

**Communication**

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

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**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: Volland *et al.*, 1992a, 1992b, Elimert *et al.*, 1997; *Arabidopsis* LS: Volland *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 derivatives. 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.

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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 ( $1 \times 10^6$  dpm/ml) heterologous probe from potato AGPase large subunit cDNA (*sAGPLI*, courtesy of Dr. Bernd Müller-Röber). Approximately  $3.0 \times 10^5$  recombinant phages were screened. Plaque filters were hybridized in a solution containing  $6\times$  SSC,  $5\times$  Denhart's solution, 0.1% SDS and 50  $\mu$ g/ml calf thymus DNA for 16 h at 60°C and washed with  $1\times$  SSC-0.1% SDS several times. The filters were exposed to X-ray film at  $-70^\circ\text{C}$  prior to autoradiography. Two positive clones (*iAGPLI-a* and *iAGPLI-b*) were subcloned into the *KpnI* 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  $\mu$ g of total RNA was loaded on 1% agarose gel containing  $1\times$  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 ( $10\times$  SSC). The membranes were hybridized by  $32^P$ -labeled ( $1 \times 10^6$  dpm/ml) probes of total cDNA fragments generated by *KpnI* restriction digestion of *iAGPLI-a* and *iAGPLI-b*. Hybridization was performed in a solution containing  $5\times$  Denhardt's solution, 0.1% SDS, 100 mg/ml salmon sperm DNA, and  $5\times$  SSC for 20 h at 60°C. After hybridization, the blots were washed three times in  $1\times$  SSC-0.1% SDS at 60°C prior to exposure to X-ray film at  $-70^\circ\text{C}$ .

**Genomic DNA blot analysis** Thirty  $\mu$ g of genomic DNA isolated from sweet potato tuber by CsCl gradient ultracentrifugation (Sambrook *et al.*, 1989) was digested with *XbaI* and *EcoRI*. 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  $10\times$  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'-CGATTTCGTGGGATGTTAT-3' (sense) and 5'-ACCATAGC-CAATGGCCA-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

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1  CT TTG ACT GTA GAA GCA CCG ATA TTG GAG AGG CGT CGG GCA AAC 44
2  L T V E A P I L E R R R A N 14
3  CCT AAG AAT GTG GCT GCA ATC ATA CTG GGA GGG GGT GCA GGG ACA 89
4  P K N V A A I I L G G G A G T 29
5  CAA CTA TTC CCT CTC ACC AAC CGA GCT GCA ACC CCT GCT GTT CCA 134
6  Q L F P L T N R A A T P A V P 44
7  CTT GGA GGA TGC TAT AGG TTG ATA GAC ATT CCG ATG AGC AAC TGC 179
8  L G G C Y R L I D I P M S N C 59
9  ATC AAC AGC GGG GTT AAC AAG ATC TTT GTG CTG ACC CAG TTC AAT 224
10 T N S G V N K I F V L T Q F N 74
11 TCT GCT TCT CTT AAC CGT CAC ATT TCC CGT ACC TAC TTT GGC AAT 269
12 S A S R T Y 89
13 GGT GTG AGC TTC GGA GAT GGA TTT GTT GAG GTG CTG GCT GCA ACT 314
14 G V S F G D G F V E V L A A T 104
15 CAA CAA CAA GGG GAA ACG GGG ATG AAG TGG TTT CAG GGA ACT GCA 359
16 Q T Q G E T G M W F Q G T A 119
17 GAT GCT GTT AGA CAG TTT ACA TGG GTT TTT GAG GAC GCG AAG AAC 404
18 D A V R Q F T W V F E D A K N 134
19 AAG GAC ATT GAT AAT ATA GTT ATT CTG TCA GGA GAT CAA CTT TAT 449
20 K D I D N I V I L S G D Q Q Y 149
21 CCG ATG GAT TAC ATG GAC TTA GTG CAG AAT CAC ATC GAA CGC AAT 494
22 R M D Y M D L V Q N H I E R N 164
23 TCT GAT ATT ACT CTT TCA TGT CCG ACC GAT GTT GAG GAT AGC CGA GCA 539
24 S T T L S C A T V G 179
25 TCG GAC TTT GGG CTG GTG AAA ATT GAC CTT GAG GCG CCA GTT GTC 584
26 S D F G L V K I D R R G R V V 194
27 CAG TTT TGC GAG AAA CCT AAA GGC ACT GAT CTA AAA GCA ATG CAA 629
28 Q F C E K P K G T D L K A M Q 209
29 GTA GAT ACT ACT CTC TTG GGA TTG CCA CCG CAA GAT GCC AGA TTA 674
30 V D T T L L G L P P Q D A R L 224
31 AAT CCT TAT ATT GCT TCG ATG GGG GTT TAT GTC TTT AAG ACG GAT 719
32 N P Y I A S M G V Y V F K T D 239
33 GTC CTT TTG CGG CTC CTG AGG TGG AGA TAT CCG ACA TCC AAC GAC 764
34 V L R L L R L R A W R Y P T S N D 254
35 TTT GGA TCC GAG ATT CTT CCT GCT GCT GTG ATG GAG CAC AAT GTT 809
36 F G S E I L P S A A V M E H N V 269
37 CAA GCT TAC ATT TTC AGA GAC TAC TGG GAG GAC ATC GGG ACA ATA 854
38 Q A Y I F R D Y W E D I G T I 284
39 AAA TCT TTC TAC GAC GCT AAC TTG GCC CTA ACT GAA GAG TTC CCG 899
40 K S F Y D A N L A L T E E F P 299
41 AAG TTT GAG TTC TAT GAT CCA AAG ACA CCT TTC TAC ACG TCC CCA 944
42 K F E F Y D P K T P F V T S P 314
43 AGA TTC CTA CCG CCA ACC AAA ATT GAT AAC TGC AAG ATC AAG GAT 989
44 R F L P P T K I D N C K I K D 329
45 GCA ATA ATC TCC CAC GGG TGT TTC TTA CGA GAG TGC AGT GTG GAA 1034
46 A I I S H G C F L R E C S V E 344
47 CAC TCC ATC ATT GGT GAA AGG TCA CCG CTA GAT TGT GGT GTT GAA 1079
48 H S I I G E R S R L D C G V E 359
49 CTG AAG GAT ACT TTA ATG ATG GGA GCA GAT ACC TAT GAA ACA GAA 1124
50 L K D T L M M G A D T Y E T E 374
51 TCT GAA ATT GCC TCG CTT CTG GCA GAT GGA AAA GTC CCG ATT GGG 1169
52 S E I A S L L A D G K V P I G 389
53 GTT GGA GAA AAC ACA AAA ATT AGG AAT GCT ATC AAT GAC AAG AAT 1214
54 V R I G K D V V I M N K D G V 419
55 CAA GAC TCA GAC CCG CCC GAT GAA GGT TTC TAC ACT AGA TCA GGG 1304
56 Q D S D R P D E G F Y I R S 434
57 ATT ACT ATT ATA ATG GAG AAA GCA ACC ATT CCT GAT GGA ACC GTC 1349
58 T I I I M E S A T I P D G T V 449
59 ATA TAA TAT CAA CGA AGA ACG ATT CGT GGG ATG TTA TGT TTC GGT 1394
60 I *
CTAGGATGAATGCGGTTTTCCTTCCTCCCGGAGAAACAAAGATGGCAACCAACCAAGAT
GTTTTGTTGAAGTTTACCTGGACTTGTGAAAGAGGAAGCGGAGGCGAGTTGAAGCAGA
GGGAATATGAATAAACTGGGATGATCCATATCTGGTGCATCTTCTGCTATTGTGACA
ATATCATATCATATCATATGTAAGATATGTTATATTTTCGATGATAATGATGTAAGGT
TGGCCATTTGGCTATGGTTGTGTAAGAAAAA 1661

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

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1  A GGA GAT CAA CTT TAT CGC ATG GAT TAC ATG GAC TTA GTG CAG 43
1  G D Q L Y R M D Y M D L V Q 14
44 AAT CAC ATC GAA CGC AAT TCT GAT ATT ACT CTT TCA TGT GCC ACA 88
15 N H I E R M N S C A T 29
89 GTT GGG GAT AGC CGA GCA TCG GAC TTT GGG CTG GTG AAA ATT GAC 133
30 V G D S R A S D F G L V K I D 44
134 CGA AGA GGC CGA GTT GTC CAG TTT TCG GAG AAA CCT AAA GGC ACT 178
45 R R G R V V Q F C E K P K G T 59
179 GAT CTA AAA GCA ATG CAA GTA GAT ACT ACT CTC TTG GGA TTG CCA 223
60 E H K A M Q V D T T L L G L P 74
224 CCG CAA GAT GCC AGA TTA AAT CCT TAT ATT GCT TCG ATG GGG GTT 268
75 P Q D A R L N P Y I A S M G V 89
269 TAT GTC TTT AAG ACG GAT GTC CTT TTC CGG CTC CTG AGG TGG AGA 313
90 Y V F K T D V L F R L L R W R 104
314 TAT CCC ACA TCC AAC GAC TTT GGA TCC GAG ATT CTT CCT GCT GCT 358
105 Y P T S N D F G S E I L P A A 119
359 GTG ATG GAG CAC AAT GTT CAA GCT TAC ATT TTC AGA GAC TAC TGG 403
120 V M E H N V Q A Y I F R D Y W 134
404 GAG GAC ATC GGG ACA ATA AAA TCT TAC GAC GCT AAC TTG GCC 448
135 E D I G T I K S F Y D A N L A 149
449 CTA ACT GAA GAG TTC CCG AAG TTT GAG TAT GAT CCA AAG ACA 493
150 L T E F P K F E F Y D P K T 164
494 CCT TTC TAC ACA TCC CCC ACA TTC CTC CGG CCA ACC AAA ATT GAT 538
165 P F S P R F L P F T K I D 179
539 AAC TGC AAG ATC AAG GAT GCA ATT ATC TCC CAC GGG TGT TTC TTA 583
180 N C K I K D A I I S H G C F L 194
584 CGA GAG TGC ACT GTG GAA CAC TCC ATC ATT GGT GAA AGG TCA CGG 628
195 R E C T V E H S I I G E R S R 209
629 CTA GAC TGT GGT GTT GAA CTG AAG GAT ACT TTA ATG ATG GGA GCA 673
210 L D C G V E L K D T L M M G A 224
674 GAT AAC TAT GAA ACA GAA TCT GAA ATT GCC TCG CTT CTG GCA GAT 718
225 D N Y E T E S E I A S L L A D 239
719 GGA AAA GTC CCG ATT GGG GTT GGA GAA AAC AAA ATT AGG AAT 763
240 G K V P I G V G E N T K I R N 254
764 GCT ACT ATT GAC AAG AAC GTA AGG ATT GGG AAA GAC GTT GTC ATC 808
255 A I I I D R N V R I I G K D V V I 269
809 ACG AAT AAA GAT GGT GTT CAA GAA TCA GAC CGC CCC GAT GAA GGC 853
270 T N K D G V G E S G F D 284
854 TTC TAC ATC AGA TCA GGG ATT ACT ATT ATA ATG GAG AAA GCA ACC 898
285 F Y I R S S I T M E A T 299
899 ATT CGT GAT GGA ACC GTC ATA TAG TAT CAA CGG AGC AGG CAC CGG 943
300 I R D G T V I *
TGGGATCACAGGAGACGATTCTATGGACGTTATGTTTCAGTCTAGGATGATCGCGGTTT
TGAAGAAACAAAAGTGGCAGGCCAAGATGTTTGTGTAAGTTTACCTGGACTTGTGAAA
AAGAGGAGAGAGCGGAGTGAAGCAGAGGGAATAAACTGAGGGATGCATCCATATCTGG
TGCATTTCTGCTATGTACAATATCATATCATATCATATGTAAGATATAGTATATTTTC
GATGATAAATGTAAGGTTGGCCATTGGCTATGTTGTAAGGTTCTGATGCTTAA
AAAGTTAAACATGTTTCTTATTCATTCACAAAAA 1277

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**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.

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 SGITHIMEKATI 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 (*zAGPL*) 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

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sAGPL1 1 NKIKPGVAYSVITTENDTQTVEFDMPLERRRRANPKDVAAILVGGEGTK 50
iAGPLI-a 1 -----LT.EA.I.....N...I....A..Q 30
iAGPLI-b -----

sAGPL1 51 LFPLTSRTATPAVPVGGCYRLIDIPMSNCINSAINKIFVLTQYNSAPLNR 100
iAGPLI-a 31 .....N.A.....L.....GV.....F..S... 80
iAGPLI-b -----

sAGPL1 101 HIARTYFGNGVSFGDGFVEVLAATQTPGEAGKKWFQGTADAVRKFIVWFE 150
iAGPLI-a 81 .....Q..T.M.....Q..T.... 130
iAGPLI-b -----

sAGPL1 151 DAKNKNENIENVLSGDHLYRMDYMLVQNHDRNADITLSCAPAEDSRAS 200
iAGPLI-a 131 .....B.D...I....Q.....D.....E..S.....TVG.... 180
iAGPLI-b 1 -----Q.....D.....E..S.....TVG.... 36

sAGPL1 201 DFGLVKIDSRGRVQFAEKPKGFDLKMAGVDVTLVCLSPQDAKKSPIYAS 250
iAGPLI-a 181 .....R.....C.....T.....L..P...RLN... 230
iAGPLI-b 37 .....R.....C.....T.....L..P...RLN... 86

sAGPL1 251 MGVIYFKTCDVLLKLLKWSYPTSNDFSGEIIIPAAIDYNYQAYIFKDYWD 300
iAGPLI-a 231 .....R..R..R.....L...VMEH.....R..... 280
iAGPLI-b 87 .....FR..R.R.....L...VMEH.....R..... 136

sAGPL1 301 IGTIKSFYNASIALTQEFPEFQYDPKTPFYTSRFLPPTKIDCNCKIKDA 350
iAGPLI-a 281 .....D.N...E...K.E..... 330
iAGPLI-b 137 .....D.N...E...K.E..... 186

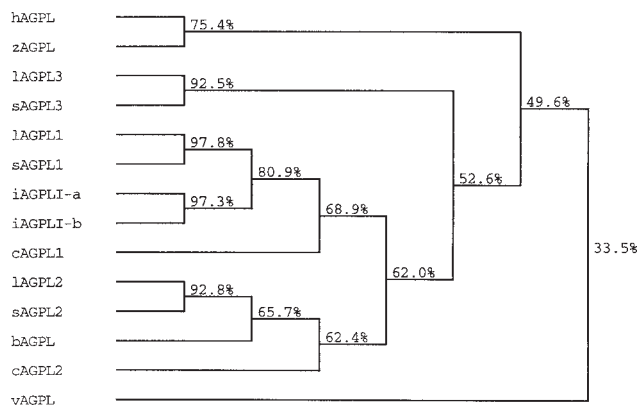
sAGPL1 351 IISHGCFRLDCSVEHSIVGERSRLDCGVELKDTFMMGADYYQTESEIASL 400
iAGPLI-a 331 .....E.....I.....L.....T.E..... 380
iAGPLI-b 187 .....E.T.....I.....L.....N.E..... 236

sAGPL1 401 LAEGKVPIGIGENTKIRKCIIDKNAKIGKNVSIINKDGVQAEADRPPEGFY 450
iAGPLI-a 381 ..D.....V.....NA.....VR...D.V.M.....DS...D.... 430
iAGPLI-b 237 ..D.....V.....NA.....VR...D.V.T.....S...D.... 286

sAGPL1 451 IRSGIILILEKATIRDGTVI 470
iAGPLI-a 431 .....T.M.....P.... 450
iAGPLI-b 287 .....T.M..... 306

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**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.

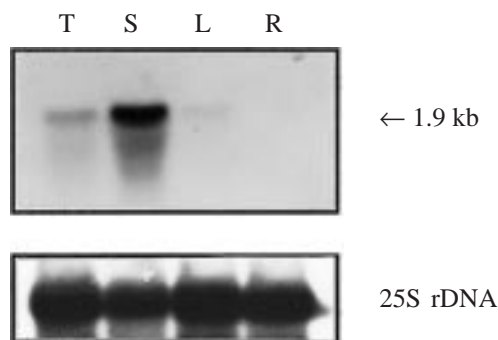


**Fig. 3.** Dendrogram was generated by the MacDNASIS software program. h, barley; z, maize; l, tomato; s, potato; i, sweet potato; b, sugar beet; v, bean; c, oriental melon.

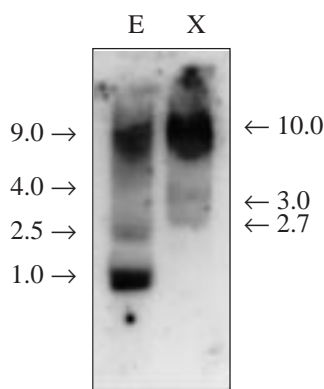
compared to all AGPases. The amino acid sequence identities of tomato and potato *iAGPL* in each group share 92–98% homology. This degree of homology is due to their belonging in the same family. By contrast, isoforms in the same species (for example, *sAGPL1*, 2, and 3) are grouped differently and exhibit 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 *EcoRI* and *XbaI* which did not cleave the internal area of coding region. For hybridization, 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). *EcoRI* digestion yielded 1.0 kb, (a doublet), 2.5 kb, 4.0 kb and 9.0 kb bands (Fig. 5). *XbaI* 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  $^{32}\text{P}$ -labeled ( $1 \times 10^6$  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, *XbaI* and E, *EcoRI*. 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 ( $\text{D}^{413}$ ) 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  $\text{K}^{113}$ ,  $\text{K}^{403}$ ,  $\text{K}^{441}$  for *iAGPLI-a* and  $\text{K}^{263}$  and  $\text{K}^{301}$  for *iAGPLI-b*. These clones also contain an allosteric activator site of SGITIMEKATI

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 *sAGPLI* 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 *sAGPLI* were differentially expressed in different tissues, each AGP large subunit gene would have preferential expression in tissues depending on its role, although each gene belongs to the same group based on sequence identity.

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## 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., Thorbjørnsen, 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 Müller-Röber, 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., Somerville, 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 pyrophosphorylase 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, J. R. and Preiss, J. (1982) Pyrophosphorylases in *Solanum tuberosum*. III. Purification, physical, and catalytic properties of ADP glucose pyrophosphorylase 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.