Volume 16 Number 2 1988 Nucleic Acids Research

Characterization and heterospecific expression of cDNA clones of genes in the maize GSH S-transferase multigene family

Gregory Grove, Ray P.Zarlengo, Kurt P.Timmerman, Nan-qian Li, Ming F.Tam[1] and Chen-Pei D.Tu*

Department of Molecular and Cell Biology, Pennsylvania State University, University Park, PA 16802, USA and [1]Institute of Molecular Biology, Academia Sinica, Taipei 11529, Taiwan

Received October 29, 1987; Accepted December 17, 1987

ABSTRACT

We have isolated from a constructed λgt11 expression library two classes of cDNA clones encoding the entire sequence of the maize GSH S-transferases GST I and GST III. Expression of a full-length GST I cDNA in E. coli resulted in the synthesis of enzymatically active maize GST I that is immunologically indistinguishable from the native GST I. Another GST I cDNA with a truncated N-terminal sequence is also active in heterospecific expression. Our GST III cDNA sequence differs from the version reported by Moore et al. [Moore, R. E., Davies, M. S., O'Connell, K. M., Harding, E. I., Wiegand, R. C., and Tiemeier, D. C. (1986) Nucleic Acids Res. 14:7227-7235] in eight reading frame shifts which result in partial amino acid sequence conservation with the rat GSH S-transferase sequences. The GST I and GST III sequences share ~45% amino acid sequence homology. Both the GST I and the GST III mRNAs contain different repeating motifs in front of the initiation codon ATG. Multiple poly(A) addition sites have been identified for these two classes of maize GSH S-transferase messages. Genomic Southern blotting results suggest that both GST I and GST III are present in single or low copies in the maize (GT112 RfRf) genome.

INTRODUCTION

Glutathione S-Transferases (GST; EC 2.5.1.18) are abundant proteins in maize, comprising over 1% of the total soluble protein of etiolated maize tissues (1). Although little is known of its biological function, maize GST has been demonstrated to detoxify a number of commonly used herbicides via a pathway starting with conjugation of the herbicide to glutathione. This ability renders maize tolerant to application of weed killers such as alachlor (2-chloro-2',6'-diethyl-N-[methoxymethyl]acetanilide) and atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) (1-3).

In rats and humans, GST is also an abundant protein, apparently involved in xenobiotics metabolism and possibly transport of physiological compounds such as heme and bilirubin (for a recent review, see ref. 4). In both plants and animals, GST is a mixture of isozymes, each of which is a dimer consisting either of identical subunits or of two distinct but structurally similar

subunits. Rat liver GST subunits can be resolved into at least five classes based on electrophoretic mobility (M.W. 24,000-28,000) (5,6). Further heterogeneity has been demonstrated within some of these classes by sequencing of cDNA and genomic clones. Thus the GST gene superfamily in mammals is quite complex (7-17, Qian, B., Lai, H.-C. J. and Tu, C.-P. D., manuscript submitted).

In maize, at least three distinct isozymes have been identified (1, Timmerman, K. P. and Tu, C.-P. D., manuscript in preparation). Two of these, GST I and GST III, have been purified and shown to be homodimers (1). cDNA clones for both GST I and GST III subunits have been cloned independently in our lab and elsewhere (18-20) using different cloning strategies. For GST I, the sequences of the clones from the two laboratories are nearly identical except that our sequence clarifies an ambiguous position and contains a complete 3' noncoding region and a longer 5' noncoding region. For GST III, however, our sequence analysis revealed a number of frame-shift differences between the two GST III clones. These differences result in substantial differences in the deduced amino acid sequence of the GST III polypeptide, although both terminate at the same codon. It is likely that the sequence reported below is correct because the resulting amino acid sequence includes a substantial region of residues in common with GST I and, interestingly, which are also partially conserved in several mammalian GST isozymes. We also report on the heterospecific expression of both a complete and a partial GST I cDNA in *E. coli*.

MATERIALS AND METHODS

Isolation of corn poly(A) RNA

Corn poly(A) RNA was isolated essentially as described previously (21) for rat tissues. Briefly, seedlings or leaves from corn line GT112 RfRf were homogenized in a guanidinium-thiocyanate buffer containing 7.5% polyvinyl-polypyrolidone (PVPP) (Sigma). Total extracted RNA was banded on CsCl gradients and poly(A) RNA was isolated by chromatography on oligo(dT)-cellulose column twice (20).

Isolation of maize GST I and GST III protein and antibodies

The isolation of maize GST I and GST III is described elsewhere (Timmerman & Tu, manuscript in preparation). N-terminal sequences were determined on an Applied Biosystems Model 470A gas phase microsequenator. Antibodies against GST I and GST III were obtained from New Zealand white female rabbits after five weekly injections.

cDNA library construction

Double-stranded, blunt-ended cDNA was synthesized from maize poly(A) RNA with a cDNA synthesis kit from Amersham Corp. which uses the procedure of Okayama and Berg (23) as modified by Gubler and Hoffman (24). The resulting cDNA molecules were purified by phenol/chloroform extraction twice, ether extraction twice, and ethanol precipitation.

EcoRI sites in the cDNA were methylated in 20 µl containing 200 mM Tris-HCl (pH 7.9), 1 mM EDTA, 1 mM DTT, 10 mM NaCl, 0.08 mM S-adenosyl methionine (freshly diluted into H_2O) and 20 units EcoRI methylase. The reaction was incubated 30 min at 37°C. 50 µl of TE buffer was added and the mixture extracted with phenol/chloroform. The cDNAs were then chromatographed on a Sepharose CL-4B column (buffer was 10 mM Tris-HCl [pH 7.9], 1 mM EDTA, 100 mM NaCl) to enrich for full length molecules. Fractions (3 drops) containing the first two-thirds of the cDNA peak were pooled and precipitated with two volumes of ethanol in the presence of 5 µg of tRNA.

After recovery of the cDNA from ethanol and subsequent washing with 70% ethanol, EcoRI linkers were ligated to the cDNA molecules in a total volume of 10 µl containing 50 mM Tris-HCl (pH 7.7), 10 mM $MgCl_2$, 5 mM DTT, 4% polyethylene glycol, 1 mM ATP, EcoRI linkers (at a 25/1 molar ratio to cDNA ends) and 400 units T4 DNA ligase. The incubation was started in a 16°C water bath which was transferred to a 4°C cold room overnight. After overnight incubation, 50 µl of H_2O was added to the reaction and the mixture incubated 10 min at 65°C. After cooling to room temperature, 5x EcoRI buffer and 80 units EcoRI was added and the mixture incubated 4-5 hours at 37°C. The reaction was then extracted two times with phenol-chloroform, one time with ether and then chromatographed as before on Sepharose CL-4B to remove excess linkers. Appropriate fractions (leading 2/3 of the peak) were pooled and ethanol precipitated with addition of 5 µg of tRNA. Following recovery of the cDNA from ethanol, the pellet was washed with 70% ethanol and dissolved in 1-5 µl of H_2O depending on the estimated weight of the cDNA.

Approximately 25 ng of cDNA was ligated to 1 µg of λgt11 arms in 5 µl containing 50 mM Tris-HCl (pH 7.7), 10 mM $MgCl_2$, 5 mM DTT, 4% polyethylene glycol, 1 mM ATP and 200 units T4 DNA ligase under incubation conditions described above for linker ligation. After overnight ligation, the DNA was packaged with the Gigapack Gold kit exactly according to manufacturer's instructions and then plated (15 x 150 mm plates). After development of plagues (ca. five hours at 42°C), each plate was soaked two hours at room temperature with 10 ml SM buffer (86 mM NaCl, 8 mM $MgSO_4$, 50 mM Tris-HCl [pH

7.5], 0.01% gelatin). The buffer containing the λgtll library was then removed from the plates with a pipet and Pipet-Aid (Drummund), pooled, centrifuged 15 min at 6000 rpm at 4°C. The supernatant was recovered and stored at 4°C over 0.3% chloroform until screening.

Screening the cDNA library

The maize λgtll cDNA library was screened with GST I and GST III antisera and ^{125}I-Protein-A (Amersham) according to published procedures (25).

Sequence analysis

Phage DNA was isolated from positive plaques according to Maniatis et al. (26). The maize DNA inserts were excised from the phage DNA by EcoRI digestion. Smaller fragments, representing only part of the maize insert, were also isolated using other appropriate restriction digestions. These complete and partial fragments were subcloned into M13 mp19 (27). Sequence analysis was carried out with single-stranded M13 DNA by the dideoxynucleotide chain-termination method of Sanger using dATP-αS^{35} (28,29).

Isolation of maize genomic DNA

This is based on a procedure provided by Professor D. T.-H. Ho of Washington University (personal communication). Leaves, from nearly mature plants or from seedlings, were harvested, washed, weighed, frozen in liquid nitrogen and homogenized to a powder in a Waring blender containing liquid nitrogen. Excessive homogenization should be avoided since this may result in nuclei breakage. Powdered tissue was suspended in 4 ml/g tissue of nuclei isolation buffer (20 mM Tris-HCl [pH 7], 20 mM KCl, 20 mM $MgCl_2$, 600 mM mannitol, 40% glycerol, 10 mM β-mercaptoethanol, 0.2% PVPP. The suspension was filtered through four layers of cheesecloth into a beaker on ice. All liquid was squeezed from the cheesecloth. The filtrate was centrifuged in a 150 ml Corex bottle at 16,000 x g for 20 min at 4°C. the nuclear pellet, still green, was gently resuspended in lysis buffer (4 ml/10 g tissue; 50 mM Tris-HCl [pH 8], 20 mM EDTA, 1% SDS, 300 mM mannitol) and kept on ice 30 min. 0.05 volumes of Pronase (10 mg/ml stock in 20 mM Tris-HCl [pH 7.5], self-digested 1 hour at 37°C) was added and the mixture incubated 20 min at 37°C. Another 0.05 vol. Pronase was added and the incubation continued 20 min longer at 37°C. Finely ground CsCl was added to a density of 1 g/ml and dissolved by gentle inversion. The solution was kept on ice for 1 hour, then centrifuged at 16,000 x g for 30 min at 15°C. After centrifugation, as much floating material as possible was removed from the top of the supernatant. The supernatant was then transferred to ultraclear tubes (Beckman)(9.5 ml/tube); 150 µl ethidium bromide (10 mg/ml stock) was added and the tubes

were centrifuged at 46,000 rpm for 24 hours at 15-20°C in a 70.1 Ti rotor. The DNA band was collected with a 20 gauge syringe by puncturing the tube just below the band. The DNA was diluted to 30 ml with 4.45 M CsCl in 50 mM

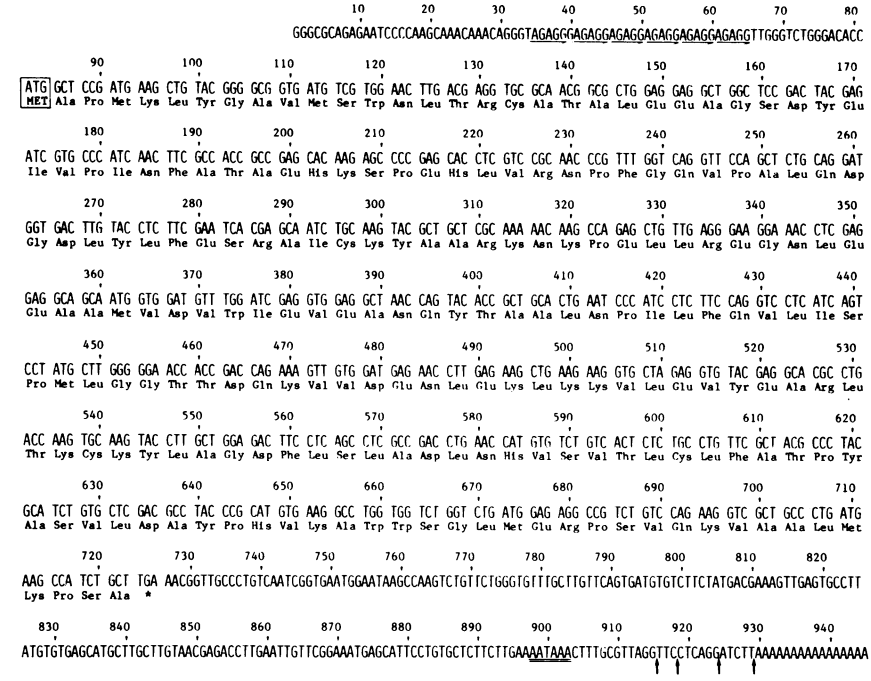

Figure 1. Sequencing strategy, nucleotide sequence and amino acid sequence of the maize GST I cDNA clone in pGTC2. The nucleotides are numbered one through 945 at every 10th position. The initiation codon ATG, confirmed from N-terminal sequence analysis is drawn in a box. The first in phase termination codon TGA is marked by an "*." The open reading frame of 214 amino acids has a calculated MW≈23802 and an estimated pI of 6.06. The pentanucleotide repeats (AGAGG) are underlined with half arrows. The AATAAA signal is labelled with double underlines. The vertical arrows downstream from AATAAA are positions of poly(A) addition identified from cDNA clones pGTC22, pGTC12, pGTC25 (pGTC35), and pGTC2, respectively. The restriction sites used for subcloning in DNA sequencing are labelled with closed circles. The associated arrows represent extents of DNA sequences determined.

Tris-HCl (pH 8), 25 mM EDTA, and 200 µg/ml ethidium bromide, distributed into four new ultraclear tubes and centrifuged again at 46,000 rpm for 24 hours at 15-20°C. The DNA bands were collected as before and extracted gently several times with several volumes each time of isoamyl alcohol until the phases were no longer pink. The DNA solution was dialyzed overnight against several changes of 10 mM Tris-HCl (pH 7.9), 1 mM EDTA, 10 mM NaCl. After dialysis, sodium acetate (pH 5.4) was added to a final concentration of 0.3 M and the DNA was precipitated with two volumes of ethanol for 1 hour at -20°C. The precipitate was collected by centrifugation in an HB-4 rotor for 20 min at 9,000 x g, washed with 70% ethanol and dissolved in 200-500 µl H_2O. The solution was centrifuged a few seconds to remove particulate materials. From 60 g of leaves (nearly mature), 240 µg genomic DNA was obtained with A260/A280 ratio = 1.83.

Genomic Southern blotting

Genomic blots were done according to published procedures (26). Probes were labeled with a random primer oligolabeling kit according to the manufacturer's instructions (Pharmacia).

Construction of GST I expression plasmids

The GST I inserts were excised from two different λgt11 DNAs by EcoRI digestion, electrophoresed in, and recovered from a 3.5% polyacrylamide gel and then cloned into the high copy number plasmid pUC19 which had been linearized by EcoRI digestion are treated with phosphatase. Plasmid DNA from pGTC 2 was then isolated and the cDNA insert excised from plasmid DNA by EcoRI digestion. The insert, after gel purification, was blunt-ended by treatment with the Klenow fragment of E. coli DNA polymerase I. Phosphorylated NcoI linkers were ligated to insert DNA under ligation conditions described above. Excess linkers were digested with NcoI which also cleaved the 5' non-translated region (79 nucleotides, Figure 1) at a site just before the ATG codon of the DNA insert itself. The resulting truncated cDNA containing the full coding sequence, with NcoI sites at both ends was purified by gel electrophoresis and then ligated into the NcoI site of the tac promoter-based expression vector pKK233-2 (Pharmacia). Transformants of E. coli strain JM105 were selected and several were tested by rapid alkaline extraction (30) for the presence of the cDNA inserts in both the correct and incorrect orientation. The EcoRI insert of the partial GST I cDNA clone pGTC25, which began with nucleotide 85 in Figure 1, was ligated to EcoRI treated pKK223-3 DNA (Pharmacia P. L. Biochemicals) for direct expression of the GST I activity. Enzyme activity assay against 1-chloro-2,4-dinitrobenzene,

purification of GST I from E. coli cultures and Western blot analysis were carried out with published procedures (31-33, Timmerman and Tu, manuscript in preparation).

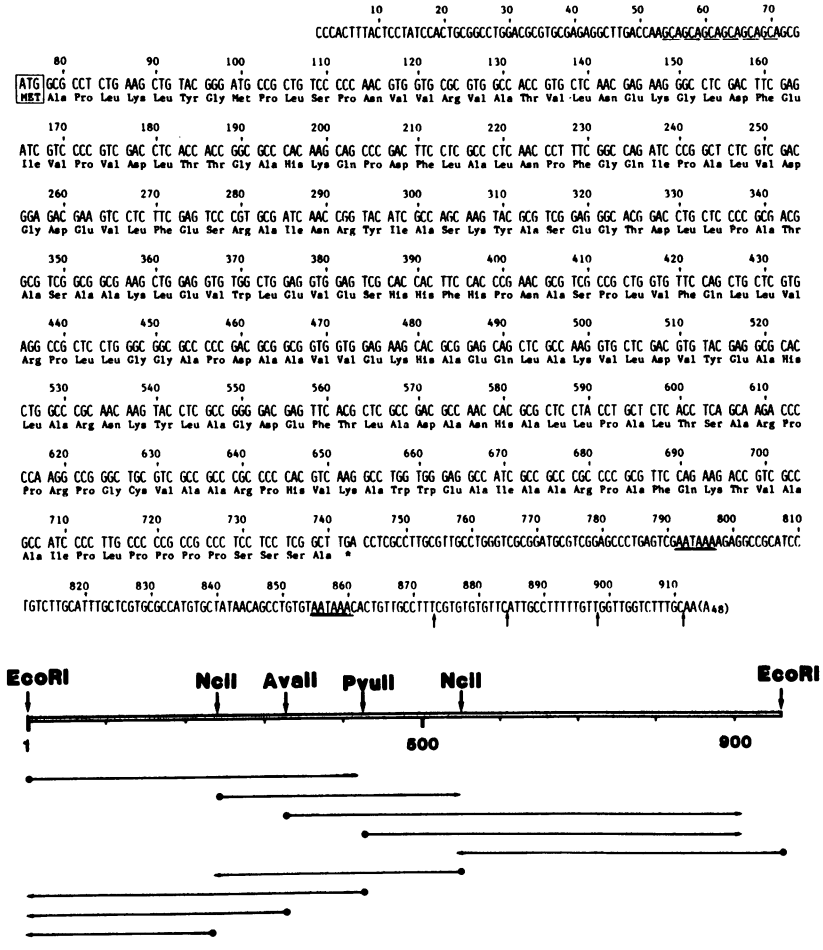

Figure 2. Sequencing strategy, nucleotide sequence and amino acid sequence of the maize GST III cDNA clone in pGTC27. The nucleotide sequence is numbered one through 911 at every 10th position. The initiation codon ATG, confirmed from N-terminal sequence analysis, is drawn in a box. The first in phase termination codon TGA is marked by an "*." The open reading frame of 222 amino acid has a calculated MW≅23949 and an estimated pI of 6.34. The GCA repeats in front of the initiation ATG codon are underlined. The two AATAAA sequences are labelled with double underlines. The vertical arrows downstream from AATAAA are positions of poly(A) addition identified from cDNA clones pGTC1, pGTC9, pGTC20, and pGTC27, respectively. The restriction sites used for subcloning in DNA sequencing are labelled with closed circles. The associated arrows represent extents of DNA sequences determined.

RESULTS AND DISCUSSION

Isolation of cDNA clones for maize GST I and GST III

The maize λgt11 library was screened initially with an antiserum against a mixture of the GST I and GST III isozymes (Timmerman & Tu, in preparation). Thirty-six positive plaques were purified to the isolated, single-plaque stage. Of the 36, 18 were also positive when screened with antiserum against the purified GST I isozyme. The 18 negative clones were presumed to be GST III and this was proven by DNA sequencing (see below). Five clones from each of the two groups were selected for more detailed analysis by DNA sequencing.

Sequence heterogeneity of GST I and GST III cDNA clones

The cDNA inserts from the λgt11 clones were isolated and subcloned into M13 mp19 DNA. All inserts, from both classes of clones, were of similar size, approximately 900-1000 bp. About 200-300 nucleotides from both the 5' and 3' ends of those 10 clones were sequenced and compared. One in each group was sequenced completely and shown in Figure 1 (pGTC2, GST I) and Figure 2 (pGTC27, GST III).

Among the GST I clones, four of the five are identical over the common regions sequenced. The fifth has a T instead of a G at position 124 (Figure 1) , leading to the substitution of Leu for Val at amino acid residue 15 of the GST I polypeptide. The N-terminal portion of the coding sequence matched precisely with the determined N-terminal sequences Ala-Pro-Met-Lys-Leu-Tyr-Gly-Ala-Val-Met-Ser-?-Asn-Val-Thr-Arg-?-Ala. In a GST I gene cloned elsewhere (pMON9000, ref. 19), from a different strain of maize, there is a G at nucleotide 124, in agreement with four of the clones analyzed in this study. Compared to pMON9000, there are two differences in the coding sequence other than at nucleotide 124. These differences, neither of which affect the amino acid sequence, are at positions 442 and 607 where pGTC2 has a T and a G respectively in place of a G and a T in pMON9000. There are several differences in the 5' and 3' non-coding regions, one of which is noteworthy. The pentanucleotide sequence AGAGG is repeated six times in pGTC2 in the 5' non-coding region with an extra G between the first two repeats (nucleotides 35-65 in Figure 1). In the genomic clone of pMON9000, the pentanucleotide sequence is repeated seven times (19). The unassigned amino acid in pMON9000 (19) at position 116 should be a Gln from our sequence in Figure 1.

A sequence of a cDNA clone for GST III (designated GST IIIA) has been previously reported (20). In the present work, we report a sequence for GST III and also of the identification of apparent errors in the previously

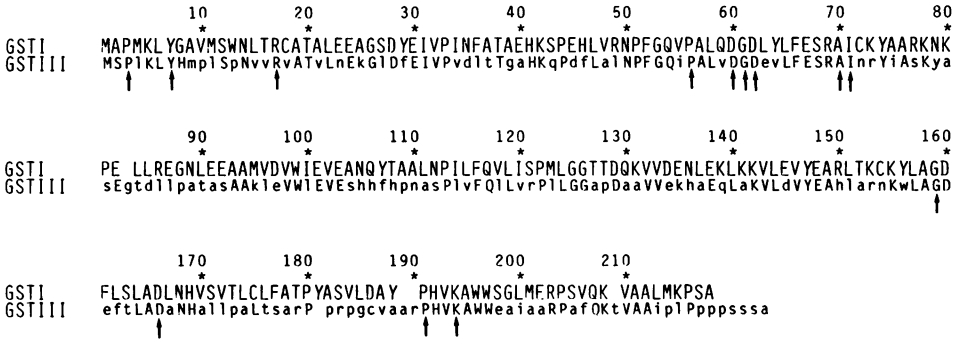

Figure 3. Conservation of amino acid sequences between GST I and GST III. The GST I sequence is represented by capital letters. Amino acid sequences in GST III that are divergent from those in GST I are represented in lower case letters. Vertical arrows mark amino acid residues that are conserved in rat GSTs (8,9).

reported sequence. Among the partial sequences of five independent clones in this study there are no differences. The deduced N-terminal amino acid sequences matched those determined from purified GST III up to 28 cycles except for the poor resolution in cycle 17. One GST III clone (pGTC27) was sequenced entirely and compared to GST IIIA (Figure 2). The sequences (i.e. pGTC27 and GST IIIA) are different and include a significant number of deletions or additions within the coding sequence. The pGTC27 insert is missing a G between G407 and T408 and contains seven extra nucleotides not reported in GST IIIA; C454, G519, C599, C614, G625, G628 and C644. These differences obviously have a profound effect on the deduced amino acid sequence. Both sequences terminate at the same stop codon, however, since the sum of the deletion/additions is a multiple of 3. The internal amino acid sequence between positions 112 and 189 is substantially altered. GST III sequence in Figure 2 is presumably the correct sequence since the resulting deduced amino acid sequence contains a region of residues highly homologous to GST I and also homologous to a number of mammalian GSTs as well (Figure 3 and ref. 34).

In addition to the presumed mistakes in GST IIIA, there are seven differences in coding regions of pGTC27 and GST IIIA, namely G for A at 374, C for T at 396, G for C at 405, C for G at 406, G for C at 476, G for C at 527, and C for T at 673. The substitutions at 374 and 527 are neutral based on our deduced reading frame. The substitutions at 396, 405 and 406, 476 and 673 result in, respectively, substitution of His for Tyr, Ala for Arg, Glu for

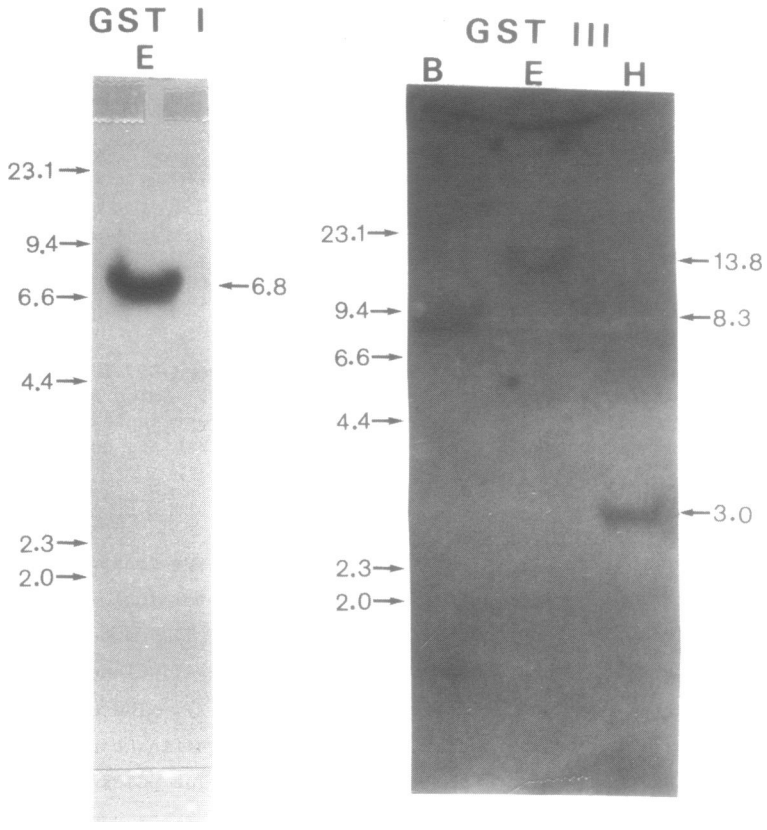

Figure 4. Genomic Southern Blotting. Twenty µg of purified maize genomic DNA (GT112 RfRf) were restricted respectively with EcoRI, BamH-1 and Hind III and separated by agarose gel electrophoresis (0.8%). After transferring to nitrocellulose filters hybridization was carried out with ^{32}P-labelled GST I cDNA insert (Panel A) or with ^{32}P-labelled GST III cDNA insert (Panel B) at 42°C for 24 hours in the presence of 50% formamide and 10% dextran sulfate. The filters were washed overnight in 0.1 X SSC at 60°C before autoradiography. Numbers represent Hind III digested λDNA markers or positive bands in kb units.

Asp, and Ala for Val. Within the noncoding regions, the most noteworthy difference is the number of repeats of the triplet GCA just before the start codon. GST IIIA contains four repeats whereas pGTC27 has six repeats (nucleotides 54-71) plus a GCG sequence just before the ATG codon. Among a number of other one-base substitutions or deletion/additions in the non-coding regions is a more substantial deletion of seven bases (CGTTCGC) from pGTC27 which are present 11-17 nucleotides beyond the stop codon of GST IIIA (between

nucleotides 758 and 759). Some differences could be originated from the different corn lines used in the two laboratories.

Imprecise poly(A) addition sites as revealed by cDNA sequencing

Among the GST I and GST III cDNAs that we have sequenced and contain stretches of poly(A) sequences, we found that the sites of poly(A) addition are not precise. For example, in GST I cDNAs poly(A) have been added at nucleotides 914 (pGTC22), 917 (pGTC12), 923/924 (pGTC25, pGTC35) or 928 (pGTC2) in Figure 1. They correspond to 13, 16, 22/23, 27 nucleotides downstream of the putative poly(A) addition signal AATAAA. The poly(A) additions among GST III clones are just as imprecise. They are located at 13 (pGTC1), 24/25 (pGTC9), 38 (pGTC20) or 51 (pGTC27) nucleotides downstream from the nearest poly(A) addition signal AATAAA (nucleotides 855-860 in Figure 2). A second AATAAA (792-797) is located upstream, however. This imprecise poly(A) addition in maize GST I and GST III cDNAs is contrasted by the relative constant poly(A) addition sites observed with independently isolated rat and human GST cDNAs (9-11,16,17, Rhoads, D. M., Zarlengo, R. P., and Tu, C.-P. D., unpublished results). Whether this is due to a lack of a second component of the polyadenylation signal in the maize GST genes or a recognition pattern in plants which is different from the animal system remains to be investigated (35,36).

Genomic blot results

Purified, ^{32}P-labeled GST I and GST III cDNA inserts were used to probe Southern blots of restricted maize genomic DNA. For GST I, only one band in each of the two restricted samples hybridized to the probe; a 6.8 kb EcoRI fragment (Figure 4A) and a 1.4 kb HindIII fragment (data not shown). Similarly the GST III probe (Figure 4B) hybridized to only a single fragment from a BamHI digest (8.3 kb), an EcoRI digest (13.8 kb) and a HindIII digest (3.0 kb). Thus in the maize strain used for this work (GT112 RfRf), the GST I and III genes are each probably present in only one copy per genomic equivalent.

Genomic DNA blots for GST I and III have been published previously (19,20), although from different maize strains. As with the results described above, only one GST III fragment was detected for BamHI (6.7 kb), EcoRI (6.5 kb) and HindIII (3.0 kb). Interestingly, only the latter appears to be the same size as that determined for GT112 RfRf as reported above. For GST I, two different strains were tested previously (neither GT112 RfRf). Each of three restriction enzyme digestions gave different results for each strain. Further, there were 2-4 bands in each digest indicating the possibility of

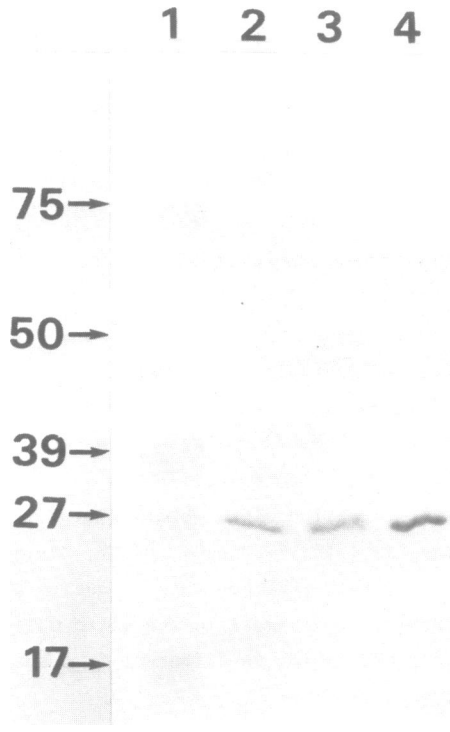

Figure 5. Western blot analysis of E. coli-expressed GST I protein with antiserum made against purified maize GST I. Lanes contain the following samples: 1, prestained molecular weight standards; 2, E. coli-expressed GST I purified by Orange A-agarose column chromatography (containing two other bands on silver staining of $NaDodSO_4$-polyacrylamide gels, not shown); 3, purified maize GST I and GST III; 4, purified maize GST I.

more than one copy of the GST I gene in these strains. As noted above, however, this does not appear to be the case with GT112 RfRf.

Heterospecific expression of GST I cDNAs in E. coli

One of the two expression plasmids, designated as pGTC2-KK, contained the full coding sequence of GST I (pGTC2) in the NcoI site of vector pKK233-2. The other, designated as pGTC25-KK, contained a truncated cDNA (pGTC25) in which the initiation codon after the ribosome-binding signal on pKK223-3 was Met-4 of Figure 1. Both of them expressed GST activity against CDNB as the substrate. Furthermore, the Western blot analysis of GST activity partially purified (specific activity, ~38 units/mg) from sonicated E. coli (pGTC2-KK) extract by Orange A-agarose affinity chromatography and of electrophoretically

pure corn GST I and III (~70 units/mg) markers (Figure 5) clearly showed that polypeptides of the correct electrophoretic mobilities were synthesized in E. coli and that they cross-reacted specifically with antiserum prepared against purified GST I protein. Earlier results only showed that GST I cDNA can be expressed from yeast expression vectors in yeast by activity assay (18,19). Our results also suggest that the precise N-terminal structure (at least the three N-terminal amino acids Met-Ala-Pro) is not essential for CDNB conjugation activities. The level of CDNB activity in sonicated E. coli crude extract is only 35% of that from the full-length GST I expression construct, however. Therefore, it is now feasible to study the structure and sequence relationship of a maize GST by domain replacement and site-directed mutagenesis.

ACKNOWLEDGEMENTS

We thank Dr. Charles D. Boyer for planting the corn lines, Dr. G. E. Scott for corn seeds (GT112 RfRf), and Eileen McConnell for typing the manuscript. This research project has been supported by United States Department of Agriculture Competitive Research Grants 83-CRCR-1-1250 and 86-CRCR-1-2148, and in part by The Pennsylvania State University Agricultural Experiment Station. C.-P. D. Tu is a recipient of a Research Career Development Award (K04 ES00140) from the United States Public Health Service. This publication has an assigned journal series No. 7827 from the Pennsylvania Agricultural Experiment Station.

*To whom correspondence should be addressed at: Rm 6, Althouse Lab, Pennsylvania State University, University Park, PA 16802, USA

REFERENCES

1. Mozer, T. J., Tiemeier, D. C., and Jaworski, E. G. (1983) Biochemistry 22, 1068-1072.
2. Shimabukuro, R. H., Frear, D. S., Swanson, H. R., and Walsh, W. C. (1971) Plant Physiol. 47, 10-14.
3. Frear, D. S. and Swanson, H. R. (1970) Phytochemistry 99, 2123-2132.
4. Mannervik, B. (1985) Adv. Enzymol. Relat. Areas Mol. Biol. 57, 357-417.
5. Tu, C.-P. D. and Reddy, C. C. (1985) J. Biol. Chem. 260, 9961-9964.
6. Hayes, J. D. (1984) Biochem. J. 214, 839-852.
7. Abramovitz, M. and Listowsky, I. (1987) J. Biol. Chem. 262, 7770-7773.
8. Lai, H.-C. J. and Tu, C.-P. D. (1986) J. Biol. Chem. 261, 13793-13799.
9. Lai, H.-C. J., Grove, G., and Tu, C.-P. D. (1986) Nucleic Acids Res. 14, 6101-6114.
10. Ding, G. J.-F., Ding, V. D. H., Rodkey, J. A., Lu, A. Y. H., and Pickett, C. B. (1986) J. Biol. Chem. 261, 7952-7957.
11. Satoh, K., Kitahara, A., Soma, Y., Inaba, Y., Hatagama, I., and Sato, K. (1985) Proc. Natl. Acad. Sci. (USA) 82, 3964-3968.

12. Suguoka, Y., Kano, T., Okuda, A., Salai, M., Kitagawa, T., and Muramatsu, M. (1985) Nucleic Acids Res. 13, 6049-6057.
13. Ding, G. J.-F., Lu, A. Y. H., and Pickett, C. B. (1985) J. Biol. Chem. 260, 13268-13271.
14. Telakowski-Hopkins, C. A., Rodkey, J. A., Bennett, C. P., Lu, A. Y. H., and Pickett, C. B. (1985) J. Biol. Chem. 260, 5820-5825.
15. Tu, C.-P. D., Lai, H.-C. J., Li, N., Weiss, M. J., and Reddy, C. C. (1984) J. Biol. Chem. 259, 9434-9439.
16. Lai, H.-C. J., Li, N., Weiss, M. J., Reddy, C. C., and Tu, C.-P. D. (1984) J. Biol. Chem. 259, 5536-5542.
17. Pickett, C. B., Telakowski-Hopkins, C. A., Ding, G. J. F., Argenbright, L., and Lu, A. Y. H. (1984) J. Biol. Chem. 259, 5182-5188.
18. Wiegand, R. C., Shah, D. M., Mozer, T. J., Harding, E. I., Diaz-Collier, J., Saunders, C., Jaworski, E. G., and Tiemeier, D. C. (1986) Plant Mol. Biol. 7, 235-243.
19. Shah, D. M., Hironaka, C. M., Wiegand, R. C., Harding, E. I., Krivi, G. G., and Tiemeier, D. C. (1986) Plant Mol. Biol. 6, 203-211.
20. Moore, R. E., Davies, M. S., O'Connell, K. M., Harding, E. I., Wiegand, R. C., and Tiemeier, D. C. (1986) Nucleic Acids Res. 14, 7227-7235.
21. Tu, C.-P. D., Weiss, M. J., Karakawa, W. W., and Reddy, C. C. (1982) Nucleic Acids Res. 10, 5407-5419.
22. Bantle, J. A., Maxwell, I. H., and Hahn, W. E. (1976) Anal. Biochem. 72, 413-427.
23. Okayama, H. and Berg, P. (1982) Mol. Cell Biol. 3, 161-170.
24. Gubler, V. and Hoffman, B. S. (1983) Gene 25, 263-269.
25. Young, R. A. and Davis, R. W. (1983) Science 222, 778-782.
26. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, New York).
27. Messing, J. (1983) Methods Enzymol. 101, 20-78.
28. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. (USA) 74, 5463-5467.
29. Biggin, M. D., Gibson, T. J., and Hong, G. F. (1983) Proc. Natl. Acad. Sci. (USA) 80, 3963-3965.
30. Birnboim, H. C. and Doly, J. (1979) Nucleic Acids Res. 7, 1513-1523.
31. Habig, W. H. and Jakoby, W. B. (1981) Methods Enzymol. 77, 231-235.
32. Asaoka, K. (1984) J. Biochem. (Tokyo) 95, 685-696.
33. Towbin, H., Staehelin, T., Gordon, L. (1979) Proc. Natl. Acad. Sci. (USA) 76, 4350-4354.
34. Rhoads, D. M., Zarlengo, R. P., and Tu, C.-P. D. (1987) Biochem. Biophys. Res. Commun. 145, 474-481.
35. Berget, S. M. (1984) Nature 309, 179-182.
36. Mason, P. J., Elkington, J. A., Lloyd, M. M., Jones, M. B., and Williams, J. G. (1986) Cell 46, 263-270.