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Plant Gene Register

A cDNA Encoding Starch Branching Enzyme I from Maize Endosperm[1]

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ADP-Glc pyrophosphorylase (EC 2.7.7.27), starch synthases (EC 2.4.1.21), and SBEs (EC 2.4.1.18) are the key enzymes in the pathway of plant starch biosynthesis. Starch is a polymer of Glc that exists as two fractions, amylose and amylopectin, in maize (*Zea mays* L.) kernel amyloplasts. The essentially linear polymer amylose contains α-1,4-linked Glc, whereas the branched polymer amylopectin contains 5% α-1,6-linked Glc in addition to linear regions of α-1,4-linked Glc. Amylopectin synthesis requires the action of SBE, which catalyzes the formation of α-1,6-linkages (Borovsky et al., 1976; Boyer, 1985). The branching process involves two steps with the hydrolysis of an internal 1,4-bond and the formation of a 1,6-bond using the linear chain (six to seven Glc units). Thus, branching enzymes are thought to interact with starch synthases in formation of amylopectin (Boyer and Preiss, 1981).

Three SBE isozymes differing in enzymatic, chromatographic, and immunological properties have been resolved in maize endosperm, SBE I, SBE IIa, and SBE IIb (Boyer and Preiss, 1978a, 1978b; Boyer and Fisher, 1984; Guan and Preiss, 1993; Takeda et al., 1993). Recently, analysis of SBE I, SBE IIa, and SBE IIb revealed that SBE I may preferentially branch long chains of α-glucan, whereas SBE IIa and SBE IIb may play a different role in branching short chains during starch biosynthesis (Takeda et al., 1993).

We previously reported the cloning of a cDNA encoding SBE II from maize endosperm (Fisher et al., 1993). Using antibodies to purified SBE I protein from maize endosperm, Baba et al. (1991) isolated a partial-length cDNA encoding the SBE I isoform. This cDNA lacks the entire open reading frame, because no ATG codon was found 5′ of the known plastid signal peptide cleavage site. Based on similarity to the rice SBE I-like cDNA (*rbe1*; Baba et al., 1991), it was hypothesized that the SBE I cDNA lacked only two bases of the coding region.

As part of our long-term goal to characterize genetic loci encoding maize SBE isoforms, we isolated an apparently full-length SBE I cDNA that includes the entire coding region and 5′ and 3′ untranslated regions. Based on the sequence data presented by Baba et al. (1991), two synthetic oligonucleotide primers were synthesized and used to amplify maize genomic DNA. After amplification, a single DNA fragment of the expected molecular size (427 bp) was subcloned, sequenced, and shown to represent an authentic SBE I sequence (Table I). Three previously described λgt10 cDNA libraries (Fisher et al., 1993) constructed from endosperm poly(A)$^+$ RNA 14, 22, and 29 d after pollination were screened by hybridization with this PCR product.

Table I. *Characteristics of SBE I cDNA from maize endosperm*

Organism:	
	Zea mays L. (W64A X 182E).
Gene Product:	
	SBE isoform I (1,4-α-D-glucan 6-α-D-(1,4-α-D-glucanotransferase); EC 2.4.1.18); starch biosynthesis.
Clone Type; Designation:	
	cDNA, full length; λ29-III-1; pA2–4 (pBluescript).
Source:	
	cDNA libraries in λgt10 constructed from maize endosperm poly(A)$^+$ mRNA isolated 14, 22, and 29 d after pollination.
Techniques:	
	Two synthetic oligonucleotide primers were synthesized and used to PCR amplify maize genomic DNA (oligonucleotide 1, 5′-GACTGAATTCCTGCGCAGGAGGCA-GAGCTT-3′; oligonucleotide 2, 5′-GATCGAATTCCATAGATACGTGGAGCAGCA-3′). After amplification, the single band of the expected molecular size (427 bp) was subcloned, sequenced, and used to screen endosperm cDNA libraries by hybridization. A full-length clone of 2789 bp was subcloned into pBluescript II SK−; double-stranded DNA was sequenced with synthetic oligonucleotide primers; both strands of a full-length clone (λ29-III-1; pA2–4) were sequenced.
Method of Identification:	
	Sequence identity to other SBE clones (maize SBEI cDNA and deduced amino acid sequence identity with partial SBE I peptide sequence previously determined by Baba et al. [1991]).
Structural Features of Protein:	
	Open reading frame of 822 amino acids; calculated M_r of mature protein of 85,974; putative 63-amino acid transit peptide N-terminal to 759-amino acid mature protein sequence.
Subcellular Location:	
	Amyloplast.

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Abbreviation: SBE, starch branching enzyme.

After purification of several positive isolates, a full-length cDNA was isolated, subcloned into plasmid pBluescript II SK^- (Stratagene), and sequenced on both strands by primer walking.

DNA sequence analysis of this *sbe1* cDNA revealed a size of 2789 bp with 99% DNA sequence identity with the partial maize *sbe1* cDNA (Baba et al., 1991). It includes a 5′ untranslated region and an ATG translation start codon in frame with a large open reading frame encoding the SBE I plastid transit peptide and mature protein. The 5′ sequences and some additional bases in the 3′ untranslated region including a poly(A) tail were not reported by Baba et al. (1991). The putative transit peptide for routing of the protein to the amyloplast (63 amino acids) precedes a mature protein of 759 amino acids, with residue identity the same as that reported by Baba et al. (1991).

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