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Plant Gene Register

An O-Acetylserine (Thiol) Lyase cDNA from Spinach[1]

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Cys biosynthesis represents the essential step of incorporation of reduced sulfur into an organic compound in microorganisms and plants. OAS-TL (EC 4.2.99.8; also called Cys synthase) catalyzes the formation of L-Cys from free or bound sulfide and *O*-acetyl-L-Ser. Both the substrate *O*-acetylserine and the product Cys of this reaction are postulated to be involved in the regulation of sulfur uptake and assimilation (Giovanelli et al., 1980; Neuenschwander et al., 1991). OAS-TL has been purified to apparent homogeneity from spinach (*Spinacia oleracea* L.) leaf extracts (Murakoshi et al., 1985) and spinach chloroplasts (Droux et al., 1992) and consists of two identical subunits of 35 kD with a pyridoxal phosphate cofactor. Isoforms of OAS-TL occur in the cytosol, chloroplasts, and mitochondria of spinach leaves (Lunn et al., 1990). We report here the complete cDNA sequence for a nuclear-encoded plastid isoform of OAS-TL from spinach and its expression in different organs.

A spinach young leaf cDNA library was screened with a rabbit polyclonal antiserum originally generated against a soluble chloroplast protein fraction that had been fractionated for RNA binding activity by Suc gradient centrifugation and heparin affinity chromatography. Four independent cDNA clones were isolated from 500,000 plaques. The sequences of the cDNA insert ends showed that the four clones differed only in length. The complete nucleotide sequence of the longest cDNA insert (designated plOAS-TL) was determined for both strands. A comparison of the predicted amino acid sequence with sequences in the GenBank/EMBL data base revealed extensive homology to the proteins encoded by the *Escherichia coli* and *Salmonella thyphimurium cysK* and *cysM* genes, which encode Cys synthase activity (Byrne et al., 1988). Amino acid homology to OAS-TL from *Capsicum annuum* chromoplasts (Römer et al., 1992) is 70%, including a putative chloroplast transit sequence (von Heijne et al., 1989), and 77% for the mature proteins. The sequence deduced from plOAS-TL is consistent with the total amino acid composition as well as an amino terminal peptide sequence reported for the purified, mature OAS-TL from spinach chloroplasts (Droux et al., 1992). This sequence begins at amino acid position 52 of the plOAS-TL cDNA clone, which would indicate a mature subunit of 35.7 kD. Therefore, the cDNA clone reported here probably encodes a plastid-localized OAS-TL. The amino acid sequence of plOAS-TL shows 69% identity to that of a spinach cDNA encoding an OAS-TL that has been suggested to be a cytosolic isoform (Saito et al., 1992). The latter sequence contains no transit peptide, and its alignment starts at a position similar to that of the suggested mature OAS-TL protein from plastids.

Table I. *Characteristics of an OAS-TL cDNA from spinach*

Organism:	*Spinacia oleracea* L. cv Marathon.
Function:	Encodes a subunit of the homodimeric enzyme OAS-TL (EC 4.2.99.8) that catalyzes the synthesis of L-Cys from *O*-acetylserine and free or bound sulfide.
Source:	cDNA library in λZAP II constructed using poly(A)$^+$ RNA isolated from young spinach leaves (<1 cm).
Techniques:	Immunoscreening, restriction fragment subcloning, dideoxy sequencing of both strands; comparison by Intelligenetics Data base and Macvector sequence analysis software.
Features of cDNA Structure:	Total length of 1446 bp, open reading frame from nucleotide 43 to 1213, representing a full-length clone. Potential polyadenylation signal at nucleotide 1407.
Structural Features of Deduced Amino Acid Sequence:	Open reading frame encodes a protein of 390 amino acids. The first 52 amino acids represent a putative chloroplast transit peptide, suggesting a mature protein of 338 amino acids (M_r = 35.853) with a predicted isoelectric point of 4.9.
Antibodies:	Rabbit polyclonal antiserum available.
Expression Characteristics:	Transcript of approximately 1.5 kb. Highest steady-state concentration of mRNA in young leaves, followed by mature leaves, roots, and shoots.

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The GenBank accession number for the sequence reported in this article is L05184.

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Abbreviation: OAS-TL, *O*-acetylserine (thiol) lyase.

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