Plant Physiol. (1993) 103: 1463–1464

Plant Gene Register

Isolation and Sequencing of Tomato Fruit Sucrose Synthase cDNA[1]

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Suc synthase (EC 2. 4. 1. 13) catalyzes the reversible reaction: Suc + UDP ↔ UDP-Glc + Fru. In plants, its main physiological function is to cleave Suc and generate UDP-Glc, which is used in such diverse pathways as starch synthesis, cell wall component synthesis, and energy generation (Huber and Akazawa, 1986; Sung et al., 1988). It has been demonstrated that Suc synthase is a key enzyme during the early stages of tomato fruit development. Its activity positively correlates with tomato fruit relative growth rate and starch accumulation in the early stages of fruit development (Robinson et al., 1988; Wang et al., 1993). It has been proposed that Suc synthase activity is a valuable biochemical marker of tomato fruit sink strength (Sun et al., 1992).

To gain a better understanding of the role and regulation of Suc synthase in tomato fruit development, we isolated a Suc synthase cDNA clone, designated TOMSSF, from a cDNA library prepared from mRNA of tomato (*Lycopersicon esculentum* Mill. cv VF 36) pistils from flowers at anthesis (Gasser et al., 1989) (Table I). The cDNA is 2725 bp long and contains an open reading frame encoding a protein of 805 amino acids with an ATG at position 63 and the stop codon TGA at position 2478. The DNA sequence of the 5′ noncoding open reading frame and 3′ noncoding regions of TOMSSF has 97%, 97%, and 75% sequence similarity to the analogous regions of the potato Suc synthase cDNA clone isolated from a developing tuber cDNA library (Salanoubat and Belliard, 1987). Mismatches in the 5′ noncoding region were found at positions −2 and −3 relative to the ATG initiation codon. The CTATAATGG sequence, within which the translation initiation codon resides, is unique compared with its counterparts in other plant Suc synthase sequences as well. The predicted amino acid sequence is very similar to the Suc synthase sequences of other plants. There is a C-rich region in the 3′ untranslated region (from 2569–2599 bp; C content in this region is 87%). Hydropathy analysis revealed that the deduced protein did not contain a hydrophobic region at the amino terminal and does not contain a signal sequence (Von Heijne, 1986). The molecular mass of the predicted protein is 92.4 kD, which is approximately equal to that of the Suc synthase subunit determined by western blot analysis of SDS-PAGE-separated proteins from tomato fruits using a polyclonal antibody raised against maize Suc synthase (Notle and Koch, 1993). Thus, we found no evidence for proteolytic processing of the Suc synthase protein.

Received June 28, 1993; accepted July 19, 1993.

The GenBank accession number for the sequence reported in this article is L19762.

LITERATURE CITED

Gasser CS, Budelier KA, Smith AG, Shah DM, Fraley RT (1989) Isolation of tissue-specific cDNAs from tomato pistils. Plant Cell **1:** 15–24

Huber SC, Akazawa T (1986) A novel sucrose synthase pathway for sucrose degradation in cultured sycamore cells. Plant Physiol **81:** 1008–1013

Notle KD, Koch KE (1993) Companion-cell specific localization of sucrose synthase in zones of phloem loading and unloading. Plant Physiol **101:** 899–905

Robinson NL, Hewitt JD, Bennett AB (1988) Sink metabolism in tomato fruit. Plant Physiol **87:** 727–730

Salanoubat M, Belliard G (1987) Molecular cloning and sequencing of a sucrose synthase cDNA from potato (*Solanum tuberosum* L.): preliminary characterization of sucrose synthase mRNA distribution. Gene **60:** 47–56

Sun JD, Loboda T, Sung SJ, Black CC (1992) Sucrose synthase in wild tomato, *Lycopersicon chmielewskii*, and tomato fruit sink strength. Plant Physiol **98:** 1163–1169

Sung SJ, Xu DP, Gelloway CM, Black CC (1988) A reassessment of glycolysis and gluconeogenesis in higher plants. Physiol Plant **72:** 650–654

Von Heijne G (1986) A new method for predicting signal sequence cleavage sites. Nucleic Acids Res **14:** 4683–4690

Wang F, Sanz A, Brenner ML, Smith AG (1993) Sucrose synthase, starch accumulation, and tomato fruit sink strength. Plant Physiol **101:** 321–327

Wang MB, Boulter D, Gatehouse JA (1992) A complete sequence of rice sucrose synthase-1 (*RSs1*) gene. J Plant Mol Biol **19:** 881–885

Werr W, Frommer WB, Maas C, Starlinger P (1985) Structure of the sucrose synthase gene on chromosome 9 of *Zea mays* L. EMBO J **4:** 1373–1380

Yu WP, Wang AY, Tuang RH, Sung HY, Su JC (1992) Isolation and sequences of a rice sucrose synthase cDNA and genomic DNA. J Plant Mol Biol **18:** 139–142

[1] Supported in part by the Minnesota Agricultural Experimental Station. Paper No. 20671, Scientific Journal Series.

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Table I. *Characteristics of a tomato fruit Suc synthase cDNA (TOMSSF)*

Organism:
Lycopersicon esculentum Mill. cv VF 36.
Gene; Gene Product:
TOMSSF; Suc synthase (EC 2.4.1.13).
Source:
cDNA library in λgt10 vector constructed from poly(A$^+$) RNAs of tomato pistils from flowers at anthesis (Gasser et al., 1989).
Techniques:
Screening of the cDNA library with the 1.5-kb *Eco*RI to *Hind*III fragment of the potato Suc synthase cDNA, λ10a (Salanoubat and Belliard, 1987), as a probe. Complete dideoxy sequencing of both strands using a combination of subclones and nested deletions.
Method of Identification:
Deduced protein sequence shares 98% homology to a potato Suc synthase sequence (Salanoubat and Belliard, 1987).
Expression:
Polyadenylated transcript of approximately 2800 bases. Expression predominantly in tomato pistils and developing fruit with very low levels of expression in roots and stem base. No detectable expression in leaves, apical buds, or stamens.
Structural Features of the Gene:
The translation initiation codon is located within a CTATAATGG sequence. Translation start site at base 63. Stop site at base 2478. There is a C-rich region in the 3′ untranslated region (from 2569–2599 bp, C content in this region is 87%). Polyadenylation signal AATAAG is located at 2671.
Codon Usage:
All coding codons are used.
(G + C) Content:
45.6% in the coding region.
Features of the Protein:
Open reading frame of 805 amino acids. No consensus signal sequence. The predicted molecular mass and isoelectric point of the mature protein are 92.42 kD and 5.85, respectively. It shows 98%, 81%, 74%, 73%, 65%, 73%, and 65% similarity to Suc synthase amino acid sequences of potato (Sanaloubat and Belliard, 1987), mung bean (GenBank D10266), maize (Werr et al., 1985), barley (GenBank X65871), rice ss1 (Wang et al., 1992), rice ss2 (Yu et al., 1992), and *Arabidopsis* (GenBank X60987), respectively.
Gene Number:
One copy per haploid genome as determined by Southern blot analysis.
Antibodies:
Polyclonal antibodies against maize Suc synthase (Notle and Koch, 1993), cross-react with the tomato Suc synthase protein.