Plant Gene Register

A cDNA Clone Encoding the 27-Kilodalton Subunits of Glutathione S-Transferase IV from Zea mays

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The GST (EC 2.5.1.18) family of enzymes are well known for their role in the detoxification of various xenobiotic compounds in both plant and animal systems. These enzymes facilitate the addition of GSH, γ-glutamylcysteinylglycine, to an electrophilic site of a suitable substrate, yielding a GSH conjugate.

In maize (Zea mays), the GSH-conjugation reaction is responsible for the metabolism of several classes of pesticides to nonphytotoxic forms (Lamoureux and Rusness, 1989). Multiple GSTs have been identified in maize and include GST isozymes I, II, III, and IV (Timmerman, 1989; Irzyk and Fuerst, 1993). These isozymes differ with respect to molecular mass, subunit composition (hetero- or homodimeric), and substrate specificity. Nucleotide sequences and deduced amino acid sequences have previously been reported for maize GSTs I, II, and III (Moore et al., 1986; Wiegand et al., 1986; Grove et al., 1988; Bridges et al., 1993).

Herbicide safeners are compounds used to protect crops, such as maize, from herbicide injury. In maize, both GST activity and herbicide metabolism, via GSH conjugation, are stimulated by herbicide safener treatment (Viger et al., 1991). We have previously identified and purified a GST isozyme from maize that is induced by treatment with the herbicide safener benoxacor [4-(dichloroacetyl)-3,4-dihydro-3-methyl-2H-1,4-benzoxazine; Peek et al., 1988] and designated this isozyme as maize GST IV (Irzyk and Fuerst, 1993). Native GST IV is a homodimer composed of two 27-kD subunits and can conjugate GSH to chloracetamide and S-triazine substrates but not to 1-chloro-2,4-dinitrobenzene, a model GST substrate.

The open reading frame encodes a 223-amino acid polypeptide with a predicted molecular mass of 24,570 D and a pl of 6.1. The deduced amino acid sequence of GSTIV protein (Irzyk and Fuerst, 1993) is nearly identical (99% homology) with the nucleotide sequence for the 27-kD subunit GST IV protein (Irzyk and Fuerst, 1993). The PCR primers corresponded to nucleotide positions +279 to +304, priming in the sense direction, and to positions +720 to +742, priming in the antisense direction. The PCR product was sequenced prior to use for library screening.

Sequencing determined that the 1003-bp clone contained an open reading frame coding for a 223-amino acid polypeptide with a predicted molecular mass of 24.6 kD. The deduced amino acid sequence of GST27 shares 56% identity with the 29-kD subunit of maize GST I (Grove et al., 1988), 44% identity with the 26-kD subunit maize GST III (Grove et al., 1988), and 100% identity with the 27-kD subunit GST IV (Bridges et al., 1993). Similarly, the nucleotide sequence determined for GST27 is nearly identical (99% homology) with the nucleotide sequence for the cDNA clone of the 27-kD subunit of GST II (Bridges et al., 1993). Maize GST II is a heterodimer composed of a 29-kD GST I subunit and a 27-kD subunit. Our results demonstrate that this smaller subunit of GST II is identical with the 27-kD subunits of GST IV. The observation that

**Table I. Characteristics of the GST27 cDNA clone from Zea mays seedlings**

| Organism: | Zea mays cv Blizzard. |
| Source: | cDNA library in AZAP II constructed using poly(A)+ RNA isolated from shoots of 4-d-old maize seedlings. |
| Techniques: | Library screened with an approximately 450-bp PCR product generated from Z. mays cv Blizzard cDNA. Both strands of the cDNA clone were subjected to automated nucleotide sequence analysis using fluorescent deoxyribonucleotide triphosphate technology. |
| Method of identification: | Comparison of the deduced amino acid sequence with published maize GST sequences. |
| Characteristics of the cDNA: | The cDNA contains a 98-nucleotide untranslated 5' region, a 669-nucleotide open reading frame, and a 136-nucleotide untranslated 3' region ending with an 18-nucleotide polyadenylation tract. |
| Characteristics of the Deduced Amino Acid Sequence: | The open reading frame encodes a 223-amino acid polypeptide with a calculated molecular mass of 24,570 D and a pl of 6.1. |

Table I. Characteristics of the GST27 cDNA clone from Zea mays seedlings

Abbreviation: GST, glutathione S-transferase.
maize GSTs I and II can utilize 1-chloro-2,4-dinitrobenzene as a substrate (Mozer et al., 1983) but maize GST IV cannot (Irzyk and Fuerst, 1993) suggests that subunit composition of the native enzymes may influence substrate specificity.

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LITERATURE CITED


