Food Sci. Biotechnol. Vol. 16, No. 3, pp. 343 ~ 348 (2007)

Food Science and Biotechnology

Changes in the Expression of ADP-Glucose Pyrophosphorylase Genes During Fruit Ripening in Strawberry

Jeong-Il Park and In-Jung Kim[1]*

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Yuseong, Daejeon 305-701, Korea
[1]Faculty of Biotechnology, College of Applied Life Sciences, Cheju National University, Jeju 690-756, Korea

Abstract Starch contents play important roles in determining the fruit quality. Stawberry accumulates starch in the early stages and then mobilized into soluble sugars during fruit ripening. To date the molecular studies on the ADP-glucose pyrophosphorylase (AGPase), a key enzyme of starch biosynthesis, were not reported. cDNAs encoding small (FagpS) and large (FagpL1 and FaspL2) AGPase subunits were isolated from strawberry (*Fragaria* × *ananassa* Duch. cv. Niyobou). Both *FagpS* and *FagpL1* cDNAs have open reading frames deriving 55-58 kDa polypeptides, where *FagpL2* contains a partial fragment. Sequence analyses showed that FagpS has a glutamate-threonine-cysteine-leucine (ETCL) instead of a glutamine-threonine-cysteine-leucine (QTCL) motif found in all the dicot plants except for *Citrus*. In fruits, *FagpS* and *FagpL1* were expressed in all stages with a little change in the amounts of transcripts. In the case of *FagpL2*, we were not able to detect any signal from all stages of fruit development and all tissues except for very a weak signal from the leaf. The results indicate that *FagpL1* and *FagpL2* show ubiquitous and leaf-specific expression patterns, respectively. The studies suggest that the starch contents in strawberry might be controlled by the expression of AGPase gene at both the transcriptional and post-transcriptional levels during fruit development.

Keywords: ADP-glucose pyrophosphorylase, fruit, gene expression, starch, strawberry

Introduction

Levels of starch are major quality-determining factors of crops (1). Sugars are mainly composed of glucose, fructose, and sucrose accounting for more than 99% of the total sugars in ripe strawberry fruit (2). Generally, sugar contents increase and starch contents decrease as a fruit ripens. During fruit development the photoassimilate in leaves was found to be exported to the fruit, a relatively strong sinks. Sucrose is the main carbohydrate translocated to the fruit. The major carbohydrate reserve material is starch in the fruit (3).

ADP-glucose pyrophosphorylase (AGPase) plays a central role in starch synthesis in higher plants. The evidence for this has been obtained by mutant studies and by transgenic experiments (4, 5). Through the analysis of photosynthetic partitioning, it has been reported that AGPase is the main rate-limiting step for starch synthesis. Both 3-phosphoglycerate (3-PGA) and inorganic pyrophosphate are important factors regulating the rate of starch biosynthesis (6).

Plant AGPase is a heterotetrameric enzyme which is composed of two small and two large subunits (7, 8). The presence of both subunits is essential for normal enzymatic activity, which is supported by several evidences including mutants lacking one of the subunits (4, 9), and by analysis of transgenic plants (5).

Multiple cDNA clones encoding AGPase have been isolated from numerous plants. It is generally recognized that higher plants have multiple genes encoding large AGPase subunits, which are expressed in a developmental stage- and tissue-specific manner (10). Distinct from large subunits, small AGPase subunits are encoded by lower copies of genes in plants, and their amino acid sequences are highly conserved regardless of plant species. Furthermore, their expression patterns are not tissue specific.

In many fleshy fruits, the breakdown of starch to glucose, fructose, or sucrose is a characteristic of a ripening event. For strawberry, cell enlargement in fruit was accompanied by the loss of starch grain. Therefore, it is necessary to illuminate more clearly the function of AGPase which is involved in the control of starch content during fruit ripening and which has an effect on the fruit quality including texture and storage duration. In this research, we studied on the sequence characteristics and expression profiles of AGPase in the fruit development stage.

Materials and Methods

Plant materials The strawberries (*Fragaria* × *ananassa* Duch. cv. Niyobou) were cultivated under greenhouse conditions, and the fruits were harvested at four stages of development as determined by maturity and external fruit color. The developmental stages were as follows: green (G), white (W), turning (T), and red (R) stages.

Preparation of the AGP probe by reverse transcription-polymerase chain reaction (RT-PCR) amplification The total RNA was isolated from the strawberry fruits and leaves with an Extract-A-plant^{TM} RNA isolation kit (Clontech, Palo Alto, CA, USA). Poly(A)$^+$ RNA was purified from the total RNA with a Poly A Tract mRNA isolation kit (Promega, Madison, WI, USA). First-strand cDNA was synthesized from poly(A)$^+$ RNA by reverse transcriptase (Promega) using random hexamers as primer. For the PCR reaction of the small AGPase subunit, the sense primer (SU2: 5'-GA GA[A/G] CA[A/G] TTG [C/A]AA GC[A/T]

*Corresponding author: Tel: 82-64-754-3357; Fax: 82-64-756-3351
E-mail: ijkim@cheju.ac.kr
Received July 18, 2006; accepted January 15, 2007

ATG-3') and the antisense primer (SL1: 5'-GT ACC AAT ATC TTC CCA GTA-3') were used. For the PCR reaction of large AGPase subunits, the sense primer (LU2: 5'-CC[A/C/T] ATG AG[C/T] AA[C/T] TG[C/T] [A/T]T [C/T] AA-3'), and the antisense primer (LL2: 5'-CC[A/G/T] AT[A/G] TC[C/T] TCC CA[A/G] TA[A/G] TC-3') were used. These PCR primers were synthesized on the basis of the conserved regions of the previously published sequences of small and large AGP subunits. RT-PCR fragments were obtained from the combination of PCR primers and were then inserted into the pGEM-T vector (Promega). These fragments were used as probes for the screening of full-length cDNA clones of AGPase and then was radioactively labeled with a random primer labeling kit (Promega) with [α-^{32}P]dCTP for the probe preparation.

Isolation and sequence analysis of cDNA clones A strawberry fruit and leaf cDNA library from poly $(A)^+$ RNA was constructed using the Zap-cDNA synthesis and the Gigapack® II gold cloning kits (Stratagene, La Jolla, CA, USA) according to the manufacturer's instruction manual. Approximately 2×10^6 recombinant phages were screened by using a ^{32}P-labeled RT-PCR product as a probe. The filters were pre-hybridized for 1-2 hr at 42°C in 30% formamide, 5×Denhardt's solution, 5×SSPE, and 100 mg/mL denatured salmon sperm DNA. After the pre-hybridization, the filters were incubated with ^{32}P-labeled PCR products for 24 hr under the same conditions. The filters were then washed once in 2×SSC, 0.05% sodium dodecyl sulfate (SDS) at room temperature, once with 1× SSC, 0.05% SDS at 42°C, and once in 0.1×SSC, 0.1% SDS for 15 min at 65°C. After isolation of positive clones, the phagemids were excised *in vivo* by means of a helper phage.

The longest cDNA for each of small or large AGPase subunits, respectively, was sequenced on both strands with T3, T7, and sequence-specific primers. Multiple sequence alignments were performed with the CLUSTALX(1.8) computer program (11). Aligned sequences from the deduced protein sequences were used to build a phylogenic tree according to the TreeView 1.6.6 program.

Genomic DNA gel blot analysis Genomic DNA gel blot analyses were performed using genomic strawberry DNA digested with *Eco*RI and *Hin*dIII. The genomic strawberry DNA was isolated from a young leaf tissue in a large-scale method using cetyltrimethylammonium bromide (12). The DNA (10 μg) was digested with restriction enzymes *Eco*RI and *Hin*dIII. Restriction fragments were then separated by electrophoresis through 0.8%(w/v) agarose gels before transferring them onto positively charged Hybond-N membranes (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). The hybridization to the labeled probe with [α-^{32}P]dCTP and the random primer were done overnight in a new prehybridization buffer. The filters were washed at high stringency conditions, once at 42°C for 15 min in 2×SSC and 0.05% SDS, once at 65°C for 15 min in 1×SSC and 0.1% SDS, and twice at 65°C for 15 min in 0.1×SSC and 0.1% SDS. The probes labeled with [α-^{32}P]dCTP were not hybridized with any AGPase genes other than corresponding genes under this hybridization condition. Filters were exposed at -70°C to Fuji RX film.

RNA gel blot analysis Total RNA was isolated from three organs (leaves, flowers, and roots) and fruits in four developmental stages (G, W, T, and R). The RNA was fractionated on 1.2%(w/v) denaturing agarose gel. Prehybridization, hybridization, and washing were performed as described in the Genomic DNA gel blot analysis. After the filters (Hybond-N from Amersham Biosciences) were stripped of probe according to the manufacturer's instructions, the same blot was hybridized with a partial cDNA of 18S rRNA labeled with [α-^{32}P]dCTP.

Results and Discussion

Isolation of *AGPase* cDNA clones from strawberry cv. Niyobou Using the PCR-amplified 250 bp (SU2-SL1) cDNA fragment of AGPase small subunit as a probe, we screened a fruit cDNA library under high stringency conditions and isolated 10 positive clones. Sequencing analysis led to the identification of cDNAs encoding AGPase small subunits (*FagpS*). Similar efforts using the 670 bp PCR product cDNA fragment (LU2-LU2) as a probe resulted in the isolation of 16 positive clones from a fruit and leaf cDNA libraries, which led to the identification of two different AGPase large subunit cDNAs (*FagpL1* and *FagpL2*). As cultivated strawberry is a hybrid, that was supposed to have at least two or more for small, and more for large subunits of AGPase. However, we didn't obtain any additional isoforms from strawberry fruit and leaf cDNA libraries. Also, other degenerate primers for isolation of the additional small and large subunits of plant AGPase could not amplify any PCR products in probe preparation.

The sizes of the *FagpS* (GenBank accession No. AY518343), *FagpL1* (AY518344), and *FagpL2* (AY518345) cDNAs are 1872, 2065, and 1257 bp, respectively. Two cDNAs encoding *FagpS* and *FagpL1* contained complete coding sequences, while the *FagpL2* cDNA only had a partial fragment. To obtain the full length *FagpL2* clone, we isolated other positive clones out of 2,000,000 plaques of strawberry fruit and leaf cDNA libraries, respectively. However, we could not isolate any clones having full length clone.

The deduced amino acid sequence of *FagpS* is shown in Fig. 1. The amino acid sequence of the FagpS protein shared 52% homology with that of a large subunit, FagpL1. The amino acid sequences of two large AGPase subunits showed 63% homology with each other (Fig. 1).

AGPase is localized to the plastids and is initially synthesized as large precursor polypeptides containing an N-terminal plastid targeting leader sequence (15). The N-terminal sequence of the mature, small subunit protein from a spinach leaf has been determined by the Edman degradation method to be VSDSQNSQ (7). A similar sequence VSDSKNSE was also found in the primary sequences of FagpS (Fig. 1), indicating that the preceding residues comprise the plastid targeting leader sequence. The putative plastid targeting signal of FagpS was also predicted by the TargetP (16) and PSORT (17) algorithms. In the case of FagpL1, the preceding residues comprise the plastid targeting leader sequence (Fig. 1). The underlined

```
FagpL1      MDSWCVTLK-----------PNTHLRQPTQAGLCCGANGFLGQRIRESFGNRGWVHGSEK
FagpL2      ------------------------------------------------------------
FagpS       MASSSMAANGVPTLRLSTSNIATNQTQKTNRGLSFSGSHLSGTKIPTPATCLRTCSPSPS

FagpL1      TRPGVVSSVVTTKDFETTLKVPTYHRPRVDPKNVASIILGGGAFTQLFPLTRRAATPAVP
FagpL2      ------------------------------------------------------------
FagpS       TRR--APLVVSPKAVSDSKNSETCLDP-DASRSVLGIILGGDGRTRLYPLTKKRAKPAVP

FagpL1      VGGCYRLIDIPMSNCINSNINKIFVLTQFNSTSLNRHLARTYFGNGINFG-DGFVEVLAA
FagpL2      ------------------------------------------------------VEVLAA
FagpS       LGANYRLIDIPVSNCLNSNVSKIYVLTQFNSASLNRHLSRAYASNMGGYKNEGFVEVLAA
                                                                  ******

FagpL1      TQTSGEAGMDWFQGTADAVRQFVWVFEDAKNRNVENILILSGDHLYRMDYMDFVQSHVDS
FagpL2      TQTPGESGKKWFQGTADAVRQFHWLFEDARSKDIEDVLILSGDHLYRMDYMDYIQNHRQS
FagpS       QQSPENP--NWFQGTADAVRQCLWLFEEH---NVLEFLVLAGDHLYRMDYEKFIQAHRET
             *:. :.  .*********** *:**:    :: :.*:*:********* .::* * ::

FagpL1      NADITLSCAVVGDSRASDYGLVKIDSRGKIIQFAEKPRGAGLKAMQSDTTLLGFSPQDAL
FagpL2      GADITISCLPMDDSRASDFGLMKIDKKGKVLSFSEKPKGNDLKAMAVDTTVLGLSVEEAL
FagpS       DADITVAALPMDEKRATAFGLMKIDDEGRIIEFAEKPKGEQLKAMKVDTTILGLDDERAK
            .****::.  :.:.**: :**:***..*:::.*:***:*  ****  ***:**:. : *

FagpL1      KSPYVASMGVYVFKTDILLELLKKSYPNSNDFGSEIIPAAVEE-RNVQAYIFIDYWEDIG
FagpL2      KKPYIASMGVYVFKKEILLNLLRWRFPTANDFGSEIIPASANE-FFMKAYLFNDYWEDIG
FagpS       EMPYIASMGIYVVSKNVMLDLLREKFPGANDFGSEVIPGATSIGLRVQAYLYDGYWEDIG
            : **:****:**...:::*:**:  :* :******:**.:..    ::**:: .******

FagpL1      TIQSFYDANLALTEE-FPKFQFYDPKTPFFTSPRFLPPTKIDNSRVVDAIISHGCFLQEC
FagpL2      TIRSFFEANLALTEH-PNKFSFYDAAKPMYTSRRNLPPSKIDGSKIVDSIISHGSFLTDC
FagpS       TIEAFYNANLGITKKPIPDFSFYDSSSPIYTQPRYLPPSKMLDADITDSVIGEGCVIKNC
            **.:*::***.:*:.   .*.***. .*::*. * ***:*: .: :.*::*..*..: :*

FagpL1      FVQSSIVGERSRLDYGVELKDSIMMGADSYQTESEIAALLARGKVPIGIGRNTKIRLCIV
FagpL2      LIEHSVVGIRSRINTNVHLKDTVMLGADYYETDSEVLSLLAEGRVPVGIGENTKIKDCII
FagpS       KIHHSVIGLRSCISEGAVIEDTLLMGADYYETDVDRRLMAKKGSVPIGIGKNSHIKRAII
             :. *::* ** :. .. ::*::::*** *:*: :   :  .* **:***.*::*: .*:

FagpL1      DLNAKIGKDVIIMNKDGIQEADRPEEGFYIREESLSLWRRE--------
FagpL2      DKNARIGNNVVIANTEGVQEADRSSEGFYIRSGVTVILKNSTIEDGLSI
FagpS       DKNARIGDNVKIIKSDNVQETARETDGYFIKSGIVTVIKDAWIPSGTVI
            * **:**.:* * :.:.:**: *  :*::*:.    : :
```

Fig. 1. Alignment of deduced amino acid sequences of FagpS, FagpL1, and FagpL2. The sequences were aligned using the CLUSTAL X program. An asterisk indicates identical amino acids. Arrowheads indicate the putative proteolytic cleavage site. Underlined sequences are important residues for substrate binding (RAATPAV and KRAKPAV) at the N-terminal region, and for allosteric activator binding at the C-terminal region. A region corresponding to QTCL in dicot AGPase enzymes is boxed.

sequence of the KRAKPAV in FagpS and the RAATPAV in FagpL1 is related to the substrate binding sequence. In addition, the underlined sequence of the C-terminal region has a high homology to the allosteric activator binding site.

Genomic DNA blot analysis Genomic DNA gel blot analyses were performed using strawberry genomic DNA digested with *Eco*RI and *Hin*dIII. As shown in Fig. 2, the blot patterns revealed 2 to 7 bands with major band(s), which indicates that the gene for *FagpS*, *FagpL1*, and *FagpL2* presents in a few copies in the strawberry genome. The different patterns of three cDNAs indicate that the cDNAs did not cross-hybridize with one another at the high stringency condition. *F.* × *ananassa* as a hybrid of two parents, *F. virginiana* and *F. chiloensis*, has a polyploidic genome. Thus weaker cross-hybridizing bands in blots might result from the polyploidic genome of *F.* × *ananassa*.

Comparison of strawberry AGPase with other plant AGPases The phylogenetic relationship among small AGPase subunits is presented in Fig. 3A. They can be

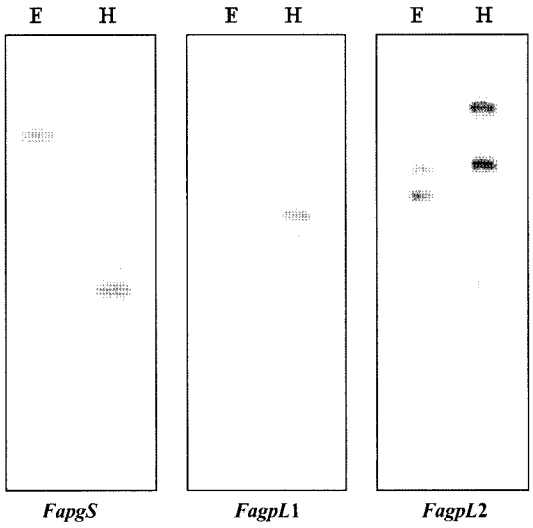

Fig. 2. Genomic Southern blot of strawberry AGPases. The genomic DNA (20 mg/lane) was digested with either *Eco*RI (R) or *Hin*dIII (H), separated on 0.8% agarose gel, transferred to a nylon membrane, and hybridized with radioactively labeled cDNA specific fragments of *FagpS*, *FagpL1*, and *FagpL2*, respectively.

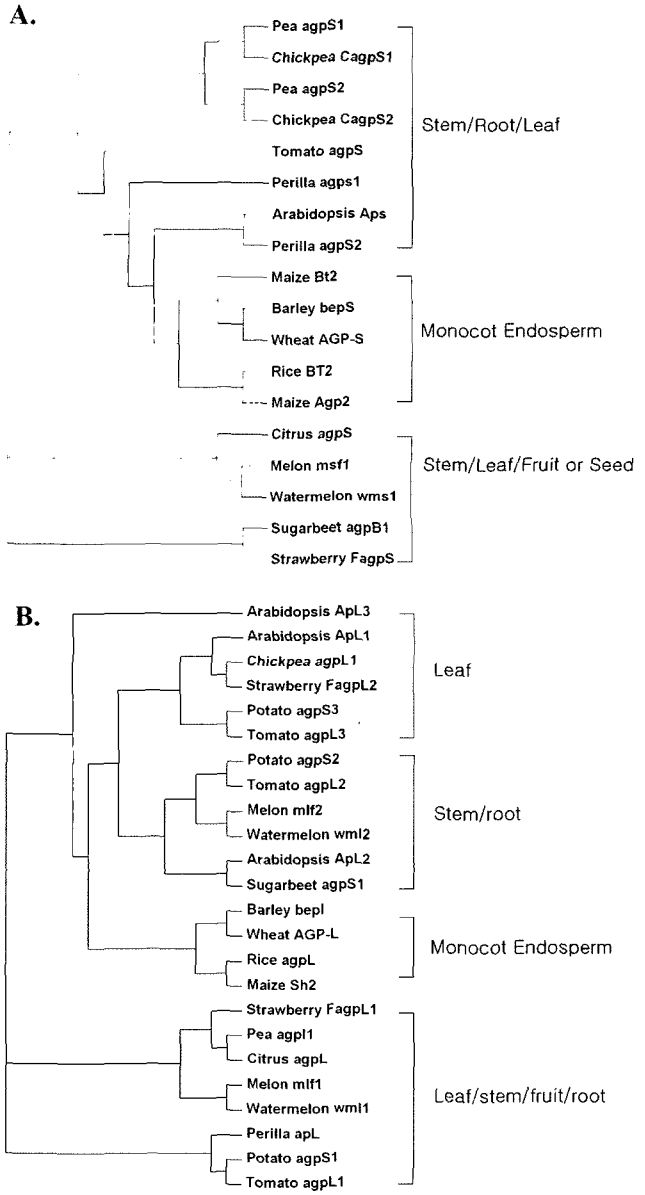

Fig. 3. Dendrogram for small and large AGPase subunit proteins from diverse sources. This tree was generated by the TreeView 1.6.6 and the alignment program (CLUSTAL X version 1.83). A. The amino acid sequences of the small subunits were obtained from *Arabidopsis* (GenBank accession No. X73365), barley (AY634681), chickpea (AF356004 and AF356005), *Citrus* (AF184597), maize (AY032604 and AF334959), melon (AF030382), pea (X96764 and X96765), *Perilla* (AF249915 and AF249916), rice (AY028315), sugar beet (X78899), tomato (L41126), watermelon (AF032471), wheat (X66080), and strawberry (AY518343, this work). B. The large subunits were obtained from *Arabidopsis* (AF117570, X73364, and X73366), barley (X67151), chickpea (AF356002), *Citrus* (AF184598), maize (S48563), melon (AF030383 and AF030384), pea (X96766), *Perilla* (AF249917), rice (AY028314), sugar beet (X78900), potato (X76136, X74982, and X61187), tomato (U88089, U85496, and U85497), watermelon (AF032472 and AF032473), wheat (Z21969), and strawberry (AY518344 and AY518345, this work).

divided into two major groups or branches such as the cereal monocot endosperm and all non-endosperms. Although the primary sequence of strawberry FagpS is closely related to the sugar beet, the FagpS polypeptide contains a glutamate-threonine-cysteine-leucine (ETCL) instead of a glutamine-threonine-cysteine-leucine (QTCL) motif at the amino terminal region. It was known that all small dicot subunits, except for only one case of *Citrus*, contain a conserved amino acid (QTCL) motif at the amino terminal region of the small subunit, which is related to the enzyme activity (18). This is the first report of small subunits containing an ETCL motif among small dicot subunits. Especially, the Cysteine^{12} residue within the QTCL motif plays an important role in heat stability and reductive activation through the formation of an intermolecular disulfide bridge between the two, small subunits. It was reported that the surrounding sequence of Cysteine^{12} affects the heat stability of small, recombinant subunits in maize (18). The replacement of Glutamine to Glutamate in maize AGPase led to a significant decrease in heat stability (18), which suggests that strawberry FagpS is less stable at a high temperature than other dicot enzymes. In the case of other non-climacteric fruits like *Citrus*, the small subunit FagpS lacked a Cysteine^{12} residue. We happened to observe that the unique sequence characteristics of two non-climacteric fruit-producing plants, strawberry and *Citrus*, differ from the QTCL motif of other dicot plants. A comparison of the deduced amino acid sequence suggested the evolutionary link between strawberry FagpL2 and chickpea agpL1. Strawberry FagpL2 showed the highest similarity with chickpea CagpL1 (90%), while FagpL1 has a strong similarity with watermelon wml1 (74%), potato agpS1 (73%), and *Citrus* agpL (71%).

The phylogenetic relatedness among large AGPase subunits is presented in Fig. 3B. The tree shows that large subunit sequences can be categorized into four groups including one group of monocot endosperm and three groups of dicots according to the tissue types expressed. FagpL1 is a member of the leaf/stem/fruit/root group, while FagpL2 belongs to the leaf category. Thus we expected that *FagpL1* ubiquitously expresses in the tissues and *FagpL2* shows a leaf-specific expression pattern.

RNA expression of strawberry AGPases The spatial expression profiles of *FagpS*, *FagpL1*, and *FagpL2* from cv. Niyobou were investigated by an RNA gel blot analysis using the total RNA isolated from three tissues (leaves, flowers, and roots) and fruits in four developmental stages (G, W, T, and R).

FagpS was weakly expressed steady-state levels in all tissues and differentially according to fruit developmental stages (Fig. 4). The transcripts decreased as the fruits matured, slightly increased at the turning stage, and declined a little at the full red stage. Similar expression patterns were observed in the *agpS* of *Citrus* (19), which suggests that the expression patterns have a relationship with the non-climacteric respiration pattern. Interestingly, the profile of *FagpL1* also shows a very similar expression pattern to *FagpS*. The slightly increase of *FagpS* and *FagpL1* in transcription level at the turning stage imply that strawberry fruits may accumulate starch in the later stage. However starch contents decrease as a strawberry fruit ripens (2). A similar pattern was reported by Souleyre *et al.* (13). The starch breakdown could lead to a higher fruit sugar content at maturity. Both levels of starch and AGPase transcript

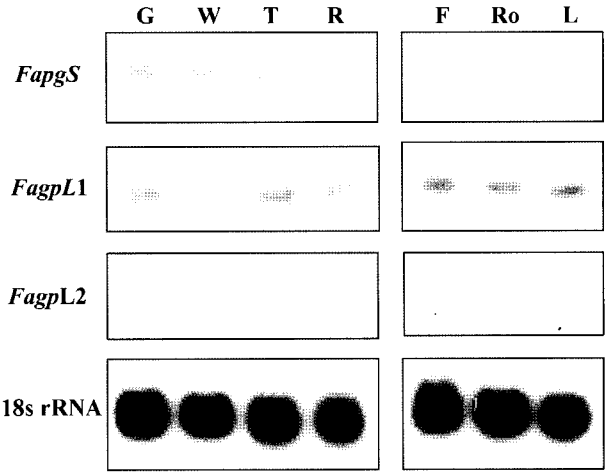

Fig. 4. Northern blot analysis of *FagpS*, *FagpL1*, and *FagpL2* at four stages of fruit development and in various tissues of strawberry cv. Niyobou. The total RNA (20 μg/lane) was separated on denaturing agarose gel, transferred to nylon membrane, and hybridized with ^{32}P-labeled probes. G, green fruits; W, white fruits; T, turning fruits; R, red fruits; F, flowers; Ro, roots; L, leaves.

decreased in the early developmental stages of G and W stages. In the residual stages, both patterns were diverged into different manners. Thus the results suggest that the starch biosynthesis is regulated by AGPase both at the transcriptional level and at the post-transcriptional level during fruit development.

In the case of *FagpL2*, we cannot detect any signal of all tissues in the same condition despite the longer exposure time, except for a very weak signal in leaves, that is, the expression of *FagpL2* showed a leaf-specific pattern. The leaf-specific pattern expressed by *FagpL2* was consistent with *AgpL3* in tomato (10). The presence of large, multiple subunit genes, which display significant sequence diversity, suggest that the two large subunits have different allosteric regulatory and catalytic properties. When considering the expression patterns of the large isoforms of strawberry, FagpS may mainly form a heterotetramic structure with FagpL1 during starch synthesis in developing fruits and other tissues.

The studies indicate that strawberry FagpS has a unique amino acid characteristic which is different from other dicot enzymes, and that the two non-climacteric fruits, strawberry and *Citrus*, have similar expression patterns. The results suggest that AGPase might have evolutionary relatedness among non-climacteric fruit-producing plants, and that the expression has some connection with the respiration pattern.

The result suggest that AGPase gene might be a target modulating the starch and sugar contents at the early developmental stages in strawberry fruits by metabolic engineering, which might lead to a higher fruit quality as a food. Recent work showed that fruit-specific down-regulation of the AGPase gene results in the increase in sugar and the decrease in starch contents in strawberry (20). Similar phenomena were observed in transgenic *Vicia narbonensis* (21), oilseed rape (22), and potato (5) plants as well as the changes of protein species and content. Furthermore the sugar content decreased slightly in strawberry during stored at cold condition (23). The increased sugar content induced higher water content in seeds, and therefore increased seed maturation duration and yield. Thus the increase of water contents at the harvesting stage in fleshy fruits such as strawberry might lead to the increased storage duration by retardation of dehydration. The studies described above indicate that AGPase has an effect on the fruit quality by regulating starch and soluble sugar contents.

Acknowledgements

We are deeply grateful to Professor Won-Il Chung at Korea Advanced Institute of Science and Technology for generously discussion and providing plant samples.

References

1. Choi ID, Son JR, Hong HC, Lee JH, Kim KJ. Indigestible carbohydrate contents and physical properties of Goami2 harvested at the maximized milling quality. Food Sci. Biotechnol. 15: 254-259 (2006)
2. Manning K. Soft fruit. pp. 347-376. In: Biochemistry of Fruit Ripening. Seymour GB, Taylor JE, Tucker GA (eds). Chapman and Hall, New York, NY, USA (1993)
3. Kim NH, Yoo SH. Molecular structure and gelatinization properties of turnip starch (*Brassica rapa* L.). Food Sci. Biotechnol. 14: 470-473 (2005)
4. Lin TP, Caspar T, Somerville C, Preiss J. Isolation and characterization of a starchless mutant of *Arabidopsis thaliana* (L.) Heynh lacking ADP glucose pyrophosphorylase activity. Plant Physiol. 86: 1131-1135 (1988)
5. Müller-Röber B, Sonnewald U, Willmitzer L. Antisense inhibition of the ADP-glucose pyrophosphorylase in transgenic potatoes leads to sugar-storing tubers and influences tuber formation and expression of tuber storage proteins. EMBO J. 11: 1229-1238 (1992)
6. Neuhaus HE, Kruckeberg AL, Feil R, Gottlieb L, Stitt M. Dosage mutants of phosphoglucose isomerase in the cytosol and chloroplasts of *Clarkia xantiana*: II. Study of the mechanisms which regulate photosynthate partitioning. Planta 178: 110-122 (1989)
7. Morell MK, Bloom M, Knowles V, Preiss J. Subunit structure of spinach leaf ADP-glucose pyrophosphorylase. Plant Physiol. 85: 182-187 (1987)
8. Okita TW, Nakata PA, Anderson JM, Sowokinos J, Morell M, Preiss J. The subunit structure of potato tuber ADP-glucose pyrophosphorylase. Plant Physiol. 93: 785-790 (1990)
9. Cross JM, Clancy M, Shaw JR, Greene TW, Schmidt RR, Okita TW, Hannah C. Both subunits of ADP-glucose pyrophosphorylase are regulatory. Plant Physiol. 2135: 137-144 (2004)
10. Park SW, Chung WI. Molecular cloning and organ-specific expression of three isoforms of tomato ADP-glucose pyrophosphorylase gene. Gene 206: 215-221 (1998)
11. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25: 4876-4882 (1997)
12. Clark MS. Plant Molecular Biology: A Laboratory Manual. Springer-Verlag, Berlin Heidelberg, New York, NY, USA. pp. 5-8 (1997)
13. Souleyre EJF, Iannetta PPM, Ross HA, Hancock RD, Shepherd LVT, Viola R, Taylor MA, Davies HV. Starch metabolism in developing strawberry (*Fragaria×ananassa*) fruits. Physiol. Plant. 121: 369-376 (2004)
14. Dinar M, Stevens MA. The relationship between starch accumulation and soluble solids content of tomato fruits. J. Am. Soc. Hortic. Sci. 106: 415-418 (1981)
15. Choi SB, Kim KH, Kavakli IH, Lee SK, Okita TW. Transcriptional expression characteristics and subcellular localization of ADP-glucose pyrophosphorylase in the oil plant *Perilla frutescens*. Plant Cell Physiol. 42: 146-153 (2001)

16. Emanuelsson O, Nielsen H, von Heijne G. ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites. Protein Sci. 8: 978-984 (1999)
17. Nakai K, Horton P. PSORT: a program for detecting sorting signals in proteins and predicting their subcellular localization. Trends Biochem. Sci. 24: 34-36 (1999)
18. Linebarger CRL, Boehlein SK, Sewell AK, Shaw J, Hannah LC. Heat stability of maize endosperm ADP-glucose pyrophosphorylase is enhanced by insertion of a cysteine in the N terminus of the small subunit. Plant Physiol. 139: 1625-1634 (2005)
19. Kim IJ, Noh SJ, Lee BH, Jo JK, Kim YS, Chung WI. Molecular characterization of cDNA clones for ADP-glucose pyrophosphorylase from *Citrus*. Biochim. Biophys. Acta 1518: 324-328 (2001)
20. Park JI, Lee YK, Chung WI, Lee IH, Choi JH, Lee WM, Ezura H, Lee SP, Kim IJ. Modification of sugar composition in strawberry fruit by antisense suppression of an ADP-glucose pyrophosphorylase. Mol. Breeding 17: 269-279 (2006)
21. Weber H, Rolletschek H, Heim U, Golombek S, Gubatz S, Wobus U. Antisense-inhibition of ADP-glucose pyrophosphorylase in developing seeds of *Vicia narbonensis* moderately decreases starch but increases protein content and affects seed maturation. Plant J. 24: 33-43 (2000)
22. Vigeolas H, Möhlmann T, Martini N, Neuhaus E, Geigenberger P. Embryo-specific reduction of ADP-Glc pyrophosphorylase leads to an inhibition of starch synthesis and a delay in oil accumulation in developing seeds of oilseed rape. Plant Physiol. 136: 2676-2686 (2004)
23. Mo EK, Sung CK. Effect of *Pichia anomala* SKM-T and *Galactomyces geotrichum* SJM-59 dipping on storage property and sensory quality of strawberry. Food Sci. Biotechnol. 14: 487-492 (2005)