Mol. Cells, Vol. 10, No. 1, pp. 108–112

Communication

Molecules and Cells

Isolation and Characterization of Polymorphic cDNAs Partially Encoding ADP-glucose Pyrophosphorylase (AGPase) Large Subunit from Sweet Potato

Sang Sook Lee, Jung Myung Bae, Mi Sook Oh, Jang Ryol Liu, and Chee Hark Harn[†,*]

Plant Cell and Molecular Biology Research Unit, Korea Research Institute of Bioscience and Biotechnology, Taejon 305-600, Korea.

(Received on October 23, 1999)

cDNA clones encoding sweet potato AGPase large subunit (iAGPLI) from the cDNA library constructed from the tuberous root were isolated. Two clones were characterized and named *iAGPLI-a* and *iAGPLI-b*. They were 1,661 bp and 1,277 bp in length and contained partial open reading frames of 450 and 306 amino acids, respectively. Both nucleic acid and amino acid sequence identities between *iAGPLI-a* and *iAGPLI-b* were 83.8% and 97.3%, respectively. Based on the amino acid sequence analysis, iAGPLI-a and iAGPLI-b share the highest sequence identity (81%) with potato AGPase large subunit. The *iAGPLI-a* and *iAGPLI-b* genes were expressed predominantly in the stem and weakly in the tuberous root, and no transcript was expressed in other tissues. The sweet potato genome contains several copies of the *iAGPLI* gene.

Keywords: ADP-Glucose Pyrophosphorylase Large Subunit; Starch Biosynthesis; Sweet Potato.

Introduction

Biosynthesis of starch in all plants and glycogen in bacteria require ADP-glucose as a main substrate for elongating the polymers. ADP-glucose is generated by the catalytic reaction of ADP-glucose pyrophosphorylase (AGPase) which converts glucose 1-phosphate and ATP to ADP-glucose and pyrophosphate. AGPase in plants is a heterotetramer composed of two large subunits and two small subunits encoded by different genes (Preiss, 1991). Several isoforms exist in plants with the sizes of the small subunits ranging between 50–56 kDa, and the sizes of the large subunits range between 51–60 kDa (Lin *et al*., 1988; Morell *et al*., 1987; Sowokinos and Preiss, 1982). By contrast, bacterial AGPase is expressed by a single gene forming a homotetramer with a subunit mass of 48 kDa. As an allosteric enzyme in enteric bacteria, AGPase is activated by fructose-1,6-bisphosphate and inhibited by AMP. The plant enzyme is activated by 3-PGA and inhibited by Pi (Preiss, 1978), although the AGPase, in a few plants such as barley, showed insensitivity to the allosteric regulation by 3-PGA/Pi (Kleczkowski *et al*., 1993).

cDNA clones encoding the large subunit (LS) have been isolated from many plants (corn LS: Bhave *et al*., 1990, Giroux *et al*., 1995; potato LS: La Cognata *et al*., 1995, Müller-Röber *et al*., 1990, Nakata *et al*., 1991; sugar beet LS: Müller-Röber *et al*., 1995; wheat LS: Olive *et al*., 1989; barley LS: Villand *et al*., 1992a, 1992b, Elimert *et al*., 1997; *Arabidopsis* LS: Villand *et al*., 1993; tomato LS: Park and Chung, 1998; oriental melon LS: Park *et al*., 1998). Analysis of the deduced amino acid sequence identity among cDNA clones revealed that small subunits are highly conserved (Bae and Liu, 1997) while the large subunits are relatively divergent.

Sweet potato is an important agricultural crop used both for food and for its derivaties. In order to investigate the biochemical and molecular mechanisms of the biosynthetic machinery of starch formation in sweet potato tubers, we have cloned and characterized cDNA clones encoding a large subunit of AGPase from sweet potato.

[†] Present address: Biotechnology Center Nongwoo Seed Research Institute, Kyonggi-do 442–372, Korea.

* To whom correspondence should be addressed.
Tel: 82-337-883-7055; Fax: 82-337-883-1193
E-mail: chharn@nongwooseeds.co.kr

Abbreviations: AGPase, ADP-glucose pyrophosphorylase; AGPL, ADP-glucose pyrophosphorylase large subunit; AGPS, ADP-glucose pyrophosphorylase small subunit; LS, large subunit; PGA, phosphoglycerate; SS, small subunit.

Materials and Methods

Plant materials Sweet potatoes (*Ipomoea batatas* cv. White Star) were grown in a green house for one year. Leaves, stems, roots and tuberous roots were harvested at the same time from mature plants.

Isolation of cDNA clones and sequencing A cDNA library constructed from poly (A)+ RNA of tuberous root was screened by using a 32^P-labeled (1×10^6 dpm/ml) heterologous probe from potato AGPase large subunit cDNA (*sAGPL1*, courtesy of Dr. Bernd Müller-Röber). Approximately 3.0×10^5 recombinant phages were screened. Plaque filters were hybridized in a solution containing 6× SSC, 5× Denhart's solution, 0.1% SDS and 50 μg/ml calf thymus DNA for 16 h at 60°C and washed with 1× SSC-0.1% SDS several times. The filters were exposed to X-ray film at −70°C prior to autoradiography. Two positive clones (*iAGPLI-a* and *iAGPLI-b*) were subcloned into the *Kpn*I site of Bluescript vector and sequenced by the dideoxynucleotide chain termination method using Sequenase II (USB, Cleveland, OH). All sequencing reactions were performed with custom synthesized oligonucleotide primers. To confirm correct sequences, overlapping subclones were sequenced on both strands and subjected to automated sequencing. Sequence homologies were analyzed with MacDNASIS (Hitachi Software Engineering America) and the BLAST network service (National Center for Biotechnology Information).

RNA gel blot analysis Total RNA was isolated from leaf, stem, root, and tuber tissues of sweet potato as described by Chomczynski and Sacchi (1987) with modification. Thirty μg of total RNA was loaded on 1% agarose gel containing 1× MOPS buffer and 0.66 M formaldehyde. After electrophoresis at 80 V for 2 h, the gel was transferred to a nylon membrane in blotting buffer (10× SSC). The membranes were hybridized by 32^P-labeled (1×10^6 dpm/ml) probes of total cDNA fragments generated by *Kpn*I restriction digestion of *iAGPLI-a* and *iAGPLI-b*. Hybridization was performed in a solution containing 5× Denhardt's solution, 0.1% SDS, 100 mg/ml salmon sperm DNA, and 5× SSC for 20 h at 60°C. After hybridization, the blots were washed three times in 1× SSC-0.1% SDS at 60°C prior to exposure to X-ray film at −70°C.

Genomic DNA blot analysis Thirty μg of genomic DNA isolated from sweet potato tuber by CsCl gradient ultracentriguation (Sambrook *et al.*, 1989) was digested with *Xba*I and *Eco*RI. Neither of the restriction enzymes are present within the cloned cDNA sequence. Restriction fragments were separated on 0.8% agarose gel at 80 V for 6 h. The gel was soaked for 20 min in a denaturation solution (1.5 M NaCl and 0.5 M NaOH) and neutralized for 40 min in a neutralization solution (1.5 M NaCl and 0.5 M Tris·HCl, pH 8.0). DNA was then blotted onto nylon membranes in 10× SSC and the membranes were hybridized by the same conditions used for RNA gel blot analysis (above). The primers used for generating PCR fragments for hybridization probes are 5′-CGATTCGTGGGATGTTAT-3′ (sense) and 5′-ACCATAGC-CAAATGGCCA-3′ (antisense) for 280 bp of *AGPLI-a* at 3′ terminus, and 5′-AGTTTAC-CTGGACTTGTG-3′ (sense) and 5′-AACCATAGCCAA-ATGGCC-3′ (antisense) for 177 bp of *AGPLI-b* at 3′ terminus.

Results

Characterization of large subunit AGPase cDNA clones Approximately 300,000 recombinant phages were screened and two positive clones were isolated (*iAGPLI-a*, 1661 bp and *iAGPLI-b*, 1277 bp) as shown in Figs. 1A and 1B. The respective open reading frames ended with the TAG termination codon at position 1,353 bp and 920 bp, flanked by 3′ untranslated regions of 308 and 357 bp. The polyadenylation regions were located at 1652 (*iAGPLI-a*) and 1267 bp (*iAGPLI-b*). The deduced amino acid sequence of both forms contained highly conserved Lys residues, which are allosteric activator binding sites for the large subunit of spinach AGPase (Morell *et al.*, 1988). iAGPLI-a exhibited Lys residues at 113, 403 and 441 amino acids and iAGPLI-b exhibited two residues at 259 and 297 amino acids. The first Lys residue at 113 amino acid of iAGPLI-a was not present in iAGPLI-b because the iAGPLI-b is 144 amino acids shorter than iAGPLI-a. Both clones also contained Aspartic acid 413 (D^{413}), a putative

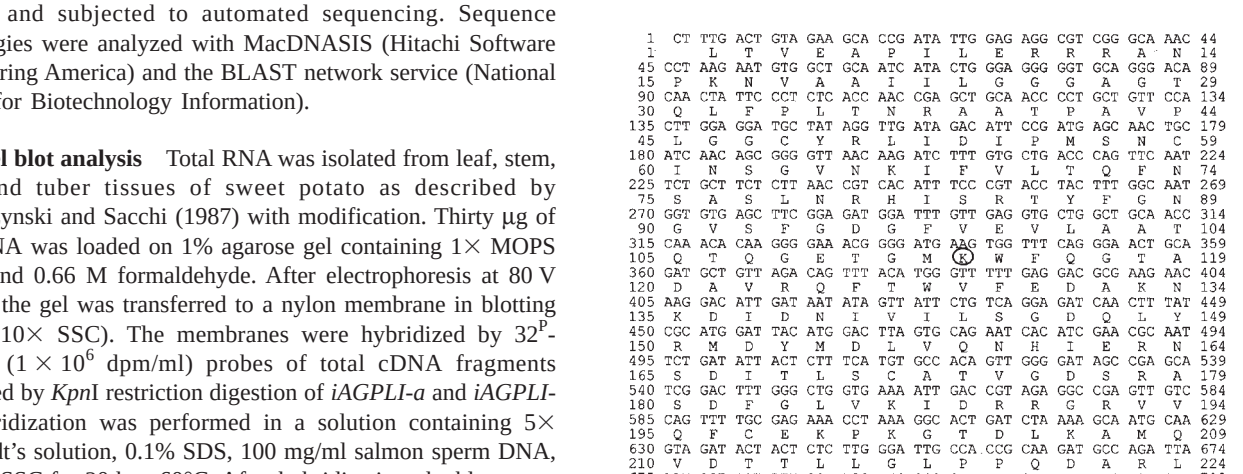

Fig. 1A. Nucleotide sequence and its derived amino acid sequence of *iAGPLI-a* (accession number: AJ252316).

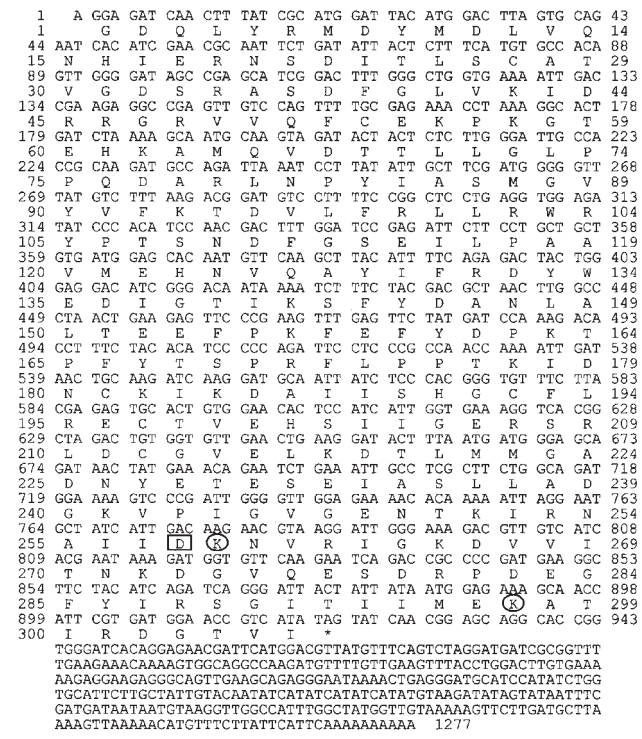

Fig. 1B. Nucleotide sequence and its derived amino acid sequence of *iAGPLI-b* (accession number: AJ245392). The asterisk indicates the stop codon. Circles indicate amino acids corresponding to allosteric activator binding sites, and the box is a putative binding site of 3-PGA.

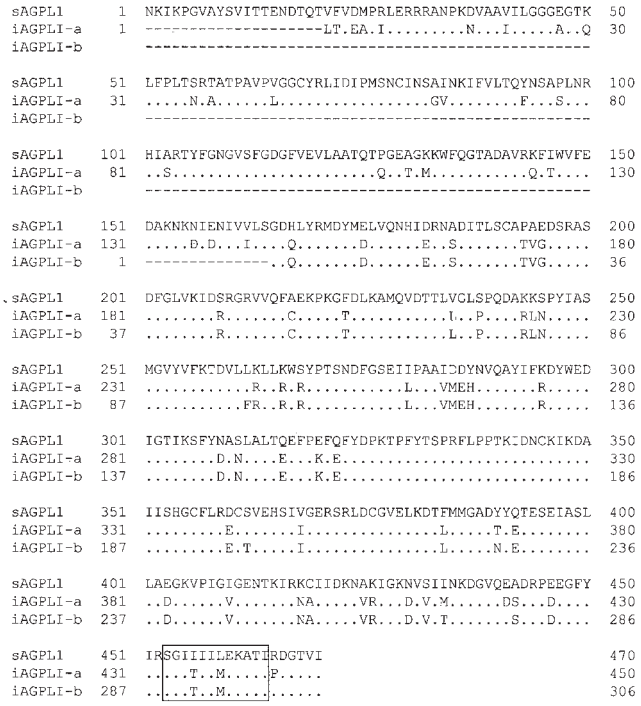

Fig. 2. Amino acid sequence alignment of AGPase large subunits from sweet potato (iAGPLI-a and b) and potato (sAGPL1). Dashes (-) and dots (.) indicate gaps and identity, respectively. The allosteric activator site at the C-terminal end of the proteins is marked by a box.

binding site of 3-PGA found in potato AGPase (Greene *et al.*, 1996), at 402 (iAGPLI-a) and 258 (iAGPLI-b) amino acid. *iAGPLI-a* and *iAGPLI-b* share a DNA base pair identity of 83.8% (data not shown). The nucleotide divergency lies on only noncoding region at 3′ terminus after stop codon. Both clones contained partial open reading frames of 450 and 306 amino acid, respectively (Fig. 2). iAGPLI-a and -b share 81% amino acid sequence identity with potato AGPase large subunit while the amino acid sequence identity between a and b is 97.3% due to a difference of 6 amino acids. They also contained an allosteric activator site of SGITIIMEKATI at position 433 and 289 amino acids, respectively.

Amino acid sequence alignment of AGPase large subunits The deduced amino acid sequences from cDNA clones encoding AGPase large subunits were aligned and sequence identities were calculated. Several distinct groups of AGPase large subunit were classified (Fig. 3). Barley (hAGPL) and maize (zAGPLE) represent a group (I) of monocot plants with 75% homology. Most AGPLs in dicot plants can be grouped into three large subunits from potato and tomato AGPLs, designated by the numbers of 1 (group II), 2 (III) and 3 (IV). Bean AGPase large subunit (vAGPL, group V) is a distinct isoform showing the least identity

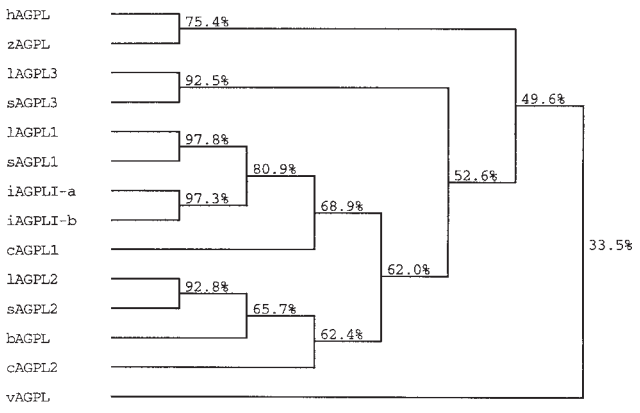

Fig. 3. Dendrogram was generated by the MacDNASIS software program. h, barley; z, maize; l, tomato; s, potato; i, sweet potato; b, sugar beet; v, bean; c, oriental melon.

compared to all AGPases. The amino acid sequence identities of tomato and potato iAGPL in each group share 92–98% homology. This degree of homology is due to their belonging in the same family. By contrast, isoforms in the same species (for example, sAGPL1, 2, and 3) are grouped differently and exhibit lower identities. Sweet potato large subunits (iAGPLI-a and -b) belong to group II.

Northern and Southern blot analysis RNA gel blot analysis was performed using total RNA extracted from leaf, stem, root and tuberous root. The *iAGPLI-a* transcript of 1.9 kb was detected predominantly in the stem but also weakly in the tuberous root (Fig. 4). Northern blot hybridized with the probe from *iAGPLI-b* generated the same results (data not shown). For Southern blot analysis, genomic DNA was digested with *Eco*RI and *Xba*I which did not cleave the internal area of coding region. For hybridizaton, PCR probes obtained at 3′ terminus from *iAGPLI-a* was used. The same results were generated by PCR probes at *iAGPLI-b* and/or a total size of *iAGPLI-a* and *-b* (data not shown). *Eco*RI digestion yielded 1.0 kb, (a doublet), 2.5 kb, 4.0 kb and 9.0 kb bands (Fig. 5). *Xba*I digestion generated 2.7, 3.4 and 10.0 kb (more than two bands) bands indicating that the sweet potato genome contained several copies of the *iAGPLI* gene.

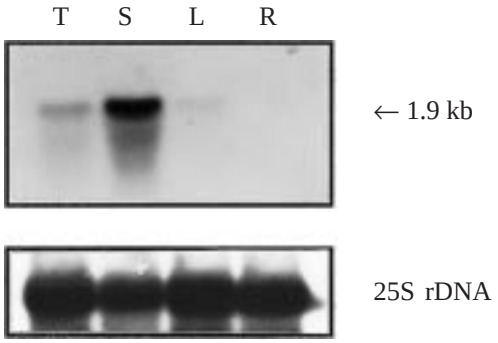

Fig. 4. Northern analysis of *iAGPLI-a* gene expression in various tissues. Total 30 μg was loaded in 1% agarose gel and blotted in nylon membrane. The membranes were hybridized by 32^{P}-labeled (1×10^{6} dpm/ml) probes of total cDNA from *iAGPLI-a*. L, leaf; S, stem; R, root; T, tuberous root.

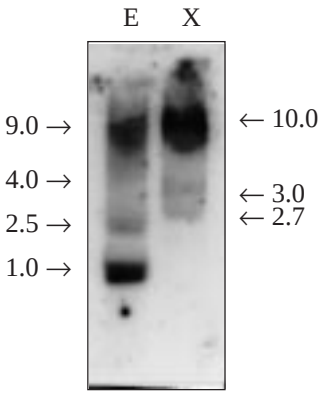

Fig. 5. Southern blot analysis of genomic DNA from sweet potato. Thirty μg of genomic DNA was digested with X, *Xba*I and E, *Eco*RI. A PCR fragment (1359–1648 bp) of *iAGPLI-a* was used as a probe for Southern hybridization. Hybridization with the PCR fragment (1041–1217 bp) of *iAGPLI-b* generated the same results (data not shown).

Discussion

We have isolated two cDNA clones (*iAGPLI-a* and *iAGPLI-b*) which encode the AGPase large subunit from sweet potato. Based on the amino acid sequence comparison with the full length clone of potato AGPase large subunit (sAGPL1), these two clones contained partial sequences of the sweet potato AGPase large subunit. Attempts to find the 5′ terminus for the full length of each clone were not successful (data not shown).

The sequence identities among potato AGPL isoform 1, 2 and 3 are approximately 50–62%. This indicates that potato has at least three distinct isoforms of the AGPase large subunit and each isoform would play a unique role in generating ADP glucose. Sweet potato may have several distinct isoforms like other plants thus far characterized. In contrast with the distinct isoforms, the sequence identity between iAGPLI-a and iAGPLI-b is 97.3%. Besides, the nucleotide sequences are almost identical along the DNA sequence except for the 3′ UTR region. A single gene of *iAGPLI* may have been diverged into iAGPLI-a and iAGPLI-b in the same manner that tomato and potato AGPase large subunits have been differentiated from the same gene through evolution. The heterogeneity of the original gene (*iAGPLI*) may have occurred during evolution especially in sweet potato (*Ipomoea batatas*) plant, which is hexaploidy (6x; 2n = 90). The cDNAs encoding small subunits of AGPase from sweet potato, which were previously isolated, also showed heterogeneity of 1% difference in amino acid sequences (Bae and Liu, 1996), indicating that sweet potato genome contains the extra gene copies for each AGP small subunit gene. Therefore, each gene encoding AGPase large and small subunit isoform seem polymorphic in sweet potato genome because of the polyploidy nature. The presence of several copies of each gene shown in Southern blots (Fig. 5 as well as in the data of Bae and Liu, 1996) support this idea. Taken together, our data suggest that each AGPase large and small subunit gene of polyploid plants such as potato (4x; 2n = 48) would also be polymorphic, although no evidence has been reported yet.

Potential binding sites for substrates and allosteric effectors in the AGPase has been revealed by chemical modification studies (Ball and Preiss, 1994; Greene *et al*., 1996a; 1996b). Aspartic acid 413 (D^{413}) is a putative binding site of 3-PGA, an allosteric activator of AGPase. This residue is present in all AGPase thus far characterized, as well as in iAGPLI-a and iAGPLI-b (Figs. 1A and 1B). Pyridoxal phosphate, an analog of 3-PGA, interacts with three highly conserved Lys residues in large subunits. These are considered as allosteric activator binding site of spinach AGPase (Morell *et al*., 1988). Both clones contain the Lys residues K^{113}, K^{403}, K^{441} for iAGPLI-a and K^{263} and K^{301} for iAGPLI-b. These clones also contain an allosteric activator site of SGITIIMEKATI

which is also present in the AGPase small subunit as SGIVTVIKDAL where the sequence identity is moderate (Müller-Röber *et al*., 1990). A further study of structure-function analysis will help to understand the regulation mechanism of AGPase with two distinct large and small subunits.

The expression levels of *iAGPLI-a* and *iAGPLI-b* were predominant in stems, whereas the potato *sAGPL1* were mainly expressed in tubers (La Cognata *et al*., 1995). Since the cDNA library was constructed with the mRNA isolated from the tuberous root, the predominant expression in stems is unusual. However, minute levels of transcripts, representing a stem iAGPLI, must have been expressed in the tuberous root and picked up when the cDNAs were synthesized. Considering that the *iAGPLI* and *sAGPL1* were differentially expressed in different tissues, each AGP large subunit gene would have preferentially expression in tissues depending on its role, although each gene belongs to the same group based on sequence identity.

Acknowledgments We would like to thank Young Ock Ahn for technical help on manuscript preparation. This work was sponsored by a grant from Korea Ministry of Science and Technology (KG1111).

References

Bae, J. M. and Liu, J. R. (1996) Molecular heterogeneity of small subunit ADP-glucose pyrophosphorylase cDNAs isolated from tuberous roots of sweet potatoes. *Mol. Cells* **6**, 521–527.

Bae, J. M. and Liu, J. R. (1997) Molecular cloning and characterization of two novel isoforms of the small subunit of ADP-glucose pyrophosphorylase from sweet potato. *Mol. Gen. Genet.* **254**, 179–185.

Bhave, M. R., Lawrence, S., Barton, C., and Hannah, L. C. (1990) Identification and molecular characterization of shrunken-2 cDNA clones of maize. *The Plant Cell* **2**, 581–588.

Chomczynski, N. and Sacchi, N. (1987) Single step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. *Anal. Biochem.* **161**, 156–159.

Elimert, K., Luo, C., Dejardin, A., Villand, P., Thorbjornsen, T., and Kleczkowski, L. (1997) Molecular cloning and expression of the large subunit of ADP-glucose pyrophosphorylase from barley (*Hordeum vulgare*) leaves. *Gene* **189**, 79–82.

Giroux, M., Smith-White, B., Gilmore, V., Hannah, L. C., and Preiss, J. (1995) The large subunit of the embryo isoform of ADP-glucose pyrophosphorylase from maize. *Plant Physiol.* **108**, 1333–1334.

Kleczkowski, L. A., Villand, P., Luthi, E., Olsen, O.-A., and Preiss, J. (1993) Insensitivity of barley endosperm ADP-glucose pyrophosphorylase to 3-phosphoglycerate and orthophosphate regulation. *Plant Physiol.* **101**, 179–186.

La Cognata, U., Willmitzer, L., and Muller-Rober, B. (1995) Molecular cloning and characterization of novel isoforms of potato ADP-glucose pyrophosphorylase. *Mol. Gen. Genet.* **246**, 538–548.

Lin, T. P, Caspar, T, Somervill, C., and Preiss, J. (1988) Isolation and characterization of a starchless mutant of *Arabidopsis thaliana* (L.) Heynh. lacking ADP glucose pyrophosphorylase activity. *Plant Physiol.* **86**, 113–1135.

Morell, M., Bloom, M., Knowles, V., and Preiss, J. (1987) Subunit structure of spinach leaf ADP glucose pyrophosphorylase. *Plant Physiol.* **85**, 182–187.

Morell, M., Bloom, M., and Preiss, J. (1988) Affinity labeling of the allosteric activator sites of spinach leaf ADP glucose pyrophosphorylase. *J. Biol. Chem.* **263**, 633–637.

Müller-Röber, B., Kossman, J., Hannah, L. C., Willmitzer, L., and Sonnewald, U. (1990) Only one of two different ADP-glucose pyrophosphorylase genes from potato responds strongly to elevated levels of sucrose. *Mol. Gen. Genet.* **224**, 136–143.

Müller-Röber, B., Nast, G., and Willmitzer, L. (1995) Isolation and expression analysis of cDNA clones encoding a small and a large subunit of ADP-glucose phrophosphorylase from sugar beet. *Plant Mol. Biol.* **27**, 191–197

Nakata, P. A., Greene, T. W., Anderson, J. M., Smith-White, B. J., Okita, T. W., and Preiss, J. (1991) Comparison of the primary sequences of two potato tuber ADP-glucose pyrophosphorylase subunits. *Plant Mol. Biol.* **17**, 1089–1093.

Olive, M. R., Ellis, R. J., and Schuch, W. W. (1989) Isolation and nucleotide sequences of cDNA clones encoding ADP-glucose pyrophosphorylase polypeptides from wheat leaf and endosperm. *Plant Mol. Biol.* **12**, 525–538.

Park, S. W. and Chung, W. I. (1998) Molecular cloning and organ-specific expression of three isoforms of tomato ADP-glucose pyrophosphorylase gene. *Gene* **206**, 215–221.

Park, S. W., Kahng, H. Y., Kim, I. J., Park, J. O., and Chung, W. I. (1998) Molecular cloning and characterization of small and large subunits of ADP-glucose pyrophosphorylase from oriental melon. *J. Plant Res.* **111**, 59–63.

Preiss, J. (1978) Regulation of ADP-glucose pyrophosphorylase. *Adv. Enzymol. Relat. Areas. Mol. Biol.* **46**, 317–381.

Preiss, J. (1991) Biology and molecular biology of starch synthesis and its regulation. *Oxford Surv. Plant Mol. Cell. Biol.* **7**, 59–114.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sowokinos, JR. and Preiss, J. (1982) Pyrophosphorylases in Solanum tuberosum. III. Purification, physical, and catalytic properties of ADP glucose pyrophoshorylase in potatoes. *Plant Physiol.* **69**, 1469–1466.

Villand, P., Aalen, R., Olsen, O. A., Lüthi, E., Lönneberg, A., and Kleczkowski, L. A. (1992a) PCR amplification and sequence of cDNA clones for the small and large subunits of ADP-glucose pyrophosphorylase from barley tissues. *Plant Mol. Biol.* **19**, 381–389.

Villand, P., Olsen, O. A., Kilian, A., and Kleczkowski, L. A. (1992b) ADP-glucose pyrophosphorylase large subunit cDNA from barley endosperm. *Plant Physiol.* **100**, 1617–1618.

Villand, P., Olsen, O. A., and Kleczkowski, L. A. (1993) Molecular characterization of multiple cDNA clones for ADP-glucose pyrophosphorylase from *Arabidopsis thaliana*. *Plant Mol. Biol.* **23**, 1279–1284.