

Note

A Structural Gene Encoding β -Amylase of Barley

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A structural gene encoding the β -amylase that is abundant in the starchy endosperm of ungerminated barley seeds was isolated and characterized. It was 3825 bp in length. In the sequence of the structural gene, a sequence identical to that of the cDNA was found to contain seven exons and six introns.

β -Amylase (α -1,4-glucan maltohydrolase; EC 3.2.1.2) catalyzes the liberation of β -maltose from the nonreducing ends of starch and related 1,4- α -glucans.¹⁾ The enzyme has been purified from various higher plants, e.g., barley, soybean, and sweet potato, and microorganisms.²⁾ The β -amylase is a major protein in the starchy endosperm of ungerminated barley seeds.³⁾ Numerous studies have been reported on the purification and properties of barley β -amylase.²⁾ We have reported that polymerase chain reaction (PCR) amplification of mRNA from developing barley (*Hordeum vulgare* L. cv. Haruna) endosperm was used to clone and sequence the full-length cDNA encoding β -amylase.⁴⁾ The β -amylase cDNA was 1775 bp in length. The β -amylase was deduced to be composed of 535 amino acids, and its molecular weight was calculated to be 59,610. In the case of sweet potato β -amylase, Yoshida *et al.* have isolated a nuclear gene encoding β -amylase that is abundant in tuberous roots, and characterized it.⁵⁾ As for barley β -amylase, Kreis *et al.* have used a characterized β -amylase cDNA clone⁶⁾ to demonstrate, by DNA restriction-fragment-length polymorphism analysis, that β -amylase genes were present not only on the long arm of chromosome 4 but also on the short arm of chromosome 2.⁷⁾ But no studies of the isolation and sequence of a nuclear gene encoding β -amylase have been reported to date. To our knowledge this is the first that a structural gene encoding β -amylase of barley has been cloned and sequenced.

About 1000 barley seeds were germinated in darkness at 20°C

for 7 days in vermiculite, and 65 g of young leaves were taken. From 10 g of the young leaves, total DNA was isolated⁸⁾ and about 1 mg of DNA was recovered.

PCR amplification was used to clone a structural gene encoding β -amylase of barley. An LA PCR kit was obtained from the Takara Shuzo and was used as recommended. The reaction mixture contained 10 μ g of DNA, 20 pmol of each primer, 2.5 mM of dNTP, and 2.5 units of *Ex Taq* DNA polymerase. Based on the 5'-end and the 3'-end sequences of the β -amylase cDNA from cultivar Haruna two-rows,⁴⁾ a pair of 20-mer oligodeoxynucleotides was designed. A *Bam*HI recognition sequence (GGATCC) was added to the 5'-end of each primer and two extra bases (AA)

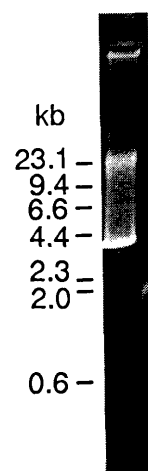


Fig. 1. Analysis of DNA Product from PCR.

Numerals on the left margin refer to *Hind*III-digested λ DNA markers.

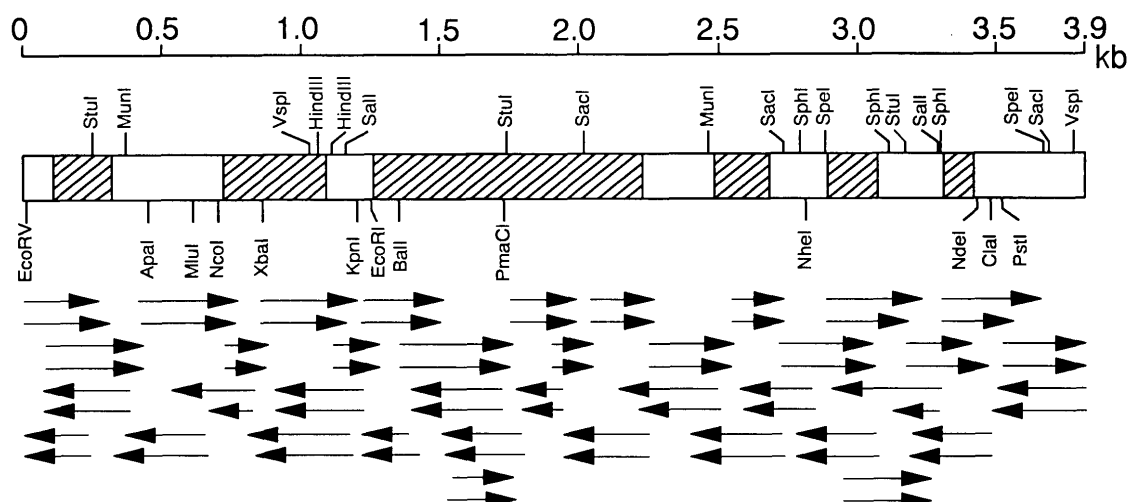


Fig. 2. Restriction Map and DNA Sequencing Strategy for the Cloned 3.9-kb Fragment.

The 3.9-kb *Bam*HI fragment was digested with various restriction enzymes, and DNA fragments were subcloned into pUC118 and pUC119 for DNA sequencing. The direction and extent of sequencing are shown by horizontal arrows for the subclones used. Open boxes and the boxes with oblique lines indicate exon and intron, respectively.

Abbreviation: PCR, polymerase chain reaction.

The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases with the accession number D49999.

A DNA band corresponding to a reasonable size (3.9 kb) for the structural gene was detected on a 0.7% agarose gel (Fig. 1). The PCR product was digested with *Bam*HI and inserted into the

VI

Small letters indicate the sequences of introns, and the intron sequences, numbered I through VI, are boxed. The deduced amino acid sequence is shown below the nucleotide sequence. Asterisks mark a stop codon. The two consensus sequences of polyadenylation (AATAAA) are underlined.

*Bam*HI site of pUC118. Figure 2 shows the restriction map of the 3.9-kb *Bam*HI fragment and the DNA sequencing strategy. DNA sequencing was done using the dideoxy method⁹⁾ with M13 (–21 and reverse) dye-primers and dye-terminators with a DNA sequencer (Model 373A, Applied Biosystems).

The nucleotide sequence of the 3.9-kb fragment is shown in Fig. 3. It was 3825 bp in length. In the sequence of the structural gene, the sequence identical to that of the cDNA⁴⁾ was found to contain seven exons and six introns as in the case of the nuclear gene encoding β -amylase of sweet potato.⁵⁾ The length of introns ranged from 113 to 971 bp, and the border sequences of all six introns matched the GT-AG rule.¹⁰⁾ Whether the cloned structural gene was derived from chromosome 2 or chromosome 4 remains to be elucidated.

Cloning and sequencing of a genomic DNA including promoter and terminator regions encoding β -amylase of barley are now in progress in our laboratory and will be reported elsewhere.

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