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Anomalous electrophoretic behaviour of the glutathione *S*-transferase Ya and Yk subunits isolated from man and rodents

A potential pitfall for nomenclature

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1. GSH *S*-transferases are dimeric enzymes. The subunits in the rat are resolved into six types, designated Yf, Yk, Ya, Yn, Yb and Yc, by discontinuous SDS/polyacrylamide-gel electrophoresis [Hayes (1986) Biochem. J. **233**, 789–798]. 2. The relative electrophoretic mobility of the Ya and Yk subunits is dependent on the amount of cross-linker (*NN'*-methylenebisacrylamide) in the resolving gel. At low degrees of cross-linking, C_{Bis} 0.6% (w/w), the Yk and Ya subunits possess a faster anodal mobility than do the Yf, Yn, Yb and Yc subunits (i.e. order of mobility Yk > Ya > Yf > Yn > Yb > Yc), whereas at higher degrees of cross-linking, C_{Bis} 5.0% (w/w), Yf subunits possess the fastest mobility (i.e. order of mobility Yf > Yk $\geqslant$ Yn > Yb $\geqslant$ Ya > Yc). 3. Resolving gels that contain low concentrations of cross-linker [C_{Bis} 0.6% (w/w)] allow the resolution of a hitherto unrecognized polypeptide that is isolated by *S*-hexyl-GSH–Sepharose affinity chromatography. This new polypeptide, which we have designated Yb*, is normally obscured by the main Yb band in resolving gels that comprise concentrations of cross-linker of at least C_{Bis} 1.6% (w/w). 4. The Ya- and Yb-type subunits in guinea pig, mouse, hamster and man were identified by immuno-blotting and their apparent M_r values in different electrophoresis systems were determined. The Ya subunits in all species studied possess a variable cross-linker-dependent mobility during electrophoresis. 5. Since the transferase subunits are currently classified according to their mobilities during SDS/polyacrylamide-gel electrophoresis, it is apparent that the variable electrophoretic behaviour of the Ya and Yk subunits may lead to the mis-identification of enzymes.

INTRODUCTION

The GST enzymes are a highly complex group of multifunctional detoxication proteins that are thought to help combat chemically induced carcinogenesis (Smith *et al.*, 1977; Chasseaud, 1979).

Several affinity matrices have been described that greatly facilitate the purification of GST enzymes. *S*-Hexyl-GSH–Sepharose (Mannervik & Guthenberg, 1981) is the most widely employed matrix, as its use results in high recovery of GST activity. Despite the specificity of this gel, work has shown that at least one enzyme, GST K (YkYk protein), binds to *S*-hexyl-GSH–Sepharose poorly, but can be isolated by using the GSH–Sepharose matrix described by Simons & Vander Jagt (1977) (Hayes, 1986).

The GST enzymes are dimeric and, since hybrid forms have been identified in both the rat and man (Hayes *et al.*, 1981; Beale *et al.*, 1982; Boyer *et al.*, 1983; Stockman *et al.*, 1985), the enzymes that make up this family are best defined by their individual subunit composition. To date, at least five subunit types have been described in rat liver, designated Yk, Ya, Yn, Yb and Yc (Bass *et al.*, 1977; Hayes *et al.*, 1981; Scully & Mantle, 1981; Beale *et al.*, 1983; Hayes & Chalmers, 1983; Hayes, 1986).

Extrahepatic GST enzymes also exist. Several groups of workers have described an additional GST subunit type in rat heart, spleen, kidney and placenta that has been variously called Yα (Tu *et al.*, 1983), Yp (Kitahara *et al.*, 1984) or Yf (Hayes, 1984, 1986). This polypeptide type is closely related to those contained in transferase λ purified from human lung (Hayes & Mantle, 1986). The Yf subunit may also be identical with the low-M_r GST subunit found in high concentration in pre-neoplastic nodules (Satoh *et al.*, 1985) and referred to as subunit 7 (Meyer *et al.*, 1985; Jensson *et al.*, 1985) or P21 (Eriksson *et al.*, 1983; Farber, 1984).

There is disagreement about the M_r values quoted for GST subunits in the rat (cf. Hayes *et al.*, 1981; Scully & Mantle, 1981; Pickett *et al.*, 1982, 1984; Tu *et al.*, 1982). However, since it is the relative electrophoretic mobility of GST monomers that permits their identification, the discrepancies in calculated subunit size are generally considered to be of little importance. Hence it is currently recognized that the relative anodal mobilities of the rat GST subunits during SDS/polyacrylamide-gel electrophoresis are as follows: Yf > Yk > Ya > Yn > Yb > Yc (Hayes, 1986). Estimates of the size of human GST subunits also differ significantly (Pattinson, 1981; Hayes *et al.*, 1983; Stockman *et al.*, 1985; Vander Jagt *et al.*, 1985; Singh *et al.*, 1985), but the problem of subunit identification has been compounded because of the polymorphism displayed by the enzymes in man.

Abbreviation used: GST, GSH *S*-transferase.

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We now show that the concentration of *NN'*-methylenebisacrylamide in the gel can profoundly affect the mobility of Ya and Yk monomers. Conditions are described that allow the relative positions of Yf, Yk, Ya, Yn and Yb subunits to change. One consequence of this altered electrophoretic resolution has been the identification of a new type of GST subunit, Yb*; this possesses a marginally faster mobility than that of the main Yb band. Similar changes have been found in the mobilities of the GST subunits from other species, including man.

MATERIALS AND METHODS

Chemicals

Serva Coomassie Blue R-250 (research grade) was obtained from Uniscience, London S.W.18, U.K. The other chemicals used were purchased from BDH Chemicals, Poole, Dorset, U.K., or Sigma Chemical Co., Poole, Dorset, U.K., and were analytical or electrophoresis grade.

Isolation of GST enzymes

The enzymes from the tissues of male Wistar rats were obtained by methods described previously (Hayes & Mantle, 1986; Hayes, 1986). The use of *S*-hexyl-GSH–Sepharose 6B to isolate the transferases from various tissues has been described previously (Hayes & Mantle, 1986).

Mouse GST enzymes were purified from the livers of male laca mice by chromatography on *S*-hexyl-GSH–Sepharose 6B. The affinity-purified material was resolved into five peaks of activity, designated GST P1–P5, by h.p.l.c. on Bio-Gel HPHT hydroxyapatite (Hayes *et al.*, 1986). We have reported that GST P1–P3 enzymes are similar; GST P1 and GST P2 both comprise YfYf subunits, whereas GST P3 contains Yf subunits and a new polypeptide with a slightly faster electrophoretic mobility (Ye). GST P4 contains YaYa monomers, and GST P5 comprises YbYb subunits (Hayes *et al.*, 1986).

Electrophoresis

Discontinuous SDS/polyacrylamide-gel electrophoresis was performed with the buffer systems described by Laemmli (1970). The resolving gels comprised 10%, 12% or 15% (w/v) polyacrylamide and the concentration of cross-linker *NN'*-methylenebisacrylamide was varied. The stacking gel used throughout this study comprised 5.75% (w/v) acrylamide and 0.15% (w/v) *NN'*-methylenebisacrylamide. The following polypeptide markers were used: cytochrome *c* (M_r 12300), myoglobin (M_r 17200), soya-bean trypsin inhibitor (M_r 20100), trypsinogen (M_r 24000), chymotrypsinogen A (M_r 25700), carbonic anhydrase (M_r 29000), ovalbumin (M_r 45000), albumin (M_r 66300) and ovotransferrin (M_r 77000).

Immuno-blotting

This was performed as described previously (Hayes & Mantle, 1986), with antisera raised against GST L (YaYa, from rat liver), GST A (Yb_1Yb_1, from rat liver), GST AA (YcYc, from rat liver), GST λ (YfYf, from human lung) and GST ϵ (B_1B_1, from human liver).

RESULTS AND DISCUSSION

Variable mobility of the Ya subunit isolated from the rat

GST pools from various rat tissues, prepared by *S*-hexyl-GSH–Sepharose affinity chromatography, have previously been shown to contain different subunits when analysed by immuno-blotting (Hayes & Mantle, 1986). However, it was found that these samples gave different electrophoretic band patterns, when analysed in Dublin, from those previously observed in Edinburgh. The Edinburgh laboratory (J.D.H.) routinely uses a resolving gel comprising 12% (w/v) polyacrylamide that contains 0.32% (w/v) *NN'*-methylenebisacrylamide (i.e. C_{Bis} 2.6%) to examine GST preparations (Hayes & Clarkson, 1982), whereas the Dublin laboratory (T.J.M.) routinely uses 15% (w/v) polyacrylamide resolving gels containing 0.09% (w/v) *NN'*-methylenebisacrylamide (i.e. C_{Bis} 0.6%) (Sheehan, 1985).

Fig. 1(*a*) shows that, when the hepatic Ya, Yb and Yc subunits, and the cardiac Yf, Yn, Yb and Yc subunits, each set purified from rat organs by *S*-hexyl-GSH–Sepharose affinity chromatography, are subjected to SDS/polyacrylamide-gel electrophoresis in 12% (w/v) polyacrylamide gels containing C_{Bis} 2.6%, subunit Yf (the fastest-running band in the cardiac GST pool) has

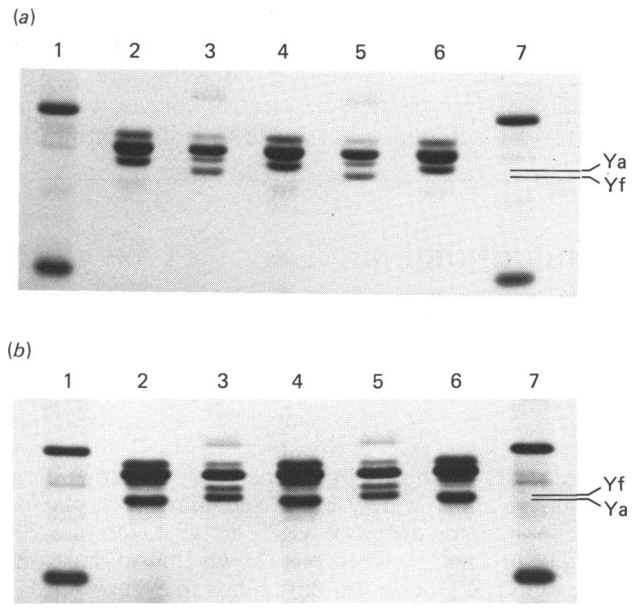


Fig. 1. Comparison of the electrophoretic patterns of GST subunits obtained with polyacrylamide gels containing different concentrations of cross-linker

The GST enzymes in rat liver (comprising Ya, Yb and Yc subunits) and rat heart (comprising Yf, Yn, Yb and Yc subunits) were purified by *S*-hexyl-GSH–Sepharose affinity chromatography. The enzyme pools were analysed by SDS/polyacrylamide-gel systems that incorporated resolving gels comprising (*a*) 12% (w/v) polyacrylamide containing 0.32% (w/v) *NN'*-methylenebisacrylamide or (*b*) 15% (w/v) polyacrylamide containing 0.086% (w/v) *NN'*-methylenebisacrylamide. In both panels the same protein samples were run. In each track approx. 20 μg of protein was applied as follows: tracks 1 and 7, mixtures of carbonic anhydrase and trypsin inhibitor; tracks 2, 4 and 6, liver GST enzymes; tracks 3 and 5, heart GST enzymes.

Table 1. *NN'*-Methylenebisacrylamide-dependent variations in the apparent M_r of rat-GST subunits

SDS/polyacrylamide-gel electrophoresis was performed as described in the text. The Ye, Yf and Yn GST subunits were isolated from extrahepatic tissues (spleen, lung and testis). The remaining subunits were purified from rat liver. The gels incorporated various concentrations of cross-linker; acrylamide/*NN'*-methylenebisacrylamide ratios of 175:1, 62:1, 38:1, 20:1, 13:1 and 8.2:1 were employed. The proteins used to calibrate the gels are listed in the Materials and methods section. Of those used, ovalbumin (M_r 45000), carbonic anhydrase (M_r 29000), trypsin inhibitor (M_r 20100) and myoglobin (M_r 17200) proved to be the most reliable markers. The subunits are listed according to their mobility in gels containing C_{Bis} 2.6%. Comparisons within a column show that Ya subunit migrates faster than Yf subunit at C_{Bis} 0.6% but co-migrates with Yb subunit at C_{Bis} 5–12%. Abbreviations: N.C., not calculated; N.R., not resolved.

	10^{-3} × Apparent M_r					
C_{Bis} (%) ...	0.6†	1.6	2.6‡	5.0	7.7	12
T (%) ...	15	12	12	12	12	12
Transferase monomer						
Ye	24.0	N.C.	24.2	24.2	N.C.	N.C.
Yf	24.0	25.1	24.8	24.9	24.5	24.6
Yk	22.8	25.0	25.0	26.3	26.0	25.8
Ya	23.0	25.5	25.5	27.0	26.6	26.3
Yn	24.8	N.C.	26.0	26.5	N.C.	N.C.
Yb*	25.8	N.R.	N.R.	N.R.	N.R.	N.R.
Yb (i.e. Yb_1 or Yb_2)	26.1	27.3	26.3	27.0	26.6	26.3
Yc	27.1	28.6	27.5	28.0	27.5	27.3

† System used in Dublin.
‡ System used in Edinburgh.

a higher mobility than subunit Ya (the fastest-running band in the hepatic GST pool). Using this degree of cross-linking the relative mobility of the GST polypeptides is Yf > Ya > Yn > Yb > Yc. By contrast, Fig. 1(*b*) shows the electrophoretic pattern obtained from the same samples run on a 15% (w/v) polyacrylamide gel containing C_{Bis} 0.6%; under these conditions the order of the Yf and Ya subunits is reversed. When a small amount of cross-linker is employed the relative mobility of the GST subunits is Ya > Yf > Yn > Yb > Yc. Although alterations in the concentration of acrylamide (10, 12 or 15%, w/v) used in the resolving gel significantly altered the R_F values of GST subunits, the relative positions of the Yf and Ya subunits did not change if the acrylamide/*NN'*-methylenebisacrylamide ratio was kept constant. However, the relative mobility of Yf and Ya subunits was altered by manipulating the concentration of *NN'*-methylenebisacrylamide in the resolving gel (i.e. C_{Bis} 0.6, 1.6, 2.6, 5.0, 7.7 or 12.0%).

The use of M_r marker proteins shows that the changes in the relative positions of Ya and Yf subunits, after SDS/polyacrylamide-gel electrophoresis, is primarily due to alterations in the mobility of the Ya subunit, not of the Yf subunit (Table 1). There are significant differences in the M_r of individual subunits calculated from the various gels, but these are assumed to represent the inherent imprecision of the method.

Effects of *NN'*-methylenebisacrylamide on the rat Yk subunit

GST K (YkYk) is a cytosolic enzyme in the rat that possesses poor affinity for *S*-hexyl-GSH–Sepharose. However, it can be isolated by virtue of the fact it binds to GSH–Sepharose (Hayes, 1986). Yk subunits are not present in Fig. 1, but Fig. 2 shows that, like that of Ya subunits, the electrophoretic mobility of Yk subunits varies according to the concentration of *NN'*-methylenebisacrylamide in the resolving gel.

The fact that Ya and Yk subunits share sequence homology (Hayes, 1986) as well as immunochemical cross-reactivity (Hayes & Mantle, 1986) suggests that the variable mobility of Ya and Yk subunits is due to a common structural feature, or features, possessed by these two polypeptides. The basis of this alteration in electrophoretic mobility is not clear. The variable behaviour is not thought to be due to binding of *NN'*-methylenebisacrylamide during electrophoresis, since incubation of the Ya subunit with the unpolymerized cross-linking agent [i.e. 2% (w/v) *NN'*-methylenebisacrylamide in the sample mixture applied to the gel] before electrophoresis did not alter its mobility. Further, 1.5% (w/v) concentrations of *NN'*-methylenebisacrylamide did not significantly inhibit (i.e. <10%) the GSH–1-chloro-2,4-dinitrobenzene-conjugating activity of GST K (YkYk), GST F (YaYa), GST C (Yb_1Yb_2) or GST AA (YcYc) under standard assay conditions.

It seems more likely that the variable mobilities could be accounted for by a hydrodynamic effect, a consequence of sieving by the polyacrylamide matrix, since the anomalous behaviour is most pronounced, or reaches a plateau, at a C_{Bis} of 5.0% (Table 1); at this relative concentration of *NN'*-methylenebisacrylamide, sieving by the polyacrylamide gel is maximal (Maizel, 1971; Gelfi & Righetti, 1981; Hames, 1981). This possibility suggests that the Ya and Yk subunits may not adopt a random-coil configuration after denaturation in the presence of SDS. In this context it may be noteworthy that the rat GST subunits that possess a 'blocked' *N*-terminus are the polypeptides that display a cross-linker-dependent electrophoretic mobility. Both the Ya subunit (Hayes *et al.*, 1981) and the Yk subunit

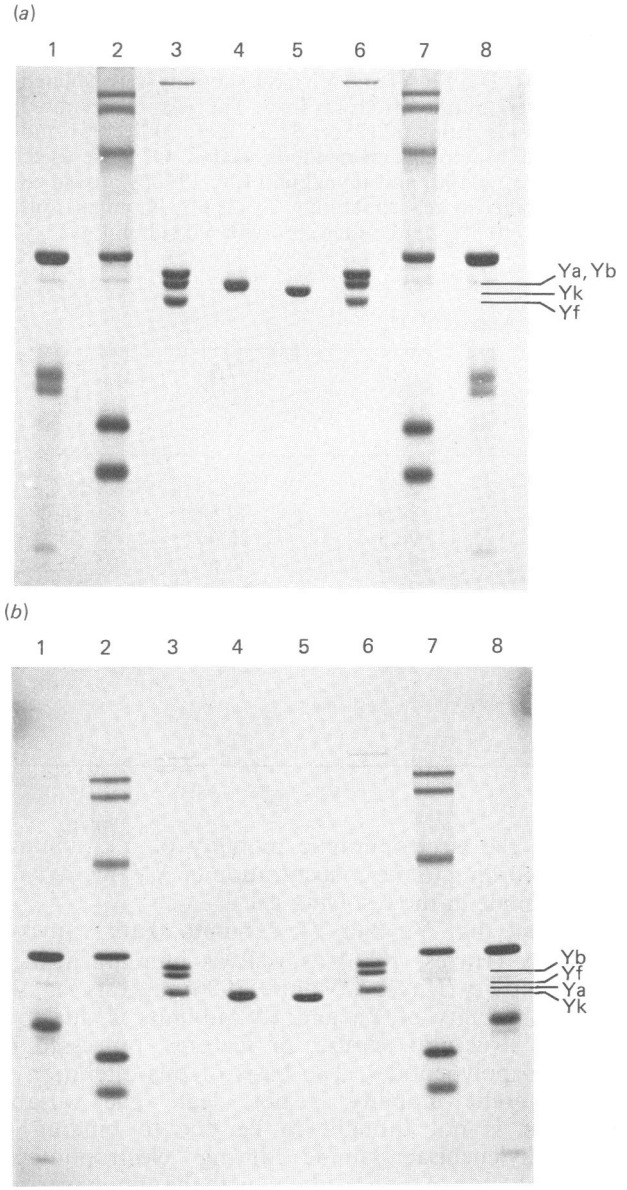


Fig. 2. Variable mobility of GSY Yk subunits in different polyacrylamide-gel matrices

Electrophoresis was carried out as described in the text with (*a*) 12% (w/v) polyacrylamide gels containing 0.60% (w/v) *NN'*-methylenebisacrylamide or (*b*) 15% (w/v) polyacrylamide gels containing 0.086% (w/v) *NN'*-methylenebisacrylamide. In both panels the same protein samples were applied as follows: tracks 1 and 8, 20 μg of a mixture of carbonic anhydrase and trypsin inhibitor; tracks 2 and 7, 40 μg of a mixture containing ovotransferrin, albumin, ovalbumin, chymotrypsinogen A, myoglobin and cytochrome *c*; tracks 3 and 6, 20 μg of rat lung GST enzymes purified by *S*-hexyl-GSH–Sepharose affinity chromatography (major lung GST enzymes comprise Yf, Yb and Yc subunits, minor polypeptide Ye); track 4, 10 μg of GST F (YaYa); track 5, 10 μg of GST K (YkYk).

(J. E. Fothergill, B. Dunbar & J. D. Hayes, unpublished work) possess a blocked *N*-terminus, whereas the Yf polypeptide, also termed Yp, the Yb polypeptide and the Yc polypeptide possess a proline residue at the

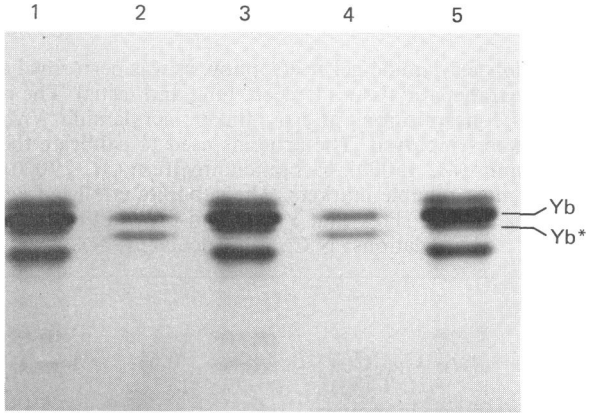


Fig. 3. Identification of the GST Yb* polypeptide in rat liver

Electrophoresis was performed in 15% (w/v) polyacrylamide gels comprising 0.086% (w/v) *NN'*-methylenebisacrylamide. The samples applied were as follows: tracks 1, 3 and 5, 20 μg of liver GST enzymes purified by *S*-hexyl-GSH–Sepharose affinity chromatography; track 2, 5 μg of GST P (Yb_1Yn); track 4, 5 μg of GST S (Yb_2Yn). The liver GST subunit that migrates between the Ya and Yb bands and is distinct from the Yn subunit found in transferases P and S is indicated by Yb*.

N-terminus (Frey *et al.*, 1983; Ding *et al.*, 1985; Telakowski-Hopkins *et al.*, 1985; Suguoka *et al.*, 1985).

Identification of Yb* polypeptides in the rat

When the rat hepatic *S*-hexyl-GSH–Sepharose-affinity-purified GST pool was examined with an SDS/polyacrylamide-gel system that contained low concentrations of cross-linker (i.e. C_{Bis} 0.6%), a polypeptide was resolved that possessed a marginally faster mobility than the major Yb-type subunit (Fig. 3). This polypeptide, which we have referred to as Yb* (Table 1), has not been identified before, because it is recovered with the main Yb band when resolving gels that possess a greater degree of cross-linking are employed. Examination of individual purified GST enzymes from rat liver showed that the Yb* polypeptide was associated with certain of the neutral/acidic enzymes that were isolated from the N3 pool (see Hayes & Chalmers, 1983; Hayes, 1986).

At present the number of Yb-type subunits in rat liver has not been formally established, but it is reasonable to assume the existence of a minimum of five polypeptides, Yb_1–Yb_5 (Hayes & Chalmers, 1983; Tu & Reddy, 1985). The identity of subunit Yb* has not yet been established, but it appears to be separate from subunits Yb_1, Yb_2 and Yb_3. To facilitate a uniform nomenclature, it will ultimately be desirable to refer to subunit Yb* as Yb followed, in subscript, by the appropriate subunit number (e.g. Yb_4). The identification of subunit Yb*, and the electrophoretic conditions required for its resolution, should prove very useful in discriminating between Yb-type subunits. The Yb* subunit is present in rat colon, rat spleen and rat thymus (results not shown) as well as hamster liver (Fig. 4).

Variable mobility of GST subunits in other species

The possibility that other species possess GST subunits displaying mobilities that vary according to the

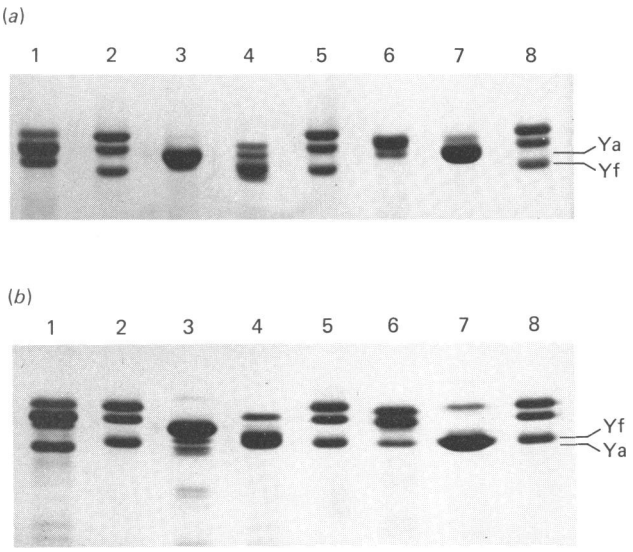


Fig. 4. Variable electrophoretic mobility of GST subunits in species other than the rat

Electrophoresis was performed with (*a*) 12% (w/v) polyacrylamide gels containing 0.32% (w/v) *NN'*-methylenebisacrylamide or (*b*) 15% (w/v) polyacrylamide gels containing 0.086% (w/v) *NN'*-methylenebisacrylamide. The transferase samples were all purified by *S*-hexyl-GSH–Sepharose affinity chromatography. In both panels the same protein samples (approx. 20 μg in each case) were applied as follows: track 1, rat liver GST enzymes; tracks 2, 5 and 8, rat lung GST enzymes; track 3, guinea-pig liver GST enzymes; track 4, mouse liver GST enzymes; track 6, hamster liver GST enzymes; track 7, human liver GST enzymes (this specimen contained GST μ). The positions of the Ya and Yf subunits are indicated.

amount of cross-linker in the resolving gel was investigated. In these experiments the rat lung transferases served as an 'internal standard', since the major polypeptides that these enzymes comprise (Yf, Yb and Yc subunits) display electrophoretic mobilities that are relatively constant (Table 1); rat lung also contains Ye subunits. Fig. 4 demonstrates that the electrophoretic band patterns of hepatic GST enzymes obtained from guinea pig, mouse, hamster and man varied considerably according to the amount of cross-linker employed.

Immunochemical identification of Yf, Ya, Yb and Yc subunits in man and rodents

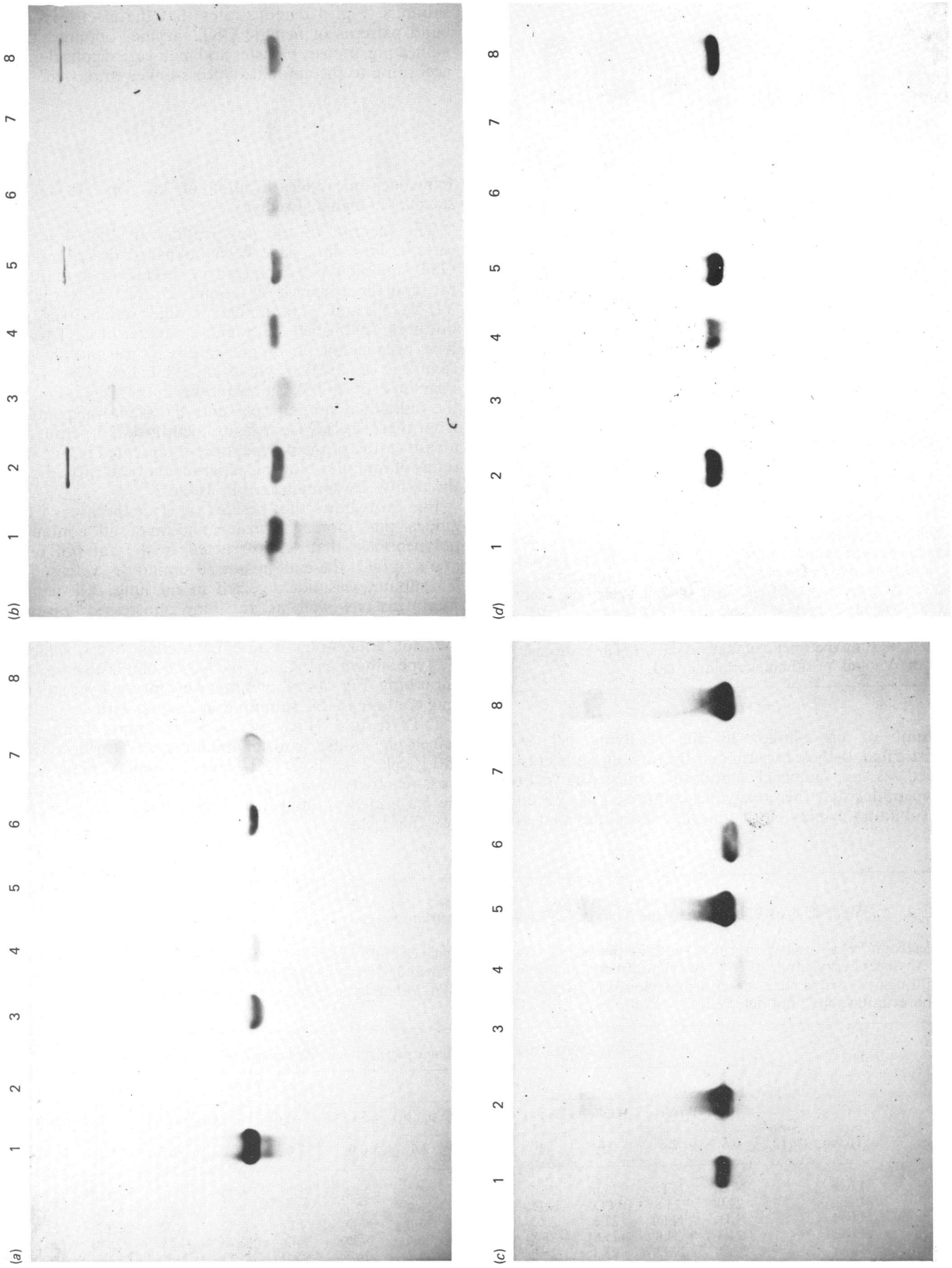
The identity of the polypeptides in Fig. 4 is not certain. Few data have been published describing the GST subunit types expressed in species other than the rat, and the existence of subunits equivalent to the rat Yf, Ya, Yb and Yc monomers in other rodent livers has not been established. Although a number of GST forms have been isolated from guinea pig, mouse and hamster (Smith *et al.*, 1980; Irwin *et al.*, 1980; Lee *et al.*, 1981; Pearson *et al.*, 1983), the variations in the size quoted for the individual subunits prevents the GST polypeptides from these species from being identified. To permit the identification of the polypeptides represented in Fig. 4, a series of immuno-blotting experiments were carried out; the results are summarized in Table 2.

Fig. 5(*a*) shows that besides rat liver the livers from guinea pig, mouse, hamster and man all contained polypeptides that cross-reacted with anti-[GST L (YaYa)] IgG; these all possessed similar M_r values. The Ya subunit was not detected in rat lung. All hepatic specimens, as well as rat lung, possessed Yb-type subunits (Fig. 5*b*). By contrast, rat and hamster livers, but not guinea-pig, mouse or human livers, express Yc-type subunits (Fig. 5*c*); the Yc subunit is also present in rat lung. Fig. 5(*d*) shows that only mouse liver and rat lung contain a GST subunit that reacted with anti-[GST λ (YfYf)] IgG (Fig. 5*d*). GST subunits from rat, guinea-pig, mouse, hamster and human liver probed with anti-[GST ϵ (B_1B_1)] IgG gave a similar pattern of cross-reactivity to that found in Fig. 5(*a*), indicating that the Ya and B_1 subunits are immunochemically related (see Table 2).

Table 2. Apparent sizes of Yf-, Ya-, Yb- and Yc-type GST subunits in various species

Electrophoresis was performed with either 12% (w/v) polyacrylamide gels that contained 2.6% (w/w) *NN'*-methylenebisacrylamide or 15% (w/v) polyacrylamide gels that contained 0.6% (w/w) *NN'*-methylenebisacrylamide. Immunoblotting was carried out as described previously (Hayes & Mantle, 1986). Values in parentheses represent weakly positive results. Abbreviation: N.D. not detected.

			$10^{-3} \times M_r$ of immunoreactive polypeptides with antisera raised against											
			Rat enzymes								Human enzymes			
			GST Y fYf		GST L (YaYa)		GST A (Yb_1Yb_1)		GST AA (YcYc)		GST λ		GST ϵ (B_1B_1)	
Species	Tissue	C_{Bis} (%) ...	0.6	2.6	0.6	2.6	0.6	2.6	0.6	2.6	0.6	2.6	0.6	2.6
Rat	Liver		N.D.	N.D.	23.0	25.5	26.1	26.3	27.1	27.5	N.D.	N.D.	(23.0)	(25.5)
	Lung		24.0	24.8	N.D.	N.D.	26.1	26.3	27.1	27.5	24.0	24.8	N.D.	N.D.
Guinea pig	Liver		N.D.	N.D.	23.2	25.3	25.5	26.0	N.D.	N.D.	N.D.	N.D.	23.2	25.3
Mouse	Liver		24.3	24.8	(25.8)	(25.8)	26.1	26.4	N.D.	N.D.	24.3	24.8	(25.8)	(25.8)
Hamster	Liver		N.D.	N.D.	23.3	26.0	25.7	26.7	26.5	27.0	N.D.	N.D.	23.3	26.0
Man	Liver		N.D.	N.D.	(23.3)	(25.9)	26.8	26.5	N.D.	N.D.	N.D.	N.D.	23.3	25.9


(b)
1 2 3 4 5 6 7 8
(d)
1 2 3 4 5 6 7 8
(a)
1 2 3 4 5 6 7 8
(c)
1 2 3 4 5 6 7 8

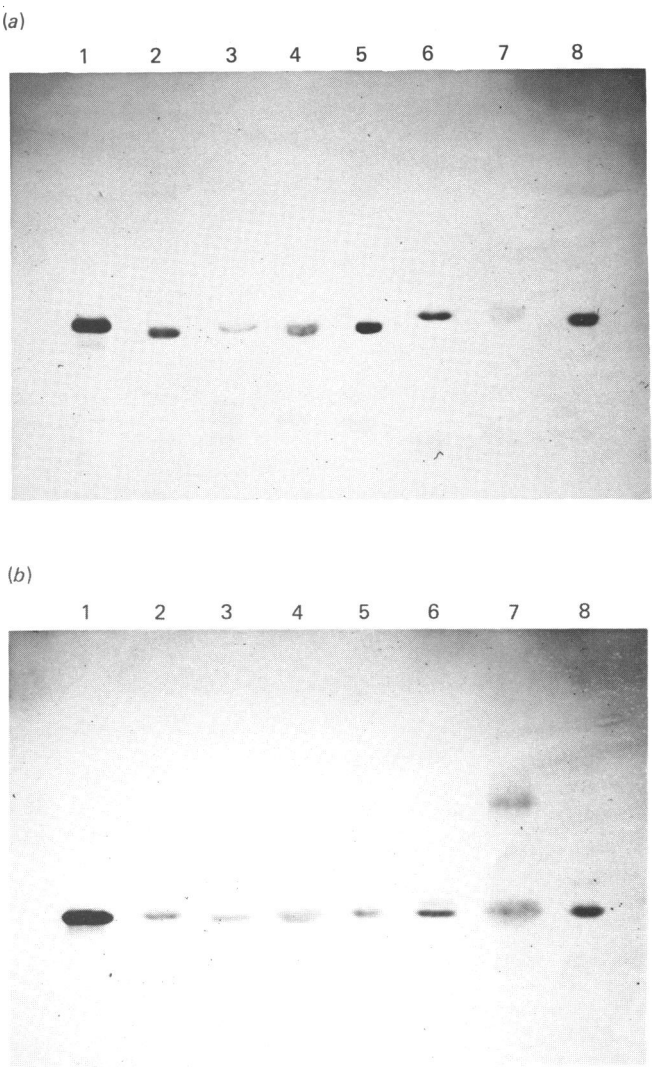


Fig. 6. Use of immuno-blotting to examine the relative electrophoretic mobilities of GST Yf and Ya subunits in different polyacrylamide-gel matrices

Electrophoresis was performed in either 12% polyacrylamide gels containing 0.32% cross-linker (*a*) or 15% polyacrylamide gels containing 0.086% cross-linker (*b*). The GST subunits were transferred to nitrocellulose and simultaneously probed with anti-(GST YaYa) and anti-(GST YfYf). The GST samples blotted in panels (*a*) and (*b*) were from: 1, rat liver; 2, rat lung; 3, guinea-pig liver; 4, mouse liver; 5, rat lung; 6, hamster liver; 7, human liver; 8, rat lung. Tracks 2, 4, 5 and 8 contained Yf subunits; tracks 1, 3, 4, 6 and 7 contained Ya subunits. Note that in (*a*) the GST Ya subunit in human liver shows little cross-reactivity with antiserum raised against rat GST YaYa but that significantly stronger cross-reactivity is observed in Fig. 5(*a*) and in (*b*) of this Figure.

Immunochemical identification of the GST subunits in guinea pig, hamster and man displaying cross-linker-dependent electrophoretic mobilities

The results in Fig. 5 showed that the samples of guinea-pig, hamster and human liver GST examined contain Ya but not Yf subunits, whereas, conversely, rat lung contains Yf but not Ya subunits. It was therefore possible to determine whether the Ya subunit in species other than the rat displays a cross-linker-dependent electrophoretic mobility by probing these samples directly by using combinations of antisera directed against Yf and Ya subunits. This experimental approach could not be applied to the mouse liver GST forms since this organ expresses both Ya and Yf subunits; the mouse Ya and Yf subunits were purified to allow their electrophoretic characteristics to be established (see below). Immuno-blots of gels, loaded as in Fig. 4 and containing either C_{Bis} 0.6% or C_{Bis} 2.6%, were constructed with combinations of either anti-[GST λ (YfYf)] IgG and anti-[GST L (YaYa)] IgG or anti-[GST λ (YfYf)] IgG and anti-[GST ϵ (B_1B_1)] IgG. The blots show that the relative positions of the rat Yf subunit and the Ya subunits of guinea pig, hamster and man change according to the amount of cross-linker in the resolving gel (Fig. 6).

Cross-linker-dependent electrophoretic behaviour of purified mouse GST subunits

The possibility that the relative mobility of the mouse Ya and Yf subunits can change according to the degree of cross-linker employed in the resolving gel was investigated with purified isoenzymes. The mouse enzymes, designated GST P1 (YfYf), P3 (YeYf), P4 (YaYa) and P5 (YbYb), were prepared, from the *S*-hexyl-GSH–Sepharose affinity pool, by using h.p.l.c. on hydroxyapatite (Hayes *et al.*, 1986). 'Dot blotting' has previously been used to show that GST P1 and P3 both react with anti-(rat YfYf) IgG whereas GST P4 and P5 cross-react with antisera raised against rat Ya and Yb subunits respectively (Hayes *et al.*, 1986). In gels that contained C_{Bis} 0.6% subunits of GST P1, P3, P4 and P5 all appeared as single bands; subunits of GST P1 and P3 co-migrated with the rat Yf subunit, subunits of GST P4 migrated between the rat Yf and Yb subunits, and subunits of GST P5 co-migrated with the rat Yb subunit (Fig. 7). At higher concentrations of *NN'*-methylenebisacrylamide the mobility of subunits of GST P1, relative to rat Yf subunit, was unaffected whereas GST P3 was resolved into two bands (i.e. Ye and Yf). Similar electrophoretic changes were observed in the Ye and Yf subunits in rat lung. By contrast, the relative mobility of GST P4 (YaYa) decreased as C_{Bis} was increased from 0.6% to 5.0% (Fig. 7). Under all conditions the GST P5 subunits and the rat Yb subunit co-migrated.

Fig. 5. Identification of GST Yf, Ya, Yb and Yc subunits in the livers of various species

The GST enzymes from rat, guinea pig, mouse, hamster and human liver were prepared and analysed by SDS/polyacrylamide-gel electrophoresis (with gels incorporating C_{Bis} 2.6%) as described in the legend to Fig. 4. Panels (*a*), (*b*), (*c*) and (*d*) show the nitrocellulose blots of the polyacrylamide gel probed with anti-(GST YaYa), anti-(GST Yb_1Yb_1), anti-(GST YcYc) and anti-(GST YfYf) respectively. The gel was loaded with approx. 20 μg of GST enzymes in each track as follows: track 1, rat liver GST enzymes; tracks 2, 5 and 8, rat lung GST enzymes; track 3, guinea-pig GST enzymes; track 4, mouse GST enzymes; track 6, hamster GST enzymes; track 7, human GST enzymes.

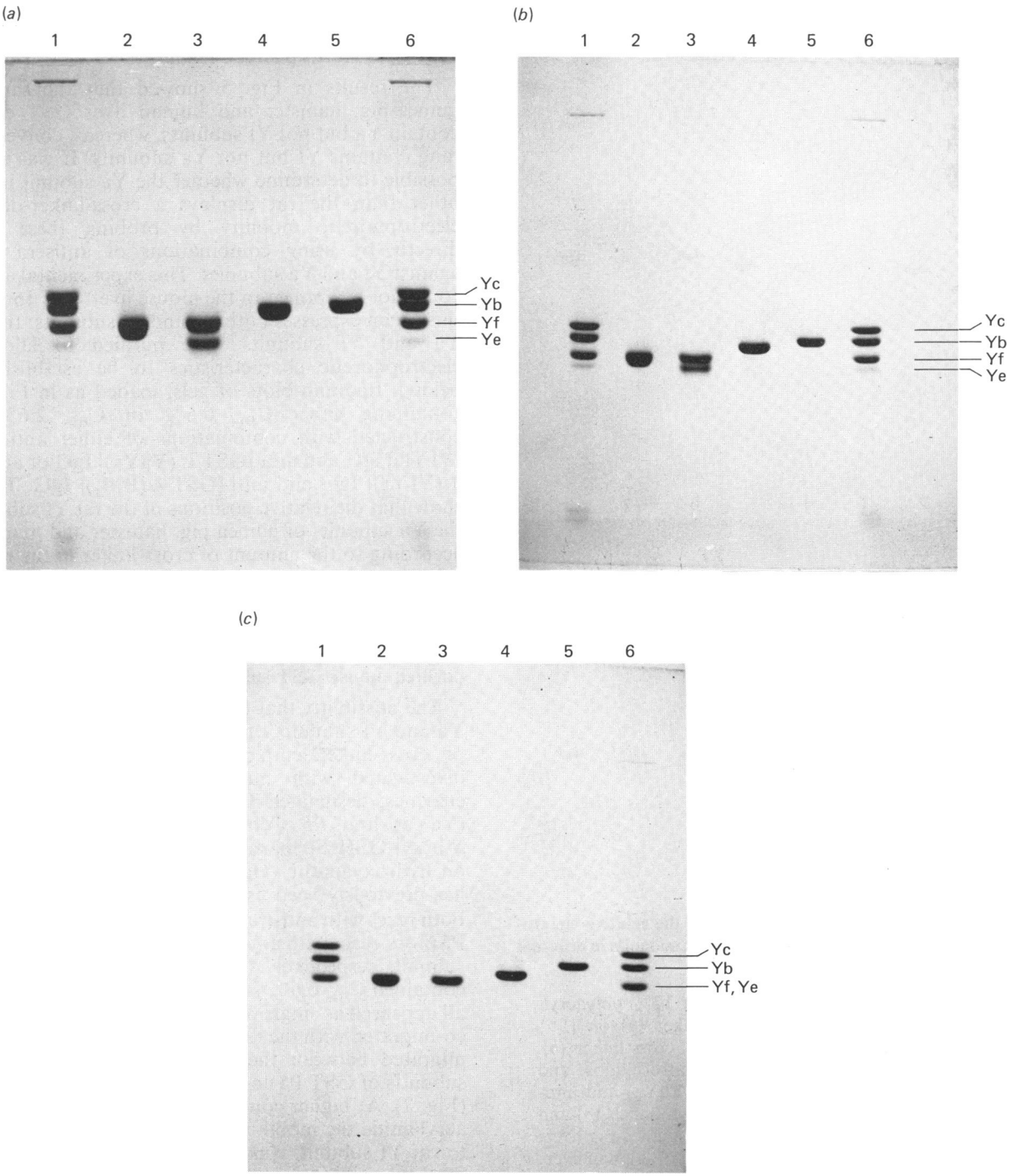


Fig. 7. Electrophoretic behaviour of purified mouse GST subunits

Electrophoresis was performed in polyacrylamide gels that contained various degrees of cross-linking: (*a*) C_{Bis} 5.0%; (*b*) C_{Bis} 2.6%; (*c*) C_{Bis} 0.6%. The polyacrylamide gels were each loaded as follows: track 1, rat lung GST enzymes (major subunits Yf, Yb and Yc); track 2, mouse GST P1; track 3, mouse GST P3; track 4, mouse GST P4; track 5, mouse GST P5; track 6, rat lung GST enzymes.

CONCLUSION

The use of SDS/polyacrylamide-gel electrophoresis has played a central role in determining the quaternary structure of GST enzymes. Although most workers in the field employ the discontinuous buffer system devised by Laemmli (1970), no attempt has been made to standardize the polyacrylamide gels used to study these isoenzymes. Generally, in protein studies, the ratio of monomeric acrylamide to cross-linking agent (*NN'*-methylenebisacrylamide) is often held at 30 to 0.8 or, less commonly, 30 to 1.6 (Davis, 1964; Maizel, 1971).

However, gels with ratios of acrylamide to cross-linker of 30 to 0.18 (Anderson *et al.*, 1973) or 30 to 0.15 have also been described (Tegtmeyer *et al.*, 1975). The characteristics of the polyacrylamide matrix used by the various research groups studying the transferases are seldom described, but differences do exist in the portion of total monomer represented by *NN'*-methylenebisacrylamide.

The data presented show that the degree of cross-linking used in the resolving gel is an important variable that can profoundly influence the electrophoretic separation of GST subunits isolated from various species. It is apparent that the influence of *NN'*-methylenebisacrylamide on the relative mobilities of GST subunits can lead to misclassification of these polypeptides. The differences in the mobilities of Ya and Yk subunits (i.e. group I GST subunits) observed in different electrophoretic systems may be exploited to facilitate their identification.

We have found that in certain circumstances individual samples that comprise several GST subunits migrated as a single polypeptide band during one set of electrophoretic conditions, whereas the same sample could be resolved into multiple bands under conditions that, though different, did not change the average R_F value of the subunits. For example, the mouse GST P3 sample contains two polypeptides (YeYf), which are clearly resolved in gels comprising C_{Bis} 5.0% but cannot be distinguished in gels comprising C_{Bis} 0.6%. Under the latter conditions GST P3 appears to contain the same subunit composition as the mouse GST P1 (YfYf). By contrast, when gels containing C_{Bis} 0.6% are used the hamster Yb and Yc subunits are clearly resolved, whereas these subunits possess similar mobilities in gels comprising C_{Bis} 2.6% (Fig. 4).

The choice of the 'best' electrophoretic conditions will vary according to the application. For example, the use of gels incorporating C_{Bis} 0.6% will prove a useful aid to elucidating the quaternary structures of the group II GST enzymes in the rat (i.e. Yb-, Yb*- and Yn-containing enzymes), but will be unhelpful during the study of mouse GST Ye and Yf subunits. Our data show that several concentrations of cross-linker should be used during the electrophoretic characterization of GST isoenzymes; possibly the use of resolving gels incorporating C_{Bis} 0.6% and C_{Bis} 2.6% should be recommended.

The differences in the M_r values of GST subunits calculated by different groups of workers can probably be attributed to the use of different marker proteins; chymotrypsinogen A (M_r 25700) and carbonic anhydrase (M_r 29000) are most commonly used for this purpose. In the present study chymotrypsinogen A exhibited a slower mobility than would be predicted from its M_r value in all the electrophoresis systems examined (Fig. 2). Indeed, on occasions chymotrypsinogen and carbonic anhydrase were found to have co-migrated during electrophoresis. We would therefore not recommend the use of chymotrypsinogen A as an M_r calibration protein.

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