Isolation, properties and tissue distribution of rat glutathione transferase E

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A simple small-scale purification procedure is described for GSH transferase E. This enzyme is shown to be a dimer of subunits of apparent M_r 28500, to have an isoelectric point of pH 7.0, GSH transferase activity towards certain alkyl epoxides and alkyl halides, and to be the most active Se-independent GSH peroxidase so far described. It is present in a number of tissues, although at a low concentration. It is relatively abundant in the epididymis and the adrenal gland, but undetectable in lactating mammary gland and skeletal muscle. Its previously observed lability is confirmed.

Glutathione transferase E (5-5) *Rat liver* *Purification* *Subunit size* *Tissue distribution*

1. INTRODUCTION

The first glutathione (GSH) transferase to be purified was GSH epoxide transferase [1], later referred to as GSH transferase E [2]. It was defined by its activity towards certain compounds containing an oxirane ring in the terminal position of an alkyl chain, e.g., EPNP. However, in contrast with other GSH transferases, GSH transferase E has been little studied, perhaps because it is present in small amounts, labile and difficult to purify and also because it is not detected by CDNB, the most commonly used substrate for GSH transferase isoenzymes.

This paper describes a rapid small-scale method for its purification, further defines its physical and enzymic properties and describes its tissue distribution.

Abbreviations: GSH, reduced glutathione; CDNB, 1-chloro-2,4-dinitrobenzene; EPNP, 1,2-epoxy-3-(*p*-nitrophenoxy)propane; TBPO, *trans*-4-phenyl-3-buten-2-one; PNPB, *p*-nitrophenethyl bromide; PNBC, *p*-nitrobenzyl chloride; CuOOH, cumene hydroperoxide; PAGE, polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

2.1. *Enzyme assays and SDS–PAGE*

Activities of GSH transferases towards CDNB, EPNP, PNPB, TBPO, PNBC, and CuOOH were determined as previously described [2,3]. Fractions were analysed for purity by SDS–PAGE [4] protein being detected with Coomassie blue or a silver stain [5].

2.2. *Purification of GSH transferase E*

The soluble supernatant fraction obtained from a homogenate of 50 g liver from Wistar-strain rats, inbred at the Courtauld Institute of Biochemistry, was passed through an *S*-hexyl-glutathione affinity [6] column in 0.05 M Tris adjusted to pH 7.8 with HCl. The flow-through and a 0.2 M NaCl wash were combined, made 1 mM in EDTA, 5 mM in GSH and the protein precipitating between 30 and 65% saturated ammonium sulphate collected and dissolved in 10 ml of a buffer containing 25 mM KH_2PO_4, 25 mM KCl, 1 mM EDTA, 3 mM GSH adjusted to pH 7.0 with KOH. It was then applied to a Sephadex G-100 column (2.5 × 98 cm) equilibrated in the same buffer. A fraction was obtained containing EPNP–GSH transferase activity

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Table 1

Summary of purification of GSH transferase E

Step	Procedure	Total protein (mg)	Total activity (μmol/min)	Specific activity (μmol/min/mg protein)
I	Soluble supernatant	2569	526	0.20
II	*S*-Hexylglutathione affinity column			
	(a) Flow through	742	271	0.37
	(b) 0.2 M NaCl wash	1675	427	0.25
III	Ammonium sulphate	1081	183	0.17
IV	Sephadex G-100	175	144	0.82
V	Isoelectric focusing	5.3	45	8.49
VI	CM–Sepharose	0.4	10.2	25.50

which was then subjected to isoelectric focusing in a sucrose density gradient containing 0.75% (w/v) Ampholines of pH range 5–8 and 0.25% (w/v) Ampholines of pH range 3–10 (fig.1). The main peak with EPNP–GSH transferase activity, which focused at approx. pH 7.0, was collected and applied to a CM–Sepharose column (1.1 × 80 cm) equilibrated with a buffer containing 25 mM NaH_2PO_4, 1 mM GSH, 1 mM EDTA adjusted to pH 6.3 with NaOH and the material adsorbed to the column was eluted at a rate of 15 ml per h with a linear gradient of NaCl. Purified GSH transferase E was eluted with approx. 0.04 M NaCl.

3.RESULTS

3.1. *Purification*

Table 1 shows a summary of the purification of GSH transferase E and fig.1–3 illustrate some of the steps in the purification procedure. Step II using the *S*-hexylglutathione affinity column has the value of allowing GSH transferase E to pass through unadsorbed while retaining most of the activity due to other GSH transferases. Steps III and IV bring about useful separations based on solubility and molecular size. Considerable separation of GSH transferase E from much protein and other GSH transferases is brought about in step V by isoelectric focusing (see fig.1). Final purification is achieved by gradient elution from CM–Sepharose in step VI, illustrated in fig.3, which shows the separation of GSH transferase E from all other remaining GSH transferases as well as a protein of apparent M_r 30000 and unknown biological activity.

One can gain an idea of the tissue content of an enzyme by comparing its specific activity of the starting material with that of the purified product, however, it is difficult to do this in the case of GSH transferase E for two reasons. Firstly, it is not

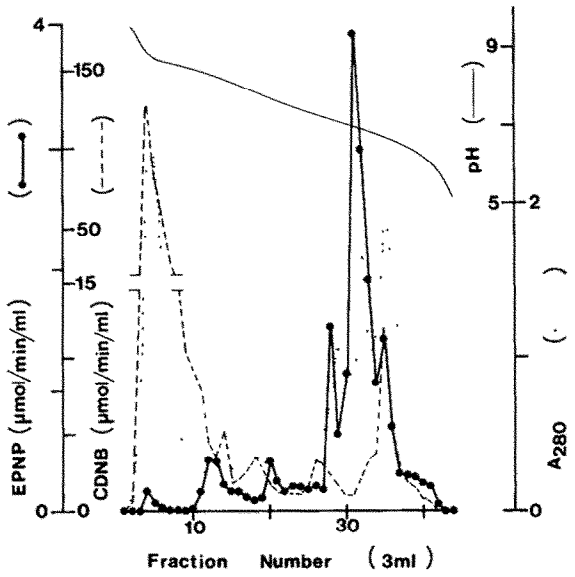

Fig.1. Isoelectric focusing. GSH transferases not retained on the affinity column and partially purified by ammonium sulphate precipitation and Sephadex G-100 chromatography were subjected to isoelectric focusing in a sucrose density gradient as described in section 2.

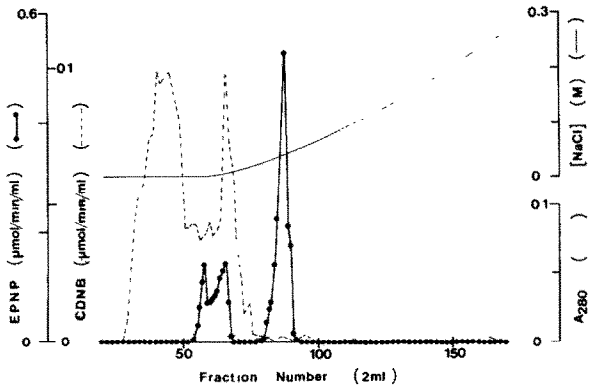

Fig.2. Cation-exchange chromatography. The GSH transferase E-containing fraction obtained by isoelectric focusing (fig.1) was separated on CM–Sepharose as described in section 2.

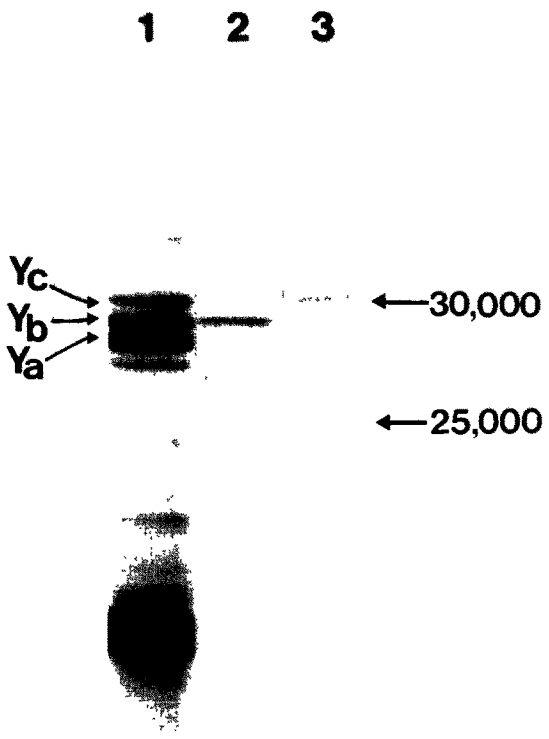

Fig.3. SDS–PAGE. Lane 1, GSH tranferase E-containing fraction from isoelectric focusing; lanes 2 and 3, fractions 88 and 92, respectively, obtained by gradient elution from CM–Sepharose as in fig.2.

Table 2

Specific activities of GSH transferase E

Substrate	Activity (μmol/min per mg protein)
1,2-Epoxy-3-(*p*-nitrophenoxy)propane	25.5
Cumene hydroperoxide	12.5
p-Nitrobenzyl chloride	4.3
p-Nitrophenethyl bromide	4.2
1-Chloro-2,4-dinitrobenzene	<0.15
trans-4-Phenyl-3-buten-2-one	<0.001

possible to determine the activity in the starting material due to GSH transferase E since other much more abundant GSH transferases also have EPNP–GSH transferase activity (0.5–1.0 units/mg) and, as shown above, it is not until the final step that EPNP–GSH transferase activity from other sources is separated from GSH transferase E. Secondly, the activity which is associated with GSH transferase E is very labile, there being irreversible losses of up to 75% during both steps V and VI.

3.2. *Characterisation*

GSH transferase E has an isoelectric point of pH 7.0 (see fig.1) and gives a single band on SDS–PAGE with an apparent M_r of 28500 (see fig.3). (M_r values of 23500 and 26500 have also been quoted for subunits of this size. On the basis

Table 3

GSH transferase E content of various rat tissues

Tissue	GSH transferase E (μmol/min per g tissue)
Epididymis (whole)	2.5
Female adrenal gland	1.3
Male adrenal gland	1.2
Liver	0.7
Testis	0.2
Epididymal fluid	0.2
Seminal vesicles	0.2
Lactating mammary gland	not detected
Skeletal muscle	not detected

of SDS–PAGE M_r 28500 is now regarded to be more accurate [7].) Its enzymic activity with a number of substrates is shown in table 2, the best substrates among those tested being EPNP and CuOOH.

3.3. *Tissue distribution*

The soluble supernatant fractions of a number of tissues were subjected to isoelectric focusing and the fraction with a high ratio of EPNP–GSH transferase activity to CDNB–GSH transferase which focused at pH 7.0 was taken to represent GSH transferase E. Results are shown in table 3 where it is seen that, while the epididymis and the adrenal gland are relatively rich in activity, the lactating mammary gland and skeletal muscle have none and tissues such as the liver and the testis have intermediate values.

4. DISCUSSION

The yield of protein associated with GSH transferase E, namely 0.015% of the total soluble supernatant protein, contrasts with the yields of GSH transferases B_1, B_2, AA, A, C and 'D', which we find to be approx. 2.0, 2.0, 0.4, 1.0, 2.0 and 0.5% of soluble supernatant protein, respectively. Low abundance of GSH transferase E may be compensated for, to some extent, by the greater specific activity of the native protein: it appears that at least 90% of the activity of the native enzyme may be lost during purification. This lability has been remarked upon previously [1].

Like other GSH transferases, GSH transferase E is not substrate specific, but catalyses a range of different reactions involving GSH such as oxirane ring opening, nucleophilic displacement and organic peroxide reduction. With respect to the last named, its specific activity as a GSH peroxidase with CuOOH as substrate is higher than that of any other GSH transferase so far tested.

On SDS–PAGE the subunits of GSH transferase E have an apparent M_r of 28500 which is very close to that of the Y_b band associated with GSH transferases A, C and 'D'; enzymes with which GSH transferase E appears to differ in most other respects. In the new nomenclature of [7] which is based on subunit composition, GSH transferase E is assumed, for the time being, to be a homodimer and is referred to as GSH transferase 5-5 while GSH transferases B_1, B_2, AA, A, C and 'D' are referred to as GSH transferases 1-1, 1-2, 2-2, 3-3, 3-4 and 4-4, respectively.

In conclusion, this work has confirmed the existence of GSH transferase E, produced an alternative and more brief method of purification, and added to its known properties. The problem of stabilizing its activity remains to be resolved. Its tissue distribution is of interest, but the significance which can be attached to it, as with the other GSH transferase isoenzymes, is unknown.

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