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*Plant Gene Register*

# Molecular Cloning of a Cysteine Synthase cDNA from *Arabidopsis thaliana*

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Cys biosynthesis in higher plants occurs by a process similar to that known in microorganisms (Kredich, 1987). Cys synthase [*O*-acetylserine (thiol)-lyase, EC 4.2.99.8] catalyzes the formation of Cys from *O*-acetylserine and free or carrier-bound sulfide. Cys synthase has been purified from various plants and microorganisms and has been shown to have a molecular mass of 52 to 70 kD. The plant enzyme consists of two identical monomers, each with a molecular mass of approximately one-half of the intact enzyme and a tightly bound co-factor, pyridoxal phosphate.

Multiple isoforms of Cys synthase are present in plant cells (Brunold et al., 1989; Lunn et al., 1990; Rolland et al., 1992) consistent with the idea that each cell compartment has the ability to form Cys. Recently, several cDNA clones for these different forms of Cys synthase have been isolated (Saito et al., 1992, 1993; Youssifian et al., 1993; H. Hesse, unpublished data). The molecular genetic organization, regulation of Cys biosynthesis in plant cells, and the exact roles of the various isoforms of Cys synthase have not been clarified. It is therefore important to identify subcellular isoforms of Cys synthase to analyze their expression in plant tissue as well as to improve the understanding of Cys biosynthesis for both sulfur assimilation and subcellular interaction.

To study the regulatory mechanism by which Cys synthase is regulated, a cDNA library of λZAPII (Stratagene) made from RNA isolated from flowers of *Arabidopsis thaliana* was screened. A cDNA clone was isolated by screening the library with a radiolabeled genomic DNA fragment encoding 300 bp of a Cys synthase gene from *A. thaliana* that was amplified by PCR using primers derived from consensus sequences of the published Cys synthase sequences from spinach and pepper (Römer et al., 1992; Saito et al., 1992). A cDNA subsequently designated cytACS 1 was sequenced. The cytACS 1 cDNA is 1278 bp in length and contains an open reading frame encoding a polypeptide that is 324 amino acids in length with a predicted molecular mass of 34.6 kD. The features of this cDNA are summarized in Table I.

Sequence comparison of the predicted amino acid sequence with other plant Cys synthase polypeptide sequences shows that all Cys synthase proteins between different species such as spinach, wheat, and watermelon are 70 to 75% similar (Saito et al., 1992; Youssifian et al., 1993; Noji et al., 1994). Recently, a cDNA sequence of a cytosolic Cys synthase isoform from *A. thaliana* was published (Hell et al., 1994). The deduced amino acid sequence of cytACS 1 is 84.8% similar to the predicted amino acid sequence of the cytosolic Cys synthase cDNA isolated by Hell et al. (1994).

Southern analysis indicated that the corresponding gene is present in low frequency in *A. thaliana*, which is supported by the isolation of the two cytosolic cDNA isoforms. Northern blot analysis from leaves and roots of *A. thaliana* showed that a 1.3-kb transcript was constitutively expressed in these tissues.

**Table I.** *Characteristics of cytosolic Cys synthase from A. thaliana*

| | |
|---|---|
| Organism: | |
| | *Arabidopsis thaliana* L. cv Columbia. |
| Genome Location: | |
| | Nuclear genome. |
| Gene Copy Number | |
| | Low copy. |
| Gene Product: | |
| | Cys synthase (*O*-acetylserine [thiol] lyase, EC 4.2.99.8). |
| Source: | |
| | A cDNA library in Uni-ZAPII XR constructed with poly(A)$^+$ RNA isolated from flowers. |
| Sequencing Technique: | |
| | Dideoxy nucleotide chain-termination method was used to completely sequence both strands. |
| Method of Identification: | |
| | Screening with a PCR-amplified genomic fragment from *A. thaliana*. Nucleotide and amino acid sequence comparison with other plant Cys synthases. |
| Expression: | |
| | Northern blot detected the 1.3-kb Cys synthase transcript in all examined tissues in equal amounts. |
| Features of the Predicted Amino Acid Sequence: | |
| | The open reading frame encodes 324 amino acid residues with a molecular mass of 34.6 kD. |

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The EMBL accession number for the sequence reported in this article is X81679.

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