Soybean Root Nodule cDNA Encoding Glutathione Reductase

Xiaoyan Tang and Mary Alice Webb*
Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907

GSH is widely distributed in organisms and has diverse functions in protein synthesis, sulfur storage, and protection against a variety of stresses (Meister and Anderson, 1983). GR (EC 1.6.4.2) is a flavoprotein that catalyzes the reduction of GSSG to reduced GSH using NADPH as the reducing cofactor. It is a key enzyme in the GSH-ascorbate cycle, which provides protection against oxidative stress, particularly in photosynthetic tissues of plants (Foyer and Halliwell, 1976). Tobacco transformed with the gor gene, encoding GR in Escherichia coli, showed mixed results in resistance to oxidative stress (Aono et al., 1991; Foyer et al., 1991). Recently, a cDNA clone encoding GR was isolated from a cDNA library of pea leaves (Creissen et al., 1991). An N-terminal leader sequence encoded by the pea cDNA was consistent with targeting of the gene product to the chloroplast.

In root nodules, enzymes of the GSH-ascorbate cycle have been suggested to function in peroxide scavenging and protection against other oxidative stresses (Dalton et al., 1986). In soybean, GR activity was much higher in nodules than in uninfected roots and was enhanced in nodules exposed to higher than normal pO2 (Dalton et al., 1991). GR activity was also about 4-fold higher in effective nodules than in ineffective nodules, with a range of factors controlling effectiveness (Dalton et al., 1993). Soybean (Glycine max) nodules contain both homo-GSH as well as GSH, and the combined concentration of thiol tripeptides in nodules was calculated to be about 1 mM (Dalton et al., 1993). Recent studies examining the role of GSH in relation to pathogen defense responses (Wingate et al., 1988; Edwards et al., 1991) have implications for GSH function during nodule development as well.

A full-length cDNA encoding soybean nodule GR (Table I) was isolated serendipitously in screening a soybean nodule cDNA library with antibodies against nodule allantoinase. The deduced amino acid sequence of the cDNA was found to share a high degree of homology to sequences for GR in other organisms and was 90% similar and 83% identical to pea GR (Creissen et al., 1991). The soybean GR sequence also had an N-terminal leader sequence characteristic of a plastid transit peptide, which would target the gene product to nonphotosynthetic plastids in root nodules.

---

Table 1. Characteristics of the cDNA clone encoding GR from soybean nodules

| Organism: | Glycine max (L.) Merr. cv Williams 82. |
| Location in Genome: | Nuclear genome. |
| Gene Product and Function: | Glutathione reductase (EC 1.6.4.2); reduction of GSSG to GSH. |
| Source: | A cDNA expression library was constructed from poly(A)+ RNA isolated from soybean root nodules 24 d after inoculation of seeds. |
| Method of Identification: | Similarity of deduced amino acid sequence to GR in pea (90%) and other organisms. |
| Features of cDNA: | 2099 bp in length; translational start site at nucleotide 59 and stop site at nucleotide 1691; an 18-nucleotide poly(A) tail at the 3' end. |
| Structural Features of the Deduced Protein: | The 1632-bp open reading frame encodes a deduced polypeptide of 544 amino acids, including an N-terminal leader sequence of 50 to 60 residues characteristic of a plastid targeting sequence. Cys residues forming redox-active disulfide bridge and Arg residues required for binding NADPH are conserved. |
| Expression: | mRNA of about 2.1 kb was present in poly(A)+ RNA isolated from soybean leaves and root nodules. Transcript was detected in total RNA isolated from root nodules between 8 and 24 d after inoculation. Expression in infected region of nodules was shown by in situ hybridization. |

A full-length cDNA encoding soybean nodule GR (Table I) was isolated serendipitously in screening a soybean nodule cDNA library with antibodies against nodule allantoinase. The deduced amino acid sequence of the cDNA was found to share a high degree of homology to sequences for GR in other organisms and was 90% similar and 83% identical to pea GR (Creissen et al., 1991). The soybean GR sequence also had an N-terminal leader sequence characteristic of a plastid transit peptide, which would target the gene product to nonphotosynthetic plastids in root nodules.

---

1 This work was supported by a grant from the Purdue Research Foundation. This is Purdue Agricultural Experiment Station journal paper No. 13556.

* Corresponding author; fax 1-317-494-5896.

Abbreviation: GR, glutathione reductase.
LITERATURE CITED


