Cloning and Sequencing of Chickpea cDNA Coding for Threonine Deaminase

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TD (EC 4.2.1.16) is the enzyme catalyzing the conversion of L-Thr to α-ketobutyrate, the first step of the pathway leading to the biosynthesis of the essential amino acid, L-Ile. Regulation of TD activity by Ile was the first recognized instance of allosteric feedback regulation by the end product of a biosynthetic pathway (Umbarger, 1956). The substrate L-Thr is a feedback inhibitor of aspartokinase and homoserine dehydrogenase (Umbarger, 1978; Jones and Fink, 1982), two of the key regulatory enzymes of the branched chain amino acid pathway. The role of TD in plant cell proliferation and differentiation influenced by Ile, Leu, and Met has been reported earlier from our laboratory (Basu et al., 1989). To extrapolate these biochemical data to the molecular level, we attempted to isolate cDNA for TD. Here we report the cloning and complete nucleotide sequence of TD cDNA from *Cicer arietinum* L. and expression of TD transcript in different parts of the plant.

The TD genes from *Escherichia coli* ilv A (Cox et al., 1987), yeast ilv1 (Kielland-Brandt et al., 1984), and tomato (Samach et al., 1991) have been cloned and characterized. Homologous domains conserved in evolution, denoted as C 1–5 and R 1–7, have been identified and characterized (Taillon et al., 1988). The 1.1-kb *PstI* fragment of pC519 containing ilv1 gene (Kielland-Brandt et al., 1984) from yeast was used as a probe to isolate the chickpea homolog. The cDNA library was constructed in λ ZAP II using poly(A)+ RNA from immature seeds of chickpea. Several rounds of plaque hybridization were carried out using the probe to isolate the homologous clone. The clone for TD cDNA, pCiTD2, is 1872 bp long and its deduced polypeptide contains a 590 amino acids. The N terminus of the deduced polypeptide contains a typical two-domain transit peptide consistent with chloroplast lumen targeting sequences (Keegstra et al., 1989), indicating chloroplast localization of the mature protein (Table I). The N-terminal domain (45 residues) is rich in Thr and Ser (37%), and the rest of the sequence (46 residues) contains 8 regularly spaced Pro and 19 other hydrophobic residues. The cleavage site of transit peptide has not been identified. The deduced amino acid sequence shows homology with the reported sequences of TD from the following sources: tomato, 61% (Samach et al., 1991); yeast, 46% (Kielland-Brandt et al., 1984); and *E. coli*, 37% (Cox et al., 1987). Homology in C and R-type conserved domains is markedly higher compared to the other regions of the protein (Table I). Also, the homology in the first half (N-terminal) of the protein is higher than in the second half. Another prominent characteristic is the presence of a putative Ser/Thr deaminase PIP binding site at positions 128 to 147 of the deduced polypeptide sequence. In our study, northern analysis of the total RNA from different parts of chickpea plant shows higher transcript levels in flowers than in other organs. The overexpression of TD in flowers has also been reported in tomato (Samach et al., 1991).

ACKNOWLEDGMENT

We wish to thank Dr. Kielland-Brandt (Carlsberg Laboratory, Denmark) for sending us ilv1, pC519.

Table I. Characteristics of chickpea cDNA encoding TD

| Feature | Organism: *Cicer arietinum* L. | Function: Biosynthesis of Ile. | Cloning Technique: Screening of cDNA library constructed from poly(A)+ RNA isolated from developing seeds of chickpea with a 1.1-kb *PstI* fragment of ilv1 gene as heterologous probe from yeast. | Sequencing Technique: cDNA clone was in vivo excised from λ ZAP II recombinant phage and strand sequenced using both Sequenase and synthetic primers. | Method of Identification: Homology in the complete sequence, conserved domains and N-terminal region with reported TD sequences from *E. coli*, yeast, and tomato. | Features of cDNA: 1872 bp long, sequence for transit peptide, marked homology with reported TD sequences from various sources in C and R-type conserved domains. | Features of Predicted Amino Acid Sequence: N-terminal domain rich in Thr, presence of chloroplast lumen targeting sequence, presence of Ser/Thr PIP binding site. |

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1 This work was supported by a grant from Department of Biotechnology, government of India, to S. G.-M. and a fellowship from the Council of Scientific and Industrial Research, New Delhi, to S.J.J.

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Abbreviation: TD, threonine deaminase.
Received August 19, 1994; accepted September 26, 1994. 
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The EMBL accession number for the sequence reported in this 
article is X78575.

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