A new member of the cytosolic *O*-acetylserine(thiol)lyase gene family in *Arabidopsis thaliana*

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Abstract A cDNA, *Atcys-3A, encoding* *O*-acetylserine-(thiol)lyase has been isolated from *Arabidopsis thaliana*. The deduced peptide sequence showed a high level of similarity with the bacterial counterpart, and a remarkable percentage of identity with other higher plant *O*-acetylserine(thiol)lyase genes. Sequence comparision and Southern blot analysis suggested that *Atcys-3A* was a new and different to the previously reported member of the cytosolic gene family in *Arabidopsis*. The *Atcys-3A* expression was activated by sulfur limitation, requiring a carbon and nitrogen source for maximal expression. A similar pattern of regulation was observed at the *O*-acetylserine(thiol)lyase activity level. Northern blot analysis also showed an organ-specific expression of *Atcys-3A*.

Key words: *O*-Acetylserine(thiol)lyase; Transcriptional regulation; Sulfur deprivation; Enzyme activation; *Arabidopsis thaliana*

1. Introduction

Sulfur plays an important role in the regulation of plant growth and development. In its reduced form is involved in the biosynthesis of primary and secondary metabolites and in the synthesis of coenzymes. Even oxidized sulfur metabolites are necessary for the synthesis of plant sulpholipids in the intact chloroplast membrane. L-cysteine is the principal starting metabolite for the synthesis of other sulfur-containing compounds. The last step for L-cysteine biosynthesis from *O*-acetyl-L-serine and sulfide is catalyzed by *O*-acetylserine(thiol)lyase (*O*-acetylserine sulfhydrylase, OASSase, EC 4.2.99.8). The enzyme is an homodimer of 60–70 kDa and contains two molecules of pyridoxal 5′-phosphate as cofactor [1].

Two OASSase isoenzymes, called A and B, were found in bacteria, encoded by *cysK* and *cysM* genes, respectively [2–4]. Both genes are transcriptionally activated, requiring sulfur limitation and *O*-acetyl-L-serine as inducer [5]. In plants, the existence of *O*-acetylserine(thiol)lyase activity has been demonstrated in all three cell compartments involved in protein synthesis: cytosol, chloroplasts and mitochondria [6]. Various plant genes coding for the cytosolic [7,8], plastidic [9–12] and mitochondrial [13] isoforms have been recently isolated and characterized. The deduced amino acid sequences show a high level of similarity with the bacterial counterpart. Furthermore, it has been demonstrated that the plant enzyme is functional in bacteria, being possible the cloning of a plant gene by complementation in an *E. coli* cys-auxotroph [8]. Very recently, it has been shown the existence of different nuclear gene families encoding cytosol and chloroplast isoforms in the genomic organization of *Arabidopsis thaliana* [14].

The present study was focused on the isolation and characterization of *O*-acetylserine(thiol)lyase genes from plants. We have identified a new member of the cytosolic gene family, *Atcys-3A*, coding for this enzyme in *A. thaliana*. Furthermore, we have analyzed the regulation of *O*-acetylserine(thiol)lyase in different growth conditions and plant organs at the activity and gene expression levels.

2. Materials and methods

2.1. cDNA cloning and sequencing

A cDNA library in λYES vector prepared from *Arabidopsis* plants [15] was screened with a cDNA fragment with significant homology to bacterial *cysK* gene, provided by the Arabidopsis Biological Resource Center (Columbus, OH). This cDNA fragment was isolated at random by Höfte et al. [16] in a project aimed to stablish an inventory of expressed sequence tags in *Arabidopsis*. Hybridization with the ^{32}P-labelled probe was performed at 42°C for 18h and final washes in 0.5 × SSC, 0.1%SDS. On an screening of 5×10^5 plaques, 8 positive clones were isolated. The clone designed *Atcys-3A* contained the longest insert (1.3 kb) and was selected for further analysis. The *Atcys-3A* insert was subcloned into the *Eco*RI site of Bluescript II KS plasmid vector. DNA sequencing was performed on both strands by the dideoxynucleotide chain termination method using different synthetic primers. All sequence data manipulations were carried out using GCG software.

2.2. Southern and Northern blot analysis

For Southern analysis, 15 μg total DNA was digested with *Eco*RI, *Eco*RV, *Hin*dIII and *Xho*I, fractionated on a 0.8% agarose gel, and transferred onto a nylon membrane (Hybond N, Amersham), and then hybridized with ^{32}P-labelled *Atcys-3A* cDNA insert. For Northern analysis, 20 μg total RNA isolated from seedlings grown in different conditions or different parts of mature plants, were denatured, fractionated on a 1.5% formaldehyde-agarose gel, and transferred onto a nylon membrane (Hybond N, Amersham). The blots were hybridized sequentially with ^{32}P-labelled *Atcys-3A* cDNA insert and maize 17S DNA for detection of the *Arabidopsis* 18S rRNA. Hybridization for both Southern and Northern analysis was performed at 42°C for 18 h and final washes in 0.2 × SSC, 0.1%SDS at 60°C for 15 min.

2.3. Determination of O-acetylserine(thiol)lyase activity

Arabidopsis seedlings were grown in Hoagland liquid medium supplemented with carbon (5 g/l sucrose) at 25°C and continuous light. Sulfur-deprived (-S) and nitrogen-deprived (-N) seedlings were obtained by replacing the SO_4^{2-}, NO_3^- and NH_4^+ salts in the Hoagland medium with Cl^- and K^+ salts, respectively. Seedlings grown with no sucrose added to the medium were referred as (-C) seedlings. 10-day-old seedlings were ground in liquid nitrogen with a mortar and pestle, and resuspended in 50 mM phosphate buffer, 1 mM EDTA, 1 mM DTT, 10 μM pyridoxal 5′-phosphate and 0.5 mM PMSF (pH 7.5). The homogenates were centrifuged at 15,800 × *g* for 10 min at 4°C, and the

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SSDI 0014-5793(95)00255-3

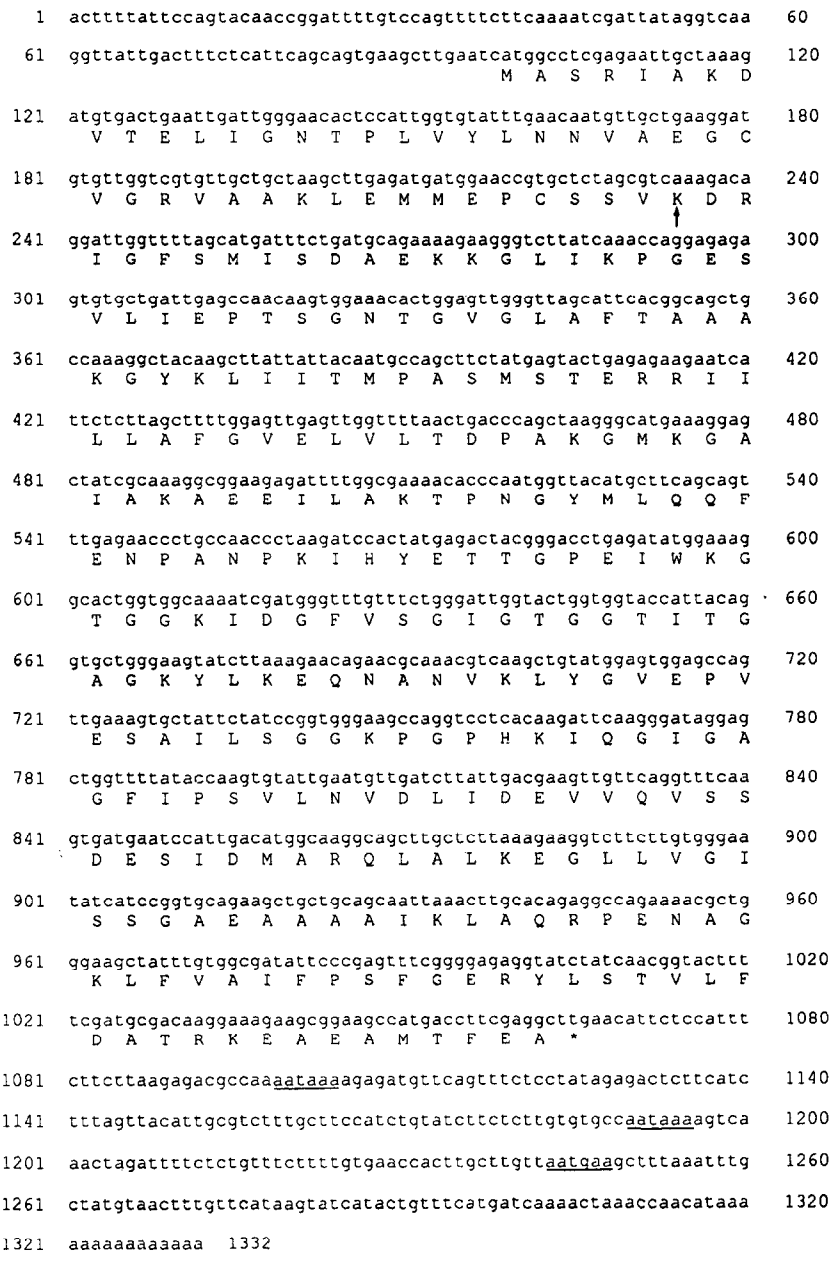

Fig. 1. Nucleotide and deduced amino acid sequence of the *Atcys-3A* cDNA encoding *A. thaliana* *O*-acetylserine(thiol)lyase. Numbering begins at the first nucleotide of the sequence. Potential polyadenylation signals are underlined. The arrow shows the pyridoxal 5′-phosphate binding site.

resulting supernatants were used as crude extracts and assayed for enzyme activity as previously described [1]. The amount of total protein was determined by the method of Bradford [17].

3. Results and discussion

3.1. Isolation and sequence analysis of a new cDNA encoding for cytosolic O-acetylserine(thiol)lyase from A. thaliana

We are interested to determine and characterize the levels of regulation of cysteine biosynthesis in photosynthetic organisms. For this purpose, we first attempted the isolation of genes encoding *O*-acetylserine(thiol)lyase isoenzymes in plant model systems. We have cloned several cDNAs encoding OASSase from an *Arabidopsis* cDNA library, and the clone *Atcys-3A* containing the longest insert was selected for further analysis. The *Atcys-3A* cDNA clone consisted of a 1,332-bp-long nucleotide sequence and revealed an open reading frame of 322 amino acids. The 5′ untranslated region was 98 bp long, and the 268-nucleotide 3′ untranslated region contained three putative polyadenylation signals and a short poly(A) tail (Fig. 1).

The deduced peptide sequence showed a high level of similarity (around 70%) with the bacterial counterpart all over the open reading frame. When compared to *O*-acetylserine(thiol)lyase A encoded by *cysK* genes in *E. coli* and *S. typhimurium* [2,3], the homology was higher than to the isoenzyme B encoded by *cysM* genes [4]. In addition, Atcys-3A deduced protein showed a remarkable percentage of identity with other higher plant OASSase proteins, being more similar

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  1 MASRIAKDVTELIGNTPLVYLNNVAEGCVGRVAAKLEMMEPCSSVKDRIG 50
    ||||||||||||||||| ||||||||||||||||||||||||||||||||
  1 masriakdvtelignt_llvylnnvaegcvgrvaaklemmepcssvkdrig 50

 51 FSMISDAEKKGLIKPGESVLIEPTSGNTGVGLAFTAAAKGYKLIITMPAS 100
    ||||||||||||||||||||||||||||||||:|||||||||||||||||
 51 fsmisdaekkglikpgesvlieptsgntgvgiaftaaakgykliitmpas 100

101 MSTERRIILLAFGVELVLTDPAKGMKGAIAKAEEILAKTPNGYMLQQFEN 150
    ||||||||||||||||||||||||||||||||||||||||||||||||||
101 msterriillafgvelvltdpakgmkgaiakaeeilaktpngymlqqfen 150

151 PANPKIHYETTGPEIWKGTGGKIDGFVSGIGTGGTITGAGKYLKEQNANV 200
    ||||||||||||||||||:||| |||||||| |||||..||:..|||
151 panpkihyettgpeiwkgtgg_kidifvsgigtg..itgag_silknrtanv 198

201 KLYGVEPVESAILSGGKPGPHKIQGIGAGFIPSVLNVDLIDEVVQVSSDE 250
    |||||||||||| ||||||||||||||||||||||||||||||||||||
199 klygvepvesail_iggkpgphkiqgigagfipsvlnvdlidevvqvssde 248

251 SIDMARQLALKEGLLVGISSGAEAAAAIKLAQRPENAGKLFVAIFPSFGE 300
    ||||||||||||:|||||||.|||||||||||||||||||||||||||||
249 sidmarqlalkegflvgissgaaaaaaiklaqrpenagklfvaifpsfge 298

301 RYLSTVLFDATRKEAEAMTFEA 322
    |||||||    |..:
299 rylstvl_smrqgkkrkp..... 315
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Fig. 2. Comparison of deduced amino acid sequences of cytosolic *O*-acetylserine(thiol)lyase genes from *A. thaliana*. Sequences are Atcys-3A in upper case letters (this work), and At.OAS.5–8 in lower case letters [14]. The sequence mismatching is underlined.

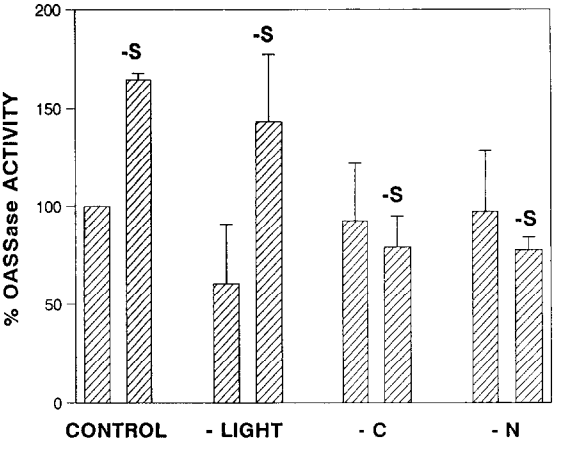

Fig. 3. Effects of different growth conditions on *O*-acetylserine(thiol)lyase activity level in *A. thaliana*. Plant seedlings were grown in + S or − S Hoagland liquid medium supplemented with carbon (5 g/l sucrose) at 25°C and continuous light (Control conditions), or in the absence of light (-Light), carbon source (-C), or nitrogen deprived (-N), as indicated. Further details are described in section 2. Extracts were prepared from 10-day-old seedlings and OASSase specific activity was measured as indicated in section 2. The activity level of +S control seedlings was set as 100% (1.9 ± 0.2 U/mg). The values are averages of at least four independent experiments, and the error bars represent standard deviations.

to the cytosolic forms (identity higher than 80%) [7,8] than to the chloroplast (70%) [9–11], and mitochondrial ones (61%) [13]. One interesting finding was that the amino acid sequence of the chloroplast wheat gene was more related to all the cloned cytosolic isoforms (Table 1). The bacterial and higher-plant enzymes share several conserved motifs along the sequence [18]. The Lys residue in the conserved SVK motif has been involved in the binding site for pyridoxal 5′-phosphate [10,19–20]. All these features were present in the Atcys-3A deduced peptide sequence (Fig. 1).

While our work was in progress, Hell et al. [14] reported the isolation of two cDNA clones coding for compartment specific *O*-acetylserine(thiol)lyase isoforms. Sequence comparision showed that *Atcys-3A* cDNA belonged to the cytosolic gene family in *Arabidopsis*, being a distinct member to the *At.OAS.5–8* cDNA reported before (Table 1). We have performed DNA sequencing on both strands, cheking very carefully the mismatching positions with the *At.OAS.5–8* cDNA sequence. However, we can not rule out the possibility of errors in the nucleotide sequence published by Hell et al. [14]. The Atcys-3A deduced protein was 7 amino acid longer, showing 21 different residues along the sequence and with the highest difference in the last 15 amino acids at the C-terminal (Fig. 2).

Southern blot analysis of genomic DNA revealed several crossreactive bands (not shown), with a pattern very close to the genomic organization previously observed [14]. This finding suggested that the cytosolic isoform was encoded by a multigene family, and was consistent with the isolation of these two different members (this work and [14]).

3.2. *Regulation of* O-*acetylserine(thiol)lyase in different growth conditions and plant organs*

To get a better understanding of the regulation of *O*-acetylserine(thiol)lyase in higher plants, we have analyzed the activity level and gene expression in *Arabidopsis* seedlings grown under different conditions. OASSase activity was up-regulated by sulfur starvation, requiring carbon and nitrogen sources to be effective. This activation seemed not to be light-dependent (Fig. 3). Increasing of specific activity by sulfur depletion have been also observed in cultured tobacco and *C. reinhardtii* cells and

Table 1
Homologies (identities) among the deduced amino acid sequences of cysteine synthases

	1	2	3	4	5	6	7	8	9	10	11	12
1. At-3A	–	95(93)	92(84)	93(83)	90(82)	85(70)	85(72)	77(61)	73(56)	73(56)	69(45)	69(44)
2. At-5-8	–	–	90(81)	90(80)	87(78)	85(69)	85(70)	74(58)	72(54)	71(54)	67(42)	67(42)
3. Sp-cyt	–	–	–	93(86)	92(83)	88(74)	86(76)	78(61)	74(55)	73(56)	69(45)	70(44)
4. Watmelo	–	–	–	–	92(84)	86(73)	86(75)	75(58)	73(55)	73(55)	69(44)	69(44)
5. Wheat	–	–	–	–	–	86(71)	85(74)	77(60)	73(54)	72(54)	67(42)	67(42)
6. Sp-chl	–	–	–	–	–	–	86(76)	75(62)	76(59)	75(58)	69(41)	69(41)
7. Pepper	–	–	–	–	–	–	–	72(62)	71(56)	70(55)	68(42)	68(42)
8. Sp-mit	–	–	–	–	–	–	–	–	69(49)	67(48)	67(43)	66(44)
9. EcoliA	–	–	–	–	–	–	–	–	–	98(96)	66(44)	65(44)
10. SaltyA	–	–	–	–	–	–	–	–	–	–	66(42)	65(42)
11. EcoliB	–	–	–	–	–	–	–	–	–	–	–	97(94)
12. SaltyB	–	–	–	–	–	–	–	–	–	–	–	–

The sequences are from *Arabidopsis* cytosolic isoforms (1. This work and 2. Hell et al. [14]); 3. spinach cytosolic [7]; 4. watermelon [8]; 5. wheat [12]; 6. spinach chloroplast [10]; 7. pepper [9]; 8. spinach mitochondrial [13]; 9. *E. coli* isoenzyme A [2]; 10. *S. tythimurium* isoenzyme A [3]; 11. *E. coli* isoenzyme B [4]; and 12. *S. typhimurium* isoenzyme B [4].

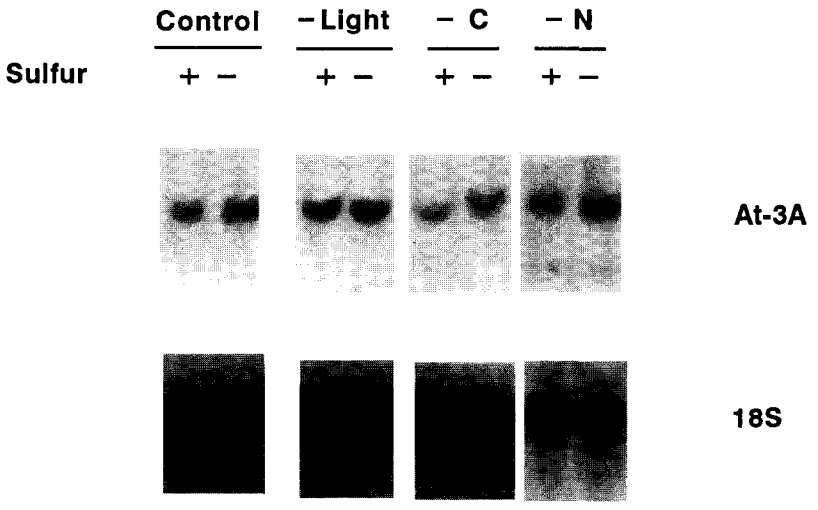

Fig. 4. Effects of different growth conditions on *A. thaliana Atcys-3A* gene expression. Total RNA was isolated from 10-day-old seedlings grown as described in Fig. 3, and Northern blots were performed as indicated in section 2. The results from a single representative experiment are presented.

maize leaves [21–24]. Furthermore, the maximun level of activation by sulfur limitation required CO_2 in the culture medium of *Chlamydomonas* [23]. It has also been suggested a regulatory coupling of nitrogen and sulfur assimilation pathways in plants to balance the flow of these components in protein synthesis [25]. *Arabidopsis* seedlings appeared to have this mechanism, as suggested by the regulation of *O*-acetylserine(thiol)lyase activity. This activity regulation resembled the transcriptional activation of *cysK* and *cysM* genes in bacteria. Both genes were activated under sulfur limitation and in the presence of the N-and C-containing metabolite, *O*-acetyl-L-serine [5]. To determine wether the *O*-acetylserine(thiol)lyase regulation in *Arabidopsis* was at the mRNA level, we performed Northern blot analysis of total RNA extracted from seedlings grown in the conditions above described. *Atcys-3A* was expressed under all studied conditions, but at different intensity. Indeed, we could observe an about 2-fold *Atcys-3A* mRNA accumulation by sulfur starvation, when compared to the level of the 18S rRNA. This activation was light- independent, and the maximun level of *Atcys-3A* gene expression required a carbon and nitrogen source in the culture medium (Fig. 4). Very recently, Ruffet et al. [26] have shown that in spinach chloroplasts the level of serine acetyltransferase, the enzyme involved in the *O*-acetylserine biosynthesis, is very low compared to the amount of *O*-acetylserine(thiol)lyase. Thus, the C- and N-limitation together with the low level of serine acetyltransferase, could produce a net deficiency in the precursor *O*-acetylserine. Similarly to bacterial sulfur genes, the absence of the inducer *O*-acetylserine might negatively regulate the *Arabidopsis* cytosolic *O*-acetylserine(thiol)lyase expression.We have to point out that the activity data represented the addition of different isoenzymes and we have only analyzed the expression pattern of the cytosolic isoform. However, the similarity between *O*-acetylserine(thiol)lyase activity and *Atcys-3A* mRNA expression could suggest that the other isoenzymes were similarly regulated.

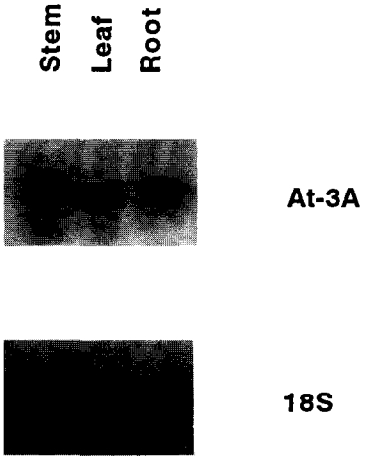

Fig. 5. Organ-specific expression of *Atcys-3A* in *A. thaliana*. Plants were grown on moist vermiculite supplemented with Hoagland medium at 25°C and under 16 h light–8 h dark photoperiod. RNA was isolated from various parts of mature plants and Northern blots were performed as indicated in section 2. The results from a single representative experiment are shown.

We have also analyzed the expression pattern of this gene in different organs of mature plants. Northern blot analysis of total RNA extracted from roots, stems and leaves of *Arabidopsis* plants showed an organ-specific expression of *Atcys-3A* (Fig. 5). The transcript was most abundant in roots, being also present in similar levels in stems and leaves. This type of expression was expected for a cytosolic gene. Similar pattern of expression was also detected for the other member of the cytosolic *O*-acetylserine(thiol)lyase gene family in *Arabidopsis* [14]. It is interesting to note that in spinach the expression of the cytosolic *cysA* gene was constitutive in leaves and roots [7]. Furthermore, the expression of the plastidic wheat gene was surprisingly highest in roots [12].

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