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Plant Gene Register

cDNA Encoding Tropinone Reductase-II from *Hyoscyamus niger*

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Two stereospecific NADPH-dependent reductases, TR-I and TR-II, constitute a branching point in the biosynthesis of tropane alkaloids. TR-I catalyzes the stereospecific reduction of tropinone to tropine (Koelen and Gross, 1982), whereas TR-II reduces tropinone to pseudotropine (Dräger et al., 1988). We previously characterized TRs that had been purified from cultured roots of *Hyoscyamus niger* (Hashimoto et al., 1992) and showed that the two TRs had both common and different biochemical and kinetic properties. To obtain a better understanding of the structure and evolutionary relationship of these reductases, we isolated cDNA clones coding for TR-II from *H. niger* (Table I).

An internal amino acid sequence was found in both TR-I and TR-II that had been purified from the cultured roots of *Datura stramonium* and *H. niger*, respectively (Nakajima et al., 1993). An oligonucleotide probe corresponding to this sequence was synthesized and used to screen the cDNA library from cultured roots of *H. niger*. DNA sequencing analysis revealed that all four of the isolated cDNA clones encoded the TR-II polypeptide, probably due to the low concentration of the TR-I transcript in this genus. None of the cDNA clones contained a full ORF; some lacked the amino-terminal part and others the carboxy-terminal part and the 3′ nontranslated region. Because the nucleotide sequence of the overlapping part (0.4–0.8 kb) matched perfectly, we concluded that these clones were derived from a single gene. The combined nucleotide sequence (1049 bp) contained a 783-bp ORF coding for a polypeptide composed of 260 amino acids, and the calculated mol wt of 28,436 agreed well with the molecular mass for the TR-II subunit (29 kD) that had been purified from *H. niger* (Hashimoto et al., 1992).

The isolated cDNA was expressed in *Escherichia coli* as a fusion protein to β-galactosidase under the control of the *lac* promoter. The fusion protein was induced by isopropyl-β-D-thiogalactopyranoside, and the bacterial lysate was assayed for TR activities as described elsewhere (Nakajima et al., 1993). The fusion protein catalyzed the same highly stereospecific reduction of tropinone as the TR-II from *H. niger*; pseudotropine was the sole reaction product detected.

The deduced amino acid sequence of TR-II from *H. niger* is highly homologous to that from *D. stramonium* (Nakajima et al., 1993). The two TR-II proteins share a total of 242 amino acid residues (93%), which includes 6 residues that are strictly conserved in all 20 of the short-chain dehydrogenases that have been characterized so far (Persson et al., 1991).

Table I. *Characteristics of TR-II cDNA from H. niger*

Organism:
Hyoscyamus niger.

Techniques:
A cDNA library was constructed in pcDNAII (Invitrogen, San Diego, CA) with mRNA isolated from cultured roots of *H. niger* that had been grown in Gamborg's B5 medium supplemented with 3% (w/v) Suc at 25°C in the dark for 3 d. The library was screened with a synthetic oligonucleotide probe with a sequence (5′-AAYTTYGARGCIGCITAYCA-3′; Y, T or C; R, A or G; I, inosine) that represented the internal amino acid sequence Asn-Phe-Glu-Ala-Ala-Tyr-His found in a proteolytic peptide fragment of TR-II from *H. niger*.

Method of Identification:
The deduced amino acid sequence contained all four of the internal amino acid sequences (5, 23, 26, and 29 residues long) that had been determined for TR-II purified from *H. niger*. The β-galactosidase-fusion protein of the isolated cDNA expressed in *E. coli* converted tropinone to pseudotropine, but not to tropine.

Expression Characteristics:
A moderate amount of TR-II transcript was detected in cultured roots. A small amount was found in roots of intact plants. None was found in aerial parts of the plants.

Features of the Protein:
260 amino acid residues; mol wt 28,436; 6 residues characteristic of short-chain dehydrogenases (Gly^{16}, Gly^{22}, Asp^{66}, Gly^{139}, Tyr^{159}, and Lys^{163}) are conserved.

Antibody:
Mouse polyclonal antiserum.

Subcellular Localization of the Protein:
Not tested.

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Abbreviations: ORF, open reading frame; TR, tropinone reductase.

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