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Plant Gene Register

ADP-Glucose Pyrophosphorylase Large Subunit cDNA from Barley Endosperm

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AGP[1] carries out the first committed step of starch biosynthesis in bacteria and plants. The plant enzyme is a heterotetramer composed of two subunit types, encoded by different genes. AGP from maize endosperm and potato tuber, as well as from leaves of all plants examined (including barley), is allosterically activated by 3-phosphoglyceric acid and inhibited by Pi (1, 4). The barley endosperm enzyme, on the other hand, is highly active without 3-phosphoglyceric acid and is only slightly affected by the activator and inhibitor (2). Because AGP is assumed to regulate the rate of starch formation, this characteristic of the barley enzyme may prove useful in attempts to increase starch synthesis, e.g. in potato tubers, by genetic engineering.

To initiate such experiments, we isolated and sequenced a full-length cDNA clone encoding the large subunit of AGP from barley endosperm (Table I). A cDNA library, made from barley starchy endosperm, was screened using as the initial probe a DNA fragment obtained by amplification of starchy endosperm cDNA using degenerate primers (6). Later, probes made from the 5′ ends of isolated partial clones were used. The lengths of about 50 consecutively isolated clones were analyzed, and 12 clones were partially sequenced. Except for chimeric artifacts and differences in length, all clones appeared to be identical with bepl10, the full-length clone shown in Figure 1. However, for one partial clone, the polyadenylation site was 67 bp downstream from the site in bepl10. This extra sequence is also included in the figure.

When compared with clones for the same enzyme in other plants and tissues, the amino acid sequence of bepl10 has the highest identity (91%) to WE:AGA7, a large subunit partial clone from wheat endosperm (3). Identities to maize endosperm (5) and potato tuber (T.W. Okita, EMBL Data Library) clones are 67 and 69%, respectively. With respect to fragments of transcripts for the large subunit from leaves (wheat [3], barley, and *Arabidopsis thaliana* [P. Villand, unpublished data]), identities are about 60%. There is also a considerable identity (45–50%) to small subunit clones from leaves, endosperm, and potato tubers.

Table I. *Characteristics of cDNA of AGP Large Subunit from Barley Starchy Endosperm*

Organism:
Hordeum vulgare L. var *disticum* cv Bomi.
Gene Product:
ADP-glucose pyrophosphorylase (EC 2.7.7.27), large subunit; starch biosynthesis.
Clone Type; Designation:
cDNA, full-length; bepl10.
Source:
cDNA library in λZAPII vector, custom made by Clontech from starchy endosperm harvested 12 d after pollinatiion.
Techniques:
Library screened with a 550-bp DNA fragment obtained by amplification of cDNA from starchy endosperm as described (6), followed by second and third screenings with 5′ ends of partial clones to obtain full-length clones: double-stranded plasmid dideoxynucleotide sequencing of subclones with synthetic oligonucleotide primers.
Method of Identification:
Sequence homology to other AGP large subunit clones; western blot analysis of cDNA expressed in *Escherichia coli*; comparison to partially purified protein from barley endosperm.
Expression Characteristics:
Expressed in the starchy endosperm and in the roots of barley.
Codon Usage:
No obvious bias in codon usage.
Structural Features of Protein:
Open reading frame of 527 amino acids; calculated M_r 58,188.
Antibodies:
Antisera to the synthetic peptide CIIDMNARIGRDVVISN (amino acids 476–492 in bepl10) available.
Subcellular Location:
Presumably amyloplast, assembled with small subunit protein.
Location on Chromosome for Corresponding Gene:
Long arm of chromosome 5, single-copy gene.
EMBL Accession No.:
X67151.

[1] Abbreviation: AGP, ADP-glucose pyrophosphorylase.

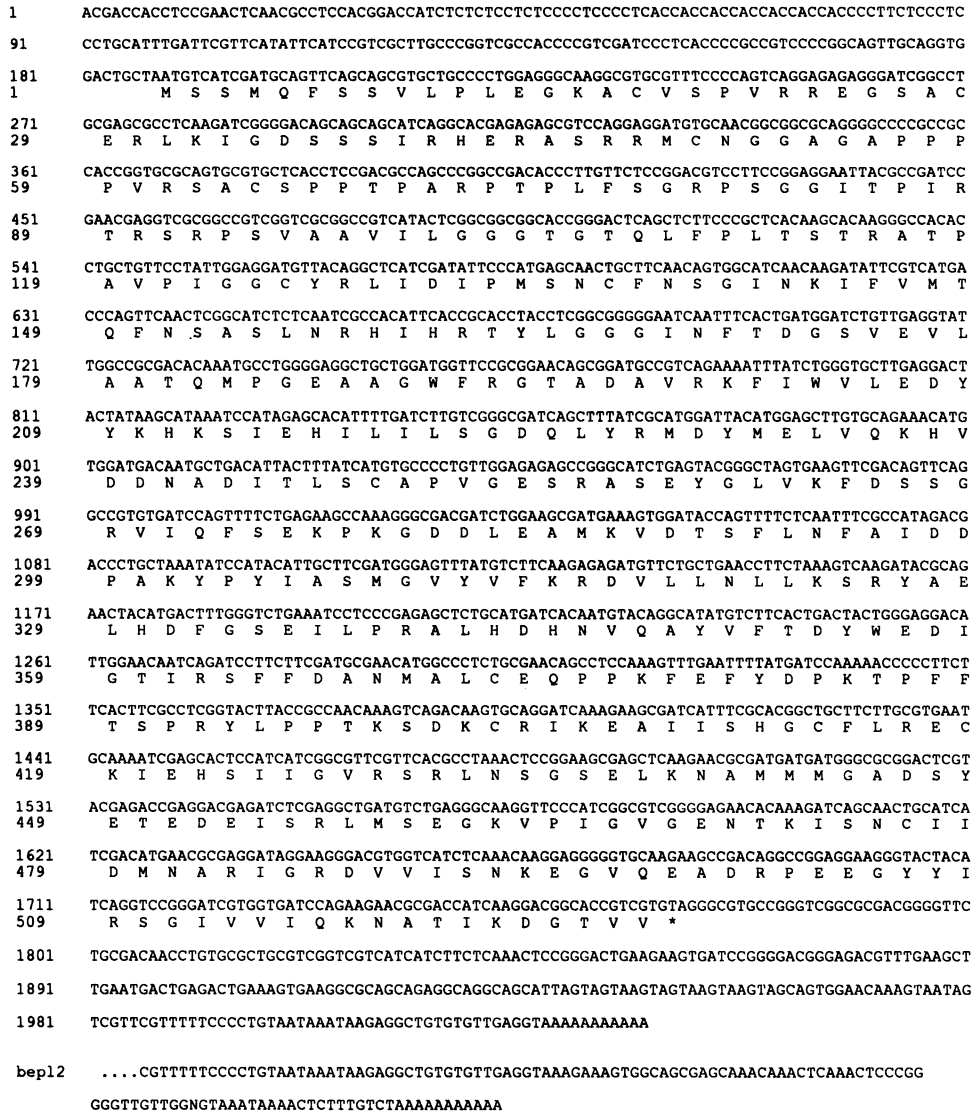

Figure 1. Nucleotide sequence and deduced amino acid sequence of bepl10, a full-length cDNA clone for AGP large subunit from barley endosperm. Alternative polyadenylation is demonstrated by the additional 3′ sequence of a second clone, bepl2.

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