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Characterization of the *Arabidopsis thaliana* cDNA Encoding Dihydroorotase (Accession No. **AF000146**)

Lan Zhou (1), François Lacroute (2), and Robert Thornburg (1) (*)

(1) Department of Biochemistry Biophysics, 2232 Molecular Biology Building, Iowa State University, Ames, Iowa 50011

(2) Centre de Génétique Moléculaire, Centre Nationale de La Recherche Scientifique, 22 Avenue de la Terrasse, 91198 Gif sur Yvette, Cedex, France

*Corresponding Author;
FAX 1-515-294-0453
Email thorn@iastate.edu

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Dihydroorotase (L-4,5-dihydroorotate amidohydrolase, E.C. **3.5.2.3**, catalyzes the reversible enzymatic conversion of carbamoyl aspartate (ureidosuccinate) into dihydroorotic acid via an intermolecular amide condensation. This is one of the very few enzymes used to form an amide bond without a directly coupled energy source such as ATP to drive the reaction. Dihydroorotase is the third enzyme in the highly conserved de novo pyrimidine biosynthetic pathway. Like other enzymes on the interior of a biochemical pathway, dihydroorotase is not feedback regulated by end product inhibition in *E. coli* (Sander and Hebe, 1971), *S. cerevisiae* (Lacroute, 1968), or in *C. pepo* (Lin and Lovatt, 1986). The catalytic mechanism of action of the mammalian and most bacterial dihydroorotases is thought to be similar to that of a zinc protease (Christopherson and Jones, 1980).

The *E. coli* enzyme has been cloned and overexpressed (Brown and Collins, 1986). The enzyme has a tightly bound catalytic Zn(II) ion at the active site (Washabaugh and Collins, 1984) and also has two weakly bound structural Zn(II) ions per subunit that are not essential for activity (Washabaugh and Collins, 1986). In the eukaryotic CAD enzyme, the structural Zn(II) ion is required for activity; however, the two weakly bound structural Zn (II) ions are not present (Simmer *et al.* , 1990). The *E. coli* enzyme is also a dimer (Washabaugh and Collins, 1984) while the enzyme is a monomer in certain parasitic protozoa (Krungrai *et al.* , 1990).

Sequences encoding dihydroorotase (or CAD) have been isolated from *Homo sapiens* (Simmer *et al.* , 1990), *Mesocricetus auratus* (golden hamster; Bein *et al.* , 1991), *Drosophila melanogaster* (Freund and Jarry, 1987), *D. discoideum* (Faure *et al.* , 1989), *S. cerevisiae* (Guyonvarch *et al.* , 1988), *Ustilago maydis* (Spanos *et al.* , 1992), *E. coli* (Bäckström *et al.* , 1986), *Methanococcus jannaschii* (Bult *et al.* , 1996), and *Arabidopsis thaliana* (Nasr *et al.* , 1994).

The genetic organization of the genes encoding dihydroorotase is variable. In mammals (Simmer *et al.* , 1990), *Drosophila melanogaster* (Freund and Jarry, 1987) and *Dictyostelium discoideum* (Faure *et al.* , 1989), the dihydroorotase enzymatic activity is part of a large multifunctional enzyme termed CAD. This

protein is encoded by a single gene and contains the enzymatic activities of the first three enzymes of pyrimidine biosynthesis, Carbamoyl phosphate synthase (EC [2.7.2.9](#)), Aspartyl transcarbamoylase (EC [2.1.3.2](#)) and Dihydroorotase ([3.5.2.3](#)). In plants, *S. cerevisiae* (Guyonvarch *et al.* , 1988), *Chlorella* (Dunn *et al.* , 1977) and some fungi (Spanos *et al.* , 1992), on the other hand, the dihydroorotase is encoded as a monomeric protein that is not physically linked with other enzymes.

In plants, expression of dihydroorotase has been examined during germination of wheat grains, the enzymatic activity of dihydroorotase drops by 50% during the first 30 minutes following imbibition and remains low until about 12 hours after imbibition, when the activity begins to rise. By two days after germination, the activity is four-fold higher than in dry seeds (Mazus and Buchowicz, 1972). Thus, the gene encoding dihydroorotase must be developmentally regulated during seedling development. Whether the dihydroorotase gene is also environmentally expressed in plants is not currently known.

DNA sequence reactions were performed using the Applied Biosystems Prism Dye-deoxy Cycle Sequencing Kit. The reactions were run on an Applied Biosystems Prism 377 DNA sequencer, Perkin-Elmer Corp. Sequence was initiated from known vector sequences. On the basis of these runs, primers specific to the dihydroorotase sequence were constructed to extend the cDNA sequence. DNA sequences were performed in duplicate or triplicate for each run. Each strand was completely sequenced including sequencing through all restriction enzyme sites and the entire sequence of the cDNA confirmed on the opposite strand. The sequence was deposited in GenBank with accession number [AF000146](#).

The cDNA encoding dihydroorotase is 1362 nucleotides long. The cDNA is full length. A methionine start codon was found at [86..88]. The region surrounding the methionine start codon (CAGATGA) is in poor compliance with the consensus eukaryotic translation start region (Kozak, 1986). None of the nucleotides surrounding the start codon is consensus. The pyrimidine at position -3 and substitutions at -1, -2, and +4 should result in a poor rate of translation initiation for the *Arabidopsis* dihydroorotase. An ochre stop codon is located at [1217..1219]. A consensus polyadenylation signal (AATAAA) was observed at [1325..1330], 18 nucleotides upstream from the polyadenylation site [1348].

The *Arabidopsis thaliana* Dihydroorotase is a 377 amino acid protein that shares high identity with prokaryotic dihydroorotase enzymes and moderate identity with the eukaryotic enzymes. The three conserved domains found among other dihydroorotases (Guyonvarch *et al.* , 1988) are also conserved in the *Arabidopsis* cDNA: Domain A (42-DWHLHLRDGDL-52), Domain B (67-AIVMPNLKPPVTS-79) and Domain C (276-FLGTDSAPHERSRK-289). The histidines of Domain A are thought to coordinate the catalytic zinc (Brown and Collins, 1991). Based upon this identity, we speculate that the catalytic mechanism of the plant enzyme is similar to that of the *E. coli* and *S. cerevisiae* enzymes.

Table 1. Characteristics of the *Arabidopsis thaliana* cDNA for Dihydroorotase

Organism:

Arabidopsis thaliana cv Columbia

Source:

Clone was obtained by complementation of a *S. cerevisiae* ura4 mutant (Nasr *et al.* , 1994)

Function of Gene:

Dihydroorotase (L-4,5-dihydroorotate amidohydrolase), EC [3.5.2.3](#), third enzyme of the de novo pyrimidine biosynthetic pathway, enzyme catalyzes an intramolecular condensation of carbamoyl aspartate (ureidosuccinate) to form dihydroorotate.

Method of Identification:

Sequence isolated by complementation of a *S. cerevisiae* dihydroorotase mutant; confirmed by identity with known dihydroorotases from a variety of organisms.

Characteristics of cDNA:

The cDNA is contained on a 1362 nucleotide fragment. A 85 bp 5' untranslated sequence [1 . . 85]

Poor consensus signal surrounding the translation start codon (CAGATGA) found at [83 . . 89] A 377 amino acid protein [86 . . 1216] An opal stop codon (TGA) at [1217 . . 1219]. A 131 bp 3' untranslated sequence [1217 . . 1348]. A consensus polyadenylation signal (AATAAA) found at [1325 . . 1330], which is 18 nucleotides upstream from the polyadenylation site [1348].

Features of the deduced Dihydroorotase:

A 377 amino acid protein. pI = 8.64 MW = 41,950 Protein was predicted to be 31.6% alpha-helical; 20.4% beta-pleated sheet and 48.0% unstructured.

Identity of the Arabidopsis protein with dihydroorotases from other species:

Species	% identity	Accession Number
<i>E. coli</i>	54.5	P05020
<i>S. typhimurium</i>	51.6	P06204
<i>U. maydis</i>	27.6	P31301
<i>S. cerevisiae</i>	23.9	P20051
<i>M. jannaschii</i>	22.7	P46538

In addition, FastA analysis revealed that the *A. thaliana* dihydroorotase had about 20% identity with a region of the CAD enzymes from *H. sapiens* (GenPept [P27708](#)), *Mesocricetus auratus* (GenPept [P08955](#)), *D. discoideum* (GenPept [P20054](#)), and *D. melanogaster* (GenPept [P05990](#)). This region extends approximately from amino acid 1400 to 1800 of the various CAD enzymes.

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Literature Cited

- Bäckström, D, Sjöberg, R-M, Lundberg, LG** (1986) Nucleotide sequence of the structural gene for dihydroorotase of *Escherichia coli* K-12. *Eur. J. Biochem.* **160**:77-82.
- Bein, K, Simmer, JP, Evans, DR** (1991) Molecular cloning of a cDNA encoding the amino end of the mammalian multifunctional protein CAD and analysis of the 5'-flanking region of the CAD gene. *J. Biol. Chem.* **266**:3791-3799.
- Bult, CJ, White, O, Olsen, GJ, Zhou, L, Fleischmann, RD, Sutton, GG, Blake, JA, FitzGerald, LM, Clayton, RA, Gocayne, JD, Kerlavage, AR, Dougherty, BA, Tomb, J-F, Adams, MK, Reich, CI, Overbeek, R, Kirkness, EF, Weinstock, KG, Merrick, JM, Glodek, A, Scott, JL, Geoghagen, NSM, Weidman, JF, Fuhrmann, JL, Nguyen, D, Utterback, TR, Kelly, JM, Peterson, JD, Sadow, PW, Hanna, MC, Cotton, MD, Roberts, KM, Hurst, MA, Kaine, BP, Borodovsky, M, Klenk, H-P, Fraser, CM, Smith, HO, Woese, CR, Venter, JC** (1996) Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. *Science* **273**:1058-1073.
- Brothers, VM, Tsubota, SI, Germeraad, SE, Fristrom, JW** (1978) Rudimentary locus of *Drosophila melanogaster*: partial purification of a carbamylphosphate synthase-aspartate transcarbamylase-dihydroorotase complex. *Biochem. Genet.* **16**:321-332.
- Brown, DC, Collins, KD** (1986) Dihydroorotase from *Escherichia coli*. Cloning the *pyrC* gene and production of tryptic peptide maps. *J. Biol. Chem.* **261**:5917-5919.

- Brown DC, Collins KD** (1991) Dihydroorotase from *Escherichia coli*. *J. Biol. Chem.* **266**:1597-1604.
- Christopherson, RI, Jones, ME** (1980) The effects of pH and inhibitors upon the catalytic activity of the dihydroorotase of multienzymatic protein pyr1-3 from mouse ehrlich ascites carcinoma. *J. Biol. Chem.* **255**:3358-3370.
- Dunn, JH, Jervis, HH, Wilkins, JH, Meredith, MJ, Smith, KT, Flora, JB, Schmidt, RR** (1977) Coordinate and non-coordinate accumulation of aspartate tanscarbamylase and dihydroorotase in synchronous *Chlorella* cells growing on different nitrogen sources. *Biochim. Biophys. Acta* **485**:301-313.
- Faure, M. , Camonis, J. H, Jacquet, M** (1989) Molecular characterization of a *Dictyostelium discoideum* gene encoding a multifunctional enzyme of the pyrimidine pathway. *Eur. J. Biochem.* **179**:345-358.
- Freund, JN, Jarry, BP** (1987) The rudimentary gene of *Drosophila melanogaster* encodes four enzymic functions. *J. Mol. Biol.* **193**:1-13.
- Guyonvarch, A, Nguyen-Juilleret, M, Hubert, JC, Lacroute, F** (1988) Structure of the *Saccharomyces cerevisiae* URA4 gene encoding dihydroorotase. *Mol. Gen. Genet.* **212**:134-141.
- Kozak, M** (1986) Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell* **44**:283-292.
- Krungkrai, J, Cerami, A and Henderson, GB** (1990) Pyrimidine biosynthesis in parasitic protozoa: Purification of a monofunctional dihydroorotase from *Plasmodium berghei* and *Crithidia fasciculata*. *Biochemistry.* **29**:6270-6275.
- Lacroute, F** (1968) Regulation of pyrimidine biosynthesis in *S. cerevisiae*. *J. Bacteriol.* **95**:824-832.
- Lin, F-F, Lovatt, CJ** (1986) Biosynthesis of pyrimidine nucleotides in cultured root callus of *Cucurbita pepo*. *In Vitro Cell. Dev. Biol.* **22**:1-5.
- Mazus, B, Buchowicz, J** (1972) Activity of enzymes involved in pyrimidine metabolism in the germinating wheat grains. *Phytochem.* **11**:77-82.
- Nasr, F, Berthauche, N, Dufour, M-E, Minet, M, Lacroute, F** (1994) Heterospecific cloning of *Arabidopsis thaliana* cDNAs by direct complementation of pyrimidine auxotrophic mutants of *Saccharomyces cerevisiae*. I. Cloning and sequence analysis of two cDNAs catalyzing the second, fifth and sixth steps of the de novo pyrimidine biosynthesis pathway. *Mol. Gen. Genet.* **244**:23-32.
- Sander, EG, Heeb, MJ** (1971) Purification and properties of dihydroorotase from *Escherichia coli* B. *Biochim. Biophys. Acta* **227**:442-452.
- Simmer, JP, Kelly, RE, Rinkler, AG, Jr., Zimmermann, BH, Scully, JL, Kim, H, Evans, DR** (1990) Mammalian dihydroorotase: nucleotide sequence, peptide sequences and evolution of the dihydroorotase domain of the multifunctional protein CAD. *Proc. Natl. Acad. Sci. USA* **87**:174-178.
- Spanos, A, Kanuga, N, Holden, DW, Banks, GR** (1992) The *Ustilago maydis* pyr3 gene: sequence and transcriptional analysis. *Gene* **117**:73-79.
- Washabaugh, MW, Collins, KD** (1984) Dihydroorotase from *Escherichia coli*: Purification and characterization. *J. Biol. Chem.* **259**:3293-3298.
- Washabaugh, MW, Collins, KD** (1986) Dihydroorotase from *Escherichia coli*: Sulfhydryl group-metal ion interactions. *J. Biol. Chem.* **261**:5920-5929.



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