Structure and Expression of the Potato ADP-glucose Pyrophosphorylase Small Subunit*

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ADP-glucose pyrophosphorylase (EC 2.7.7.27; AGP)1 plays a pivotal role in the biosynthesis of α,1,4-glucans in both bacteria (1) and plants (2–4). AGP mediates the synthesis of the activated glucosyl donor, ADP-Glc, from Glc-1-P and ATP which is subsequently utilized by the α-glucan synthase enzyme present in these organisms. Bacterial AGPs are encoded by a single gene locus which gives rise to a homotetrameric enzyme present with native and subunit molecular masses of 200 and 48 kDa, respectively (1). The AGPs from higher plants, however, exhibit a more complex heterotetrameric structure and are composed of two subunit types, each of which is encoded by a different gene (5–9). The catalytic activity of both the plant and bacterial AGPs are allosterically regulated by small effector molecules whose levels oscillate during normal carbon metabolism in these organisms (10). In Escherichia coli, AGP is activated by fructose-1,6-bisphosphate and inhibited by AMP and ADP, whereas the plant AGP is stimulated by 3-PGA and inhibited by P1 (10).

The functional significance of these effector molecules in modulating the AGP activity in vivo, and hence partitioning of carbon into leaf starch has been established using both intact leaves and isolated chloroplasts (11). During diurnal plant growth, leaf 3-PGA and P1 levels increase and decrease, respectively, in response to photosynthesis (11). Increases in the 3-PGA/P1 ratio coincide with active starch synthesis during the day (periods of illumination) whereas decreases in the 3-PGA/P1 ratio correlates with suppression of starch synthesis and sucrose mobilization during the night (periods of darkness). Analysis of carbon partitioning in mutants defective in carbon metabolism also supports the central role of the allosteric regulation of AGP in controlling starch synthesis in leaves (12).

Recent studies have revealed that the control of starch metabolism in non-photosynthetic storage tissues, e.g. seeds and tubers, is not identical to control in leaves. Unlike the diurnal fluctuations of starch metabolism observed in leaves, deposition and mobilization of starch in non-photosynthetic storage organs occur at different stages of plant development. In addition to these ontogenic differences, the pathways of carbon metabolism in the plastids of photosynthetic leaf tissues (chloroplast) and non-photosynthetic storage organs (amyloplast) are dissimilar. Chloroplasts can generate energy and reduced carbon compounds while amyloplasts, which may lack intact glycolytic and gluconeogenic pathways (13, 14), are totally dependent on the cytoplasm for these requirements. Therefore, 3-PGA and P1 levels are not expected to oscillate inversely within the amyloplasts in response to a diurnal cycle as evident in leaves. Moreover, amyloplasts display distinct metabolite permeability properties. In chloroplasts, the principle metabolite transport system is the triose-P/P translocator which exchanges triose phosphates (e.g. 3-PGA) produced within the chloroplast with P1 from the cytosolic pools. 13C-Labeling and direct metabolite uptake studies (15, 16) indicate that the main metabolite transport system in amyloplasts is not the triose-P/P translocator but one involving Glc-1-P (and/or Glc-6-P). Import Glc-1-P can be directly utilized as substrate by AGP. The distinct metabolic partitioning to specific stages of plant development, lack of 3-PGA/P1 oscillation, and direct transport of hexose-Ps displayed by the heterotrophic amyloplast raise the question of whether allosteric regulation of AGP operates within these storage organs. Consistent with this doubt of allosteric activation of AGP activity in storage organs, many plants contain a seed-specific form of AGP distinct from the leaf AGP. In wheat (17), barley (18), and pea (19), the seed-specific
forms show little or no activation by PS inhibition in vitro. Such tissue-specific enzyme forms may be a reflection of the distinct biochemical of starch deposition displayed by the plastids from the photosynthetic and non-photosynthetic tissues. Moreover, it has been suggested that seed-specific AGPs may be localized in the cytoplasm, unlike the plastid-localized enzyme of leaf tissue (20).

Unlike the extensive enzymological and structural data available about AGP, little information exists on the structure of the AGP genes or their regulation during development. We report here the isolation and characterization of the potato tuber sAGP gene. Our evidence indicates the same sAGP gene is expressed in both photosynthetic and non-photosynthetic tissues unlike the other plants examined to date. Spatial and temporal expression patterns of the potato sAGP suggest, however, that the sAGP gene is controlled differently in the different tissues. While the gene appears to be primarily under transcriptional control in tubers, in leaves a post-transcriptional process(s) plays a prominent role. The observed tissue-specific regulation of the sAGP gene in leaves and tubers, combined with differences in starch deposition within these tissues and may obviate the need for tissue-specific sAGP genes as expressed in other plants.

MATERIALS AND METHODS

Plant Material—Russet Burbank potato (Solanum tuberosum) plants were sprouted from tubers and grown in the greenhouse under a light/dark regime of 16 h of light and 8 h of dark. In the temporal study, tubers were harvested at 15 plants, divided into groups based on size, and stored at -80 °C until processed for RNA and antigen isolations. Two plants were harvested after a light cycle and utilized for RNA, antigen, and starch analysis in the spatial expression experiments.

Construction and Screening of the AEMBL3 Potato Genomic Library—Plant DNA was isolated from the potato leaf (22), partially digested with MboI, and size fractionated through a 1% (w/v) agarose gel to obtain insert fragments of 10-20 kb. The size-selected fragments were ligated into the BamH1 site of the AEMBL3 vector, packaged in vitro into lambda phage (22), and transferred to nitrocellulose (22). Polyclonal antibodies raised against the purified potato tuber sAGP was used for immunodetection. The cross-reactive antigen was visualized by 35S-protein A (New England Nuclear) or chemiluminescence (Amersham ECL Western blot detection system). No cross-reactivity was observed between this antisera and the potato tuber sAGP gene.

Starch Measurement—Different plant tissues were harvested, pulsed (liquid N2), lyophilized, and extracted three times in 80% (w/v) ethanol at 70 °C to remove soluble sugars. The starch pellet was recovered and quantitated using a commercial kit (Boehringer, Mannheim, FRG) according to manufacturer's instructions.

Construction of a Chimeric sAGP-GUS Gene—A 3.5-kb fragment of the sAGP genomic clone, extending from a StuI site at approximately -3500 to a HindIII site at position +51 relative to the transcriptional start site, was ligated into the β-glucuronidase (β-GUS) reporter construct pBI101.2 (Clontech) at the SalI-SmaI polylinker sites. The resulting construct was mobilized into Agrobacterium tumefaciens strain LBA4404 for use in transformation procedures (23).

Plant Transformation—Binary vectors were used to transform S. tuberosum L. FL1607 via A. tumefaciens-mediated transformation based on the procedure of Guilfoyle, et al. (22). A transgenic strain of LBA4404 was grown to late log phase at 28 °C in YEP pH 7.2 (10 g/liter yeast extract, 10 g/liter peptone, 5 g/liter NaCl) supplemented with 50 μM streptomycin sulfate, 50 μg/ml kanamycin sulfate, and 50 μM acetosyringone. Sterile explants were subcultured and co-cultivated with the bacterium. The infected leaf strips were then rinsed and plated on stage 1 media containing 200 μg/ml carbenicillin, 200 μg/ml chloramphenicol, and 50 μg/ml kanamycin sulfate. Regenerated shoots, produced utilizing the same medium minus auxin, were excised and rooted on Murashige-Skoog basal salts, pH 5.8, supplemented with 0.4 mM thiamine, 0.5 μM nicotinic acid, 0.5 μM pyridoxine, 100 μg/ml myo-inositol, 2% (w/v) sucrose, 25 μg/liter indole-3-acetic acid, 50 μg/ml kanamycin sulfate, and 4 μl/liter phytogar (Life Technologies, Inc.). Fully rooted explants were transplanted to soil and grown in the greenhouse to maturity under a light dark regime of 16 h light/8 h dark.

Fluorometric β-Glucuronidase Assay—Leaf (random sample) and tuber (25 g) tissues were homogenized in 50 mM sodium phosphate, pH 7.0, 10 mM β-mercaptoethanol, 10 mM NaEDTA, 0.1% sodium lauryl sarcosine, 0.1% Triton X-100, 0.2 mM phenylmethylsulfonyl fluoride, and polyvinylpyrrolidone (29) using a Polytron, filtered through Miracloth, and centrifuged for 10 min at 13,000 × g at 4 °C. The supernatants were stored at -80 °C until assayed. β-GUS activity was measured using fluorometric assay (30) using 4-methylumbelliferyl-β-D-glucuronomide (4-MUG) as substrate and a Hoefer Scientific Fluorometer model TKO-100.
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RESULTS

Isolation and Nucleotide Sequence Analysis of the Potato sAGP Gene—Using a potato tuber sAGP cDNA fragment as a probe (23, 24), several genomic clones were isolated from the prepared potato λEMBL3 genomic library. Five of the isolated genomic clones were characterized by restriction map and Southern blot analyses. Two overlapping clones were identified that encompassed the entire sAGP gene as well as several kilobases of the 5'-upstream and 3'-downstream regions (Fig. 1). A 1.5-kb SmaI-HindIII fragment containing the 5'-untranslated region of the gene was subcloned into pBluescript and sequenced using exonuclease III-generated templates. The structural segments of the gene and 3'-untranslated regions contained useful restriction sites which allowed the generation of a number of overlapping fragments and completion of the potato sAGP sequence (Fig. 2).

By homologous alignment of the potato tuber sAGP cDNA (23, 24) and genomic nucleotide sequences, exon and intron positions were determined. The structure of the sAGP gene is complex consisting of nine exons ranging in size from approximately 100-400 base pairs and eight introns ranging in size from about 90-900 base pairs in length (Fig. 1). All introns contain a high percentage of A + T (average = 67.1%), which is characteristic for higher plant genes and has been suggested to play a key role in plant intron splicing (31). In contrast, exons had a lower A + T percentage (average = 56.8%). The exon-intron splice site regions were analyzed (Table I) and conformed to published GT-AG border element consensus sequences (32). The first exon contained the putative ATG initiation codon which was located within the TAACAATGCGG sequence and is consistent with the plant consensus translational start site motifs (33).

Examination of the 5'-flanking sequences revealed several conserved transcriptional motifs. Two potential TATA-like elements are located at -26 and -53 bp upstream of the putative transcriptional start site as well as two possible CAAT box sequences at positions -65 and -99 (Fig. 2). In addition, several other elements similar to those described in other potato tuber genes were revealed. Sequences resembling the gSpo-A1 and Box3, elements (34), putative transcriptional regulatory motifs of a sweet potato sporamin, are found at -108 (GTGTACC) and -566 (CAATACTCA). Two stretches of sequence at -578 (TAAATTCATTTTCTCA) and -64 (AACAAATCAAGTCT) possess similarities to elements found within the patatin promoter (35). This sAGP promoter also contains several repeated sequences, e.g. ACAAA at -70, -114, and -184, as well as a 26-bp element located at -136 and -170. Functional analysis is necessary to verify the identity of these elements, and such studies are under investigation.

Primer extension analysis was performed to map the site of transcription initiation. An end-labeled 30-base oligonucleotide complimentary to positions +57 to +86 (Fig. 2) was used to prime a reverse transcriptase reaction using a poly(A)+ mRNA template isolated from tuber tissue. A single 86 nucleotide extension product was obtained mapping the transcription initiation site to a "T" located 26 bp downstream from a putative TATA element (Fig. 3). The putative transcription initiation site suggested a 1.9-kb transcript size which is consistent with that estimated from Northern analysis (see Fig. 6A). An untranslated leader sequence of 64 bases is predicted from this sequence information and is in agreement with other plant leader sequences which are generally between 40-80 nucleotides in length (33).

Potato sAGP Is a Low Copy Gene—Genomic Southern blot analyses were conducted under high and moderate stringency. In both cases, the same hybridization pattern was obtained (Fig. 4). The number and size of the hybridizing fragments were consistent with the restriction map and nucleotide sequence data. Estimations from specific hybridization signals indicated that the potato AGP is present in one to two copies/haploid genome (Fig. 4). The low copy number and lack of polymorphic fragments in potato indicated that the same sAGP gene may be expressed in leaves and other non-tuber tissues. A similar view was also suggested by the spatial distribution of sAGP transcript as determined by Northern analysis using total RNA isolated from...
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FIG. 2. Nucleotide and deduced amino acid sequence of the potato sAGP gene. Uppercase letters indicate exon sequences and lowercase letters represent introns, 5'-untranslated, and 3'-untranslated regions. Underlined sequences denote potential regulatory regions described in the text. The putative transcriptional start is in bold below a (> ) superscript.
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FIG. 2—continued
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Fig. 2-continued
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Expression of a Chimeric sAGP Promoter-β-Glucuronidase Gene in Transgenic Potato Plants—To directly confirm the expression of the same sAGP gene in leaf and tuber, as implied by the Northern blot, primer extension, cDNA, and genomic Southern blot analyses, transgenic potato plants were generated containing 3500 bp of the 5' untranslated region of the potato sAGP gene fused to a β-GUS reporter gene. Five independent transformants were analyzed for β-GUS expression in both leaf and tuber tissue. Negative controls were generated by transforming promoterless β-GUS constructs. Measurable β-GUS activity was detected only in the plants transformed with the sAGP promoter-β-GUS fusion. High levels of activity were detected in tuber tissue, averaging 50.0 nmol 4-MU/mg protein (Fig. 5). Leaf expression, although orders of magnitude lower, still had significant levels of β-GUS expression averaging 0.73 nmol 4-MU/mg protein (Fig. 5). Thus, the transgenic spatial study provides direct support for the

the different tissues (see Fig. 6A). A 1.5-kb XbaI potato tuber cDNA fragment (23, 24) was used to detect an identical sized 1.9-kb transcript present at different abundances throughout the plant. Collectively, the Southern and Northern blot analyses support the view that the same sAGP gene is expressed in multiple tissues. This view contrasts considerably with the condition in maize (36), barley (37), wheat (17), and rice (38) in which tissue-specific forms of the sAGP are observed, which are easily distinguishable by both transcript size and sequence.

Analysis of the Potato sAGP from Leaf—To further investigate whether the tuber gene was also expressed in leaf tissue, primer extension analysis was performed using the same end-labeled 30-base oligonucleotide that was employed to identify the transcriptional initiation site of the tuber gene. A single 86 nucleotide reaction product identical to that obtained in the tuber study was detected suggesting that the same transcript was present in both tissues (Fig. 3).

In addition, a Desiree' potato leaf cDNA library was screened using a Russet Burbank potato tuber sAGP cDNA probe (23, 24) under conditions similar to those previously described in the isolation of tissue-specific forms of the AGP enzyme from wheat (17). Approximately 1.8 x 10^6 recombinant phage were screened yielding 40 putative clones which hybridized to the tuber cDNA. Of the 40 potential clones, five were purified and characterized. Inserts ranged in size from 1.0–1.9 kb. One clone which contained the full-length cDNA was entirely sequenced. Sequence comparisons of the sAGP Desiree' leaf clone to both the Desiree' (39) and Russet Burbank tuber cDNAs (23, 24) revealed only two nucleotide differences suggesting that the observed divergence between the leaf and tuber sequences is most likely attributable to allelic and/or varietal differences.
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Table I

| Intron | 5'-Splice site | Length (bp) | 3'-Splice site | A + T % |
|--------|----------------|-------------|----------------|---------|
| 1      | AGCCGC         | 679         | TTAAAACAATGTAG | 66.7    |
| 2      | TTCCAG         | 284         | ATGCTGTGTCGTAG | 64.1    |
| 3      | AGCAGA         | 94          | TTGAATGATTTCACAG | 70.2    |
| 4      | ATGAGA         | 399         | AACTGCTGTTTCCAG | 66.2    |
| 5      | TTTTACAG       | 106         | AATATTACTACAG   | 64.2    |
| 6      | ATCAAG         | 890         | ATTTCTGTGTCGAC | 70.0    |
| 7      | TATAGAG        | 228         | CATTTTGTGTGAC   | 67.5    |
| 8      | GTGAGA         | 195         | TTTTCTTTTTGAC   | 67.7    |

FIG. 3. Primer extension analysis of the potato sAGP. A 30-base complimentary oligonucleotide was used to prime a reverse transcriptase reaction using poly(A)* mRNA (5 µg) isolated from either tuber (left) or leaf (right) tissue. The primer extension product (p) was analyzed together with a sequencing ladder (G, A, T, and C) obtained from the Smal-EcoRI genomic template using the same primer. A primer extension reaction was conducted in the presence of rice poly(A)* mRNA (r) was used as a control.

expression of the same sAGP gene in both photosynthetic and non-photosynthetic tissues.

Spatial and Temporal Expression of the Potato sAGP—To assess the overall expression patterns of the sAGP gene during plant development, the steady state mRNA and antigen levels were analyzed in the different tissues. Northern blot analysis revealed the highest level of the sAGP transcript in tubers followed by leaves, stolons, stems, and to a small degree roots. In contrast, sAGP antigen levels exhibited a different spatial expression pattern than the sAGP mRNA determined by Northern analysis (Fig. 6). An obvious discrepancy between mRNA and antigen levels can be observed in leaves (Fig. 6, A and B). Although transcript levels in leaves were similar to those observed in tubers, relative levels of antigen were significantly lower. To show that the observed difference between leaf transcript and antigen abundance was not due to the co-migrating large subunit of ribulose biphosphosphate carboxylase/oxygenase interfering with the antibody-antigen recognition, mixing experiments using tuber and leaf protein extracts were conducted (data not shown). Irrespective of the relative proportions of the leaf and tuber extracts used, the levels of antigens observed were simply a sum of the levels present in the two extracts. Starch levels also correlated with the antigen accumulation profile (Fig. 6C). The sAGP mRNA accumulation in potato tubers increased as the tuber developed (Fig. 7A). The sAGP antigen accumulation closely followed the mRNA accumulation profile (Fig. 7B).

DISCUSSION

To identify the regulatory elements that control the expression of the potato tuber sAGP, we have isolated the gene, determined its sequence, and examined its spatial and temporal expression during plant development. Unlike most plant genes, the isolated potato sAGP gene has a highly complex structure extending over 5.5 kb and contains eight introns that vary in length between 90–900 bp. The complexity of the potato sAGP gene is also found in two other genes that encode enzymes involved in starch metabolism. These genes, one for sucrose synthase (40) and the other for the granule-bound starch synthase (41), possess 15 and 13 introns, respectively. The presence of a large first intron and multiple introns contained within the sAGP gene suggests the possibility that they may play a role in regulating expression as observed for the first intron of the sucrose synthase (42) and alcohol dehydrogenase genes (43). Studies are in progress to determine whether the inclusion of introns and/or the 3′untranslated region can account for the observed low levels of β-GUS expression in leaves.

Analysis of the promoter region combined with primer extension analysis revealed a typical promoter structure that includes a TATA box located 26 bp upstream from the putative transcription initiation site. Several potential regulatory sequences were identified in the vicinity of the promoter region. Two sequences resembled an element shown to be important in
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The expression of patatin, a potato tuber storage protein (35). One of these elements (TAAACAATTCATTTTCTCA), located at -578, matched 14 of 19 bp of a motif which has been suggested to play a role in conferring tuber specificity and sucrose inducibility of the patatin promoter (35). Our laboratory (44) and others (39) have shown that the transcript levels of both AGP genes are increased by elevated sucrose levels. A sequence at -108 (GTGTACC) has a high degree of identity to the SP8BF motif which has been localized in both the sporamin and ß-amylase promoters of sweet potato (45). Sporamin and ß-amylase, major proteins of the tuberous roots are sucrose inducible and coordinately expressed (35, 45). Both sporamin and patatin accumulate during the period of starch deposition. Thus, the possibility exists that sucrose may play a role in coordinating the expression of these storage organ genes.

Studies with barley (37), wheat (17), and rice (38) have shown that each of these plants contain tissue-specific forms of each subunit based on tissue type, photosynthetic versus non-photosynthetic. The expression of each isozyme is easily distinguishable by both transcript size and sequence. Multiple forms of AGP have also recently been shown in pea (19) and Arabidopsis (46). In contrast, we observed similar size transcript and antigen signals in spatial Northern and Western profiles of the potato plant, indicating that the same sAGP gene may be expressed in different tissues. This view was supported by the identical primer extension products synthesized by reverse transcriptase from tuber and leaf poly(A)+ RNA (Fig. 3), the near identical sequences of the leaf and tuber sAGP cDNAs, and the expression of a ß-GUS reporter gene under the control of a single sAGP promoter in both tissues. Taken together, the results indicate that the same sAGP gene is expressed in different tissues.

The relative expression of the chimeric gene, composed of the potato sAGP promoter fused to ß-GUS, in various tissues was consistent with steady state mRNA levels observed in different tissues (data not shown) with the exception of leaf tissue (Fig. 5).

FIG. 5. Expression of a chimeric sAGP promoter-ß-GUS gene in leaf and tuber. ß-GUS activity was measured in leaf and tuber extracts of five independent transgenic potato (filled circle) plants harboring a chimeric gene consisting of 3.5 kb of the sAGP 5'-flanking region fused to the ß-GUS reporter gene of pBI101. Control plants, transformed with promoterless ß-GUS constructs (pBI101), were similarly assayed, but showed no detectable reporter gene activity.

FIG. 6. Spatial analysis of the sAGP and its correlation to starch accumulation. A, an equal amount (50 µg) of total RNA from each tissue was analyzed by Northern analysis. The 1.9-kb sAGP transcript was visualized using a 32P random primed potato tuber cDNA insert and the relative mRNA levels determined by densitometry. B, relative sAGP antigen accumulation profile during tuber development. 30 µg of soluble tuber protein, isolated from the different size tubers, was resolved on a 10% (w/v) SDS-polyacrylamide gel and transferred to nitrocellulose. The sAGP antigen was visualized using IgG prepared against the purified potato tuber sAGP and an Amersham Western blot detection system. Relative antigen levels were determined by densitometry.

FIG. 7. Temporal accumulation profile of the sAGP during tuber development. A, relative sAGP transcript accumulation pattern during tuber development. 50 µg of total RNA, isolated from different sized tubers, were analyzed by Northern blot. The 1.9-kb sAGP transcript was visualized using a 32P random primed potato tuber cDNA insert and the relative mRNA levels determined by densitometry. B, relative sAGP antigen accumulation profile during tuber development. 30 µg of soluble tuber protein, isolated from the different size tubers, was resolved on a 10% (w/v) SDS-polyacrylamide gel and transferred to nitrocellulose. The sAGP antigen was visualized using IgG prepared against the purified potato tuber sAGP and an Amersham Western blot detection system. Relative antigen levels were determined by densitometry.
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5). The relative level of β-GUS activity in leaves was much lower than anticipated based on the level of sAGP steady state mRNA which was comparable to the level of transcript in tuber tissue (Fig. 6). The low reporter gene activity in leaves may indicate the absence of an essential cis-element required for expression at the transcriptional or post-transcriptional level or the presence of a second cell type-specific form of the sAGP.

The latter view is supported by restriction length polymorphism mapping experiments which indicate the presence of two sAGP loci in the tetraploid potato genome (47).

Temporal analysis of sAGP expression in potato tubers revealed coordinately increasing transcript and antigen levels during tuber development (Fig. 7). The results are consistent with the view that sAGP expression is regulated primarily at the level of transcription in the expanding tuber. Similar transcript accumulation profiles during seed development have also been observed in rice (48), wheat (49), and maize (50). In rice, the sAGP transcript levels (48) paralleled starch deposition rates (51) in which both were at a maximum during the early phase of seed development (7–9 DAF). Likewise, in wheat, coincident increases and decreases in α-AGP mRNA correlated with the enzyme levels (52) and directly followed the rate of starch synthesis (53). Thus, in non-photosynthetic storage organs AGP role in starch deposition is controlled primarily at the transcriptional level.

In contrast to the correlation observed in developing tubers, a large discrepancy between the relative abundances of the sAGP transcript and antigen levels is readily evident in leaves (Fig. 6). Although sAGP transcripts are almost as abundant in leaves as in tubers, the corresponding leaf sAGP antigen levels are much lower. This difference between leaf transcript and antigen levels indicates that sAGP expression is regulated at least in part at the level of protein synthesis and/or protein stability. Although further experiments are required to distinguish between these possibilities, the observed difference in the primary mode of regulating sAGP activity in leaves and tubers is consistent with the type and function of starch deposition which occurs within these tissues. In leaves, starch is synthesized during the photosynthetic periods when fixed carbon is in excess and degraded during the night when fixed carbon is limited. The principal controlling step between net synthesis and degradation is mediated by AGP via its allosteric behavior (11).

Therefore, the most important regulatory process in controlling starch synthesis in leaves is allosteric regulation of AGP activity, whereas processes like transcription and translation have lesser roles. In contrast to the transient diurnal response displayed by tubers, carbon metabolism in developing tubers is directed almost exclusively to starch biosynthesis since it functions as a storage organ. Consistent with this view are the high synthetic rates of starch synthesis observed in this storage organ (54), the limited role for allosteric activation of the tuber enzyme (55), and the prominent role of transcriptional regulation of AGP. Therefore, the differential regulation of the sAGP gene in leaves and tubers eliminates the need for tissue-specific isoforms as observed in all other plants examined to date.

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