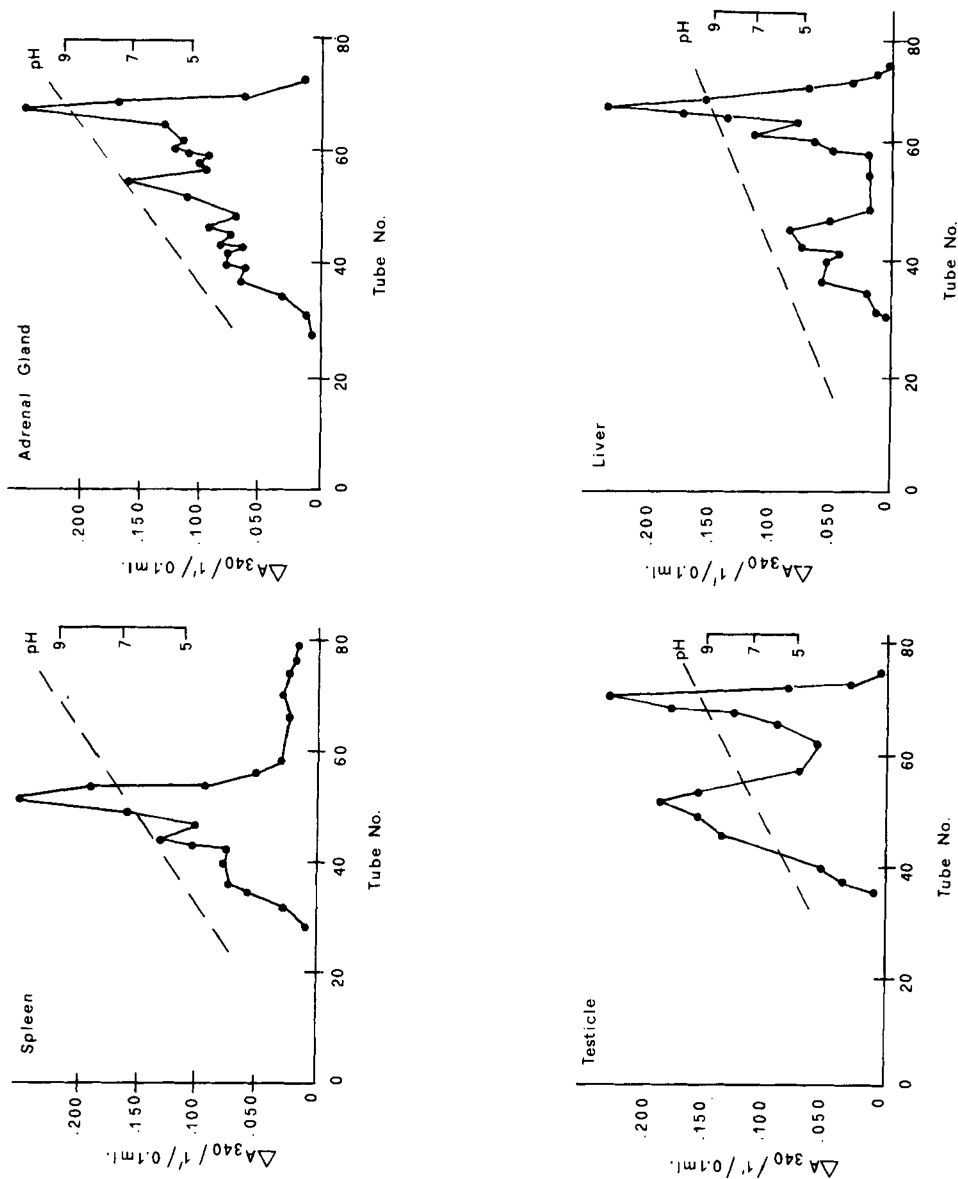

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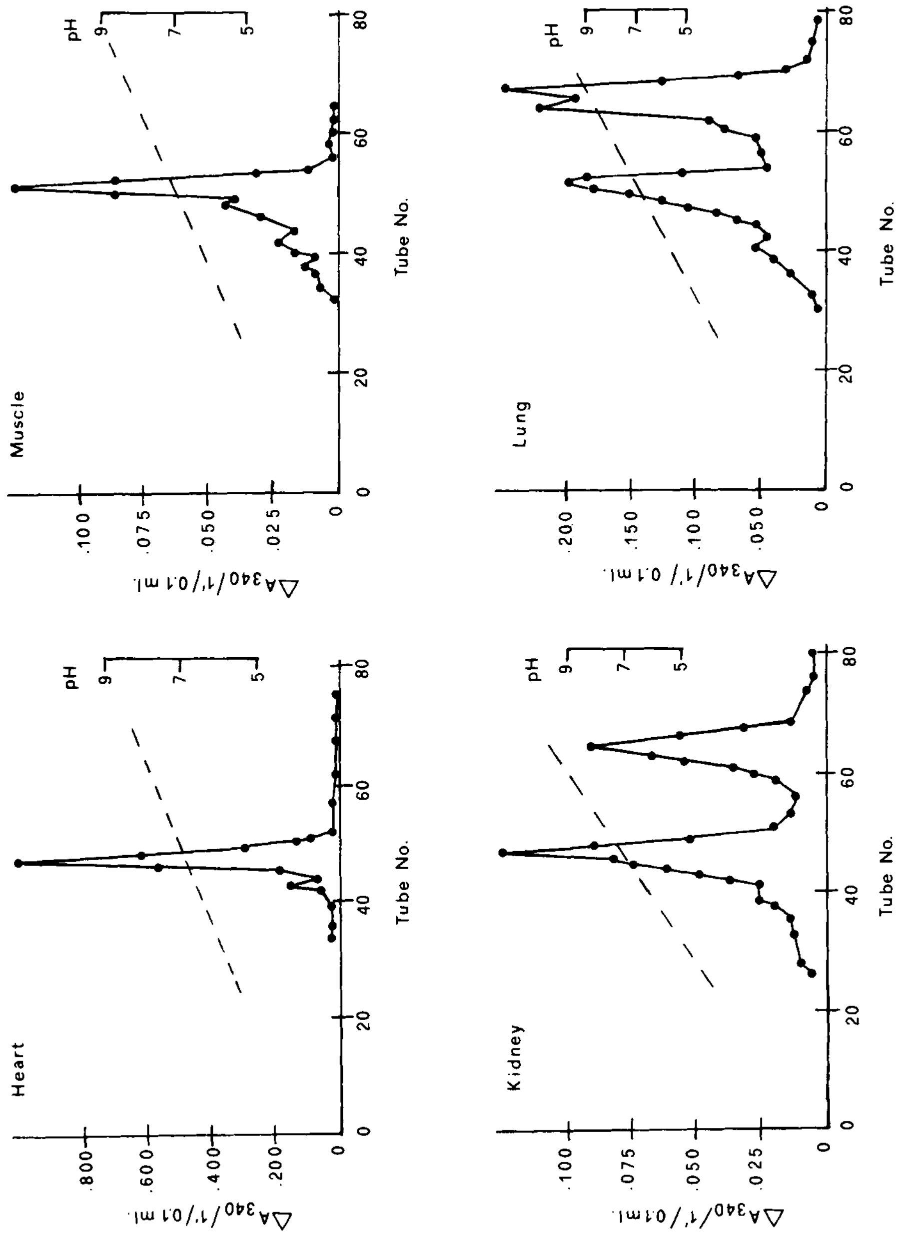

Fig.1

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TABLE I
Substrates specificity of GSH S-Transferase from different bovine tissues

Substrate	relative activity%							
	heart	muscle	kidney	testicle	lung	spleen	adrenal	liver
CDNB	100 (13.0)	100 (10.0)	100 (9.7)	100 (7.4)	100 (7.8)	100 (7.8)	100 (4.5)	100 (3.7)
DCNB	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1
p-NBCl	1.8	1.9	2.0	1.9	1.8	2.2	2.2	2.4
EA	1.5	1.6	1.7	1.4	2.1	6.3	1.7	1.5
EPNPP	1.0	0.8	1.0	1.2	1.5	2.5	1.5	2.3
TPNO	0.1	0.15	0.2	0.18	0.12	0.4	0.2	0.2
BSP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
p-NPA	1.1	1.3	1.1	3.1	0.9	1.1	1.2	1.4
CHP	1.6	3.1	9	9	14	19	21	52

n.d. = activity not detectable under given experimental conditions. In parenthesis is reported the specific activity expressed as μmoles of substrate conjugated/min/mg protein.

from cytosol isoelectric focusing) were collected in two separate pools from liver, lung, testicle and adrenal, respectively. Peroxidase activity was assayed with cumene hydroperoxide and hydrogen peroxide (Table II). The cationic fractions were able to reduce cumene hydroperoxide, but were virtually devoid of activity with hydrogen peroxide. Although also the neutral-acidic pools catalysed the reduction of cumene hydroperoxide, however such an activity must be prevalently referred to selenium-dependent glutathione peroxidase since both organic and inorganic peroxides are reduced at almost identical rates and this is, only a capability of the selenium-dependent enzyme[17].

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DISCUSSION

The results reported in the present paper show that the glutathione S-transferase activity in all bovine tissues is distributed among multiple forms distinguishable from this in electric point. However, the relative amount of each form is quite different in the various tissues. In cardiac tissues more than 90% of total activity is present in a single peak centred at pH 7.1 while in the adrenal gland the activity was dispersed in several fractions with different relative abundance.

A comparative analysis of the results indicates two main conclusions:

1) a group of neutral-acidic proteins having glutathione S-transferase activity is always present in all tissues examined;

2) only some tissues possess additional multiple forms having a basic pI. When the cationic forms are present, the ratio between the neutral-acidic and basic forms appears to significantly vary.

Therefore, summarising the data presented and those obtained by other authors, bovine tissues can be classified in two groups: those having both neutral-acidic and basic transferases such as cornea[18], liver, kidney, lung, adrenal gland and testicle, and those only provided with neutral-acidic transferase: ocular lens[11], brain cortex, red cells (Del Boccio, personal communication) cardiac tissue, muscle and spleen.

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TABLE II
Selenium dependent and selenium independent peroxidase activity in neutral-acidic and basic fractions pooled after isoelectric focusing of bovine tissues cytosol.

Tissue	Fraction	peroxidase activities			
		Total (CHP)	Se-dep (H202)	Se-ind (CHP − H202)	% Se-ind
Liver	neutral-acidic	0.35	0.22	0.13	8
	basic	1.67	0.02	1.65	92
Lung	neutral-acidic	0.38	0.27	0.11	14
	basic	0.67	0.02	0.65	86
Testicle	neutral-acidic	0.40	0.24	0.16	19
	basic	0.74	0.08	0.70	81
Adrenal gland	neutral-acidic	0.50	0.35	0.15	25
	basic	0.47	0.03	0.44	75

Activity expressed as μmoles of substrate conjugated/min/ml

It has been supposed, mainly from studies on rat liver, that the class of glutathione S-transferases enzymes represents a system able to conjugate a very large variety of harmfull exogenous compounds, by different contributions from each individual form[8]. Our result are not in agreement with this suggestion. In fact, all tissues examined do not exhibit a significant specificity in conjugating different substrates, in spite to the variable isoenzymatic pattern. Furthermore, it should be emphasize that, in bovine tissues, only CDNB is conjugated at an appreciable rate. All other compounds tested were found to be poor substrates.

However, among the various tissues examined a relevant difference can be observed in the ability of the iso-enzyme to reduce organic hydroperoxide. This correlates with the presence of cationic forms which might represent an additional system to protect the cellular structure against oxidative events. However, if this suggestion is correct, it is not clear why only in some organs this iso-enzyme should be required. Therefore, it should be of interest to determine whether distribution of the various iso-enzymes might be related to the different metabolic requirements of the various tissues to transport specific ligands.

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