Plant Physiol. (1995) 107: 1479–1480

Plant Gene Register

Nucleotide Sequence of *Arabidopsis thaliana* Arginase Expressed in Yeast[1]

Paulette M. Krumpelman, Sharyn K. Freyermuth, John F. Cannon, Gerald R. Fink, and Joe C. Polacco*

Biochemistry Department (P.M.K., S.K.F., J.C.P.) and Department of Microbiology and Immunology (J.F.C.), University of Missouri, Columbia, Missouri 65211; and Whitehead Institute, 9 Cambridge Center, Cambridge, Massachusetts 02142 (G.R.F.)

Arginase (L-Arg amidinohydrolase, EC 3.5.3.1) catalyzes the hydrolysis of L-Arg to urea and L-Orn. Orn is a precursor of Pro and polyamines and urea N is recycled by urease-catalyzed hydrolysis to ammonia (Polacco and Holland, 1993). Many plant species store much seed protein N in Arg (VanEtten et al., 1963), significant quantities of which are released and catabolized during germination (Polacco and Holland, 1993). In soybean axes arginase activity increases sharply during germination (Kang and Cho, 1990), consistent with considerable urea accumulation in urease-negative soybean seedlings (Stebbins et al., 1991). The *Arabidopsis* 12S globulin (Pang et al., 1988) and 2S albumin (Krebbers et al., 1988) seed storage proteins have a deduced Arg content of 7.0 and 6.9 mol %, respectively, whereas the Arg content of an "average" protein is 3.0 mol % (VanEtten et al., 1963). Conversion of *Arabidopsis* seed storage protein to seedling protein potentially involves Arg breakdown, consistent with a 10-fold increase in seedling arginase activity during the 0- to 6-d after germination interval (Zonia et al., 1995).

We report the nucleotide sequence of an *Arabidopsis* arginase cDNA recovered by its complementation of a yeast (*Saccharomyces cerevisiae*) arginase-deficient (*car1*) mutant (Table I). The pertinent genotype of the yeast host was *car1,dur1,ura3–52*, a nonreverting uracil auxotroph (*ura3–52*) unable to utilize either Arg (*car1*) or urea (*dur1*) as sole N source. URA^+ transformants (recovered via the method of Gietz et al., 1992) harboring members of a two-leaf-stage *Arabidopsis* cDNA library (in yeast-*Escherichia coli* shuttle vector pFL61; Minet et al., 1992) were replica plated to medium with Arg as sole N source. One URA^+ isolate grew consistently on this medium and its plasmid transmitted this trait to new host cells. We concluded that this isolate expressed an arginase because it accumulated urea in the presence of Arg and because its cDNA insert showed size and sequence similarity with arginase cloned from bacterial, yeast, and mammalian sources. Hybridization analysis indicated that it is present in a single copy in the *Arabidopsis* genome.

The cDNA contains 1357 nucleotides, with a 342-amino acid open reading frame beginning with an ATG codon at position 121. Residues 263 to 303 contain the highest identity with other arginase. However, a separate 40- to 50-residue segment of *Xenopus*, mammalian liver, yeast (*S. cerevisiae*), and *Agrobacterium* arginases contains three conserved His's, important in Mn(II) binding and catalysis (Cavalli et al., 1994). Only two of these His's (residues 161 and 187) could be identified in *Arabidopsis* arginase (based on homologous adjacent residues). Interestingly, the missing His was shown by site-directed mutagenesis to be essential for catalytic activity in rat liver arginase (Cavalli et al., 1994).

The N-terminal 79 residues exhibit no homology with other arginases. Since only plant arginases have been reported to be mitochondrial (Polacco and Holland, 1993), this region may contain a transit sequence. In agreement with this, it is rich in positively charged and hydroxylated residues (Hartl et al., 1989). We have not yet determined whether the expressed arginase is mitochondrial in *Arabidopsis* or in the yeast host. Yeast CAR1 encodes a cytoplasmic arginase (Urrestarazu et al., 1977).

ACKNOWLEDGMENTS

We thank F. Messenguy for a *car1* yeast isolate and M. Minet for the *Arabidopsis* cDNA library.

Received September 26, 1994; accepted November 2, 1994.
Copyright Clearance Center: 0032–0889/95/107/1479/02.
The GenBank accession number for the sequence reported in this article is U15019.

LITERATURE CITED

Cavalli RC, Burke CJ, Kawamoto S, Soprano DR, Ash DE (1994) Mutagenesis of rat liver arginase expressed in *Escherichia coli*: role of conserved histidines. Biochemistry **33:** 10652–10657

Gietz D, St. Jean A, Woods RA, Schiestl RH (1992) Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res **20:** 1425

Hartl F-U, Pfanner N, Nicholson DW, Neupert W (1989) Mitochondrial protein import. Biochim Biophys Acta **988:** 1–45

Kang JH, Cho YD (1990) Purification and properties of arginase

[1] Supported in part by the Missouri Agricultural Experiment Station and by grants from the National Science Foundation (IBN-911768) and the U.S. Department of Agriculture (CSRS 92–37305–7871). S.K.F. is a recipient of a National Science Foundation Plant Biology Postdoctoral Fellowship. Journal paper number 12,207 from the Missouri Agricultural Experiment Station.

* Corresponding author; e-mail bcjoecpo@muccmail.missouri.edu; fax 1–314–882–5635.

Abbreviation: URA^+, uracil protrotroph.

Table I. *Characterization of a cDNA encoding an A. thaliana arginase*

Organism:

Arabidopsis thaliana ecotype Landsberg *erecta.*

Enzyme, Function:

Arginase; hydrolysis of L-Arg to L-Orn and urea. Provides Orn for Pro and polyamine synthesis. Induced in germination; apparently involved in mobilization of storage protein N.

Source:

cDNA library of Minet et al. (1992) from two-leaf stage plantlets (grown on 1% Suc and Murashige and Skoog salts, 16-h light photoperiod). cDNA was cloned in the *Escherichia coli*-yeast shuttle plasmid pFL61, under control of the phosphoglycerate kinase promoter and transcription terminator. pFL61 contains a functional URA3 gene.

Clone Isolation:

Arginase cDNA expression was selected in yeast (*S. cerevisiae*) host (JPX8–27A) of pertinent genotype *car1,dur1,ura3–52*, a nonreverting uracil auxotroph (*ura3–52*) unable to utilize either Arg (*car1*) or urea (*dur1*) as sole N source. Of approximately 10,000 URA^+ transformants replica plated to Arg as sole N source, one isolate (23A) consistently grew. Plasmid pPMK23A, recovered from this isolate, transmitted Arg utilization to JPX8–27A cells.

Sequencing:

The *Not*I fragment of pPMK23A was subcloned into pBluescriptII KS^+ and double-stranded DNA minipreparations were sequenced by chain-termination reactions employing Sequenase (United States Biochemical) and oligonucleotide primers based on plasmid and insert sequences. Both strands of complete cDNA and deletion derivatives were sequenced.

Method of Identification:

Urea production from Arg. JPX8–27A and the URA^+ derivative 23A were grown overnight on YNB minimal medium with or without 2.5 mM Arg. Urea was determined in the supernatant of TCA (10%, w/v)-treated cells. Only 23A cells exposed to Arg produced urea.

Homology to other arginases. The cDNA contains 1357 nucleotides containing a complete 342-amino acid open reading frame beginning at nucleotide 121. Amino acid residues 263 to 303 contain the highest identity with other arginases. However, a separate 40- to 50-residue segment of mammalian, yeast (*S. cerevisiae*), and *Agrobacterium* arginases contains three conserved His's, important in Mn(II) binding and catalysis (Cavalli et al., 1994). Only two of these His's were identified in *Arabidopsis* arginase (based on the amino acid context). The third His was reported to be essential for catalytic activity in rat liver arginase (Cavalli et al., 1994). The first 79 residues, lacking homology with any animal, bacterial, or yeast arginases, may contain a transit sequence; plant arginases have been reported to be mitochondrial (Polacco and Holland, 1993). In agreement, this region is rich in positively charged and hydroxylated residues (Hartl et al., 1989). We have not yet determined whether the expressed arginase is mitochondrial in *Arabidopsis* or in its yeast host, whose own CAR1-coded arginase is cytoplasmic (Urrestarazu et al., 1977).

from soybean, *Glycine max*, axes. Plant Physiol **93:** 1230–1234

Krebbers E, Herdies L, de Clerq A, Seurinck J, Leemans J, Van Damme J, Segura M, Gheysen G, Van Montagu M, Vandekerckhove J (1988) Determination of the processing sites of an *Arabidopsis* 2S albumin and characterization of the complete gene family. Plant Physiol **87:** 859–866

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

Pang P, Pruitt R, Meyerowitz E (1988) Molecular cloning, genomic organization, expression and evolution of 12S seed storage protein genes of *Arabidopsis thaliana.* Plant Mol Biol **11:** 805–820

Polacco JC, Holland MA (1993) Roles of ureases in plant cells. Int Rev Cytol **145:** 65–103

Stebbins N, Holland MA, Cianzio SR, Polacco JC (1991) Genetic tests of the roles of the embryonic ureases of soybean. Plant Physiol **97:** 1004–1010

Urrestarazu LA, Vissers S, Wiame JM (1977) Change in location of ornithine carbamoyltransferase and carbamoylphosphate synthetase among yeasts in relation to the arginase/ornithine carbamoyltransferase regulatory complex and the energy status of the cells. Eur J Biochem **79:** 473–481

VanEtten CH, Miller RW, Wulff IA, Jones Q (1963) Amino acid composition of seeds from 200 angiospermous plant species. J Agric Food Chem **11:** 399–410

Zonia LE, Stebbins NE, Polacco JC (1995) Essential role of urease in germination of nitrogen-limited *Arabidopsis thaliana* seeds. Plant Physiol **107:** 1097–1103