Plant Gene Register

Cloning and Sequencing of a Full-Length cDNA Coding for Phenylalanine Ammonia-Lyase from Tobacco Cell Culture

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PAL (EC 4.3.1.5) plays a key role in linking primary metabolism to phenylpropanoid metabolism by catalyzing the deamination of L-Phe to produce trans-cinnamic acid. This reaction is considered a key step in phenylpropanoid metabolism. In bean (Edwards et al., 1985) and parsley (Lois et al., 1989), PAL genes are transcriptionally activated by elicitor treatment, UV light, and wounding. We reported that the addition of cytokinin to tobacco (Nicotiana tabacum L. Bright Yellow T-13) cell cultures increased PAL activity (about 2-fold) and resulted in high-level accumulations of scopoletin and scopolin (Hino et al., 1982). The increase in PAL activity was shown to result from a stimulation of de novo synthesis of the PAL enzyme, based on immunochemical studies (Nagai et al., 1988). However, the regulatory mechanism is not clearly understood. To clarify the mechanism of this cytokinin-mediated PAL induction, we have cloned a full-length PAL cDNA that corresponds to the PAL induced by cytokinin.

Total cellular RNA was isolated from cytokinin-treated cells (final concentration of 1 μg/10^6 cells/mL) by a modification of the method of Chirgwin et al. (1979). Poly(A)+ RNA was isolated by adsorption and elution from an oligo(dT)-cellulose column and used for the construction of the cDNA library. Double-stranded cDNAs were synthesized and cloned into the bacteriophage M13 mp18 or mp19. The nucleotide sequence of both strands was determined by the dideoxy chain-termination method.

In this paper, we present the sequence of the full-length cDNA encoding tobacco PAL. It contains 2136 nucleotides: 206 nucleotides of 5' untranslated region, an open reading frame of 2136 nucleotides, and 206 nucleotides of 3' untranslated region containing 19 nucleotides of poly(A). Comparison with other PAL sequences (coding region) shows 83.1% homology with the potato PAL (Joos and Hahlbrock, 1992), 82.3% with that of tomato (Lee et al., 1992), 78.9% with that of sweet potato (Tanaka et al., 1989), 77.9% with that of parsley (Lois et al., 1989), 76.7% with that of bean (Edwards et al., 1995), and 74.5% with that of tomato (Lee et al., 1992). The nucleotide sequence of the coding region was determined to be 77,400 D from the deduced amino acid sequence.

We compared the nucleotide sequence of the coding region and the deduced amino acid sequence of tobacco PAL cDNA with the partial sequences of PAL cDNA from the other plants. The homology between the nucleotide sequences was 74 to 83% for potato (Joos and Hahlbrock, 1992), tomato (Lee et al., 1992), sweet potato (Tanaka et al., 1989), parsley (Lois et al., 1989), poplar (Subramaniam et al., 1993), soybean (Frank and Vodkin, 1991), and 74.5% with that of bean (Edwards et al., 1985).

Table I. Characteristics of a PAL cDNA from tobacco cell culture

| Organism: Tobacco (Nicotiana tabacum L. Bright Yellow T-13 callus). |
| Function: Encodes a subunit of the tetrameric enzyme PAL (EC 4.3.1.5), which catalyzes the conversion of L-Phe to trans-cinnamate and NH3. |
| Source: cDNA library constructed on a template of poly(A)+ RNA from a cytokinin (1 μg/mL)-treated tobacco cell culture. |
| Techniques: A cDNA library constructed in λgt11 was screened with the 32P-labeled PAL cDNA isolated from bean (Edwards et al., 1985). Restriction fragments of the cDNA insert were subcloned into the bacteriophage M13 mp18 or mp19. The nucleotide sequence of both strands was determined by the dideoxy chain-termination method. |
| Features of cDNA Structure: Contains 2546 nucleotides: 204 nucleotides of 5' untranslated region, an open reading frame of 2136 nucleotides, and 206 nucleotides of 3' untranslated region containing 19 nucleotides of poly(A). Comparison with other PAL sequences (coding region) shows 83.1% homology with the potato PAL (Joos and Hahlbrock, 1992), 82.3% with that of tomato (Lee et al., 1992), 78.9% with that of sweet potato (Tanaka et al., 1989), 77.9% with that of parsley (Lois et al., 1989), 76.7% with that of poplar (Subramaniam et al., 1993), 75.7% with that of soybean (Frank and Vodkin, 1991), and 74.5% with that of bean (Edwards et al., 1985). |
| Feature of the Deduced Protein: Open reading frame of 712 amino acids; predicted molecular mass 77,400 D. Comparison with other PAL sequences shows 86.3% homology with the partial potato PAL (Joos and Hahlbrock, 1992), 88.0% with that of tomato (Lee et al., 1992), 86.3% with that of sweet potato (Tanaka et al., 1989), 84.5% with that of parsley (Lois et al., 1989), 83.5% with that of poplar (Subramaniam et al., 1993), 83.3% with that of soybean (Frank and Vodkin, 1991), and 84.4% with that of bean (Edwards et al., 1985). |
| Antibodies: Polyclonal antibodies against PAL from tobacco cell culture are available. |

Abbreviation: PAL, phenylalanine ammonia-lyase.

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(Frank and Vodkin, 1991), and bean (Edwards et al., 1985), and the homology between the deduced amino acid sequences was about 83 to 88% (Table I).

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LITERATURE CITED


