Note

Isolation and Characterization of Two cDNAs for Large and Small Subunits of ADP-glucose Pyrophosphorylase from Kidney Bean*

(Received March 13, 2003; Accepted April 28, 2003)

Daisuke Omoto,¹ Hiroyuki Ito,^{1,*} Yumiko Obana,¹ Kanako Matsumoto,¹ Naoto Isono,¹
 Shigeki Hamada,¹ Thomas W. Okita² and Hirokazu Matsui¹

¹*Department of Applied Bioscience, Graduate School of Agriculture, Hokkaido University
 (Kita-9, Nishi-9, Kita-ku, Sapporo 060-8589, Japan)*

²*Institute of Biological Chemistry, Washington State University
 (Pullman, Washington 99164-6340, USA)*

Abstract: Two cDNA clones for the large and small subunits of ADP-glucose pyrophosphorylase (AGPase), designated *pvagpL1* and *pvagpS1*, respectively, were isolated from developing seeds of kidney bean (*Phaseolus vulgaris* L.). Each deduced amino acid sequence showed significant identity (65–86%) with those of leguminous AGPase large or small subunits. Northern blot analysis revealed that both mRNA species accumulated abundantly at the early- to mid-stages of seed development, indicating that both genes are synchronically expressed during seed maturation. Both gene products were also detected in leaves, stems and roots of kidney bean. In leaves, the *pvagpL1* mRNA level was constant during the diurnal cycle, whereas the *pvagpS1* mRNA level decreased markedly during the dark period. These results indicate that the *pvagpL1* and *pvagpS1* genes are differentially regulated by environmental and spatial conditions.

Key words: ADP-glucose pyrophosphorylase, gene expression, kidney bean (*Phaseolus vulgaris* L.)

The sugar nucleotide, ADP-glucose, is the glucose donor in the biosynthesis of starch and is formed from glucose-1-phosphate and ATP by ADP-glucose pyrophosphorylase (AGPase; glucose-1-phosphate adenylyltransferase, EC 2.7.7.27). The plant AGPase is a heterotetrameric enzyme composed of two small and two large subunits encoded by different genes.¹⁾ The leaf isozymes are allosterically regulated in that their catalytic activities are activated by 3-phosphoglycerate (3-PGA) and inhibited by inorganic phosphate (Pi).¹⁾ In addition to biochemical regulation, AGPase activity is also regulated at the molecular level where AGPase gene expression is controlled by sucrose and/or light intensity.^{2–5)} These observations indicate that plant AGPase activity which is one of the dominant control steps of transitory starch synthesis is modulated by both transcription at the gene level and allosteric regulation at the enzyme catalytic level. We have undertaken a comprehensive study on starch biosynthesis during seed development of kidney bean.^{6–11)} Here we describe the isolation of cDNA clones encoding AGPase subunits from kidney bean and their gene expression profiles in plants.

Plant materials, total RNA species, and a cDNA library

of kidney bean developing seeds were prepared as previously described.^{8,9)} All experimental procedures in this study were the same as those described in previous studies,^{8–10)} unless otherwise stated.

Two cDNA clones for AGPase large and small subunits were isolated from a cDNA library of kidney bean developing seeds at the mid-stage. The full-length cDNA clones (X61187 for large and X61186 for small subunit) for each AGPase subunit of potato tuber were used for low-stringent screening as probes. Multiple rounds of screening of the cDNA library consisting of approximately 5×10^4 recombinant phages resulted in the isolation of four large subunit clones and three small subunit clones. Nucleotide sequences of the positive clones indicated that all of the large or small subunit clones shared identical regions. The longest large subunit clone, designated *pvagpL1*, was 2025 bp in length including an open reading frame of 1578 bp. The longest small subunit clone (*pvagpS1*) was 1885 bp that contained an ORF of 1548 bp. The proteins encoded by *pvagpL1* and *pvagpS1* (designated PvAGPL1 and PvAGPS1 respectively) corresponded to 525 and 515 amino acid residues with predicted molecular masses of 57.8 and 56.2 kDa, respectively. When analyzed by the PSORT¹²⁾ and Target P¹³⁾ network programs, both proteins were predicted to have plastid-targeting signal sequences. In plant AGPases, the N-terminal sequences of native mature proteins have only been determined by Edman degradation for the spinach leaf AGPase, to be SVTADNASETKVREIGQEKSS for the large subunit and VSDSQNSQDGLDPE for the small subunit.¹⁴⁾ Although there was no obvious sequence homology between the N-terminal regions of PvAGPL1 and spinach AGPase large subunit, the first 62–65 amino acid

*The nucleotide sequences reported in this paper will appear in the DDBJ nucleotide sequence database under the accession numbers AB103472 for *pvagpL1* and AB103473 for *pvagpS1*.

*Corresponding author (Tel. +81-11-706-2508, Fax. +81-11-706-3635, E-mail: otih@chem.agr.hokudai.ac.jp).

Abbreviations: AGPase, ADP-glucose pyrophosphorylase; PvAGPase, *Phaseolus vulgaris* L. AGPase; PvAGPL1, large subunit of PvAGPase; PvAGPS1, small subunit of PvAGPase; *pvagpL1*, cDNA for PvAGPL1; *pvagpS1*, cDNA for PvAGPS1; 3-PGA, 3-phosphoglycerate.

(A)

Fig. 1. Alignment of the primary sequences of leguminous AGPases and phylogenetic tree of leguminous AGPase small subunits.

(A) Multiple sequence alignments were determined using the ClustalW program. The sequences were obtained from the GenBank/EMBL/DBJ databases: PvAGPL1 and PvAGPS1 from *Phaseolus vulgaris* (this work, AB103472 and AB103473); PsagpL1, PsagpS1 and PsagpS2 from *Pisum sativum* (X96766, X96764 and X96765); VfAGPC and VfAGPP from *Vicia faba* (X76940 and X76941); CagpL1, CagpL2, CagpS1 and CagpS2 from *Cicer arietinum* (AF356002, AF356003, AF356004 and AF356005). Residues showing more than 90% sequence identity among all subunits are indicated as white letters on a blue background. Residues showing more than 75% identity among large subunits (upper four sequences) and more than 85% identity among small subunits (lower seven sequences) are shown as white letters on a black and brown background, respectively. (B) Alignment of the putative mature sequences of leguminous AGPase small subunits was used to construct a phylogenetic tree. The dotted lines indicate the borders between types.

residues of PvAGPL1 was predicted to be a transit signal peptide by comparison with the sequence of pea AGPase large subunit.¹⁵ In contrast, sequences homologous to the N-terminal sequence of spinach small subunit are readily observed in most plant AGPase small subunits including PvAGPS1. Based on sequence homology, PvAGPS1 was predicted to be synthesized as a preprotein with a signal sequence of 66 residues.

Since multiple cDNA clones encoding isoforms of AGPases have been isolated from various plants, it is now generally recognized that higher plants have multiple AGPase genes. To isolate different cDNA clones from *pvagpL1* and *pvagpS1*, we further screened more than 5×10^5 recombinants using *pvagpL1* or *pvagpS1* clone as a probe at low-stringency conditions, but we were unable to detect any other clones. This suggests that the other clones have a low homology to *pvagpL1* or *pvagpS1* or that both *pvagpL1* and *pvagpS1* may be major forms in the immature seeds at the mid-stage.

The primary sequences of PvAGPL1 and PvAGPS1 were compared to those of other leguminous AGPase subunits by the ClustalW program (Fig. 1). PvAGPL1 showed significant sequence identity (82%) to the pea large subunit, but much lower sequence identity to the two chickpea large subunits at a level (about 65%) comparable to that observed for large subunits from non-leguminous plant species. The PvAGPS1 sequence showed significant identity (more than 86%) and similarity (more than 91% using the BLOSUM 62¹⁶) as a matrix) to the leguminous small subunits. The phylogenetic tree indicated that leguminous small subunits are divided into three types: type 1 consists of *Psagps1* from pea,¹⁵ *VfAGPP* from fava bean¹⁷) and *CagpS2* from chickpea,¹⁸ type 2 is *Psagps2* from pea,¹⁵ *VfAGPC* from fava bean¹⁷) and *CagpS1* from chickpea,¹⁸ and type 3 is only PvAGPS1 (Fig. 1B).

The accumulation profiles of *pvagpL1* and *pvagpS1*

Fig. 2. *pvagpL1* and *pvagpS1* transcript levels in immature and mature seeds.

Each lane was loaded with 20 μ g of total RNA isolated from each tissue. S, M, L and Ma indicate small-, mid- and large-sized immature seeds and mature seeds, respectively. A DNA fragment of about 0.5 kb containing the 3'-untranslational region was prepared from *pvagpL1* or *pvagpS1* cDNA by PCR amplification and used as a probe for hybridization. The lower panel shows EtBr-stained total RNA profiles.

transcripts during seed development were analyzed by Northern blot analysis (Fig. 2). Steady state RNA levels for both genes attained their maximum levels at the early- to mid-stage of seed development. Both transcripts were absent in mature seeds. These results suggest that AGPase large and small subunit genes are regulated by similar or identical process during seed development. Interestingly, these temporal RNA accumulation patterns precede the accumulation of starch in developing kidney bean seeds. In contrast, transcripts for the specific isozyme (SSII) of starch synthases that utilize ADP-glucose synthesized by AGPase as a substrate accumulate to their maximum levels at the mid- to late-stage of kidney bean seed development (unpublished data) when starch synthesis is at their highest rates. Likewise, the mRNA levels of the specific starch-branching enzyme (PvSBE2) parallel the accumulated pattern of SSII.⁸ Thus, although AGPase catalyzes the first committed step in starch biosynthesis, the rate limiting step may be catalyzed by other enzymes in the biosynthetic pathway.

The *PsagpL1* mRNAs for pea AGPase large subunit were predominantly accumulated in sink organs such as pods and developing seeds.¹⁵ The *CagpL1* and *CagpL2* mRNAs for chickpea large subunits were mostly found in leaves and seeds, respectively.¹⁸ Unlike the accumulation patterns seen in these other legumes, the *pvagpL1* transcripts were also detected in leaves, stems, and roots (Fig. 3). In leaves, no difference was found in mRNA levels of *pvagpL1* during light/dark periods, whereas, in stems and roots, mRNA levels were much higher in the light than in the dark period. These results suggest that the *pvagpL1* gene expression is differentially regulated in the different organs of kidney bean. Moreover, in leaves the *pvagpL1* transcript levels are independent of the diurnal change of AGPase catalytic activity and, in turn, starch levels. In general, the diurnal oscillation of starch metabolism in leaves is accounted for by the allosteric regulation of AGPase. In the light, increase of 3-PGA levels by net carbon fixation and decrease of Pi levels by photophosphorylation activate AGPase activity resulting in the promotion of transitory starch synthesis. In the dark, decrease of 3-PGA levels and increase of Pi levels inactivate AGPase activity and lead to conditions favoring net starch degradation. Therefore, *pvagpL1* transcript levels in leaves are independent of the diurnal change of AGPase activity.

The accumulation profiles of *pvagpS1* in leaves, stems and roots (Fig. 3) were similar to those of *PsagpS1*¹⁵) and *VfAGPP*,¹⁷) but different from those of the other leguminous small subunits, such as *Psagps2*, *VfAGPC*, *CagpS1* and *CagpS2* whose transcripts predominantly accumulate in seeds and pods.^{15,17,18}) In small subunits from pea and fava bean, members of each type show a very similar gene expression pattern. In contrast, despite the sequence of PvAGPS1 being between the type 1 and 2 (Fig. 1B), the accumulation pattern of *pvagpS1* mRNA was close to those of type 1 members. The levels of *pvagpS1* mRNA in leaves and stems during the light period were higher than during the dark period, suggesting that *pvagpS1* transcript levels correspond to the changes in transitory starch levels. The *Arabidopsis* mutant *adgl* which encodes the

Fig. 3. Accumulation of pvagpL1 and pvagpS1 transcripts in leaves, stems and roots during light/dark periods.

The upper two panels show Northern blots while the lower two panels show the relative accumulation levels of transcripts estimated by NIH image software using the hybridization signal detected from the mid-day leaf as the standard (100%). Each lane was loaded with 20 μ g of total RNA isolated from leaves, stems and roots of kidney bean plants harvested at mid day (D) or in the middle of the night (N).

AGPase small subunit have neither small nor large subunits, indicating that the presence of small subunit protein is required for large subunit stability.¹⁹⁾ Hence, the alteration of transcript levels observed for only the small subunit, but not for the large subunit, would effectively control AGPase enzyme levels in leaves of kidney bean. To further understand the regulation of AGPase activity in kidney bean plants, our efforts are to concentrate on preparing recombinant AGPases and antiserum raised against PvAGPL1 and PvAGPS1 and on analyzing their protein and enzyme activity levels in different plant organs and under different environmental conditions. Results from these studies will lead to further insights on the relationship between AGPase gene expression, enzyme activity, starch metabolism, and plant growth and development.

This work was partly supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture, Japan (grant nos. 11660068 and 13660071 to H.I.) and by Technology and Research Premium of The Japanese Society of Applied Glycoscience (to H. I.).

REFERENCES

- 1) M.N. Sivak and J. Preiss: Synthesis of the glucosyl donor: ADPglucose pyrophosphorylase. *Adv. Food Nutr. Res.*, **41**, 43–74 (1998).
- 2) B. Müller-Röber, J. Kossman, L.C. Hannah, L. Willmitzer and U. Sonnewald: Only one of two different ADP-glucose pyrophosphorylase genes from potato responds strongly to elevated levels of sucrose. *Mol. Gen. Genet.*, **224**, 136–143 (1990).
- 3) L.N. Sokolov, A. Déjardin and L.A. Kleczkowski: Sugars and light/dark exposure trigger differential regulation of ADP-glucose pyrophosphorylase genes in *Arabidopsis thaliana* (thale cress). *Biochem. J.*, **336**, 681–687 (1998).
- 4) C.H. Harn, J.M. Bae, S.S. Lee, S.R. Min and J.R. Liu: Presence of multiple cDNAs encoding an isoform of ADP-glucose pyrophosphorylase large subunit from sweet potato and characterization of expression levels. *Plant Cell Physiol.*, **41**, 1235–1242 (2000).
- 5) X. Li, J. Xing, T.J. Gianfagna and H.W. Janes: Sucrose regulation of ADP-glucose pyrophosphorylase subunit genes transcript levels in leaves and fruits. *Plant Sci.*, **162**, 239–244 (2002).
- 6) K. Nozaki, H. Matsui, T. Tonokawa, H. Mori, H. Ito, M. Honma and S. Chiba: Amylases and branching enzyme of developing kidney bean seeds on native electrophoretic gel. *J. Appl. Glycosci.*, **45**, 117–122 (1998).
- 7) K. Nozaki, S. Hamada, T. Nakamori, H. Ito, S. Sagisaka, H. Yoshida, Y. Takeda, M. Honma and H. Matsui: Major isoforms of starch branching enzymes in premature seeds of kidney bean (*Phaseolus vulgaris* L.). *Biosci. Biotechnol. Biochem.*, **65**, 1141–1148 (2001).
- 8) S. Hamada, K. Nozaki, H. Ito, Y. Yoshimoto, H. Yoshida, S. Hiraga, S. Onodera, M. Honma, Y. Takeda and H. Matsui: Two starch-branching-enzyme isoforms occur in different fractions of developing seeds of kidney bean. *Biochem. J.*, **359**, 23–34 (2001).
- 9) N. Isono, H. Ito, T. Senoura, M. Yoshikawa, K. Nozaki and H. Matsui: Molecular cloning of a cDNA encoding granule-bound starch synthase I from kidney bean seeds and expression in *Escherichia coli*. *J. Appl. Glycosci.*, **50**, 355–360 (2003).
- 10) S. Hamada, H. Ito, S. Hiraga, K. Inagaki, K. Nozaki, N. Isono, Y. Yoshimoto, Y. Takeda and H. Matsui: Differential characterization and subcellular localization of two starch-branching enzyme isoforms encoded by a single gene in *Phaseolus vulgaris* L. *J. Biol. Chem.*, **277**, 16538–16546 (2002).
- 11) H. Yoshida, K. Nozaki, I. Hanashiro, F. Yagi, H. Ito, M. Honma, H. Matsui and Y. Takeda: Structure and physico-chemical properties of starches from kidney bean seeds at immature, premature and mature stages of development. *Carbohydr. Res.*, **338**, 463–469 (2003).
- 12) K. Nakai and M. Kanehisa: A knowledge base for predicting protein localization sites in eukaryotic cells. *Genomics*, **14**, 897–911 (1992).
- 13) O. Emanuelsson, H. Nielsen, S. Brunak and G. von Heijne: Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *J. Mol. Biol.*, **300**, 1005–1016 (2000).
- 14) M.K. Morell, M. Bloom, V. Knowles and J. Preiss: Subunit structure of spinach leaf ADPglucose pyrophosphorylase. *Plant Physiol.*, **85**, 182–187 (1987).
- 15) D. Burgess, A. Penton, P. Dunsmuir and H. Dooner: Molecular cloning and characterization of ADP-glucose pyrophosphorylase cDNA clones isolated from pea cotyledons. *Plant Mol. Biol.*, **33**, 431–444 (1997).
- 16) S. Henikoff and J.G. Henikoff: Performance evaluation of amino acid substitution matrices. *Proteins*, **17**, 49–61 (1993).
- 17) H. Weber, U. Heim, L. Borisjuk and U. Wobus: Cell-type specific, coordinate expression of two ADP-glucose pyrophosphorylase genes in relation to starch biosynthesis during seed development of *Vicia faba* L. *Planta*, **195**, 352–361 (1995).
- 18) S. Singh, S.-B. Choi, M.K. Modi and T.W. Okita: Isolation and characterization of cDNA clones encoding ADP-glucose

pyrophosphorylase (AGPase) large and small subunits from chickpea (*Cicer arietinum* L.). *Phytochemistry*, **59**, 261–268 (2002).

- 19) S.-M. Wang, W.-L. Leu, T.-S. Yu, J.-H. Long, C.-N. Wang, K. Eimert and J. Chen: Characterization of *ADG1*, an *Arabidopsis* locus encoding for ADPG pyrophosphorylase small subunit, demonstrates that the presence of the small subunit is required for large subunit stability. *Plant J.*, **13**, 63–70 (1998).

インゲンマメ ADP-glucose pyrophosphorylase cDNAs の単離と特性

尾本大輔¹, 伊藤浩之¹, 尾花由美子¹

松本加奈子¹, 磯野直人¹, 濱田茂樹¹

Thomas W. Okita², 松井博和¹

¹ 北海道大学大学院農学研究科

(060-8589 札幌市北区北9条西9丁目)

²Institute of Biological Chemistry, Washington State University
(Pullman, WA 99164-6430, USA)

植物の ADP-glucose pyrophosphorylase (AGPase) は、二つの大サブユニットおよび二つの小サブユニットから構成されるヘテロ4量体である。インゲンマメ登熟種子 cDNA ライブラリーより、両サブユニットをコードする cDNA クローン (pvagpL1 および pvagpS1) を単離した。推定アミノ酸配列は、他のマメ科植物由来 AGPase 大サブユニットあるいは小サブユニットと高い同一性を示した (Fig. 1)。ノーザン解析により、インゲンマメ植物の各器官における mRNA レベルを調べたところ、両 mRNA は登熟種子のみならず、葉、茎ならびに根においても検出された (Figs. 2, 3)。種子登熟過程における両 mRNA の蓄積は同調的であり、登熟初期から中期において顕著な蓄積が認められたが、いずれも完熟種子には検出されなかった (Fig. 2)。葉における pvagpL1 mRNA レベルは光の有無によらずほぼ一定であったが、pvagpS1 mRNA レベルは夜間激減した (Fig. 3)。これらの結果から、*pvagpL1* および *pvagpS1* 遺伝子の発現は器官特異的に異なる制御を受けることが示唆された。