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# THE BASIC GLUTATHIONE S-TRANSFERASES FROM HUMAN LIVERS ARE PRODUCTS OF SEPARATE GENES

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SUMMARY: We have characterized a second cDNA sequence, pGTH2, for the human liver glutathione S-transferases $H_a$ subunits. It is 95% homologous base-for-base to the $H_a$ subunit 1 cDNA, pGTH1, except for its longer 3' noncoding sequences. Our results indicate that the multiple basic human liver glutathione S-transferases are products of separate genes. The proposal [Kamisaka, K., Habig, W. H., Ketley, J. N., Arias, I. M., and Jakoby, W. B. (1975) Eur. J. Biochem. 60, 153-161] that deamidation may be a physiologically important process for generating glutathione S-transferases isozyme multiplicity can be all but ruled out. 

The glutathione S-transferases (GST, EC 2.5.1.18) are a family of dimeric proteins that are multifunctional in drug biotransformation, xenobiotics metabolism, and protection against peroxidative damages (for a review, see Ref. 1 and 2). At least eight classes of subunits have been identified in various rat organs and tissues; $Y_\alpha$ ($M_r$ 24,400), $Y_a$ ($M_r$ 25,600), $Y_n$ ($M_r$ 26,300), $Y_b$ ($M_r$ 27,000), $Y_c$ ($M_r$ 28,000), $Y_\beta$ ($M_r$ 26,300), $Y_\delta$ ($M_r$ 25,500), and $Y_p$ ($M_r$ 24,000) (3-10). Analyses of rat GST cDNAs for the $Y_a$, $Y_b$, $Y_c$, and $Y_p$ subunits lead to the proposal that rat GSTs are products of a supergene family (11,12). Multiple GST isozymes of overlapping substrate specificities are required to detoxify a multitude of xenobiotics in addition to serving other important physiological functions, such as protection against peroxidative damage (1-3).

Multiple basic GSTs from human livers, α-ε, which are identical immunologically, were suggested to be deamidation products from a single gene (13). Neutral and/or acidic forms have been reported to have variable biochemical and immunological properties relative to the basic isozymes (1,13-19). Recently, thirteen forms of human liver GSTs were purified using a

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combination of GSH-affinity chromatography and chromatofocusing (20). All of the isozymes exhibited both GST and GSH peroxidase activities, but they had different binding affinity toward hematin (20). They are composed of at least two classes of subunits, $H_a$ ($M_r$ 26,000) and $H_b$ (27,500), according to their relative electrophoretic mobilities to rat GSTs (21). We have demonstrated a definitive immunological cross-reactivity, between the human liver and various rat GSTs and nucleotide sequence homology between their respective cDNAs (21,22). In this communication, we present the characterization of a second $H_a$ subunit cDNA, pGTH2. Its coding sequence is designated as human GST subunit 2. We also address the question on the molecular basis of isozyme multiplicity for the basic human liver GSTs.

## EXPERIMENTAL PROCEDURES

Nucleotides and enzyme reagents: The dNTPs and ddNTPs were obtained from Pharmacia-P.L. Biochemicals (Milwaukee, WI). The four dNTPs [$\alpha$-$^{32}$P] and dATP [$\alpha$-$^{35}$S] were purchased from ICN Pharmaceuticals (Irvine, CA) and New England Nuclear (Boston, MA), respectively. Restriction endonucleases, DNA ligases, DNA polymerase I and its Klenow fragments were products of New England Biolabs (Beverly, MA) and Boehringer Mannheim (Indianapolis, IN). $^{125}$I-protein A was obtained from Amersham Corp. (Arlington Heights, IL). Antiserum against human liver GSTs (S-hexyl GSH affinity column fractions) was previously described (21). The human liver cDNA library was generously provided by Professor Harry Harris and Mr. Mitchell J. Weiss of the University of Pennsylvania. The poly(A) RNAs used in the cDNA library construction were isolated from a male liver (21).

DNA purification and sequence analyses: Phage DNAs ($\lambda$DNA and M13 DNA) were isolated from plate lysates and liquid cultures according to published procedures (23,24). Plasmid DNAs were isolated by the method of Birnboim and Doly (25) as modified (23). DNA sequencing was carried out with the dideoxy-chain termination procedure (26) using M13 mp18 and mp19 subclones (24).

Computer analysis of DNA sequence homology: DNA sequence homology among various GST cDNAs was measured by the Dot Matrix Program of Zweig (27).

## RESULTS AND DISCUSSION

Isolation and characterization of $H_a$ subunit cDNA clones.

We plated out ~250,000 plaques of the human liver cDNA library on *E. coli* strain Y1090 and screened the corresponding nitrocellulose filters with antiserum against affinity column purified human liver GSTs and $^{125}$I-protein A according to published procedures (28,29). Eleven positive plaques were obtained through three rounds of successive plaque purifications. Phage DNAs were purified from plate lysates for each plaque and restricted with EcoRI. All of them contain single EcoRI insert of ~1 kb in size and hybridize well with the nick-translated pGTH1 cDNA insert by Southern blotting procedure (30). Each EcoRI insert was subcloned into the EcoRI site of pAT153 and the EcoRI site of M13 mp18 and mp19 vectors. The M13 subclones were analyzed further by DNA sequence analysis using the dideoxy-chain termination method

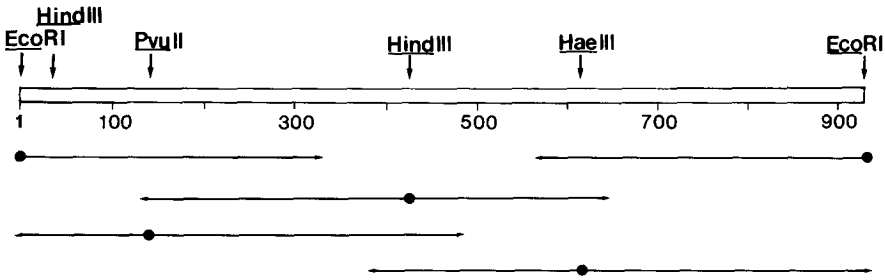


Figure 1. Sequencing strategy for the $H_a$ subunit 2 cDNA, pGTH2. The restriction sites used for M13 subcloning were identified. DNA sequences were read from the restriction sites proximal to the sequencing primer to the extent indicated by arrows. The open box represent the cDNA insert in pGTH2.

and [$\alpha$-$^{35}$S]dATP. Sequencing results revealed that 10 of the 11 $\lambda$gt11 cDNA inserts are identical to or slightly shorter than $\lambda$GTH1 cDNA. Only one is different from the $\lambda$GTH1 or $H_a$ subunit 1 sequence. This clone was designated $\lambda$GTH2 and its subclone in pAT153 as pGTH2.

The $\lambda$GTH2 cDNA insert was completely sequenced according to the strategy shown in Figure 1. Its sequence is presented in Figure 2 relative to the $H_a$ subunit 1 sequence in pGTH1 (22). There are seven and twenty-four nucleotide substitutions in the 66 nucleotide long 5' noncoding sequences and the coding sequences, respectively, between these two clones. Five out of the 11 amino acid substitutions in the 222 amino acid open reading frame are conservative with the same side chain groups (31,32). The 3' noncoding region of pGTH2 cDNA insert is significantly longer than that of pGTH1, however. In the region covered by pGTH1 cDNA 3' noncoding sequences (78 nucleotides), there are a total of 11 substitutions between the two clones. The poly(A)-addition signal in the subunit 1 cDNA AATAAA is changed into AATTAA in pGTH2 cDNA (nucleotide #795). Consequently, the mRNA for $H_a$ subunit 2 is longer. There is no AATAAA or AGTAAA sequence in the pGTH2 cDNA insert. It does not contain the poly(A) sequence which a complete cDNA clone should have. The $H_a$ subunit 1 and subunit 2 as defined by these two cDNA sequences are clearly products of two separate genes, however. These two sequences may be members of the same $H_a$ multigene family. Considering the possibility of heterodimer formation, subunits 1 and 2 may generate three different basic isozymes. The fact that pGTH1 cDNA sequences were identified approximately 10 times as frequently as the pGTH2 cDNA sequences from the same library suggest that the $H_a$ subunit 1 may correspond to the most abundant $H_a$ subunit in the human liver. The identification of a second $H_a$ subunit mRNA containing very different 3' noncoding sequences provokes serious reconsideration on the proposal that deamidation may be a physiologically important process for generating human liver GST isozymes (13). A genetic basis for human liver GST subunit

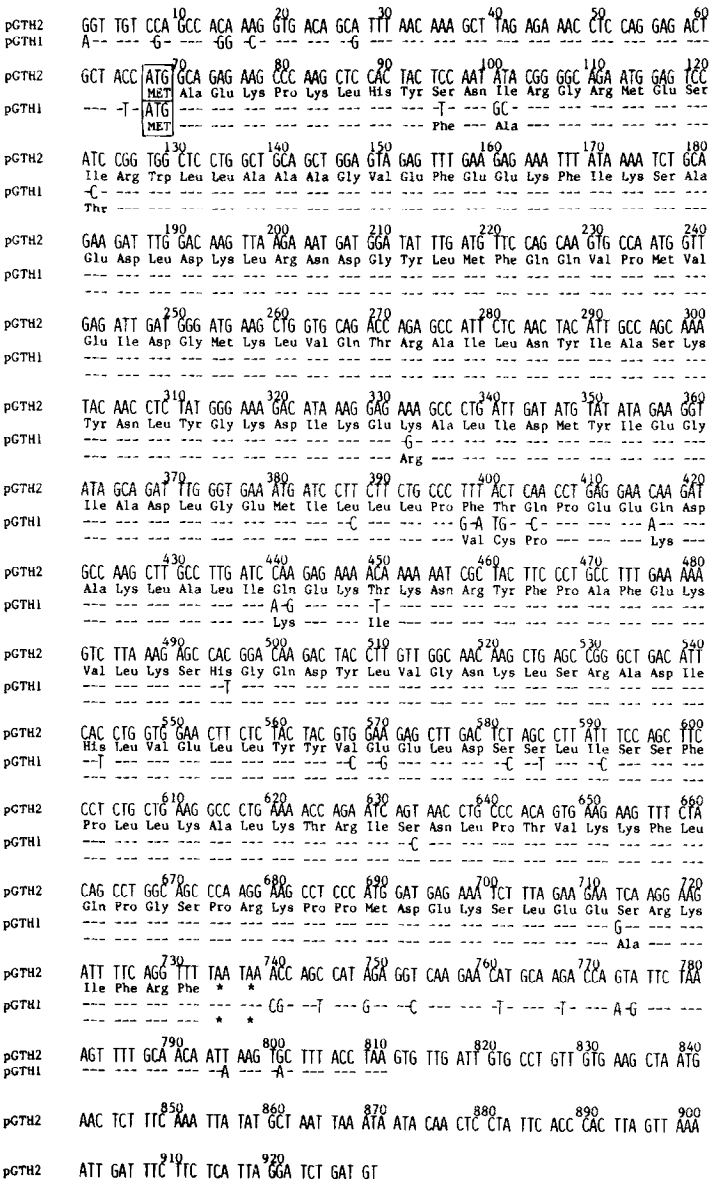

Figure 2. Comparison of nucleotide sequences of the pGTH2 ($H_a$ subunit 2) and pGTH1 ($H_a$ subunit 1) cDNAs. The putative initiation codon ATG is boxed for each cDNA. Nucleotides and amino acids of pGTH1 that are different from the pGTH2 cDNA are as indicated. The two consecutive termination codons TAA in each open reading frame are labelled with *'s.

heterogeneity or multiplicity is evident from this study and other recent reports (21,22).

Sequence relationship of human GSTs to other GSTs.

Comparison of pGTH2 cDNA sequence with those of rat and corn origins revealed significant nucleotide sequence homology. The pGTH2 nucleotide sequence and $Y_a$, $Y_c$ cDNA sequences (33-35) are ~80% identical base-for-base

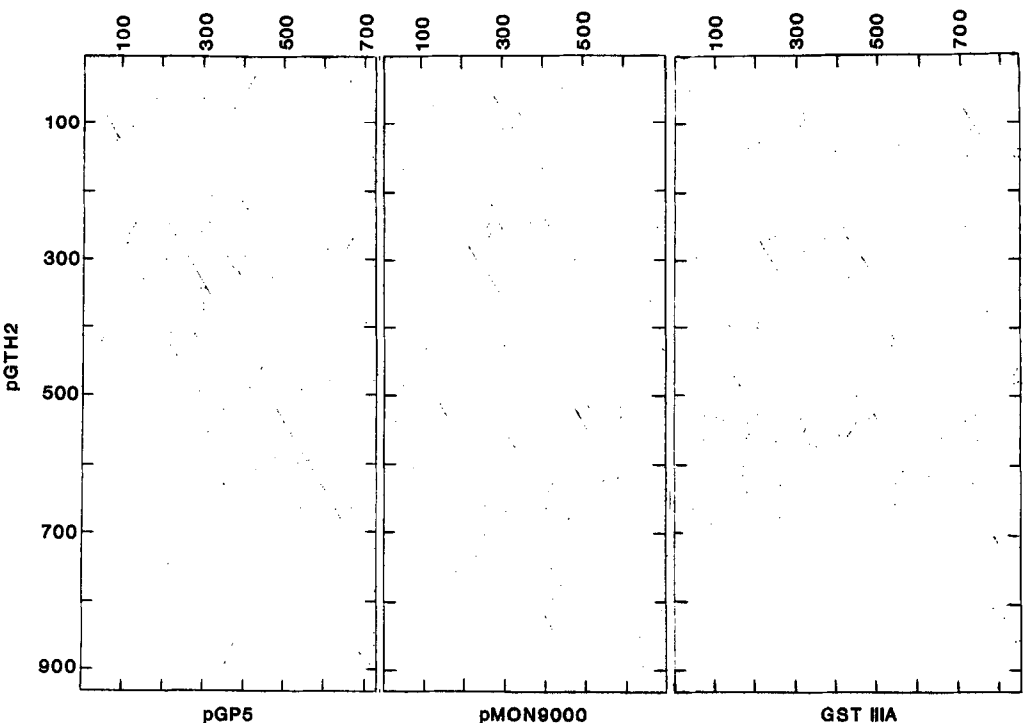


Figure 3. Nucleotide sequence homology between pGTH2 cDNA and GST cDNAs of rat and corn origins. pGP5, $Y_p$ (36); pMON9000, corn GST I (37); GST IIIA (38). Analyses were carried out by an IBM PC/AT using the Dot Matrix program of Zweig (27) with a parameter of 20 matches in a span of 25 nucleotides.

without any deletion or insertion. Dot Matrix analysis of pGTH2 cDNA sequence against pGP5 ($Y_p$) (36) and two of the corn cDNA sequences[1], pMON9000 (37) and GSTIIIA (38) is presented in Figure 3. Short segments of sequence homology definitely exist between these sequences. All of these subunits catalyze the conjugation of GSH to 1-chloro-2,4-dinitrobenzene (CDNB). Significant amino acid sequence conservation among the various rat GST subunits and human $H_a$ subunit 1 have been reported earlier (12,22). We have expanded in this communication the amino acid conservation analysis to include the corn cDNA sequences (37,38) as shown in Figure 4. There are 12 amino acids conserved throughout these GST coding sequences. An additional 24 residues belong to the same side chain groupings (31,32). Besides, 59 additional residues are conserved in three out of the four categories of GST sequences listed: $Y_a$, $Y_c$, $H_a$, $Y_p$, $Y_b$, and corn GST subunits. Therefore, ~43% of the amino acid residues in GSTs may be under evolutionary pressure to stay conserved. These are very significant conservations considering the evolutionary distance between them. The regions between amino acid positions 60 to 100 in Figure 4 appear to contain much higher percentage of these conserved residues. This

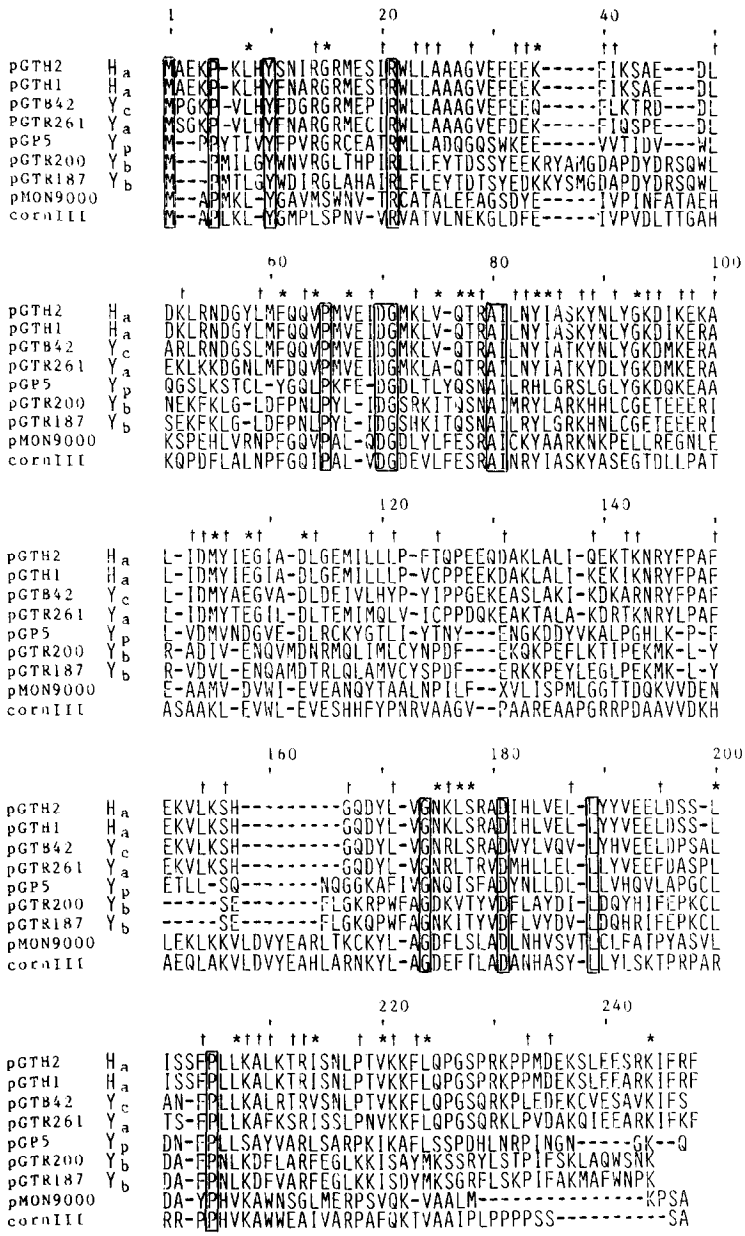


Figure 4. Amino acid sequence conservation between the human GST $H_a$ subunits 1 and 2 and GSTs of rat and corn origins. Identical amino acids are in boxes. Amino acids of the same groups (i.e. small polar, S, G, D, N; large polar, E, Q, K, R; intermediate polarity, Y, H, W; large nonpolar, F, M, L, I, V; and small nonpolar, C, P, A, T) are labelled with the asterisk, *. The daggers, †, denote amino acids of the same group in three out of the four categories: $H_a$, $Y_a$, $Y_c$; $Y_p$; $Y_b$; and corn GSTs.

region may be important for GSH binding or recognition of the hydrophobic substrates.

In conclusion, we have presented evidence that the basic human liver GSTs are products of separate genes. To fully account for the number of isozymes

observed by Vander Jagt et al. (20), more $H_a$ subunit cDNA may be isolated in the future.

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