cDNAs for Isoforms of the Δ⁹-Stearoyl-Acyl Carrier Protein Desaturase from Thunbergia alata Endosperm

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The unusual fatty acid Δ⁶-hexadecenoic acid (16:1Δ⁶) constitutes more than 80 wt% of the seed oil of Thunbergia alata Bojer ex Sims (black-eyed Susan vine) (Spencer et al., 1971). We hypothesized that this fatty acid is derived from the activity of a desaturase that is structurally related to the Δ⁹-stearoyl-ACP (18:0-ACP) desaturase (EC 1.14.99.6), the enzyme that catalyzes the insertion of the double bond of oleic acid (18:1Δ⁶) (Nagai and Bloch, 1968). Such a scenario has been demonstrated for the biosynthesis of petroselinic acid (18:1Δ⁸) in seed endosperm of Umbelliferae species (Cahoon and Ohlrogge, 1994). Using antibodies against the avocado Δ⁶-18:0-ACP desaturase, we have previously isolated a cDNA for a Δ⁶-palmitoyl (16:0)-ACP desaturase from the Umbelliferae plant coriander that is associated with petroselinic acid biosynthesis (Cahoon et al., 1992).

An identical approach was taken for the isolation of a cDNA for a putative desaturase involved in 16:1Δ⁶ formation in T. alata seed endosperm. Upon screening of a cDNA expression library prepared from poly(A)+ RNA of developing T. alata endosperm with antibodies against the avocado Δ⁶-18:0-ACP desaturase (Shanklin and Somerville, 1991), two classes of cDNAs encoding immunoreactive polypeptides were identified. The deduced amino acid sequences of these polypeptides shared considerable identity with those of known Δ⁹-stearoyl-ACP desaturases (Shanklin and Somerville, 1991; Thompson et al., 1991; Nishida et al., 1992; Sato et al., 1992; Slocombe et al., 1992; Taylor et al., 1992). Plasmids containing the longest cDNA inserts of the two classes of isolated clones were designated pTAD1 and pTAD2 (Table I). Further screening of the library with a nucleotide probe derived from pTAD2 led to the isolation of a third class of Δ⁹-18:0-ACP desaturase-like cDNAs (the corresponding plasmid containing the longest insert was designated pTAD3). Inserts of pTAD2 and pTAD3 appeared to be full length and, as such, contained coding sequence for complete plastid transit and mature peptides (based on comparisons with known Δ⁹-18:0-ACP desaturases). The cDNA insert of pTAD1, however, was apparently a partial clone that lacked nucleotide sequence for a plastid transit peptide and at least two amino acids at the N terminus of the mature peptide. The amino acid sequences encoded by the inserts of pTAD2 and pTAD3 share 86% amino acid sequence identity, and polypeptides corresponding to pTAD1 and pTAD2 share 78% amino acid sequence identity. The first 27 amino acids of the polypeptides encoded by cDNAs of pTAD2 and pTAD3 likely represent plastid transit peptide sequences (based on comparisons with previously characterized Δ⁹-stearoyl-ACP desaturases).

Table I. Characterization of Δ⁹-stearoyl-ACP desaturase cDNAs from endosperm of T. alata (black-eyed Susan vine)

| Organism: | Thunbergia alata Bojer ex Sims (black-eyed Susan vine). |
| Techniques: | cDNAs were isolated from a Uni-ZAP XR (Stratagene) expression library prepared from poly(A)+ RNA of developing T. alata endosperm. Both strands of cDNAs were sequenced using dideoxy chain-termination or automated dye-primer sequencing (Michigan State University DNA sequencing facility). |
| Method of Identification: | Comparison of deduced amino acid sequences with known sequences of Δ⁹-stearoyl-ACP desaturases. Assay of activity following expression of mature peptide encoding regions of cDNAs in E. coli. |
| Properties of cDNAs: | The cDNA inserts of pTAD1, pTAD2, and pTAD3 contain 1338, 1355, and 1440 nucleotides, respectively. |
| Features of Encoded Proteins: | The cDNAs of pTAD1, pTAD2, and pTAD3 contain open reading frames of 360, 390, and 390 amino acids, respectively. Polypeptides encoded by pTAD2 and pTAD3 share 86% amino acid sequence identity, and polypeptides corresponding to pTAD1 and pTAD2 share 78% amino acid sequence identity. The first 27 amino acids of the polypeptides encoded by cDNAs of pTAD2 and pTAD3 likely represent plastid transit peptide sequences (based on comparisons with previously characterized Δ⁹-stearoyl-ACP desaturases). |

Abbreviations: ACP, acyl carrier protein. Fatty acid nomenclature: Δ⁹ indicates that a double bond of a fatty acid is located at the 9th carbon atom relative to the carboxyl end of the acyl chain; XY indicates that a fatty acid contains carbon atoms and double bonds (e.g. 18:1).

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the above cDNAs was examined after expression of mature peptide-coding regions in *Escherichia coli* using the pET3a vector (Novagen, Madison, WI). In the case of the insert of pTAD1, coding sequence for an additional Ala and Ser was engineered onto the 5' terminus of the expressed cDNA. Soluble extracts of *E. coli* expressing each of the cDNAs catalyzed the Δ⁸ desaturation of [1-¹⁴C]18:0-ACP. As such, the desaturases encoded by the three *T. alata* cDNAs are not associated with 16:1Δ⁶ biosynthesis but instead are isoforms of the Δ⁸-18:0-ACP desaturase.

Overall, the isolation of three different Δ⁸-18:0-ACP desaturase cDNAs from a library derived from poly(A)+ RNA of *T. alata* endosperm suggests that isoforms of this enzyme can exist in a given species and in a single tissue.

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The GenBank accession numbers for sequences reported in this article are U07552 (pTAD1), U07597 (pTAD2), and U07605 (pTAD3).

**LITERATURE CITED**


Shanklin J, Somerville C (1991) Stearoyl-acyl carrier protein desaturase from higher plants is structurally unrelated to the animal and fungal homologs. Proc Natl Acad Sci USA 88: 2510–2514


Spencer GF, Kleiman R, Miller RW, Earle FR (1971) Occurrence of cis-6-hexadecenoic acid as the major component of *Thunbergia alata* seed oil. Lipids 6: 712–714
