Plant Physiol. (1993) 102: 1045–1046

Plant Gene Register

Starch Branching Enzyme II from Maize Endosperm

Dane K. Fisher, Charles D. Boyer*, and L. Curtis Hannah

Department of Horticulture, The Pennsylvania State University, University Park, Pennsylvania 16802 (D.F., C.D.B.); and Department of Vegetable Crops, The University of Florida, Gainesville, Florida 32611 (L.C.H.)

Downloaded from https://academic.oup.com/plphys/article/102/3/1045/6066992 by guest on 20 August 2022

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. ADP-Glc pyrophosphorylase and starch synthase catalyze the formation of ADP-Glc, the substrate for starch synthesis, and the polymerization of Glc into the amylose and amylopectin fractions of the starch granule, respectively. SBEs catalyze branch point formation by the cleavage and reattachment of α-1,4-linked Glc chains to α-1,6 branch points in the growing starch molecule (Borovsky et al., 1979; Boyer, 1985). Branching enzymes are proposed to interact with starch synthases in formation of amylopectin (Boyer and Preiss, 1981).

Multiple forms of SBE have been identified in maize (*Zea mays* L.) endosperm (Boyer and Preiss, 1978, 1981). Three forms of SBE, I, IIa, and IIb, from developing maize endosperm have been characterized by their level of branching activity on amylose and amylopectin and by kinetic and immunological properties (Boyer and Preiss, 1978; Fisher and Boyer, 1983; Singh and Preiss, 1985). Distinct differences have been shown between SBE I and IIa or IIb by the above parameters, but only small differences exist between SBE IIa and IIb. Genetic evidence suggests that IIa and IIb are products of separate genes (Boyer and Preiss, 1981; Hedman and Boyer, 1982). The precise role of each isoform in starch formation has yet to be determined. To further understand these enzymes, efforts to clone the genes have been undertaken. We report here the cloning of a SBE II cDNA from maize (Table I). Three λgt10 cDNA libraries were constructed from endosperm poly(A)$^+$ RNA 14, 22, and 29 DAP. A heterologous nucleic acid probe, clone pJSBE5, the cDNA for pea SBE I, was used to screen the 14-DAP library (Bhattacharyya et al., 1990). After purifying and subcloning into plasmid pBluescript II SK$^-$ (Stratagene), a full-length cDNA of 2725 bp was isolated.

Northern blots of total maize RNA isolated from endosperm tissue 12 DAP and probed with the cloned maize cDNA revealed a single transcript of approximately 2.7 kb. Deduced amino acid sequence was compared with the pea SBE I (Bhattacharyya et al., 1990), maize SBE I (Baba et al., 1991), and rice SBE I (Nakamura et al., 1992) translated cDNA sequences using Intelligenetics software. Levels of residue identity were 71, 52, and 52%, respectively. From these results, we conclude that we have cloned a second isoform of SBE from maize endosperm. This conclusion is supported by the N-terminal sequence of purified maize SBE IIb protein, which matches the cDNA predicted amino acid sequence at residues 58 to 65. The additional amino acid residues making up the N-terminal end of the deduced sequence are thought to encode a transit peptide (53 amino acids) for routing of the protein to the amyloplast. The deduced molecular mass of the mature protein from this sequence data is 84,772 D. This is slightly larger than size estimates of 80,000 D based upon SDS-PAGE analysis of

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

Organism:
Zea mays L. (W64A × 182E).
Gene Product:
SBE (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; pMA11(pBluescript).
Source:
cDNA libraries in λgt10 constructed from maize endosperm poly(A)$^+$ mRNA isolated 14, 22, and 29 DAP.
Techniques:
Libraries screened with pea (*Pisum sativum* L). SBE I clone pJSBE5 (Bhattacharyya et al., 1990); three overlapping clones subcloned into pBluescript II SK$^-$; double-stranded dideoxynucleotide sequencing of overlapping clones using various subclones and synthetic oligonucleotide primers; second screening with 5′ and 3′ ends of partial clones to obtain full-length clone; both strands of the full-length clone were sequenced (λ29-III-1; pMA11).
Method of Identification:
Sequence homology to other SBE clones; deduced amino acid (residues 58–65) identity to purified maize SBE IIb mature protein N-terminal sequence.
Structural Features of Protein:
Open reading frame of 798 amino acids; calculated M_r of mature protein of 84,772; putative 53-amino acid transit peptide N terminal to mature protein sequence.
Subcellular location:
Amyloplast.

* Corresponding author; fax 1–814–863–6139.

Abbreviations: DAP, days after pollination; SBE, starch branching enzyme.

purified SBE IIa and IIb protein (Boyer and Preiss, 1978; Singh and Preiss, 1985).

ACKNOWLEDGMENTS

We thank Dr. Cathie Martin (John Innes Institute, Norwich, UK) for providing pea clone pJSBE5 and Dr. Lynn Ingram (University of Florida) for providing the endosperm cDNA libraries.

Received December 22, 1992; accepted January 9, 1993.
Copyright Clearance Center: 0032-0889/93/102/1045/02.
The GenBank/EMBL accession number for the sequence reported in this article is L08065.

LITERATURE CITED

Baba T, Kimura K, Mizuno K, Etoh H, Ishida Y, Shida O, Arai Y (1991) Sequence conservation of the catalytic regions of amylolytic enzymes in maize branching enzyme-I. Biochem Biophys Res Commun **181:** 87–94

Bhattacharyya MK, Smith AM, Ellis THN, Hedley C, Martin C (1990) The wrinkled-seed character of pea described by Mendel is caused by a transposon-like insertion in a gene encoding starch-branching enzyme. Cell **60:** 115–122

Borovsky D, Smith E, Whelan W, French D, Kikumoto S (1979) The mechanism of Q-enzyme action and its influence on the structure of amylopectin. Arch Biochem Biophys **198:** 627–631

Boyer CD (1985) Synthesis and breakdown of starch. *In* C Neyra, ed, Biochemical Basis of Plant Breeding, Vol I. CRC Press, Boca Raton, FL, pp 133–153

Boyer CD, Preiss J (1978) Multiple forms of (1,4)-alpha-D-glucan, (1,4)-alpha-D-glucan-6-glycosyl transferase from developing *Zea mays* L. kernels. Carbohydr Res **61:** 321–334

Boyer CD, Preiss J (1981) Evidence for independent genetic control of the multiple forms of maize endosperm branching enzymes and starch synthases. Plant Physiol **67:** 1141–1145

Fisher MB, Boyer CD (1983) Immunological characterization of maize starch branching enzymes. Plant Physiol **72:** 813–816

Hedman KD, Boyer CD (1982) Gene dosage at the *amylose-extender* locus of maize: effects on the levels of starch branching enzymes. Biochem Genet **20:** 483–491

Nakamura Y, Yamanouchi H (1992) Nucleotide sequence of a cDNA encoding starch-branching enzyme, or Q-enzyme I, from rice endosperm. Plant Physiol **99:** 1265–1266

Singh BK, Preiss J (1985) Starch branching enzymes from maize. Immunological characterization using polyclonal and monoclonal antibodies. Plant Physiol **79:** 34–40

Downloaded from https://academic.oup.com/plphys/article/102/3/1045/6066992 by guest on 20 August 2022