Two immunologically distinct Yc-type subunits are present in rat lung glutathione S-transferases

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Two types of 25000- M_r subunits are present in rat lung glutathione S-transferase I (pI8.8). These subunits, designated Yc and Yc', are immunologically and functionally distinct from each other. The homodimers YcYc (pI10.4) and Yc'Yc' (pI7.6) obtained by hybridization *in vitro* of the two subunits of glutathione S-transferase I (pI8.8) were isolated and characterized. Results of these studies indicate that only the Yc subunits express glutathione peroxidase activity and cross-react with the antibodies raised against glutathione S-transferase B (YaYc) or rat liver. The Yc' subunits do not express glutathione peroxidase activity and do not cross-react with the antibodies raised against glutathione S-transferase B of rat liver. The amino acid compositions of these two subunits are also different. These two subunits can also be separated by the two-dimensional gel electrophoresis of glutathione S-transferase I (pI8.8) of rat lung.

Multiple forms of glutathione S-transferase (GST, EC 2.5.1.18) are present in rat tissues (Habig *et al.*, 1974; Bass *et al.*, 1977; Scully & Mantle, 1981). It has been suggested (Mannervik & Jensson, 1982) that the different forms of rat liver GST arise from binary combinations of four subunits Ya (M_r 22000), Yb, Yb' (both M_r 23500) and Yc (M_r 25000). We have previously demonstrated that two immunologically and kinetically distinct 22000- M_r subunits, Ya and Ya', are expressed in rat lung (Singh *et al.*, 1984). The presence of two types of 23500- M_r subunits, Yb and Yb', has also been demonstrated in liver enzymes (Mannervik & Jensson, 1982; Beale *et al.*, 1983; Frey *et al.*, 1983; Hayes, 1983). To our knowledge only one type of 25000- M_r subunit (Yc) has previously been characterized in rat tissues. In the present communication we demonstrate the existence of two immunologically and functionally distinct 25000- M_r subunits in GST I (pI8.8) of rat lung.

Materials and methods

The sources of chemicals, the antibodies against GST B (YaYc) of rat liver and the strains of rats

Abbreviation used: GST, glutathione S-transferase.

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used in this study were the same as described previously (Singh *et al.*, 1984; Partridge *et al.*, 1984). Glutathione peroxidase activity with cumene hydroperoxide and GST activities with different substrates were determined as described by Awasthi *et al.* (1975) and Habig *et al.* (1974) respectively. Protein content was determined by the method of Bradford (1976), and details of amino acid analysis are described by us previously (Dao *et al.*, 1984).

The enzyme, GST I (pI8.8), was isolated from rat lung (42g wet wt. pooled from 30 rats) by following the protocol described by us previously (Singh *et al.*, 1984). The purity and subunit composition of GST I (pI8.8) was confirmed by urea/sodium dodecyl sulphate/2-mercaptoethanol/polyacrylamide-slab-gel electrophoresis in the system described by Laemmli (1970). Hybridization *in vitro* of subunits of GST I (pI8.8) was performed by using a method similar to that described by Kitahara & Sato (1981) and Hayes *et al.* (1981). Details of this method were described by us previously (Singh *et al.*, 1984). Two-dimensional gel electrophoresis was performed by using the method of O'Farrell (1975).

Results and discussion

We have previously demonstrated that GST I (pI8.8) of rat lung is a dimer of 25000- M_r subunits

Fig. 1. Isoelectric focusing of hybridized enzymes obtained by hybridizing subunits of rat lung GST I (pI8.8). Experimental details are given in text. ○, pH gradient; ●, GST activity with 1-chloro-2,4-dinitrobenzene as substrate at 25°C; △, GSH peroxidase activity with cumene hydroperoxide as substrate at 37°C. The inset shows the urea/sodium dodecyl sulphate/2-mercaptoethanol/polyacrylamide-slab-gel electrophoresis of hybridized enzymes obtained by hybridizing subunits of rat lung GST I (pI8.8): lane a, standards, in order of decreasing M_r from origin, phosphorylase *b* (94000), bovine serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), soyabean trypsin inhibitor (20100) and α -lactalbumin (14400); lanes b, c and d, hybridized enzymes with pI 10.4, 8.8 and 7.6 respectively.

Table 1. Substrate specificities of enzymes obtained by hybridization *in vitro* of the subunits of rat lung GST I (pI8.8). One unit of enzyme utilizes 1 μ mol of substrate/min at 25°C for glutathione *S*-transferase and at 37°C for glutathione peroxidase. Abbreviation: N.D., not detected.

Substrate	GSH <i>S</i> -transferase activity (units/mg of protein)		
	pI 10.4 form (YcYc)	pI 8.8 form (YcYc')	pI 7.6 form (YcYc'')
1-Chloro-2,4-dinitrobenzene	5.50	4.42	2.20
2-Nitropyridine <i>N</i> -oxide	0.12	0.17	0.23
Ethacrynic acid	2.36	1.76	0.65
Bromosulphophthalein	0.02	0.11	0.24
Cumene hydroperoxide*	3.10	1.62	N.D.

* Glutathione peroxidase activity.

(Partridge *et al.*, 1984), and the present study confirmed this finding. However, two-dimensional electrophoretic analysis of GST I (pI8.8) in the system described by O'Farrell (1975) indicated the presence of two polypeptide spots having similar M_r values of about 25000 but differing in charge (results not presented). Upon hybridization *in vitro*, the subunits of GST I of rat lung yielded three active enzyme forms having pI values of 10.4, 8.8 and 7.6 (Fig. 1). Glutathione peroxidase activity was, however, associated with only the two of these

forms having pI values of 10.4 and 8.8 (Fig. 1). All of the three forms were dimers of 25000- M_r subunits. The M_r values of the hybridized enzymes determined by gel filtration over a column of Sephadex G-100 were found to be about 50000 and polyacrylamide-slab-gel electrophoresis under reduced and denaturing conditions indicated the presence of only 25000- M_r subunits in all of the three forms (inset in Fig. 1, lanes b, c and d).

The substrate specificities of the three enzyme forms given in Table 1 indicate significant differ-

Table 2. Amino acid composition of the enzymes obtained by hybridization *in vitro* of the subunits of rat lung GST I (pI8.8)

Values are means $\pm$ s.d. for three samples hydrolysed for 24, 48 and 96 h, and are based on an M_r value of 50000.

Amino acid residue	Composition (mol of residues/mol)	
	pI 10.4 form (YcYc)	pI 7.6 form (Yc'Yc')
Asp	35.6 $\pm$ 3.2	29.5 $\pm$ 2.2
Thr	18.6 $\pm$ 2.0	19.4 $\pm$ 1.6
Ser	47.6 $\pm$ 4.6	48.1 $\pm$ 5.2
Glu	67.8 $\pm$ 6.9	58.8 $\pm$ 6.2
Pro	10.9 $\pm$ 1.0	12.4 $\pm$ 1.8
Gly	89.8 $\pm$ 7.2	73.2 $\pm$ 8.8
Ala	35.8 $\pm$ 3.9	31.4 $\pm$ 3.1
Val	21.2 $\pm$ 2.0	19.8 $\pm$ 1.4
Met*	2.5	2.9
Ile	16.8 $\pm$ 3.4	10.9 $\pm$ 1.0
Leu	29.3 $\pm$ 3.2	19.9 $\pm$ 1.8
Tyr	14.4 $\pm$ 1.8	7.9 $\pm$ 1.5
Phe	12.1 $\pm$ 1.2	7.5 $\pm$ 0.6
Lys	42.3 $\pm$ 4.3	63.3 $\pm$ 8.8
His	48.3 $\pm$ 5.6	79.5 $\pm$ 10.2

* Values for methionine were obtained by extrapolation to zero time of hydrolysis.

ences between the more-basic (pI10.4) and the less-basic (pI7.6) forms of the hybridized enzymes. The most remarkable difference between the two is in the expression of GSH peroxidase activity: the enzyme form with pI10.4 expresses glutathione peroxidase activity, whereas the form with pI7.6 does not. Glutathione peroxidase activity of the hybridized enzyme, with pI8.8, is about half of that of the enzyme form with pI10.4. In double immunodiffusion (Fig. 2) and immunotitration studies (results not presented) it was found that only the enzyme forms with pI10.4 and 8.8 cross-reacted with the antibodies raised against GST B (YaYc) of rat liver. Taken together, these results strongly suggest that two immunologically and functionally distinct types of subunits are present in GST I of rat lung. These subunits have a similar M_r value but have significant charge differences. The more-basic subunit of GST I of rat lung may be similar to the Yc subunit of GST B of liver, because it expresses glutathione peroxidase activity and cross-reacts with the antibodies raised against GST B. The less-basic subunit, designated Yc', is immunologically distinct from subunit Yc and does not express glutathione peroxidase activity. Hybridization *in vitro* of these subunits yields the expected binary combinations of YcYc, YcYc' and Yc'Yc'. GST I is identical with the hybridized enzyme YcYc' (pI8.8), but the other

Fig. 2. Double immunodiffusion of hybridized enzymes obtained by hybridizing subunits of rat lung GST I (pI8.8) with antibodies raised against GST B (YaYc) of rat liver. The centre well contains antibodies raised against GST B of rat liver. Outer wells a, b and c contain hybridized enzymes with pI10.4, 8.8 and 7.6 respectively.

two dimers, YcYc (pI10.4) and Yc'Yc' (pI7.6), are probably not present in the lung.

The amino acid compositional differences between the Yc and Yc' subunits are also significant (Table 2). The difference index (Metzger *et al.*, 1968) between the amino acid compositions of the hybridized enzymes YcYc (pI10.4) and Yc'Yc' (pI7.6) was found to be 14.1. Thus the results of the present studies strongly suggest that Yc and Yc' subunits are indeed two distinct subunits and that one of the forms is not an artifact or a post-translational modification product derived from the other. These studies provide evidence for the presence of two distinct types of 25000- M_r subunits in rat lung GST. Whether or not GST of other tissues also have heterogeneity in their 25000- M_r subunits remains to be studied.

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