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Biochemical Genetics of Glutathione-*S*-Transferase in Man

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SUMMARY

Glutathione-*S*-transferases from liver and erythrocytes have been separated by starch gel electrophoresis and localized by a specific staining procedure. The data suggest that the most active glutathione-*S*-transferases in liver are the products of two autosomal loci, GST_1 and GST_2. Both these loci are polymorphic, and there is evidence that a common null allele exists at the GST_1 locus. The glutathione-*S*-transferase expressed in erythrocytes is the product of a third locus, GST_3, and is not polymorphic.

INTRODUCTION

The glutathione-*S*-transferases (E.C. 2.5.1.18) are a family of important detoxifying enzymes that occur in many species [1], but have been studied most extensively in rat and human liver [2, 3]. These enzymes catalyze the reaction of reduced glutathione (GSH) with a wide variety of electrophilic compounds, leading to the formation of mercapturic acids [4], a pathway by which many pharmacologically active compounds are metabolized and eliminated. In addition, the glutathione-*S*-transferases have a high binding affinity for many nonsubstrates of both endogenous (e.g., bilirubin) and exogenous (e.g., bromosulfophthalein) origins [5].

Previous studies utilizing ion exchange chromatography and isoelectric focusing have revealed multiple forms of glutathione-*S*-transferase in human liver [3, 6]. These investigations studied liver samples from only four individuals, and although some variation between different samples was noted, the lack of a suitable technique to study numerous samples under identical conditions has prevented more extensive evaluation of this heterogeneity. Other recent studies have reported single forms of glutathione-*S*-transferase in erythrocytes [7] and placenta [8].

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We recently described a new method by which glutathione-*S*-transferase isozymes may be identified after starch gel electrophoresis of tissue extracts [9]. This method has now permitted the comparison of liver and red cell samples from several hundred individuals. The data obtained from this survey suggests the existence of an extensive polymorphism of glutathione-*S*-transferase in human liver.

MATERIALS AND METHODS

Samples

Liver samples from Chinese and Indian individuals were obtained at autopsy from the General Hospital, Kuala Lumpur. Liver samples from Caucasians were collected at autopsy at the Royal Children's Hospital, Melbourne, or the Royal Canberra Hospital. All samples were stored below −20°C. In the majority of cases, death resulted from cardiovascular disease or accidental trauma. No exclusions were made on the basis of cause of death. Samples were homogenized in 50 mM Tris/HCl, pH 7.4, centrifuged at 1,500 *g* for 10 min, and the supernatants applied to Whatman 3 MM chromatography paper strips and inserted into starch gels for electrophoresis.

Red blood cell samples were anticoagulated with heparin, washed three times in isotonic saline, and stored frozen in liquid nitrogen. Thawed lysates were applied to Whatman no. 17 chromatography paper strips and inserted into starch gels for electrophoresis.

Electrophoresis

Electrophoresis was carried out horizontally in starch gels between cooling blocks. Electrophoresis of the liver samples was carried out for 16 hrs at 4 V/cm using a Tris-EDTA-borate buffer, pH 8.6. The electrode buffer was a 1:7 dilution of a stock buffer solution containing Tris, 0.9 M; boric acid, 0.5 M; and disodium EDTA, 0.02 M. The gel buffer was a 1:10 dilution of the same stock solution. Electrophoresis of erythrocyte lysates was carried out for 16 hrs at 4 V/cm in a phosphate-citrate-EDTA buffer system, pH 6.0. The electrode buffer contained NaH_2PO_4, 0.25 M; trisodium citrate, 0.15 M; and disodium EDTA, 5 mM. The gel buffer was a 1:100 dilution of the electrode buffer.

Isozyme Detection

After electrophoresis, the gels were sliced and the cut surface stained for glutathione-*S*-transferase activity as described [9].

The specific staining procedure involves two steps. (1) Eight mg of 1-chloro-2,4-dinitrobenzene (Fluka A.G., Switzerland) in 0.8 ml ethanol is combined with 14 mg of reduced GSH (Sigma Chemical, St. Louis, Mo.) dissolved in 20 ml of 0.1 M potassium phosphate, pH 6.5. This solution is taken up into a filter paper sheet, overlayed on the cut surface of the gel, and incubated at 37°C. (2) After 40 min incubation, the filter paper overlay is removed, and the gel is developed by overlaying with a solution (0.9 ml of 1% I_2 in KI diluted in 30 ml water) with an equal volume of 2% molten agar. As the agar sets, an intense blue starch-iodine color appears immediately where GSH has been conjugated to 1-chloro-2,4-dinitrobenzene by the action of glutathione-*S*-transferase.

RESULTS

Electrophoresis of liver extracts at pH 8.6 revealed multiple isozymes of glutathione-*S*-transferase that migrated toward both anode and cathode. The exact nature of these patterns varied considerably from individual to individual.

The strongly staining components migrating toward the anode could be divided into three alternate types, consisting of either a fast (more anodal) migrating zone of

activity, a relatively slow migrating zone, or both the fast and slow components together with an intermediate band with a more intense staining reaction. There is some suggestion that the fast and slow zones may be composed of major and minor components, but these are not clearly resolved. A large proportion of samples failed to exhibit these strongly staining, anodal-migrating components. In addition, most samples exhibited an additional, very weakly staining component that migrated rapidly toward the anode. The electrophoretic mobility of these anodal-migrating components in liver extracts are shown in figure 1.

The isozymes migrating toward the cathode also exhibit different patterns and are shown in figure 2. These patterns do not resolve as clearly as the anodal components, and there is evidence for some minor bands trailing behind the major areas of activity. In each individual, the major cathodal component consists of either a fast (more cathodal) zone of activity, a relatively slow migrating zone, or both the fast and slow zones with an intermediate component with elevated activity. Unlike the anodal situation, at least one of these types was observed in virtually every sample. The small number of exceptions to this (19/198) also failed to show any activity in the anodal region and were probably inactive degraded samples and so were excluded from the analysis.

The triplet patterns migrating toward the anode or the cathode are characteristic of the patterns obtained from heterozygotes for a polymorphic dimeric protein. These similarities suggest a genetic origin for the observed variation, and since we

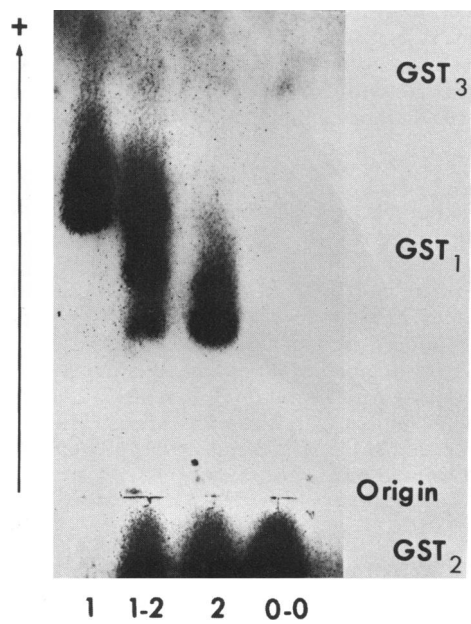

FIG. 1.—Electrophoretic separation of glutathione-*S*-transferase isozymes in human liver at pH 8.6. Relative mobilities of the products of the three loci are indicated on the *right*. Phenotypes of the *GST*$_1$ locus are given *below each sample*.

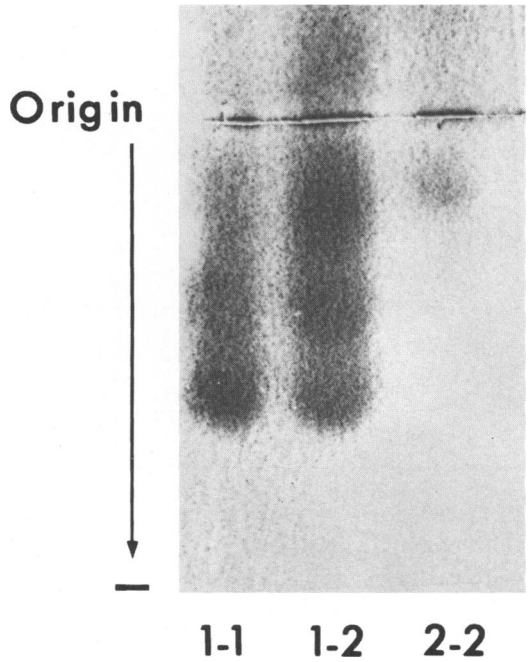

FIG. 2.—Electrophoresis of GST_2 products from liver migrating toward cathode

have observed varying combinations of the fast, slow, and triplet patterns migrating toward both the anode and the cathode, it seems likely that the strongly staining anodal and cathodal components are the products of different loci. We, therefore, propose that the strongly staining anodal components are the products of one locus (GST_1) and the cathodal components are the product of a second locus (GST_2). The electrophoretically fast, weakly staining, anodal component may be the product of a third locus. In accordance with this interpretation, the electrophoretically fast and slow components in either the anodal or cathodal directions represent allelic variation at the GST_1 and GST_2 loci, respectively. The electrophoretically fast forms have been termed type 1, and the slower forms, type 2. The apparent heterozygote triplet phenotypes have been termed 1-2. Examples of this variation have been identified according to this scheme in figures 1 and 2.

The distribution of these types in liver samples from 179 individuals from three racial groups has been compiled. The distribution of the different GST_1 types in each population sample is given in table 1. If this data is analyzed on the basis of there being two alleles at the GST_1 locus, and excluding individuals with a null phenotype, the observed and expected frequencies of each phenotype differ significantly. However, if the analysis is carried out including the individuals with a null phenotype as homozygotes for a null allele, and assuming that heterozygotes for a null allele would not be recognized, the observed and expected frequencies are in good agreement and the populations sampled appear to have achieved Hardy-Weinberg equilibrium.

TABLE 1

LIVER GST_1 PHENOTYPES FOR UNRELATED INDIVIDUALS

RACE	ANALYSIS		GST_1 1	2	2-1	0	
Chinese (no. = 96)	Two alleles	Observed	28	10	2	...	
		Expected	21.03	3.03	15.95	...	$\chi^2 = 30.5976$
	Three alleles	Observed	28	10	2	56	
		Expected	27.89	9.88	2.12	56.11	$\chi^2 = 0.0088$
Indian (no. = 43)	Two alleles	Observed	7	15	6	...	
		Expected	3.57	11.57	12.85	...	$\chi^2 = 7.9644$
	Three alleles	Observed	7	15	6	15	
		Expected	8.89	16.77	3.87	13.46	$\chi^2 = 1.9319$
Caucasian (no. = 40)	Two alleles	Observed	8	6	0	...	
		Expected	4.57	2.57	6.85	...	$\chi^2 = 14.0000$
	Three alleles	Observed	8	6	0	26	
		Expected	7.37	5.36	0.67	26.50	$\chi^2 = 0.8083$

The distribution of GST_2 phenotypes are given in table 2. Unlike the GST_1 locus, these data can be analyzed on the basis of a two-allele system, and the observed and expected phenotype frequencies are in good agreement. The gene frequencies of the common alleles at the GST_1 and GST_2 loci in the Chinese, Indian, and Australian Caucasian populations are given in table 3.

Examination of hemolysates at pH 6.0 revealed a single anodal-migrating component (fig. 3). The activity of this component was relatively weak in relation to the GST_1 and GST_2 products observed in the liver. This single component was a feature of hemolysates from 100 Australian Aborigines, 120 Australian blood donors (Caucasian), and 100 Tokelau Islanders. The single component seen in erythrocytes has a slightly faster electrophoretic mobility than the fast, weakly staining, anodal component seen in liver samples when they are compared at pH 6.0 (data not shown).

DISCUSSION

Previous reports have noted the presence of multiple forms of glutathione-*S*-transferase in human liver [3, 6]. The data presented here confirm these findings and

TABLE 2

LIVER GST_2 PHENOTYPES FOR UNRELATED INDIVIDUALS

RACE		GST_2 1	2-1	2	
Chinese (no. = 96)	Observed	62	32	2	
	Expected	63.30	29.40	3.30	$\chi^2 = 0.7671$
Indian (no. = 43)	Observed	25	17	1	
	Expected	26.01	14.98	2.01	$\chi^2 = 0.8216$
Caucasian (no. = 40)	Observed	28	11	1	
	Expected	27.99	11.03	0.99	$\chi^2 = 0.0002$

TABLE 3

FREQUENCY OF ALLELES AT THE GST_1 AND GST_2 LOCI

Population	No.	GST_1^1	GST_1^2	GST_1^0	GST_2^1	GST_2^2
Chinese	96	.1709	.0646	.7645	.8125	.1875
Indian	43	.1614	.2790	.5596	.7791	.2209
Caucasian	40	.1061	.0784	.8154	.8375	.1625

show that there is a high degree of heterogeneity between individuals. It was previously suggested that, because of the similarities in molecular weight, amino acid composition, substrate specificity, and immunological identity of the multiple forms, the glutathione-*S*-transferase isozymes in human liver may be the product of posttranslational modification of a single gene product [3]. The present data do not support that conclusion. The heterogeneity between individuals, the triplet patterns of apparent heterozygotes, and agreement with Hardy-Weinberg equilibrium all suggest a genetic interpretation is more appropriate. Unfortunately, because family data are unobtainable for such studies, complete formal proof of the present genetic interpretation is not possible.

The present data indicate the existence of at least three loci coding for glutathione-*S*-transferase in man. Three loci are expressed in the liver, although the GST_3 product is only a minor contributor to the total liver complement of glutathione-*S*-transferase, as assessed by reaction with 1-chloro-2,4-dinitrobenzene. The presence of the GST_3 product was not ascertained in all liver samples because of its low reactivity.

The present interpretation of the data indicates the presence of a null allele at the GST_1 locus. The presence of a null allele should lead to an apparent excess of homozygotes and a deficiency of heterozygotes if the heterozygotes for the null "normal" alleles (GST_1^{1-0} and GST_1^{2-0}) are identified as apparent homozygotes. This is, in fact, the case if the data for GST_1 are analyzed solely on the basis of the two observable alleles (GST_1^1 and GST_1^2). Null alleles can result from the production of an inactive or unstable product or failure to produce a product. Glutathione-*S*-transferase from human liver has been shown to be a dimer [3], and, consequently, heterozygotes for the observable alleles at each locus show a triplet electrophoretic pattern composed of homo- and heterodimers. If an inactive product or a product with altered substrate specificity was formed, heterozygotes with either the GST_1^1 or GST_1^2 alleles would be expected to express a double-banded pattern, since the electrophoretic mobility of the inactive GST_1^0 product must differ from either GST_1^1 or GST_1^2 or both. Since no double-banded patterns were observed, it is, therefore, likely that the null allele results from failure to produce a product. However, this possibility is equivocal, since the synthesis of an inactive product that is incapable of forming dimers or, alternatively, that inactivates the heterodimer would also result in the single-banded "homozygote" expression of heterozygotes with the null allele.

Considering the apparent similarities of the GST_1 and GST_2 products [3], it seems likely that these loci are the result of gene duplication. In the three racial groups studied, the high frequency of GST_1^0 and the presence of null homozygotes clearly

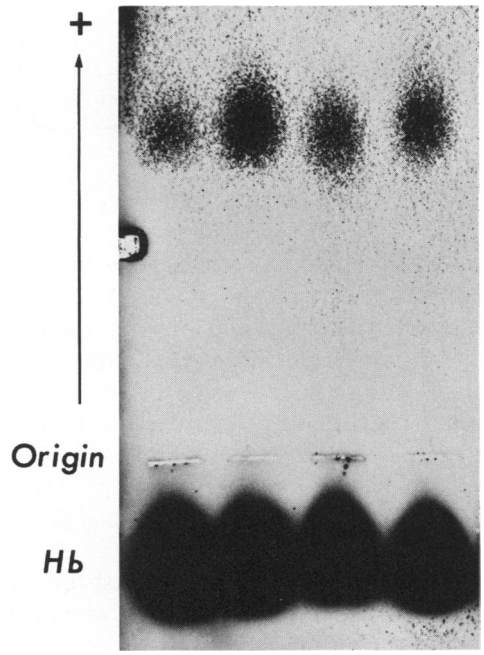

FIG. 3.—Electrophoresis of GST_3 products from erythrocytes

indicate that there is little, if any, disadvantage to individuals deficient in the products of the GST_1 locus. It is logical to consider that a recessive null allele may easily survive at a duplicate locus when the normal product is provided by the alternate locus. The theoretical aspects of similar situations have been considered [10], but the factors that prevent the fixation of a similar null allele at the alternate locus are not clear. The presence of the same alleles in approximately the same frequencies in the three racial groups studied suggests that the gene duplication and polymorphism have been present in human populations for a considerable period.

Although not specifically demonstrated in the data presented here, numerous male heterozygotes at the GST_1 and GST_2 loci were observed, indicating that these loci are autosomal.

The glutathione-*S*-transferase expressed in erythrocytes has been shown to be immunologically and physiochemically dissimilar to the major glutathione-*S*-transferases found in the liver [7]. This, together with the finding that it does not show any electrophoretic polymorphism, indicates that it is not the product of the GST_1 or GST_2 loci. During the preparation of this manuscript, a form of liver glutathione-*S*-transferase with an isoelectric point (4.63) identical with that found in erythrocytes was reported [11]. This form contributes less than 0.1% of the total glutathione-*S*-transferase activity in the human liver. Although our experiments show that, at pH 6.0, erythrocyte glutathione-*S*-transferase has a slightly faster electrophoretic mobility than the electrophoretically fast, weakly staining component from liver, we cannot be certain whether or not they are the product of the same

locus. Resolution of this problem will depend on the discovery of genetic polymorphism or on further extensive biochemical characterization of these enzymes. Until these investigations can be carried out, we suggest that the locus controlling the glutathione-*S*-transferase in erythrocytes be termed GST_3 and that the similar component in liver be tentatively assigned to that locus. A single form of glutathione-*S*-transferase has been purified from placenta [8]. Further studies are in progress to determine if this isozyme is also the product of the GST_3 locus.

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