Plant Physiol. (1995) 107: 653–654

Plant Gene Register

A cDNA Clone for an ATP-Sulfurylase from *Arabidopsis thaliana*

Dirk Klonus[1]*, Jörg W. Riesmeier, and Lothar Willmitzer

Institut für Genbiologische Forschung Berlin GmbH, Ihnestrasse 63, 14195 Berlin, Germany

Sulfate is the predominant sulfur source for plants. After uptake from the soil via specific transport proteins into plant roots, sulfate is activated by the enzyme ATP-sulfurylase to APS. ATP-sulfurylase activity in plants is detectable in green tissues and roots (Ellis, 1969; Lunn et al., 1990). Isoforms of the enzyme are localized in plastids and in the cytosol (Lunn et al., 1990; Renosto et al., 1993). In mutants of *Euglena gracilis* lacking plastids an ATP-sulfurylase could be purified from mitochondria (Li et al., 1991). There is considerable evidence that activated sulfate in the form of APS is the sulfate donor for the APS-sulfotransferase reaction leading to the reductive part of sulfur assimilation (Schmidt and Jäger, 1992). Further activation of APS is achieved by the enzyme APS kinase to 3′-phosphoadenosine-5′-phosphosulfate, the substrate for sulfate transfer reactions.

We are interested in the molecular physiology of sulfate uptake and activation by higher plants, and therefore, we have isolated two ATP-sulfurylase cDNAs from *Solanum tuberosum* by functional complementation of a yeast mutant that is deficient in ATP-sulfurylase activity (*met3*; Klonus et al., 1994). Following the same approach we have cloned three different full-length cDNAs from *Arabidopsis thaliana* encoding ATP-sulfurylases. Coincident with our work, two sequences were posted in the data base that were identical with our first clones. Here we report the nucleotide and deduced amino acid sequence of a third *A. thaliana* cDNA clone (ATMET3–1) encoding an ATP-sulfurylase (Table I).

The ATMET3–1 cDNA consists of 1706 nucleotides with an open reading frame of 1431 bp. The predicted polypeptide encoded by ATMET3–1 is 475 amino acids in length and has a calculated molecular mass of 53,638 D. The first 63 amino acids contain no acidic amino acids (Asp, Glu) and elevated levels of hydroxylated residues (Ser, Thr). These are typical features of a chloroplast transit peptide (Heinje et al., 1989). The ATMET3–1 polypeptide is very similar on the amino acid level to the other reported *A. thaliana* cDNAs: 72.7% to APS1 and 73.5% to APS3 (Leustek et al., 1994). When ATMET3–1 is compared to the deduced potato polypeptides STMET3–1 (Stmet3–2), there are 74.5% (73.7%) identical amino acids (Klonus et al., 1994). Only the deduced mature sequences without leader peptides are considered for these calculations. The ATMET3–1 sequence compared to the MET3 yeast sequence is 31% identical on the amino acid level, whereas no similarities can be found to bacterial ATP-sulfurylases. These data emphasize the existence of two unrelated families of ATP-sulfurylase in bacteria and eukaryotic organisms.

Table I. *Characteristics of Atmet3–1, an ATP-sulfurylase cDNA from A. thaliana*

Organism:
Arabidopsis thaliana Landsberg erecta ecotype.

Source:
A. thaliana whole-seedling cDNA library cloned in yeast expression vector pFL61, which carries a URA3 gene for selection in yeast. The mRNA isolated from seedlings of the two- to four-leaf stage is constitutively expressed in this plasmid under the control of the phosphoglycerate kinase promoter (Minet et al., 1992).

Techniques:
The *A. thaliana* cDNA library (Minet et al., 1992) was transformed into the *Saccharomyces cerevisiae* strain W303met3–7D (*met3,ura3,leu2*), which was deficient in ATP-sulfurylase activity. About 200,000 transformed yeast cells were plated on media lacking Met and uracil. After incubation for 14 d at 28°C, 13 colonies showed growth. Plasmids isolated from these colonies contained three groups of cDNA inserts differing in restriction pattern. They were named *ATMET3–1, ATMET3–2, ATMET3–3.*

Sequencing Techniques:
The three cDNAs were cloned into pBluescript SK− and sequenced by the dideoxy method from both ends (Sanger et al., 1977). The total sequence of *ATMET3–1* was determined by a set of subclones and some oligonucleotide primers.

Methods of Identification:
Functional complementation of a yeast mutant deficient in ATP-sulfurylase; detection of ATP-sulfurylase activity in the complemented yeast; sequence homology to potato ATP-sulfurylase cDNAs and yeast *MET3* gene.

Features of DNA Structure:
1706-bp cDNA containing an open reading frame from nucleotide 44 to 1474, a 222-nucleotide 3′ untranslated region, and a short 10-nucleotide poly(A) tail.

Features of Protein:
The open reading frame encodes a polypeptide of 475 amino acids with a predicted M_r of 53,638. The first 63 amino acids show typical composition of a plastid transit peptide.

[1] D.K. was funded by the Schering Forschungsgesellschaft.

* Corresponding author; e-mail klonus@mpimg-berlin-dahlem.mpg.de; fax 49–30–83000736.

Abbreviation: APS, 5′-adenylylphosphosulfate.

ACKNOWLEDGMENTS

We thank Dr. Michèle Minet (Centre de Génétique Moléculaire, Gif sur Yvette, France) for providing the *A. thaliana* cDNA yeast expression library.

Received August 19, 1994; accepted August 29, 1994.
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The GenBank/EMBL accession number for the sequence reported in this article is X79210.

LITERATURE CITED

Ellis RJ (1969) Sulphate activation in higher plants. Planta **88:** 34–42

Heijne G, Steppuhn J, Herrmann RG (1989) Domain structure of mitochondrial and chloroplast targeting peptides. Eur J Biochem **180:** 535–545

Klonus D, Höfgen R, Willmitzer L, Riesmeier JW (1994) Isolation and characterization of two cDNA clones encoding ATP-sulfurylases from potato by complementation of a yeast mutant. Plant J **6:** 105–112

Leustek T, Murillo M, Cervantes M (1994) Cloning of a cDNA encoding ATP sulfurylase from *Arabidopsis thaliana* by functional expression in *Saccharomyces cerevisiae.* Plant Physiol **105:** 897–902

Li J, Saidha T, Schiff JA (1991) Purification and properties of two forms of ATP sulfurylase from *Euglana.* Biochim Biophys Acta **1078:** 68–76

Lunn JE, Droux M, Martin J, Douce R (1990) Localization of ATP sulfurylase and *O*-acetylserine(thio)lyase in spinach leaves. Plant Physiol **94:** 1345–1352

Minet M, Dufour ME, Lacroute F (1992) Complementation of *Saccharomyces cerevisiae* auxotrophic mutants by *Arabidopsis thaliana* cDNAs. Plant J **2:** 417–422

Renosto F, Patel HC, Martin RL, Thomassian C, Zimmerman G, Segel IH (1993) ATP-sulfurylase from higher plants: kinetic and structural characterization of the chloroplast and cytosol enzymes from spinach leaf. Arch Biochem Biophys **307:** 272–285

Sanger F, Nicklen S, Coulsen AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA **74:** 5463–5467

Schmidt A, Jäger K (1992) Open questions about sulfur metabolism in plants. Annu Rev Plant Physiol Plant Mol Biol **43:** 325–349