Plant Physiol. (1993) 102: 1053–1054

Plant Gene Register

Starch Branching Enzyme cDNA from *Solanum tuberosum*[1]

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Starch is the main storage carbohydrate in higher plants and is composed of the two polysaccharides, amylopectin (approximately 75%) and amylose (approximately 25%). Amylopectin is a highly branched α-1,4 glucan containing α-1,6 branch points, whereas amylose is composed of long, linear α-1,4 glucans, some of which have very few α-1,6 branch points. Starch is synthesized in chloroplasts and amyloplasts by the action of three enzymic activities: ADP-Glc pyrophosphorylase (EC 2.7.7.27), starch synthase (EC 2.4.1.21), and SBE (EC 2.4.1.18) (reviewed in Preiss, 1991). ADP-Glc pyrophosphorylase catalyzes the formation of ADP-Glc, which is the Glc donor for the synthesis of amylose carried out by starch synthase, whereas SBE catalyzes the conversion of amylose to amylopectin by adding linear chains of α-1,4 glucans to amylose through α-1,6 branch points. Here we present the isolation of a branching enzyme cDNA from potato (*Solanum tuberosum*), which is an important prerequisite for further detailed studies of the mechanisms of starch biosynthesis at the enzyme and gene levels.

A full-length cDNA clone encoding SBE was isolated from a potato sprout cDNA library using a partial potato SBE cDNA clone that was originally isolated with a heterologous cDNA encoding a pea SBE (Table I). The 3114-bp DNA sequence revealed an ORF that begins with an ATG initiation codon at coordinate 121 bp and ends with a TGA codon at coordinate 2704 bp. The ORF includes 861 amino acids, which has significant similarity to SBE I from maize and rice (Baba et al., 1991; Nakamura and Yamanouchi, 1992), indicating that the 3.1-kb cDNA encodes potato SBE. The protein encoded by the ORF has a calculated M_r of 99,083, whereas the purified branching enzyme from potato tubers has been reported to have M_r values in the range from 79,000 to 103,000 (Vos-Scheperkeuter et al., 1989; Blennow and Johansson, 1991). The understanding of these discrepancies must await further analysis of the purified enzyme.

SBE has been located in the amyloplast-stroma (Kram et al., 1993), suggesting that the deduced amino-terminal sequence of SBE contains a transit peptide that targets SBE to the amyloplast. In accordance with this prediction, the SBE amino terminus has some features in common with chloroplast transit peptides (Gavel and von Heine, 1990), i.e. a high content of Ser and Thr residues and a central, positively charged domain. Moreover, the hydropathicity profiles of the amyloplast transit peptide from the potato granule-bound starch synthase (Van der Leij et al., 1991) and the amino terminus of SBE are similar, including a high increase in hydrophobicity at amino acid positions 66 to 73, just prior to the cleavage site of the synthase, which is located at amino acid 77. Thus, it is tempting to speculate that the cleavage site in SBE may also be located in that segment, perhaps at the motif I-S-A_AV (amino acid coordinates 73–76), which has resemblance to the consensus cleavage-site motif in chloro-

Table I. *Characteristics of the SBE cDNA from potato*

Organism:
Solanum tuberosum cv Dianella.
Gene Product:
SBE (α-1,4 glucan: α-1,4 glucan 6-glucosyl-transferase; EC 2.4.1.18).
Clone Type; Designation:
cDNA, full-length; pSBE8.
Source:
cDNA library in λZAPI, custom made by Stratagene from mRNA isolated from potato sprouts.
Techniques:
A partial 1400-bp cDNA clone encoding SBE from potato (obtained from Agricultural Genetics Co. Ltd.) was applied in a screening of the cDNA library yielding 15 clones. Six clones were analyzed and one of these, having a 3.1-kb insert, was sequenced on both strands using double-stranded plasmid dideoxynucleotide sequencing. The 3.1-kb cDNA clone appeared to be a full-length clone.
Features of cDNA:
The clone is 3114 nucleotides in length and consists of a 120-nucleotide 5′ untranslated region, a 2586-nucleotide ORF, and a 408-nucleotide 3′ untranslated region. The ORF potentially encodes a protein of 861 amino acids with a calculated M_r of 99,107. A repeated region is found at bp coordinates 2 to 76 and 2759 to 2830.
Method of Identification:
Similarity of the predicted amino acid sequence to the SBE I from maize and rice; 67 and 66% identical amino acids, respectively.
Expression Characteristics:
Developmental and tissue-specific expression; very high expression in growing potato tubers (Kossmann et al., 1991).
Subcellular Location:
Amyloplast-stroma (Kram et al., 1993).
Location on Chromosome:
Chromosome IV (Gebhardt et al., 1993).

[1] Part of this work was supported by the Nordic Industrial Foundation and the Danish Food Technology Programme (FØTEK).

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Abbreviations: ORF, open reading frame; SBE, starch branching enzyme.

plast transit peptides, (V/I)-X-(A/C)$_{\Delta}$A ($_{\Delta}$ indicates the cleavage point) (Gavel and von Heine, 1990).

The isolated cDNA, which appears to be full length or very close to it, may be an important tool for blocking SBE synthesis by the antisense technique. Analysis of transgenic potatoes lacking SBE activity may shed new light on the mechanisms of starch biosynthesis.

ACKNOWLEDGMENTS

We are grateful to Dorthe Pedersen for excellent technical assistance and thank Dr. Jan Marcussen and Dr. Finn Okkels for critically reading the manuscript.

Received December 23, 1992; accepted January 15, 1993.
Copyright Clearance Center: 0032-0889/93/102/1053/02.
The EMBL accession number for the sequence reported in this article is X69085.

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