A cDNA Encoding a Novel Cytochrome P450-Dependent Monooxygenase from Arabidopsis thaliana

Clint C. S. Chapple*
Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907-1153

Although more than 100 P450s have been isolated from animal and bacterial sources, it has only been in the past 5 years that genes encoding P450s have been cloned from plants. Their isolation has identified several new families of P450s, and these include cinnamate-4-hydroxylase (family CYP73) (Mizutani et al., 1993; Teutsch et al., 1993), flavonoid 3',5'-hydroxylase (CYP75) (Holton et al., 1993), and allene oxide synthase (CYP74) (Song et al., 1993), as well as a number of other P450s of unknown function, such as the avocado-ripening-related P450 (family CYP71) (Bozak et al., 1990), a putative geraniol-10-hydroxylase from Catharanthus roseus (CYP72) (Vetter et al., 1992), P450s from Solanum melongena (CYP76 and CYP77) (Toguri et al., 1993; Toguri and Tokugawa, 1994), and a P450 from Thlaspi arvense (Udvardi et al., 1994).

Recently, a mutant of Arabidopsis was identified that appeared to be defective in the activity or expression of ferulate-5-hydroxylase (Chapple et al., 1992), an enzyme that had previously been reported to be a P450 (Grand, 1984). This mutant has been named fah1 and we have since identified a T-DNA-tagged version of this mutant and have used plasmid rescue to clone the affected gene (C.C.S. Chapple, unpublished data). It was not surprising, considering that many P450s exhibit significant sequence homology, that in the course of these experiments an additional P450 was isolated, presumably by cross-hybridization of conserved sequences in the two genes.

The newly identified P450 cDNA encodes a protein of approximately 57.5 kD, consistent with the size of other P450s (Table I). The hydrophobic N terminus of the protein likely serves to anchor the protein to the ER. Amino acid sequence comparison shows that the new P450 is most similar to the avocado-ripening-related P450 (family CYP71) (Bozak et al., 1990) with an overall sequence identity of 32%. By convention, P450s sharing less than 40% sequence identity are designated as members of separate families (Nebert and Nelson, 1991). Thus, this cDNA identifies a new P450 family and has been designated CYP83.

Table I. Characteristics of a cDNA encoding a CYP83 P450 from A. thaliana

| Organism: Arabidopsis thaliana ecotype Columbia. |
| Enzyme Class: Cyt P450-dependent monooxygenase, substrate unknown. |
| Source: APRL-2 library made by Dr. Thomas Newman at the Department of Energy Plant Research Laboratory, Michigan State University. The library was constructed in λZip-Lox using pooled mRNA from etiolated seedlings, roots grown in vitro, and whole plants. |
| Cloning and Sequencing Techniques: Identified by cross-hybridization with a probe derived by plasmid rescue from a T-DNA-tagged version of a fah1 mutant of Arabidopsis, defective in ferulate-5-hydroxylase. Dye-terminator dideoxy sequencing was completed on both strands using double-stranded plasmid DNA on a DuPont Genesis 2000 sequencer and on an Applied Biosystems 373A DNA sequencer, using standard vector-based sequencing oligonucleotides or custom-synthesized oligonucleotides as appropriate. |
| Sequence Identification: Comparison of the deduced amino acid sequence to previously identified P450 sequences. |
| Sequence Features: Total length, 1551 bp. Deduced translation start site at nucleotide 8, stop site at nucleotide 1514. Open reading frame of 1506 bp encodes a protein of 57,427 D with a pl of 8.4. The deduced amino acid sequence shows a hydrophobic N-terminal tail and the conserved heme-binding sequence typical for P450s surouns Cys442. |
| Chromosomal Location: Unknown. Southern analysis indicates that this P450 is encoded by a single-copy gene in A. thaliana. |

ACKNOWLEDGMENTS

I thank Dr. Thomas Newman for supplying the λZip-Lox library and Dr. David Nelson for assigning the appropriate designation to CYP83.

Received December 21, 1994; accepted January 26, 1995.
Copyright Clearance Center: 0032-0889/95/108/0875/02. The GenBank accession number for the sequence reported in this article is U18929.

LITERATURE CITED


* E-mail chapple@biochem.purdue.edu; fax 1–317–496–1641 or 1–317–494–7897.


