Rat Liver Glutathione S-Transferases

NUCLEOTIDE SEQUENCE ANALYSIS OF A Yb_s cDNA CLONE AND PREDICTION OF THE COMPLETE AMINO ACID SEQUENCE OF THE Yb_s SUBUNIT*

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We have constructed a nearly full length cDNA clone, pGTA/C44, complementary to the rat liver glutathione S-transferase Yb, mRNA. The nucleotide sequence of pGTA/C44 has been determined, and the complete amino acid sequence of the Yb, subunit has been deduced. The cDNA clone contains an open reading frame of 654 nucleotides encoding a polypeptide comprising 218 amino acids with $M_r = 25,919$. The NH$_2$-terminal sequence deduced from DNA sequence analysis of pGTA/C44 is in agreement with the first 19 amino acids determined for purified glutathione S-transferase A, a Yb, homodimer, by Frey et al. (Frey, A. B., Friedberg, T., Oesch, F., and Kreibich, G. (1983) J. Biol. Chem. 258, 11321–11325). The DNA sequence of pGTA/C44 shares significant sequence homology with a cDNA clone, pGT55, which is complementary to a mouse liver glutathione S-transferase (Pearson, W. R., Windle, J. J., Morrow, J. F., Benson, A. M., and Talalay, P. (1983) J. Biol. Chem. 258, 2052–2062). We have also determined 37 nucleotides of the 5'-untranslated region and 348 nucleotides of the 3'-untranslated region of the Yb_s mRNA.

The Yb_s mRNA and subunit do not share any sequence homology with the rat liver glutathione S-transferase Ya, or Yc mRNAs or their corresponding subunits. These data provide the first direct evidence that the Yb_s subunit is derived from a gene or gene family which is distinct from the Ya-Yc gene family.

The rat liver glutathione S-transferases represent a family of isozymes which catalyze the conjugation of glutathione to various electrophilic ligands. These proteins also bind with high affinity various exogenous hydrophobic compounds as well as potentially toxic compounds such as bilirubin and heme (1–3). At least 10 cytosolic rat liver glutathione S-transferases have been purified and characterized to various extents (3–6). All isozymes appear to be heterodimers or homodimers comprised of subunits designated Ya, Yb, Yc, and Yn which can be distinguished electrophoretically on one-dimensional sodium dodecyl sulfate-polyacrylamide gels (4–6).

Although the nucleotide sequences of cDNA clones complementary to the Ya and Yc mRNAs have been determined (7–10), there is no sequence data to date on a cDNA clone complementary to the rat liver glutathione S-transferase Ya-Yc mRNA family. Similarly, amino acid sequence data of purified Yb subunits are also very limited. Only recently have the first 19 NH$_2$-terminal amino acids of the Yb, and Yb_z subunits of the rat liver glutathione S-transferases been reported (11). Although the Yb subunits have similar electrophoretic mobilities and are immunologically related, the chemical composition of these subunits has not been established. The construction of full length cDNA clones complementary to specific Yb mRNAs is crucial toward determining the primary structure of the Yb subunits and elucidating the mechanisms by which the Yb gene family is regulated by xenobiotics.

In earlier work from our laboratory, we described the construction and characterization of a truncated cDNA clone, pGTA/C36, which is complementary to a Yb mRNA (7). In the present study, we have utilized this clone to screen a cDNA library constructed from purified glutathione S-transferase mRNAs. We have identified a recombinant clone, pGTA/C44, which contains a nearly full length cDNA insert complementary to the Yb, mRNA. The entire nucleotide sequence of pGTA/C44 has been determined, and the complete amino acid sequence of the corresponding Yb, subunit has been deduced. Analysis of the nucleotide sequence of the Yb, cDNA clone indicates it shares significant sequence homology with a cDNA clone, pGT55, which is complementary to mouse liver glutathione S-transferase mRNA (12). However, no significant sequence homology was found between the rat liver Yb, clone and the Ya or Yc cDNA clones described previously by our laboratory (7, 10). These latter data suggest that the rat liver glutathione S-transferase Ya-Yc subunits and the Yb subunits are derived from different gene families.

MATERIALS AND METHODS AND RESULTS

DNA Sequence Analysis of pGTA/C44 and the Deduced Amino Acid Sequence of the Yb, Subunit—Sequence analysis of pGTA/C44 was carried out using the chemical sequencing procedure of Maxam and Gilbert (19). The entire nucleotide sequence of pGTA/C44 is illustrated in Fig. 1 along with the deduced amino acid sequence of the Yb subunit. The length of the cDNA insert is 1038 base pairs minus the dC tails. The

* Portions of this paper (including “Materials and Methods,” part of “Results and Discussion,” Table I, and additional Figs. 1 and 2) are presented in miniprint at the end of this paper. The abbreviations used are: ds-cDNA, double-stranded cDNA; SDS, sodium dodecyl sulfate; Pipes, 1,4-piperazinediethanesulfonic acid. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 85M-5155, cite the authors, and include a check or money order for $2.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Glutathione S-Transferase Yb₁, mRNA

13269

FIG. 1. DNA sequence analysis of pGTA/C44 and deduced amino acid sequence of the rat liver glutathione S-transferase Yb₁ subunit. Appropriate 5' and 3'-end-labeled fragments were subjected to sequence analysis using the Maxam-Gilbert chemical sequencing procedure (19). Restriction endonuclease sites (see Table I in Miniprint) used for 5'-end labeling were the Ncol, BamHI, BglII, and StuI sites. The DNA sequence was determined in the 5' and 3' directions from these sites. Restriction endonuclease sites used for 3'-end labeling were the Sphi and PstI sites. For the Sphi site sequence analysis, syntheses proceeded in both the 5' and 3' directions. For the PstI sites, both PstI fragments (746 and 290 base pairs) were isolated. 3'-end-labeled, subjected to secondary restriction endonuclease digestion, and sequenced from the end label. The sequence of all fragments was determined at least twice, and approximately 80% of the sequence has been determined in both directions.

coding region of the cDNA is flanked by a 5' untranslated region of 37 base pairs and a 3' untranslated region of 248 base pairs. The deduced amino acid sequence indicates a protein comprised of 218 amino acids with a $M_r = 25,919$. The C-terminal amino acid is lysine which is consistent with the findings of Beale et al. (20) who have determined the C-terminal sequence of glutathione S-transferase A, a Yb₁ homodimer.

Recently, Frey et al. (11) have determined the NH₂-terminal sequences of glutathione S-transferases A, C, and X. The NH₂-terminal sequences of transferases A and X are presented in Table I (see Miniprint) along with the deduced amino acid sequence obtained from DNA sequence analysis of pGTA/C44. In the first 19 amino acids, there exist five amino acid differences between transferase A and transferase X (Yb₁ homodimer). These differences occur at positions 3 (Ile-Thr), 9 (Val-Ile), 13 (Thr-Ala), 15 (Pro-Ala), and 19 (Leu-Phe). At every divergent position, the amino acid sequence deduced from pGTA/C44 agrees with the NH₂-terminal sequence determined for glutathione S-transferase A. These data indicate that the cDNA insert in pGTA/C44 is complementary to the Yb₁ mRNA rather than the Yb₂ mRNA.

Comparison of Sequence Homology of the Rat Liver Glutathione S-Transferase Yb₁, Subunit with Mouse Liver Glutathione S-Transferases—Pearson et al. (12) have constructed and characterized a cDNA clone, pGT55, which is complementary to a mouse liver glutathione S-transferase. One hundred ninety-four bp near the 5' end of the mRNA sequence were determined and are presented in Fig. 2 along with the nucleotide sequence of the corresponding region in pGTA/C44. As can be seen from this figure, there is an 85% nucleotide sequence homology between the mouse and rat sequence in this region. The NH₂-terminal amino acid sequences of the mouse liver glutathione S-transferase 8.7 and 9.3 isoenzymes are presented in Fig. 3. The NH₂-terminal sequence of glutathione S-transferase 8.7 was determined by protein sequencing techniques, whereas the NH₂-terminal amino acid sequence of glutathione S-transferase 9.3 was deduced from the nucleotide sequence of pGT55 as well as from conventional protein sequencing techniques (12). There is an 86% amino acid sequence homology between the rat liver Yb₁ subunit and the mouse liver glutathione S-transferase 9.3 subunit over the first 72 NH₂-terminal amino acids, whereas the Yb₂ subunit shares an 80% sequence homology with glutathione S-transferase 8.7 over the first 40 NH₂-terminal amino acids.

DISCUSSION

In this investigation, we have identified a cDNA clone, pGTA/C44, which is complementary to a mRNA specific for a Yb₁ subunit of the rat liver glutathione S-transferases. The DNA sequence of pGTA/C44 has been determined, and the complete amino acid sequence of the Yb₁ subunit has been deduced. The NH₂-terminal amino acid sequence deduced from the DNA sequence of pGTA/C44 agrees with the NH₂-terminal amino acid sequence of the Yb₁ subunit determined by Frey et al. (11) using conventional protein sequencing techniques. These data provide the first detailed sequence analysis of a cDNA clone complementary to the rat liver glutathione S-transferase Yb₁ mRNA family.

Although the rat liver glutathione S-transferase Ya and Yc DNA clones have a 96% nucleotide sequence homology (9, 10), the Yb₁ mRNA appears to have little sequence homology with the Ya or Yc mRNAs. Similarly, there is little amino acid sequence homology between the Yb₁ subunit and the Ya or Yc subunits. The lack of significant homology between the Ya-Yc mRNAs and the Yb₁ mRNA suggests these mRNAs are transcriptional products of distinct gene families which have evolved independently. These data are consistent with previous work from our laboratory which has demonstrated that the Ya and Yb mRNAs are regulated independently by 3-methylcholanthrene and phenobarbital (7).

The lack of significant amino acid sequence homology is not surprising given that the immunochemical and catalytic properties of the Ya, Yc, and Yb subunits are quite distinct. For example, polyclonal antibodies raised against the Yb subunit family do not cross-react with the Ya or Yc subunits. Similarly, the glutathione S-transferases comprised of Yb₁ and Yb₂ subunits have high activity toward bromosulphalein and trans-4-phenyl-3-butene-2-one, respectively, whereas the Ya and Yc subunits have high steroid isomerase
of the structural genes encoding this family of glutathione S-transferases as well as elucidate the mechanisms by which the genes are regulated by various xenobiotics.

Recently, Pearson et al. (12) reported the isolation of a cDNA clone, pGT55, which is complementary to mouse liver glutathione S-transferase 9.3 mRNA but also shares significant sequence homology with the glutathione S-transferase 8.7 isozyme. Interestingly, like glutathione S-transferase A (Yb homodimer), the mouse liver glutathione S-transferase 8.7 has high activity toward 1,2-dichloro-4-dinitrobenzene whereas glutathione S-transferase 9.3 is approximately 4-fold less active with this substrate. These data indicate that mouse glutathione S-transferase 8.7 has catalytic properties similar to the rat glutathione S-transferase Yb, subunit, whereas mouse glutathione S-transferase 9.3 is more similar to the rat Yb subunit. Finally, these data suggest that mouse glutathione S-transferases 8.7 and 9.3 have evolved from a common ancestral gene.

The construction and characterization of a full length Yb cDNA clone will facilitate the isolation and characterization of the structural genes encoding this family of glutathione S-transferases as well as elucidate the mechanisms by which the genes are regulated by various xenobiotics.

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Supplemental Material to

RAP1-14ER glutathione S-transferase:

Nucleotide sequence analysis of a Yb, mRNA clone and

determination of the complete amino acid sequence of the Yb, BURAT by

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Materials and Methods

Hybridization of cDNA and Construction of Recombinant Plasmids - To prepare a cDNA

plasmid, a cDNA clone of Yb, mRNA was prepared by polymerase chain reaction (PCR) as

described previously (7). Both the forward and reverse primers of the cDNA were synthesized as

permitted by Cuin and Hoffmann (13). The cDNA was then amplified by PCR using recombinant DNA

technology as described previously (7). Similarly, pCTA/C44 was cleaved with SalI and tagged with dCTP.

As stated previously, the 40-kb cDNA clone pCTA/C44 and the 8-kb tag d cDNA were amplified in

10 mM Tris-Cl, pH 7.6 and 90 in vitro with 2 units 3.8 k nucleotide units of

pD41. The chloroform was precipitated by the addition of 10 mM potassium acetate (Sp) and 200 of

absolute ethanol in the presence of 10 mg calf liver RNA. The precipitated RNA was collected by

centrifugation, rinsed once with 70% ethanol, dried with N2 and suspended in 10 mL H2O. The

mRNA was translated in the rabbit reticulocyte lysate translation system as described previously (46). A

portion of total translation products was dialyzed against 10% SDS-polyacrylamide gels or subjected

to in-vitro translation (46). The polyribonucleotides were identified by autoradiography.

Restriction Mapping of pCTA/C44 - A restriction map of the cDNA insert was constructed by

the method of Smith and Rhoades (15). Using 5'-tag labeled fragments, the sizes of the ends labeled

fragments generated by partial restriction endonuclease digestion were determined by 5% polyacrylamide gels.

Nucleotide Sequence Analysis - The chemical method of Maxam and Gilbert was used for DNA

sequence analysis (96). Appropriate restriction fragments were 5' or 3' end labeled and subjected to

DNA sequence analysis. Results

Construction and Characterization of a cDNA Clone Complementary to Rat Liver Glutathione S-Transferase Yb, mRNA - In previous work from our laboratory, we reported the isolation and

characterization of a cDNA clone, pCTA/C44, complementary to a glutathione S-transferase (GST).

We have utilized this clone in the present study to sequence a cDNA library constructed from poly(A) + RNA isolated by polyacrylamide gel electrophoresis (17). The cDNA sequence was

linearized with NcoI and BstHI to condition the clone for migration on a 4% polyacrylamide gel.
The labeling procedure allows for the use of a single nucleotide and facilitates the cloning of small length templates.

Although several positive clones were isolated in the library, one of the largest clones, pCTA/C44, was chosen for further study. This clone contains a cDNA insert of 1100 bp which is similar to the

size of Y b mRNA determined previously in northern blot analyses (7). The restriction endonuclease map of pCTA/C44 is presented in Figure 1. This clone is extended in both the 5' and 3' direction compared to the transcribed cDNA clone pCTA/C36. Hybrid-select transcribed DNA analysis confirmed that pCTA/C44 was complementary to a Y b mRNA. Only a single

polypeptide which co-migrates with the purified Yb subunit was observed in the total translation or in the

immunoprecipitated from the total translation (Fig. 5).

pD41

FIG 1. Restriction endonuclease map of pCTA/C44. The restriction endonuclease map of pCTA/C44 was determined by agarose and double digests as well as partial digestion of end-labeled fragments using the BamHI and NcoI procedure (16). The dotted lines in the case represents pCTA/C36 sequences. The 5' and 3' orientation of the cDNA insert in pCTA/C44 is from the NcoI site toward the BamHI site.

TABLE I

Comparison of the NH2-Terminal Sequences Deduced from the Yb, mRNA Clone with the NH2-Terminal Sequences of Purified Glutathione S-Transferase A and X

| Position | Deduced from pCTA/C44 | Glutathione S-Transferase A | Glutathione S-Transferase X |
|----------|-----------------------|----------------------------|-----------------------------|
| NH2-Terminal Sequence | NH2-Terminal Sequence | NH2-Terminal Sequence | Position |
| 1         | Pro                   | Pro                        | Pro                          |
| 2         | Met                   | Met                        | Met                          |
| 3         | Ser                   | Ser                        | Ser                          |
| 4         | Leu                   | Leu                        | Leu                          |
| 5         | Gly                   | Gly                        | Gly                          |
| 6         | Thr                   | Thr                        | Thr                          |
| 7         | Tyr                   | Tyr                        | Tyr                          |
| 8         | Asp                   | Asp                        | Asp                          |
| 9         | Val                   | Val                        | Val                          |
| 10        | Arg                   | Arg                        | Arg                          |
| 11        | Glu                   | Glu                        | Glu                          |
| 12        | Lys                   | Lys                        | Lys                          |
| 13        | Thr                   | Thr                        | Thr                          |
| 14        | His                   | His                        | His                          |
| 15        | Phe                   | Phe                        | Phe                          |
| 16        | Tyr                   | Tyr                        | Tyr                          |

* The NH2-terminal amino acid sequences of purified glutathione S-transferase A and X were determined by Frey et al. (J. Biol. Chem. 258, 11321-11323, 1983).