Plant Physiol. (1997) 113: 511–517

Pyrophosphorylases in Potato[1]

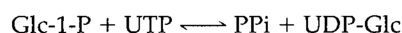
V. Allelic Polymorphism of UDP-Glucose Pyrophosphorylase in Potato Cultivars and Its Association with Tuber Resistance to Sweetening in the Cold

Joseph R. Sowokinos[2]*, Chris Thomas, and Michael M. Burrell

University of Minnesota, Department of Horticultural Science, St. Paul, Minnesota 55108 (J.R.S.); and Advanced Technologies (Cambridge) Ltd., Cambridge Science Park, Cambridge, United Kingdom CB4–4WA (C.T., M.M.B.)

UDP-glucose pyrophosphorylase (UGPase) was cloned from six American and nine European potato (*Solanum tuberosum* L.) cultivars. Restriction mapping of the different UGPase-cDNAs with *Bam*HI, *Hin*dIII, and *Eco*RI revealed that at least two mRNA populations were present in most cultivars. Staining for UGPase activity in nondenaturing gels of proteins extracted from developing potato tubers yielded two major isozymes that were highly active and appeared to be dimeric in nature. Following sodium dodecyl sulfate-polyacrylamide gel electrophoresis, all isozymes were disassociated into a single subunit with a molecular mass of 53 kD. Since UGPase has been demonstrated to be a single-copy gene in the haploid genome of potato (A.Y. Borovkov, P.E. McClean, J.R. Sowokinos, S.H. Ruud, G.A. Secor [1995] J Plant Physiol 147: 644–652), there must be allelic differences at the UGPase locus (chromosome 11). The two alleles, designated *ugp*A and *ugp*B, were identified by the absence and presence of a *Bam*HI site, respectively. The relative band intensities of the two cDNA populations following polymerase chain reaction amplification and agarose gel electrophoresis were related to a potato cultivar's ability to resist sweetening when exposed to cold temperatures.

All potato (*Solanum tuberosum* L.) cultivars presently used for the production of potato chips and fries accumulate excess free reducing sugars when exposed to cold stress (3–5°C). As raw potatoes are sliced and cooked at high temperatures in oil, reducing sugars react with the abundant free amino acids in the potato cell, forming unacceptable brown- to black-pigmented products via a nonenzymatic Maillard-type reaction (Shallenberger et al., 1959). The benefits of cold storage, i.e. less shrinkage, less disease loss, and decreased sprouting, are eclipsed by this process of cold-induced sweetening. The enzyme UGPase (UTP α-D-Glc-1-P uridylyltransferase, EC 2.7.7.9) catalyzes the first step common to the sweetening process in potato tubers via the formation of UDP-Glc (Fig. 1):



Glc-1-P + UTP ⟷ PPi + UDP-Glc

UGPase has recently received considerable attention because of its high activity in sink tissues and the key role that UDP-Glc plays as a direct or indirect precursor of Suc, starch, and structural polysaccharides in plants (see reviews by Kleczkowski, 1994; Feingold, 1982). Depending on the physiological state of potato tubers, the UGPase reaction may be channeled in vivo toward the synthesis of Glc-1-P (starch synthesis) or UDP-Glc (starch degradation) because of a metabolic coupling with other pathways of sugar metabolism (Sowokinos, 1994).

In plant tissues, UGPase is thought to be the principal source of UDP-Glc for the synthesis of Suc (Sicher, 1986) via the reaction catalyzed by Suc-P synthase (Sowokinos, 1994). Results from immunochemical experiments suggest that UGPase fulfills an important role in the synthesis of Suc in rice scutellar tissue by producing UDP-Glc as a donor of Glc residues (Kimura et al., 1992). The metabolic importance of UDP-Glc formation by UGPase was demonstrated in mutant strains of *Dictyostelium* with reduced activities of this enzyme. The morphogenesis of mutant strains was arrested during growth stages that required stored glycogen to be mobilized into UDP-Glc equivalents for use in cellulose formation (Diamond et al., 1976).

UGPase has not been considered a highly regulated step in the flux of carbons through sugar pathways via fine regulation of its catalytic activity. Although its catalatic activity in soybean nodules (Vella and Copeland, 1990) and potatoes (Sowokinos et al., 1993) does not seem to be subject to a high level of fine control by cellular metabolites and ions, its in vivo activity may be regulated by the availability of substrates. In the direction of UDP-Glc formation, purified UGPase from cv Norchip potatoes exhibited biphasic kinetics for the substrates Glc-1-P and UTP. When the concentration of Glc-1-P increased to more than 0.2 mM, the affinity of the enzyme for this important metabolite decreased nearly 9-fold (i.e. K_m increased from 0.08 to 0.68 mM; Sowokinos et al., 1993). Therefore, during periods of environmental stress (when starch is being mo-

[1] This work was from the Agricultural Experiment Station, University of Minnesota, Scientific Journal Series no. 22336.

[2] Present address: U.S. Department of Agriculture Potato Research Work Site, 311 5th Avenue NE, East Grand Forks, MN 56721.

* Corresponding author; e-mail sowok001@maroon.tc.umn.edu; fax 1–218–773–2207.

Abbreviations: ORF, open reading frame; PGM, phosphoglucomutase; UGPase, UDP-Glc pyrophosphorylase.

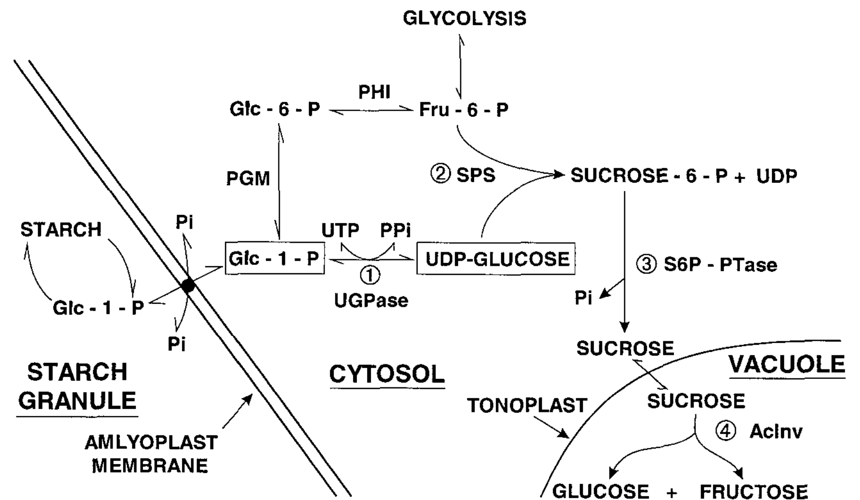


Figure 1. Enzyme reactions directly involved in free sugar formation in potato cells: 1, UGPase; 2, Suc 6-P synthase (SPS); 3, Suc 6-P phosphatase (S6P-PTase); and 4, acid invertase (AcInv). Other enzymes represented include PGM and phosphohexoseisomerase (PHI).

bilized), UGPase could impose control at the catalytic level by restricting the flow of carbons in the sucroneogenic direction (Fig. 1). Support for this hypothesis was shown in cv Desiree potatoes subjected to antisense RNA inhibition for UGPase (30% reduction of wild-type activity) and stored at 4°C and 12°C for 6 weeks. Control coefficients were calculated over a range of UGPase activities from 35 to 75 IU/g fresh weight. Control coefficient values ranging from 0.48 to 0.58 calculated by Spychalla et al. (1994) suggested that UGPase would exert significant control on the flux of carbons toward Suc during postharvest conditions.

Two nucleotide sequences of potato tuber UGPase-cDNAs have been published (Katsube et al., 1990; Spychalla et al., 1994). Their restriction maps are shown in Figure 2. The UGPase-cDNA clone isolated by Spychalla et al. (1994), designated UP2, contained 1701 bp and an ORF of 1431 bp coding for a polypeptide of 477 amino acids. The length of the ORF and the position of the polyadenylation signal corresponded exactly to that of the potato tuber UGPase cDNA clone, U4a, which was isolated by Katsube et al. (1990).

Because of the importance of UGPase in potato carbohydrate metabolism, cDNAs were isolated from genetically diverse potato clones that varied markedly in their ability to sweeten in the cold. Restriction maps were constructed on isolated cDNAs from 16 potato clones. We wanted to determine whether the polymorphism shown at the DNA level bore any relationship to the phenotypic polymorphism seen among cultivars in their ability to accumulate reducing sugars in storage. It was found that two mRNA populations (two structurally unique cDNAs) for UGPase were present in the majority of potato types analyzed. Band intensities of the two alleles, designated *ugp*A and *ugp*B, are discussed in relation to a cultivar's sweetening potential in storage.

MATERIALS AND METHODS

Potato (*Solanum tuberosum* L.) Clones Analyzed

The American cultivars and selections used in this study included Norchip, Red Pontiac, Norgold Russet, Russet Burbank, ND860-2, and ND860-2#8. European cultivars studied included Desiree, Record, Brodick, Panda, Picasso, and selection 13676. Three dihaploid clones were also in-

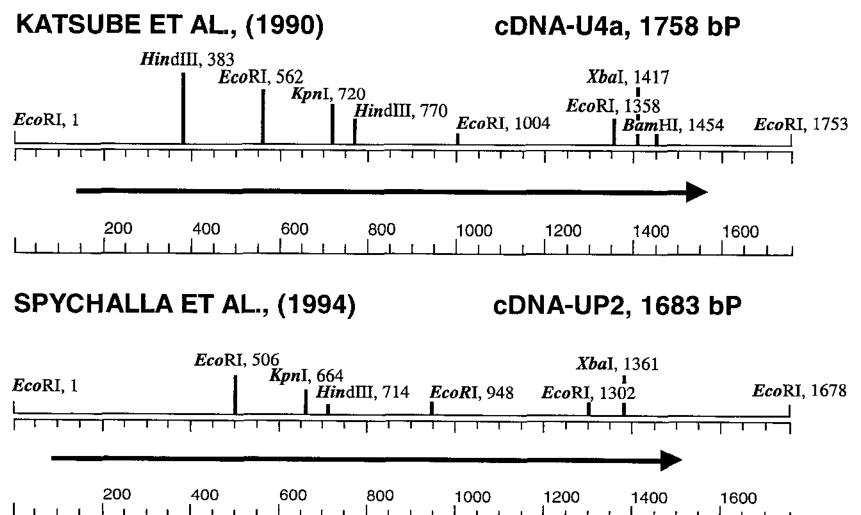


Figure 2. The restriction maps of two UGPase-cDNA clones from potato. Both cDNAs have an ORF (indicated by arrow) of 1431 bp coding for polypeptides (>99% homologous) containing 477 amino acids.

cluded in the study, PDH71, PDH97, PDH70, as well as a cDNA designated as Kat (Katsube et al., 1990). Commercial or private companies provided the mature tubers of each selection or cultivar. All tubers were stored at 4°C until utilized.

Isolation of mRNA and First-Strand cDNA Synthesis

Magnetic polystyrene beads (Dynabeads, Dynal A.S., Oslo, Norway) containing oligo(dT)$_{25}$ chains were used in the isolation of poly(A)$^+$ RNA from 100 mg of frozen potato tuber powders according to the procedure of Jakobsen et al. (1990). Following elution of poly(A)$^+$ RNA from the polystyrene beads with water at 65°C, a first-strand cDNA synthesis kit (Pharmacia) was used to form a single-stranded cDNA from approximately 4 μg of poly(A)$^+$ RNA.

Oligonucleotide Primers

Two sets of oligonucleotide primers were synthesized. Primer set A allowed the amplification of the entire UGPase ORF plus 92 additional bases (i.e. 71 bp upstream of the ATG start codon and 21 bp downstream of the TAG stop codon). The sequences of the two primers were (A)CTUDPG5′ (33 mer) (d[5′-GATCGGATCCTCCATACTCTCTGCTCCTCGAGA-3′]) and (A)CTUDPG3′ (34 mer) (d[5′-GATCGGATCCGAATTGGCACCAGCAGCTACTCTA3′]). Primer set B allowed for PCR amplification of the entire ORF of potato tuber UGPase. The sequences of these primers were (B)CTUDPG5′ (42 mer) (d[5′-GATCGGATCCATGGCTGCTGCTACTACTCTTTCTCCTGCTGA-3′]) and (B)CTUDPG3′ (43 mer) (d[5′-GATCCATGGATCCTATATATC CTCAGGTCCATTGATRTCCTTG-3′]). All primers were synthesized using the Gene Assembler Plus (Pharmacia).

PCR and Restriction Mapping

Potato cDNA (approximately 0.2 ng) was amplified in a total of 100 μL of reaction mixtures containing 1× *Taq* DNA polymerase buffer (Promega) supplemented with 1 μg of each primer, 0.02 μM of each deoxynucleotide, 0.5 μL (2.5 units) of *Thermus aquaticus* polymerase (*Taq*), and 50 μL of paraffin to retard evaporation. *Taq* was added to the PCR reaction mixtures after an initial heating to 80°C (hot start) to prevent nonspecific primer binding (D'Aquila et al., 1991). Amplification was performed for 30 cycles consisting of 1 min at 94°C (denaturation), 1 min at 58°C (annealing; first three cycles were at 54°C), and 1 min at 72°C (primer extension; time was increased to 2 min at cycle 13 and 3 min at cycle 22). Minus cDNA blanks were amplified to verify each PCR run. PCR products were subjected to restriction mapping with *Bam*HI, *Hin*dIII, and *Eco*RI and electrophoresed on 1% agarose according to the procedure of Sambrook et al. (1989). *Pst*I-digested λ phage DNA was used as a molecular mass marker.

Cloning of the PCR Products

The procedure of Girvitz et al. (1980) was used to purify UGPase-cDNAs from a low-melting agarose gel. The isolated PCR fragment was digested with *Bam*HI and electrophoresed for 2 h at 150 V. The upper DNA fragment (minus a *Bam*HI site; 1431 bp) from cv Record was ligated into the *Bam*HI site of the expression vector pGEX2T. Following transformation of *Escherichia coli* cells (XL1-Blue, Stratagene), several individual colonies were tested for the presence of the proper size of insert (in the sense orientation) using colony PCR, as described by Gussow and Clackson (1989).

Extraction of UGPase from Potato Tubers

Shortly following tuberization of six potato clones, small tubers (5–10 g) were removed and washed, and a total of 10 g was collected from longitudinal sections of three of them. All further steps were conducted at 4°C unless otherwise indicated. Ten grams of each cultivar was homogenized in a mortar and pestle with 10 mL of buffer consisting of 100 mM Tris-HCl, pH 7.5, 2 mM EDTA, 2 mM EGTA, 2 mM GSH, 1 mM PMSF, 10 μg/mL leupeptin, and 0.3% sodium bisulfite. After a 10-min grinding period, the suspension was centrifuged at 27,000*g* for 20 min. The precipitate was washed with 0.7 mL of buffer and recentrifuged, and the two supernatants were combined. Solid ammonium sulfate (60.3 g/100 mL) was added during stirring, and the pH of the suspension was maintained at 7.5. The precipitate was collected after centrifugation and dialyzed overnight against buffer (40 volumes) without sodium bisulfite with one change.

Nondenaturing PAGE

A native polyacrylamide gel was run with 1.0-mm-thick slab gels containing 7.5% acrylamide according to the procedure of Laemmli (1970).

SDS-PAGE

SDS-PAGE was run with 0.75-mm-thick slab gels containing 10% acrylamide according to the procedure of Laemmli (1970), as described previously (Sowokinos et al., 1993).

Immunoblotting

After SDS-PAGE and nondenaturing PAGE gels, proteins were transferred to PVDF membranes according to the method of LeGendre and Matsudaira (1988) and run as described previously (Sowokinos et al., 1993).

Activity Gels

UGPase activity was detected using a 1% low-melting agarose gel overlaid with a nondenaturing protein gel, according to the method described by Manchencko (1994). As the temperature of the melted agarose decreased to 45°C, 20 mL was mixed with 20 mL of a reagent solution to give a final concentration of 250 mM Tris-HCl, pH 8.5, 10 mM $MgCl_2$, 5 mM PPi, 4 mM UDP-Glc, 0.5 mM NAD^+, 20 μM Glc-1,6-diphosphate, PGM (1 unit/mL), Glc-6-P dehydrogenase (1 unit/mL), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.4 mg/mL), and phenazine

methosulfate (75 μg/mL). After the agarose mixture was solidified and overlayered with a protein gel, the reaction was continued for 25 min at room temperature in the dark. The reaction was stopped by removing the gel and allowing the agarose slab to dry on paper (3MM, Whatman).

Assay of UGPase Activity

Pyrophosphorolysis of UDP-Glc was assayed using the one-step spectrophotometric method (Sowokinos et al., 1993). In 1 mL (pH 8.0) there was 80 μmol of glycylglycine, 5 μmol of $MgCl_2$, 10 μmol of NaF, 1 μmol of UDP-Glc, 0.75 unit of Glc-6-P dehydrogenase, 1.0 unit of PGM, 20 μmol of Cys, 0.2 μmol of Glc-1,6-diphosphate, 0.6 μmol of NAD^+, and 0.01 to 0.05 unit of diluted enzyme. The reactions were initiated by the addition of 2.5 μmol of PPi. The formation of NADH (340 nm) was recorded continuously at 30°C until a loss of initial linear reaction occurred. One unit of activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol of Glc-1-P per min. Specific activity is given as IU/mg of protein. Substrates and other assay components were saturating, so that the rate of each reaction measured was linear with respect to enzyme concentration and time under the experimental conditions used.

RESULTS

Restriction analysis of UGPase-cDNAs prepared from leaf and tuber with *Bam*HI, *Eco*RI, and *Hin*dIII revealed fragments of similar size, suggesting that similar genes may be involved in both tissues (data not shown). Primer set A yielded a fragment that was approximately 90 bases longer than the fragment resulting with primer set B. The following PCRs were conducted solely with primer set B, which amplifies the ORF of potato tuber-UGPase (1431 bp). Restriction enzyme patterns of UGPase-cDNAs amplified from five potato clones are shown in Figure 3. PCR amplification yielded the expected 1431-bp fragment in the absence of restriction enzyme. The restriction patterns with *Eco*RI gave four fragments indicative of three sites in the cDNA (i.e. the upper band consists of two fragments of similar size). This is in agreement with the two restriction maps previously reported from potato (Fig. 2). Restriction with *Hin*dIII indicated the presence of at least two sites within the cDNA, whereas cv Desiree showed some polymorphism from the other four. *Bam*HI digestion revealed that two distinct cDNAs were present in the PCR products from cv Desiree and cv Brodick. The upper fragment contained no *Bam*HI restriction site and in this regard was similar to the cDNA obtained by Spychalla et al. (1994), shown in Figure 2. The lower fragment did contain a *Bam*HI site and therefore was similar to the cDNA obtained by Katsube et al. (1990) and shown in Figure 2. The fragment that had been excised (near 100 bp) was eluted from the gel.

These results suggest that both of the cDNAs previously described in potato tubers (Fig. 2) may occur simultaneously in certain cultivars. The fragment (UB) from cv Record was the result of amplifying potato UGPase-cDNA from an *E. coli* colony that had been electroporated with the vector containing only the gel-purified upper PCR fragment (minus a *Bam*HI site). This control helped to confirm that polymerase activity was specific and did not yield other DNA fragments. cDNAs from cv Norchip and cv Katsube were similar in demonstrating a single PCR product containing a *Bam*HI site. Catalysis with *Pfu* polymerase, which has 3'- to 5'-proofreading exonuclease activity, supported the idea that the two apparent cDNA populations with cv Desiree, distinguished by a *Bam*HI site, were not the result of a misreading of the base sequence (Fig. 3).

Screening of Potato Clones with *Bam*HI

Figure 4 shows the restriction patterns after *Bam*HI digestion of UGPase-cDNAs from 16 genetically diverse potato clones. The majority of the clones demonstrated mixed cDNA populations. The upper and lower fragments are designated *ugp*A and *ugp*B, respectively. The Katsube cDNA showed a single *Bam*HI site that agrees with the restriction map for the U4a-cDNA seen in Figure 2. A similar cDNA population was present in cv Norchip. DES-49 refers to a specific transformant of cv Desiree potatoes (no. 49), which had been subjected to antisense RNA inhibition for UGPase (Zrenner et al., 1993). All potatoes analyzed were tetraploids except PDH71, PDH97, and PDH70, which were haploids with two X chromosomes.

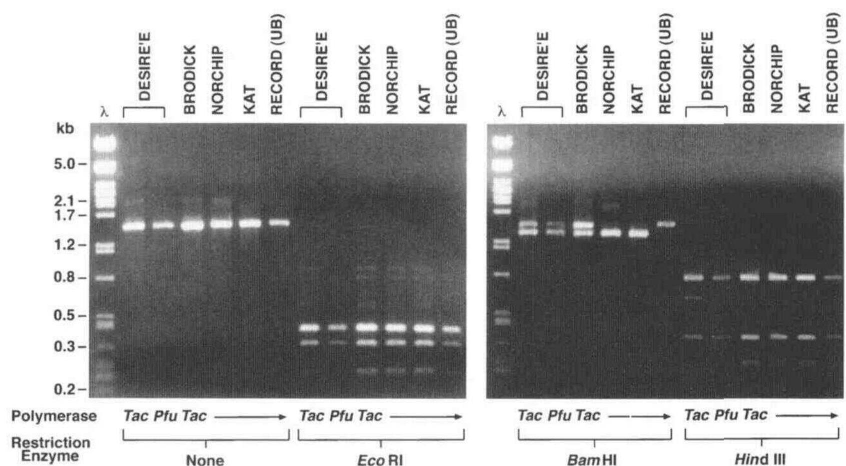


Figure 3. Restriction enzyme mapping of UGPase-cDNAs from tubers of four different potatoes. cDNAs were prepared directly from mRNA as described in "Materials and Methods." KAT refers to the cDNA previously cloned and described by Katsube et al. (1990) (Fig. 2). Record (UB) refers to the cDNA (*ugp*A allele, no *Bam*HI site) that was previously purified and cloned in *E. coli*. During PCR with cv Desiree, both *Tac* and *Pfu* polymerases were used in separate reactions.

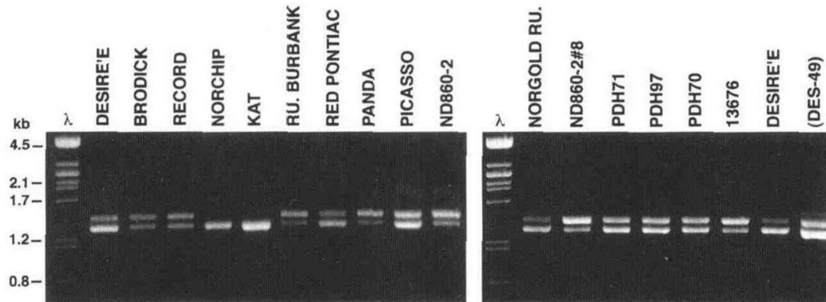


Figure 4. *Bam*HI digestion of UGPase-cDNAs from 16 genetically diverse potato tubers. cDNAs were prepared directly from mRNA as described in "Materials and Methods." The upper and lower cDNA fragments are designated *ugp*A and *ugp*B, respectively. KAT refers to the cDNA previously cloned and described by Katsube et al. (1990) (Fig. 2). DES-49 refers to a specific transformant of Desiree potatoes (no. 49) that had been subjected to antisense RNA inhibition for UGPase (Zrenner et al., 1993). The potato clones are all autotetraploids except PDH71, PDH97, and PDH70, which are haploids with two X chromosomes.

Isozymes of UGPase in Potatoes

Extracts were obtained from actively growing potato tubers of six cultivars, and proteins were separated by native and denaturing gel electrophoresis. The enzyme previously purified from cv Norchip was used as a reference. The presence of multiple antigen species for UGPase was immunodetected after separation with nondenaturing conditions (Fig. 5A). Staining for UGPase activity revealed that two of the antigens were highly active isozymes of UGPase (Fig. 5B). All cultivars yielded a basic and an acidic polypeptide, with the latter showing greater mobility. Immunodetection of UGPase subunits following SDS-PAGE revealed that each cultivar yielded a single subunit of 53 kD (Fig. 5C). Banding and activity patterns for UGPase as seen in Figure 5 were similar to those prepared from cold-stored tubers. A more detailed examination of antigen and isozyme patterns of UGPase during development and storage will be the subject of a separate investigation.

DISCUSSION

Restriction mapping of UGPase-cDNAs from potato tubers (Fig. 4) suggests that the two types of mRNA corresponding to both of the cDNAs previously described (Fig. 2) may be present in most potato cultivars. Since UGPase has been demonstrated to be a single-copy gene in the haploid genome of autotetraploid potato plants (Borovkov et al., 1995), there must be allelic differences at the UGPase locus (chromosome 11). The two alleles, designated *ugp*A and *ugp*B, are distinguished by the absence and presence of a *Bam*HI site, respectively. If the previously described cDNAs (Fig. 2) relate to these two alleles (a relationship is supported by restriction analysis), then they should have ORFs of 1431 bp that code for polypeptides with 477 amino acids and demonstrate 99% homology. The two proteins would differ by five amino acids, two of which lead to a change in charge (Table I). The cDNA (UP2) described by Spychalla et al. (1994) yields a polypeptide slightly more acidic in charge than the cDNA described by Katsube et al. (1990). During the purification of UGPase from cv Norchip potatoes, trace amounts of an acidic isoform were detected during chromatography on DEAE-Sephacel (J.R. Sowokinos, unpublished observation). Only the major, more basic isozyme was purified to homogeneity and characterized (Sowokinos et al., 1993). Although the cv Norchip tissue used in Figure 4 demonstrated only the *ugp*B allele (similar

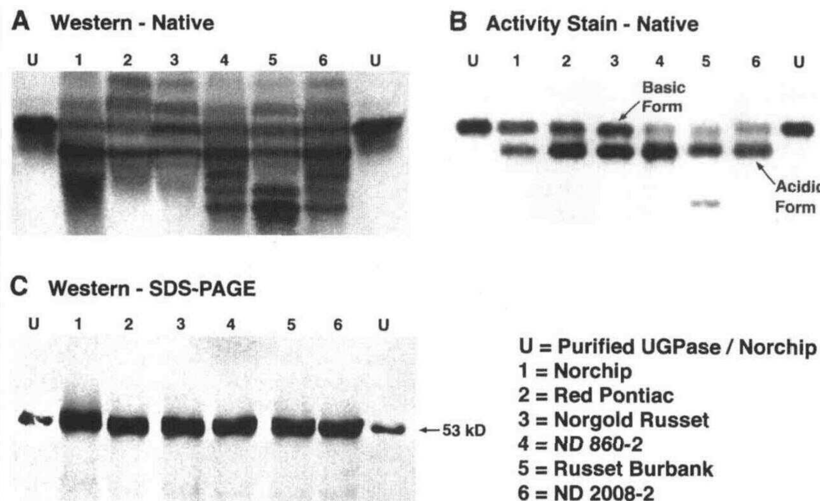


Figure 5. Comparative analysis of UGPase from six genetically diverse potato clones. A, Immunodetection of UGPase antigens after separation on a 7.5% nondenaturing polyacrylamide gel. B, The same gel as shown in A but subjected to activity staining. C, Immunodetection of UG-Pase subunits after separation on a SDS gel with 10% polyacrylamide. Potato clones used are shown on the bottom right. See "Materials and Methods" for experimental details.

in size and restriction pattern to the cDNA obtained by Katsube et al. [1990]), which should yield a basic polypeptide (Table I), it is evident that the acidic isozyme is also present in actively growing tubers (Fig. 5B). This suggests that cv Norchip tubers used to prepare cDNAs in Figure 4, which were in storage for several months, may not have contained significant levels of transcript from the *ugp*A allele.

Isozymes of UGPase have been shown to exist in slime molds (Fishel et al., 1985) and bacteria (Nakae, 1971). In plants, UGPase isozymes have been implicated in soybean nodules (Vella and Copeland, 1990) and rice cell cultures using immunocytological analysis (Kimura et al., 1992). To our knowledge, isoforms of UGPase have not previously been identified in potatoes. Figure 5B shows that two major isozymes of UGPase exist in developing potatoes. Upon western analysis with SDS-PAGE, all antigenic and isozymic forms yield a subunit 53 kD (Fig. 5C). Based on kinetic data with the purified enzyme from cv Norchip (Sowokinos et al., 1993), we believe that the two major, active forms in Figure 5B are dimeric in nature.

Although the intensity of PCR product bands does not necessarily have to correlate with the amount of its represented transcript, it became evident that a trend existed between band intensities of the two UGPase alleles and a cultivar's ability to sweeten in storage. The relative ranges of reducing sugar accumulated by potato cultivars and selections used in this study are shown in Table II. It should be noted that similar results were obtained following three separate analyses of 12 potato cultivars. In addition, the 1:1 ratio observed with the heterologous diploid clones seen in Figure 4 (PDH71, PDH97, and PDH70) indicated that there was no preferable amplification of one form over the other. Potatoes with some ability to resist sweetening at lower temperatures (3–6°C), namely ND860-2, ND860-2#8, 13676, Panda, and Russet Burbank, all demonstrated a predominance (3:1) of the allele *ugp*A (upper band, no *Bam*HI site) (Fig. 4). Cultivars that maintained a high level of sugar in the cold, namely Red Pontiac, Norgold Russet, Desiree, Picasso, and Norchip, demonstrated a predominance of the allele *ugp*B (lower band, a single *Bam*HI site). Cultivars with bands of even intensity (i.e. Brodick and Record) could not be classified into a particular sweetening category. The haploids PDH71, PDH97, and PDH70, with two X chromosomes, appeared to be heterozygous in relation to the two UGPase alleles.

Although UGPase is certainly not the only factor determining a cultivar's relative ability to sweeten in storage, the above relationship is certainly intriguing. Both of the potato UGPases previously characterized (Nakano et al., 1989; Sowokinos et al., 1993) appear to describe the basic isozyme, since their corresponding cDNA allele contained a single *Bam*HI site. Although restriction pattern and size comparisons suggest that alleles *ugp*A and *ugp*B may be similar to the cDNAs described by Spychalla et al. (1994) and Katsube et al. (1990), respectively, sequencing of the alleles is necessary to confirm this relationship. In addition, sequencing of the amino acids of the polypeptides encoded from these alleles can be used to solidify their similarity. Efforts to purify and characterize each of the resultant polypeptides from *E. coli* (as well as tuber tissue) will be conducted before developing a role or mechanism for UGPase in affecting the sweetening potential of stored potatoes.

Table I. *Charge differences between the UGPase polypeptides coded by cDNA obtained by Spychalla et al. (1994), UP2, and Katsube et al. (1990), U4a*

Amino Acid Position	cDNA		Alterations in Charge
	UP2	U4a	
5	Ala	Thr	Noncharged to noncharged polar
30	Asp	Glu	Acidic to acidic
82	Asn	Lys	Noncharged polar to basic
445	Glu	Lys	Acidic to basic
450	Ile	Val	Nonpolar to nonpolar

Table II. *The relative range of reducing sugar accumulated by the potato cultivars or selections when subjected to reduced temperature storage (3–9°C)*[a]

Low (0–10)[b]	Intermediate (11–25)	High (26–50)
	Clones	
ND860-2	Russet Burbank	Red Pontiac
ND860-2#8	Record	Norgold Russet
13676		Desiree
Panda		Norchip
Brodick		Picasso

[a] From Sowokinos et al. (1989). [b] μmol reducing sugar g^{-1} tuber fresh weight.

ACKNOWLEDGMENTS

J.R.S. would like to thank the personnel at Advanced Technologies Ltd. (Cambridge, UK), particularly Mike Burrell and Chris Thomas, for the facilities, supplies, equipment, and advice in carrying out this sabbatical research project.

Received September 23, 1996; accepted November 11, 1996.
Copyright Clearance Center: 0032–0889/97/113/0511/07.

LITERATURE CITED

Borovkov AY, McClean PE, Sowokinos JR, Ruud SH, Secor GA (1995) Effect of expression of UDP-glucose pyrophosphorylase antisense and ribozyme RNAs on the enzyme activity and carbohydrate composition of transgenic potato plants. J Plant Physiol **147:** 644–652

D'Aquila RTD, Bechtel LJ, Videler JA, Eron JJ, Gorczyca P, Kaplan JC (1991) Maximizing sensitivity and specificity of PCR by preamplification heating. Nucleic Acids Res **19:** 3749

Diamond RL, Farnsworth PA, Loomis WF (1976) Isolation and characterization of mutations affecting UDPG pyrophosphorylase activity in *Dictyostelium discoideum*. Dev Biol **50:** 169–181

Feingold DS (1982) Aldo (and keto) hexoses and uronic acids. *In* FA Loewus, W Tanner, eds, Encyclopedia of Plant Physiology, New Series, Vol 13A. Springer-Verlag, Berlin, pp 3–76

Fishel BR, Ragheb JA, Rajkovic A, Haribabu B, Schweinfest CW, Dottin RP (1985) Molecular cloning of a cDNA complementary to a UDP-glucose pyrophosphorylase mRNA of *Dictyostelium discoideum*. Dev Biol **110:** 369–381

Girvitz SC, Bacchetti S, Rainbow AJ, Graham FL (1980) A rapid and efficient procedure for the purification of DNA from agarose gels. Anal Biochem **106:** 492–496

Gussow D, Clackson T (1989) Direct clone characterization from plaques and colonies by the polymerase chain reaction. Nucleic Acids Res **10:** 4000

Jakobsen KS, Breivoid E, Hornes E (1990) Purification of mRNA directly from crude plant tissues in 15 minutes using magnetic oligodT microspheres. Nucleic Acids Res **18:** 3669

Katsube T, Kazuta Y, Mori H, Nakano K, Tanizawa K, Fukui T (1990) UDP-glucose pyrophosphorylase from potato tuber: cDNA cloning and sequencing. J Biochem **108:** 321–326

Kimura S, Mitsui T, Matsuoka T, Igaue I (1992) Purification, characterization and localization of rice UDP-glucose pyrophosphorylase. Plant Physiol Biochem **30:** 683–693

Kleczkowski LA (1994) Glucose activation and metabolism through UDP-glucose pyrophosphorylase in plants. Phytochemistry **37:** 1507–1515

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685

LeGendre N, Matsudaira P (1988) Direct microsequencing from Immobilon-P transfer membrane. Biotechniques **6:** 154–159

Manchencko GP (1994) Method of detection of specific enzymes. *In* GP Manchencko, ed, Handbook of Detection of Enzymes on Electrophoretic Gels. CRC, Boca Raton, FL, p 20

Nakae T (1971) Multiple molecular forms of uridine diphosphate glucose pyrophosphorylase from *Salmonella typhimurium.* J Biol Chem **246:** 4404–4411

Nakano K, Omura Y, Tagaya M, Fukui T (1989) UDP-glucose pyrophosphorylase from potato tuber: purification and characterization. J Biochem **106:** 528–532

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Shallenberger RS, Smith O, Treadway RH (1959) Role of sugars in the browning reaction in potato chips. J Agric Food Chem **7:** 274–277

Sicher RC (1986) Sucrose biosynthesis in photosynthetic tissue: rate-controlling factors and metabolic pathway. Physiol Plant **67:** 118–121

Sowokinos JR (1994) Post-harvest regulation of sucrose accumulation in transgenic potatoes; role and properties of potato tuber UDP-glucose pyrophosphorylase. *In* WR Belknap, ME Vayda, WD Park, eds, The Molecular and Cellular Biology of the Potato, Ed 2. CAB International, Wallingford, UK, pp 81–106

Sowokinos JR, Morgan B, Sleeper M, Shea I (1989) Establishment of glucose-forming-potential (GFP) as a tool to screen breeding clones for processing potential. *In* Proceedings of the Red River Valley Potato Research Reporting Conference. East Grand Forks, MN, pp 145–155

Sowokinos JR, Spychalla JP, Desborough SL (1993) Pyrophosphorylases in *Solanum tuberosum* IV. Purification, tissue localization, and physicochemical properties of UDP-glucose pyrophosphorylase. Plant Physiol **101:** 1073–1080

Spychalla JP, Scheffler BE, Sowokinos JR, Bevan MW (1994) Cloning, antisense RNA inhibition, and the coordinated expression of UDP-glucose pyrophosphorylase with starch biosynthesis genes in potato tubers. J Plant Physiol **144:** 444–453

Vella J, Copeland L (1990) UDP-glucose pyrophosphorylase from the plant fraction of nitrogen-fixing soybean nodules. Physiol Plant **78:** 140–146

Zrenner R, Willmitzer L, Sonnewald U (1993) Analysis of the expression of potato uridinediphosphate-glucose pyrophosphorylase and its inhibition by antisense RNA. Planta **190:** 247–252