Changes in Molecular Forms of Rat Hepatic Glutathione S-Transferase during Chemical Hepatocarcinogenesis

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ABSTRACT

Changes in molecular forms of hepatic cytosolic glutathione S-transferases (GSTs) during rat chemical hepatocarcinogenesis were investigated. GST activities toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene increased with the increased area of γ-glutamyltranspeptidase-positive foci and hyperplastic nodules induced by diethylnitrosamine followed by 2-acetylaminofluorene plus hepatectomy. Among GSTs with high activities toward 1,2-dichloro-4-nitrobenzene, which were separated by carboxymethyl Sephadex column chromatography, the activity of GST-A (YbYb') markedly increased with increased activity towards 1,2-dichloro-4-nitrobenzene in livers bearing foci and nodules and in isolated nodules and hepatomas, while activities of GST-C (YbYb') and -D (Yb'Yb') changed little. It was demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and two-dimensional gel electrophoresis that Yb as well as Ya, a subunit of ligandin (YaYa) and GST-B (YaYc), increased in livers bearing foci and nodules, while Yc as well as Yb' changed little. A new placental GST form (GST-P), which has a subunit molecular weight of 21,500 or 26,000, according to the marker proteins used, and neutral pIs of 6.8 and 6.3, is immunologically different from any form of basic GSTs and is very low in normal liver; also, it was markedly induced in livers bearing foci and nodules and in well-differentiated hepatomas but not by short-term administration of drugs such as 2-acetylaminofluorene, in contrast to GST-A and ligandin. These results indicate that GST-A and more especially GST-P could be new preneoplastic marker enzymes for chemical hepatocarcinogenesis.

INTRODUCTION

Hepatic GSTs3 (EC 2.5.1.18) are a group of multifunctional proteins involved in hepatic detoxication and are considered to play an important role(s) in chemical carcinogenesis. At least 6 or 7 forms of rat liver GST have been characterized by physicochemical properties (1, 5, 17, 18, 26, 30). Effects of several chemical agents such as PB (12, 17, 18, 28), trans-stilbene oxide (10), 3-methylcholanthrene (12), 3'-Me-DAB (4, 28), and tetrachloro-dibenzo-p-dioxan (1) on induction of certain molecular forms of GST, especially of ligandin and/or GST-B, have been well documented. However, changes in GST forms during chemical hepatocarcinogenesis have not been fully investigated.

Ligandin, one of the GST molecular forms, has been investigated as a basic azo dye- or bilirubin-binding protein (Y-protein) and was once reported to be identical with GST-B but is now separable from the latter (14, 15, 20, 26). GST molecular forms can be divided into at least 2 groups, either of which include 3 forms composed of 2 subunits (13, 21, 26). The first group, BL group, includes ligandin (YaYa), B (YaYc), and AA (YcYc) composed of 2 subunits, Ya (M, 22,000) and Yc (M, 25,000). The second group, AC group, includes A (YbYb) and D (Yb'Yb') (13, 26). Ligandin, B, AA, A, C, and D forms of GST correspond to L2, BL, B2, A2, AC, and C2, respectively, proposed by Mannervik and Jenson (26). Yb and Yb', which were named by Mannervik and Jenson (26) and correspond, respectively, to the Yb2 and Yb'2 proposed by Hayes (13), have a similar molecular size (M, 23,500), but Yb' is more acidic than Yb and differs immunologically (13, 21, 26). Two additional neutral or acidic GST forms were suggested to be present in rat liver cytosol (26). Recently, we have purified a GST form from rat placenta, tentatively named GST-P (34). It has an identical subunit (M, 21,500), slightly smaller than Ya, which is the smallest subunit in the AC and BL groups, has neutral pI(s), and differs immunologically from any of basic GSTs in the AC and BL groups.

It has been known that some enzymes such as γ-GTP and epoxide hydrolase are increased in preneoplastic hepatic tissues and are considered as hepatic preneoplastic marker enzymes (7, 8, 24, 31). In our previous reports (21, 35), we suggested that GST-A may be valuable as a new preneoplastic hepatic marker enzyme. In this report, we present evidence that GST-A, or the Yb subunit of GST-A, is induced during chemical hepatocarcinogenesis, while GST-C and -D, both of which are identical in molecular weight and immunologically related to GST-A, change little. Furthermore, we report that GST-P, which is hardly detectable in normal rat liver, is induced most markedly among GSTs in livers bearing γ-GTP-positive foci and hyperplastic nodules and that it differs from GST-A and ligandin in not being induced by the short-term administration of drugs such as AAF prior to appearance of γ-GTP-positive foci.

MATERIALS AND METHODS

Chemicals. CDNB was obtained from Aldrich Chemical Co., Milwaukee, WI; GSH was from Boehringer, Mannheim, West Germany; DCNB, DEN, and AAF were from Nakarai Chemical Co., Kyoto, Japan; CM-Sephadex C-50 was from Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals were of the highest purity available.

Treatments of Animals. Male Sprague-Dawley rats were purchased from Charles River Japan, Inc., Kanagawa, Japan; fed the basal diet (the Oriental MF diet from Oriental Yeast Co., Ltd., Tokyo, Japan); and maintained on a 12-hr-light-12-hr-dark cycle. Water and food were given ad libitum. Hepatic enzyme-altered foci or hyperplastic nodules were induced in rats weighing 160 to 180 g according to the system of Sott
and Farber (37), as shown in Chart 1, except that AAF was given for 2 weeks longer to induce larger foci and nodules. Enzyme-altered foci were counted after staining for \(\gamma\)-GTP activity as reported previously (40). AAF, 3'-Me-DAB, BHA, and PB were mixed with basal diet at 0.02, 0.06, 0.75, and 0.05%, respectively, and given to rats weighing 160 to 170 g for 2 or 4 weeks. Primary hepatomas were induced by DEN or AAF alone, as described previously (33), or by the combination of DEN and AAF, as shown in Chart 1, but without hepatectomy, followed by feeding with the basal diet for 20 or more weeks. The Yoshida ascites hepatoma AH 130 was maintained in male Sprague-Dawley rats by i.p. implantation in our laboratory and used within 8 to 8 days after inoculation.

Histological classification of hyperplastic nodules (neoplastic nodules) and hepatomas was performed according to the recommendation of a workshop on classification of specific hepatocellular lesions in rats (38).

Preparation of Tissue Extracts. Rats were starved for 18 hr before being killed by decapitation. Twenty % (w/v) liver homogenates were prepared with ice-cold 40 mM Tris-HCl buffer (pH 7.4), containing 154 mM KCl, 4 mM EDTA, and 5 mM diethiothreitol in Teflon-glass homogenizers and centrifuged at 6,500 \(\times\) g for 10 min; supernatants obtained were used for determination of total (reduced and oxidized) glutathione concentrations and then centrifuged at 105,000 \(\times\) g for 45 min; and the supernatants obtained were used for the assay and purification of GST molecular forms.

Analytical Methods. GST activities were assayed by using CDNB and DCNB, as described by Habig et al. (11). Specific activity is expressed as units/mg of protein, whereby 1 unit of enzyme was defined as the amount of enzyme needed to catalyze the conjugation of 1 \(\mu\)mol of GSH with CDNB or DCNB per min. Protein was determined by the method of Lowry et al. (25) with bovine serum albumin as a standard. Total glutathione levels in livers were determined by the method of Owens et al. (29).

Separation of GST-A from GST-C and -D by CM-Sephadex Chromatography. Basic molecular forms of GST in the AC and BL groups were separated by CM-Sephadex C-50 column chromatography, as reported previously (20), and named according to the method of Hayes et al. (13, 15). GST-C2 in our previous paper (21), which was named according to the method of Mannervik and Jenson (26), was defined as GST-D in this paper. GST-A was also separated from GST-C and GST-D, and further from GST-P, by using a minicolumn (1.0 x 5.0 cm) of CM-Sephadex C-50. Aliquots of 0.5 ml 105,000 \(\times\) g supernatants (10 mg/0.5 ml) from individual livers bearing hyperplastic nodules and hyperplastic nodules and hepatomas (above 0.1 g) large enough to be isolated by the naked eye were dialyzed against 10 mM sodium phosphate buffer, pH 7.4, and applied to the column. After the column was washed with 34 ml of the buffer to elute GST-C together with GST-D, GST-A was eluted stepwise with 0.1 M NaCl in the same buffer.

Separation of GST Subunits by Polycrylamide Electrophoresis. Subunits of GST, Ya, Yb including Yb', and Yc, were resolved by SDS-polyacrylamide slab gel electrophoresis according to the method of Laemmli (23), and further they and the Yp subunit of GST-P were separated from other proteins by 2-dimensional polyacrylamide gel electrophoresis according to the procedure of Takami and Busch (39). The marker proteins (products of Pharmacia Fine Chemicals) used as internal standards were (M, \(\times\) 10\(^3\)) phosphorylase (98/6.2), bovine serum albumin (66/6.4), ovalbumin (43/5.2), carbonic anhydrase (30/6.25), and soybean trypsin inhibitor (20/5.0). The separated GST subunits were identified by the above electrophoresis following immunoaffinity column chromatography using a Sepharose 4B column coupled with the specific anti-GST-A or -P antibody, as described previously (21).

Purification of GST-P from Rat Placenta. Details of the purification of GST-P from rat placenta will be published elsewhere. GST-P was purified to a homogeneous protein on SDS-polyacrylamide gel electrophoresis from placentas at the 15th day of gestation by sequential passing through immunoaffinity columns coupled with anti-GST-B or -C antibody. GST-P was not adsorbed to a CM-Sephadex C-50 column equilibrated with 10 mM sodium phosphate buffer, pH 6.8, and also not adsorbed to a hydroxylapatite column equilibrated with 50 mM potassium phosphate, pH 6.8, but it was adsorbed to a GSH affinity column and eluted with 20 mM GSH. Very small amounts of GSTs in AC and BL groups which were eluted together with GST-P were removed by sequential passing through immunoaffinity columns coupled with anti-GST-B or -C antibody. The antibody against GST-P as well as those against GST-A, -B, and -C was prepared as described previously (21).

RESULTS

Changes in GST Activities during an Early Stage of Hepatocarcinogenesis by DEN and AAF. During the induction of \(\gamma\)-GTP-positive foci and hyperplastic nodules by the system shown in Chart 1, GST activities towards CDNB and DCNB increased markedly from 5 to 6 weeks after the administration of DEN with appearance of large foci and nodules noticeable to the naked eye. In livers from control rats treated by the same schedule except for omission of DEN injection, very few foci appeared and GST activities toward the 2 substrates also changed little. Thus, increased GST activities seemed to be related to the induction of \(\gamma\)-GTP-positive foci. The activity of GST towards DCNB was more increased than that towards CDNB. Furthermore, the activities towards both CDNB and DCNB increased with increased area of \(\gamma\)-GTP-positive foci when examined at 6 weeks after DEN injection (Chart 2).

Molecular Forms of GST That Increased during Induction of the Foci. To detect which GST molecular forms contribute to the increased activities towards CDNB and DCNB, cytosol fractions obtained from livers bearing \(\gamma\)-GTP-positive foci and hyperplastic nodules were applied to CM-Sephadex C-50 columns, and the elution patterns of GST molecular forms were compared with those of control livers (21). It was concluded that the increased activity towards DCNB was mainly due to the increased activity of GST-A, while the increased activity towards CDNB was due to increased activities of both GST-A and ligandin. GST-C, which has a subunit in common with GST-A and
immunological properties similar to those of GST-A and also has a high specific activity towards DCNB, appeared together with GST-D in the breakthrough fractions from this column at pH 7.4. To examine more exactly which molecular form in the AC group increases with increased activity towards DCNB, we separated GST-C together with GST-D from GST-A in cytosols of livers bearing various amounts of area of γ-GTP-positive foci or in cytosols of isolated large hyperplastic nodules and hepatomas by using a minicolumn of CM-Sephadex C-50, as shown in Chart 3a. It is evident in Chart 3b that increased total activities towards DCNB are primarily due to increased activities of GST-A in most of the cases examined.

By SDS-polyacrylamide slab gel electrophoresis, GST forms in AC and BL groups can be separated into 3 subunits, Ya, Yb, and Yc, as shown in Fig. 1. It was noticed that Ya (the subunit of ligandin and GST-B) as well as Yb (the subunit of GST-A and -C) including Yb' (the subunit of GST-C and -D) was increased in livers bearing the foci and nodules (Fig. 1, Lane 3). As described precisely later, it was demonstrated by immunoaffinity column chromatography that the subunit (Yp) of GST-P, which is undetectable in normal liver (Lane 2), is induced in livers bearing foci and nodules (Lane 4), while it was unclear in the crude extract (Fig. 1, Lane 3) owing to the greatly increased Ya of ligandin.

Yb' was separable from Yb as a more acidic protein by 2-dimensional electrophoresis (Fig. 2). Yb had at least 2 charge isomers, both of which differed from Yb' immunologically, because GST-D (Yb' Yb') was not adsorbed to the anti-A antibody column, as reported previously (21). It was confirmed that Yb is markedly increased in livers bearing foci, while Yb' and Yc are increased slightly. The increase of Ya was not evident owing to its difficulty in entering the gel on first-dimensional isoelectric

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**Chart 2.** Relationships between area of γ-GTP-positive foci and GST activities toward CDNB and DCNB. Area of foci was determined as reported previously (40). GST activities in livers from rats used for Chart 1 together with other rats, similarly treated by the schedule shown in Chart 1, were determined at 6 weeks after DEN injection by using CDNB (a) and DCNB (b) as substrates. Statistical correlations in a and b are $Y = 4.66X + 364$, $r = 0.613$, $p < 0.02$ and $Y = 0.271X + 13.4$, $r = 0.781$, $p < 0.001$, respectively. Controls (●) are the same as in Chart 1. N, mean values in normal livers; bars, S.D.

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**Chart 3.** Changes in activities of GST-A and -C in livers bearing the foci and in isolated nodules and hepatomas estimated by using a CM-Sephadex C-50 minicolumn. The procedure is described in the text. In a, ○ and ● show elution patterns of GSTs in a normal liver and a liver bearing nodules, respectively. In b, activities of GST-A and GST-C including GST-D in several cytosols from livers containing hyperplastic nodules (●, ○) or in isolated hyperplastic nodules and hepatomas (●, □) are plotted against total activities towards DCNB. ○, □, activities of GST-C including GST-D; ●, □, activities of GST-A.

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**Fig. 1.** SDS-polyacrylamide gel electrophoresis of cytosolic proteins from normal liver and the liver bearing hyperplastic nodules before and after immunoaffinity column chromatography using anti-GST-P antibody. Lanes 1 and 2, 105,000 × g supernatant from normal liver; Lanes 3 and 4, the supernatant from the liver bearing nodules (Lane 4), while it was unclear in the crude extract (Fig. 1, Lane 3) owing to the greatly increased Ya of ligandin.

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M. Y. and M. Yc were estimated to be 26,000, 27,500, and 28,500, respectively, by using trypsin inhibitor (M, 20,000), carbonic anhydrase (M, 30,000), ovalbumin (M, 43,000), bovine serum albumin (M, 68,000), and phosphorylase (M, 98,000) as marker proteins, while they were estimated to be 22,000, 23,500, and 25,000, respectively, as reported by others, when α-chymotrypsinogen (M, 25,000) was used as a marker protein. The molecular weight of Yp was estimated to be 26,000 or 21,500 according to the marker proteins used (see also Ref. 34).
focusing, although it was evident on SDS-polyacrylamide gel electrophoresis, as mentioned above (Fig. 1).

Furthermore, it had been noticed on 2-dimensional electrophoresis that a protein, indicated as Yp in Fig. 2, appears under Yb' in the liver bearing the foci and nodules (Fig. 2b). This protein is hardly detectable in normal liver (Fig. 2a), has a molecular weight slightly smaller than that of Ya, and is more acidic than Ya and Yb. This protein has been identified to be the subunit of GST-P by immunoaffinity column chromatography using the anti-GST-P antibody, as shown in Fig. 1, Lane 4, and further on 2-dimensional electrophoresis following immunoaffinity column chromatography (data not shown), in the same way as GST-A was identified by using the anti-GST-A antibody (21), or as compared with the purified GST-P (34). GST-P was estimated to have a molecular weight of 26,000 in comparison with the marker proteins used by us, but it was estimated to be 21,500 when Ya is estimated to be 22,000 by using other marker proteins, as reported by others (14, 18, 26) (see also Ref. 34). It was noted that GST-P has at least 2 charge isomers with pI of 6.8 (the main protein) and 6.3 (the minor protein) (34), as is evident in Fig. 3b. It was also observed that certain proteins such as 52/6.7 and 36/6.7 (Mr x 10^3/pl), which as yet have not been identified, are increased or decreased in livers bearing foci and nodules, as indicated in Fig. 2, b and c, although most of them were changed by the short-term administration of drugs such as AAF (Fig. 2c).

Other Properties of GST-P. GST-P induced in livers bearing foci and nodules had a unique property in that it is not induced by the administration of 0.02% AAF alone in basal diet for 2 to 4 weeks prior to the appearance of the foci, as is evident in Fig. 2c. This is in contrast to GST-A (Yb) as well as ligandin (Ya), although the increased Ya subunit of ligandin is not evident in Fig. 2, b and c, owing to its difficulty to enter the gels. Similarly, Yp was not induced by the administration of 0.06% 3'-Me-DAB, 0.75% BHA, or 0.05% PB in basal diet for 2 weeks, while Ya and Yb were markedly induced, as also confirmed by SDS-polyacrylamide gel electrophoresis (data not shown).

The Yp subunit of GST-P was increased in well-differentiated hepatomas (Fig. 3b) but was negligible in poorly differentiated hepatomas such as AH 130 (Fig. 3c) and primary hepatomas induced by 3'-Me-DAB (data not shown) and also in late fetal liver (Fig. 3e), as was the Yb subunit of GST-A and the Ya subunit of ligandin (Fig. 3a, a and c).

DISCUSSION

GSTs are a group of multifunctional proteins localized mainly in the hepatic cytosol, and they participate in detoxication reactions of electrophilic compounds. Certain forms, particularly ligandin, are thought to interact with chemical carcinogen metabolites in vivo (36). An in vitro study has shown that the binding of carcinogen metabolites to nuclear DNA is inhibited by conjugation with glutathione (16). Therefore, these enzymes may be important in the inhibition (3) or even in the induction of hepatomas. Bannikov et al. (2) have demonstrated immunologically that basic azo dye-binding protein (ligandin) is absent in poorly differentiated hepatomas but is present in highly differentiated hepatomas.

We have been investigating the alterations of molecular forms of GSTs in the hepatic cytosol during chemical hepatocarcinogenesis, with special concentration on the early stages. We observed that the activity of GST-A as well as that of ligandin markedly increases with the appearance and increase of precancerous cell populations such as γ-GTP-positive foci and hyperplastic nodules. It was also demonstrated immunohistochemically that the increased activities are localized in the foci and nodules (35). This was especially evident when drugs such as the carcinogen AAF and the hepatocarcinogenic promoter PB were removed from the diet. GST-B (YaYc), which has GSH peroxidase activity, was also significantly increased in livers bearing foci and nodules (22), although this was not evident on CM-Sephadex column chromatography due to the remarkably increased GST-A (21, 22).

In our previous papers, we reported that GST-A is quite different from GST-AA, GST-B, and ligandin in the BL group of GST. GST-A is similar to GST-C and -D immunologically and in substrate specificity towards DCNB. However, it has been demonstrated in this paper that the Yb subunit of GST-A, but not the Yb' subunits of GST-C and -D, is preferentially induced in livers bearing γ-GTP-positive foci and hyperplastic nodules. Nemoto et al. (27) reported that GST-A and -C also have higher GSH S-epoxide transferase activities toward the K region of benzo(e)pyrene 4,5-oxide than do other forms. GST-E is also known to have a GSH S-epoxide transferase activity and to conjugate epoxides produced from carcinogens such as benzo(e)pyrene, but it seems to differ from molecular forms in the AC and BL groups in its molecular size and immunological properties (18). Changes in GST forms with GSH S-epoxide transferase activity during chemical hepatocarcinogenesis have not been investigated extensively.

In this paper, we have also demonstrated that GST-P, which differs immunologically from GST forms in AC and BL groups, is also markedly increased in livers bearing γ-GTP-positive foci and hyperplastic nodules. Immunohistochemically, GST-P seemed to be localized primarily in γ-GTP-positive foci, and the number or area of GST-P-positive foci increase with increased number or area of γ-GTP-positive foci with a close correlation (34). This form differs not only from other GST forms in AC and BL groups but also from other drug-metabolizing enzymes such as epoxide hydrolase, UDP-glucurononyltransferase, and cytochrome P-450 in that it is not easily induced by drugs such as AAF, 3'-Me-DAB, BHA, and PB. We have purified GST-P from the placenta, but it should be noted that GST-P is not abundant even in the placenta or in the fetal liver (Fig. 3). GST-P is also present in normal rat kidney, lung, and testis at significant but much lower levels than those in livers bearing foci and nodules.

Certain molecular forms of GSTs have GSH peroxidase activity (32). Our previous study (22) showed that selenium-dependent GSH peroxidase activity towards H2O2 markedly decreases with induction of enzyme-altered foci, but lowering of total GSH peroxidase activity to organic hydroperoxides such as cumene hydroperoxide is not so evident. Among purified forms, GST-AA, GST-B, and ligandin possess GSH peroxidase activity towards cumene hydroperoxide with specific activities decreasing in this order. GST-A possesses a very low GSH peroxidase activity. We have demonstrated that GST-B acts like GSH peroxidase in hyperplastic nodules partly to compensate for the decreased selenium-dependent GSH peroxidase activity during hepatocarcinogenesis (22).

It had been demonstrated histochemically that glutathione content was enhanced in enzyme-altered islands induced by carcinogens (6). We also confirmed through biochemical determination that the contents of total glutathione (mainly GSH) in
livers bearing the foci and nodules are significantly higher than those in control livers (22). They increased with increased number or area of foci and nodules (data not shown).

The possible roles of the increased activities of GST-A, ligandin, and GST-B with GSH peroxidase activity, and perhaps also of GST-P, and elevated GSH levels in enzyme-altered foci may be in relation to the resistance mechanism(s) of these cell populations to xenobiotics with cytotoxic activity. Farber et al. (8, 9) pointed out that preneoplastic hepatocyte foci have an important biological property of being relatively insensitive to the cytotoxic effects of hepatocarcinogens and that this mechanism of resistance might be based on decreased uptake of carcinogens into the foci and/or decreased activities of activating enzymes such as cytochrome P-450 and increased activities of inactivating (detoxicating) enzymes such as epoxide hydrase in the foci.

Several enzymes with increased activities, i.e., epoxide hydrase (24), the fetal form of UDP-glucuronyltransferase (41), and a nonspecific esterase, L-1 (19), have been known as biochemical markers for preneoplastic cell populations. GST-A, especially GST-P, both of which exist mainly in hepatic cytosol in contrast to the enzymes mentioned above, may be also good marker enzymes for preneoplasia. In fact, the increased GST-A levels were demonstrated immuno histochemically in γ-GTP-positive foci (35). GST-P has been also confirmed as being localized in the foci (34). GST-A and GST-P as well as the (iso)enzymes mentioned above, decrease during the progression of hepatocarcinogenesis from well-differentiated hepatomas to poorly differentiated hepatomas, while γ-GTP, one of the most useful hepatic marker enzymes, continues to increase or retains elevated activity. Fetal isoforms of glycosynthase such as hexokinase II and pyruvate kinase K (M2) are increased remarkably only in poorly differentiated hepatomas (33, 35).

REFERENCES


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Fig. 2. Two-dimensional electrophoresis of cytosolic proteins from normal liver and from \( \gamma \)-GTP-positive and -negative livers. a, normal liver; b, liver bearing the foci and nodules from a hepatocarcinoma rat given AAF for 4 weeks after DEN injection, as in Chart 1; c, liver from a rat given 0.02% AAF alone for 4 weeks. pH was determined by the pIs of marker proteins used in Fig. 1 and used here as internal standards. \( Y_a \), \( Y_b \), \( Y_b' \), \( Y_c \), and \( Y_p \) indicate the subunits of ligandin and GST-A, GST-B, GST-C, and GST-D, respectively. Upward and downward arrows, increased and decreased proteins other than GST subunits, respectively, as compared with those in normal liver. Two increased proteins (in b) were estimated to be 52/6.7 and 36/6.7 (M, \( \times 10^4 \) daltons), respectively.

Fig. 3. Two-dimensional electrophoresis of cytosolic proteins from fetal liver and primary and transplantable hepatomas. a, late fetal liver at the 20th day of gestation; b, well-differentiated primary hepatoma induced by AAF (0.025% in diet) for 20 weeks; c, transplantable ascites hepatoma AH 130 (harvested within 6 to 8 days after inoculation). Names of GST subunits are the same as those in Fig. 2.
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