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Translucent Tissue Defects in *Solanum tuberosum* L.

I. ALTERATIONS IN AMYLOPLAST MEMBRANE INTEGRITY, ENZYME ACTIVITIES, SUGARS, AND STARCH CONTENT[1]

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ABSTRACT

Kennebec (cv) potatoes randomly developed translucent areas in their centrally located pith-parenchymal cells during storage. These defective areas were characterized as having reduced starch concentration and increased levels of free sugars (*i.e.* sucrose and glucose) and inorganic phosphate. Electron micrographs of potato tubers stored at 10° ± 1°C for 8 months indicated that the amyloplast membrane was still intact and continuous around starch granules in both normal and prematurely sweetened tissue. The total activities of phosphorylase and sucrose-6-P synthase were elevated 5.4- and 3.8-fold, respectively, in the defective tissue compared to healthy nonsweetened tubers while there were no significant differences in the levels of sucrose synthase, UDPglucose pyrophosphorylase, invertase, or α-amylase. Total and specific activities of acid phosphatase were only slightly elevated in translucent tissue but their increase was significant ($P < 0.05$, t test) over that seen in healthy tubers. The premature sweetening in storage may have been indirectly triggered by moisture and heat stress experienced during development. Translucency eventually led to physical deterioration of the tissue.

Nonpathological stresses (*i.e.* fertility and environmental) affecting the development of potato tubers (*Solanum tuberosum* L.) can result in biochemical changes during storage which influence their market quality (6, 7, 14). Moisture stress during tuber growth is known to lead to irregular shaped tubers, chain tuberization, and the development of translucent ends (5, 6, 14). Translucent or glassy-end tubers demonstrate low solids and high levels of free sugars (6). Defective areas are clearly visible in the cut tuber at harvest and cellular destruction becomes increasingly severe in storage. The undesirable accumulation of reducing sugars from starch reserves is of critical importance to processing quality. Nonenzymic browning (Maillard reaction) results when excess sugars react with the amine groups of free amino acids during the cooking process (22).

In principally nonirrigated growing areas, a 'translucent defect' has been noted which is distinct from the 'glassy-end' condition indicated above. This defect was not visible in the raw tuber at harvest but developed during storage in the central pith-parenchymal cells. These 'translucent regions' are visibly distinct from defects arising from black heart (*i.e.* O_2 deficient tissue) or from internal heat necrosis. The mechanism of this premature sweetening process as well as the cellular regulation of carbon partitioning (*i.e.* starch-sugar) in potato tubers is presently undefined. Since translucency interfered with quality for processing, we investigated the biochemical characteristics and morphological changes of this prematurely sweetened tissue compared to unstressed tubers. It was anticipated that analysis of stressed tubers may aid in clarifying the chemical nature of this defect as well as contribute basic information about the chemical events leading to sweetening in general.

MATERIALS AND METHODS

Tissue Source and Preparation. Kennebec potatoes (*Solanum tuberosum* L.) with translucent tissue were obtained from a local warehouse. The tubers had been stored for 8 months at 10° ± 1°C with 95% RH. Tubers were cut longitudinally from the stem to the bud end and examined for centrally located, brownish-translucent areas. Tubers with such a condition were referred to as being defective. Tissue was cut into 1-cm cubes and pooled from each of the following regions: (a) normal pith-parenchymal cells from healthy tubers; (b) nontranslucent pith-parenchymal cells from defective tubers; and (c) translucent pith-parenchymal cells from defective tubers.

Fifty g of tissue selected at random from each area were frozen in liquid N_2 and lyophilized for 48 h at 30°C. The dehydrated tissue was blended and the resulting powder stored over desiccant at −20°C. These samples were used for dry matter and starch analysis.

Two hundred g of fresh tissue from each area were homogenized in an Acme Juicerator, model 6001 (Acme Juicer Manufacturing, Sierra Madre, CA)[6] at 4°C. The tissue was washed three times with 100-ml aliquots of water and adjusted to a final volume of 430 ml. Portions of each extract were frozen and

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subsequently assayed for soluble protein, free sugars (*i.e.* sucrose and glucose), and Pi. The remaining tissue was frozen in liquid N_2, stored at −20°C, and then extracted for enzyme determinations.

Analytical Techniques. *Free Sugar, Starch, and Pi Analysis.* Glucose and sucrose concentrations in potato extracts were determined using a YSI model 27 Industrial Analyzer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH) which determines β-D-glucose via a glucose oxidase-bound membrane system. The analyzer was initially calibrated using reagent grade sucrose standards supplied by YSI Co., Inc. Sucrose analysis was based on the difference between free glucose (Glc_f) and total glucose (Glc_t) in the absence and presence of excess invertase, respectively. Twenty five μl of undiluted potato extract were injected into the sample chamber (350 μl) containing 0.32 M NaH_2PO_4-Na_2HPO_4 buffer (pH 6.5). This high buffer concentration accelerated the rate of mutarotation of α- to β-D-glucose. Glucose oxidation was carried to completion and the Glc_f was displayed digitally in mg/dl. The reaction mixture was then purged from the sample chamber and fresh buffer added. Then, 25 μl of invertase (0.5 mg, 1.5×10^2 units) were injected into the sample chamber followed by 25 μl of the same potato extract used above. The hydrolysis of sucrose in the extract was run to completion in 2 to 3 min and the Glc_t reading was obtained. The per cent of sucrose was determined as indicated below:

$$\% \text{ of sucrose} = \frac{Glc_t \text{ (mg/dl)} - Glc_f \text{ (mg/dl)}}{0.526} \times \frac{V \text{ (dl)} \times 1.17}{\text{g fresh wt} \times 10}$$

where 1.17 is the correction factor obtained for calculating the actual concentration of sucrose present in the extract when invertase hydrolysis was conducted within the sample chamber. This factor was determined using the appropriate sucrose standards. If hydrolysis was conducted external to the sample chamber and an aliquot injected, this value was closer to unity. Stoichiometrically, each g of sucrose yields 0.526 g of glucose upon hydrolysis, as represented by the denominator 0.526.

Starch concentration in the desiccated tuber powders was determined as previously described by Varns and Sowokinos (28). This procedure utilized: (a) a mild alkaline (0.1 N NaOH) sonic extraction of starch from 80% ethanol washed precipitates; (b) starch hydrolysis with 1 N HCl; and (c) the determination of liberated glucose by the specific glucose oxidase system. A factor of 0.9 was used to convert glucose to starch equivalents (19).

A modification of the method of Lowry and Lopez (13) was used to assay Pi. A portion of extract from each tuber area was diluted with a 50% TCA solution to give a final concentration of 10%. This sample was then mixed for 15 min and centrifuged at 27,000*g* for 15 min to remove protein. One ml of 1% ammonium molybdate was combined with 1 ml of the TCA supernatant diluted 5-fold with water. Three ml of 0.2 N sodium acetate buffer (pH 4.3) were then added followed by 1 ml of 1% ascorbic acid. After a 20-min color development period, the blue color was read on a Klett-Summerson Photoelectric Colorimeter with a No. 66 red filter. Pi standards (1 μmol) were run simultaneously with each assay to verify the determination.

Enzyme Extraction and Assay. All extractions were conducted with the following buffer at 4°C unless otherwise indicated. Triplicate samples (10 g) from each tuber area were homogenized in a mortar and pestle with 20 ml buffer (*i.e.* 0.5% sodium bisulfite-citrate/50 mM Tris-maleate [1:4 v/v]) (pH 6.3). After a 20-min grinding and mixing period, the suspension was centrifuged at 27,000*g* for 15 min. The precipitate was washed with 20 ml of the above buffer, recentrifuged, and the two supernantants were combined. The extract was dialzyed overnight against 50 mM Tris-maleate (pH 6.3, 40 volumes) with one change. The dialysate was centrifuged to remove inactive protein. The Biuret method was used to assay for protein and crystalline BSA was used as a standard as described previously (25). Extracts were immediately measured for enzyme activity. Substrates and other assay components were saturating so that the rate of each reaction was linear with respect to enzyme concentration and time under the various experimental conditions used.

Phosphorylase. Reaction mixtures (1 ml) contained 50 mM Tris-maleate (pH 6.3), 7.5 mg amylopectin, 15 mM NaF, 10 mM glucose-1-P, and 0.05 to 0.25 unit of enzyme. Amylopectin blanks and Pi standards (1 μmol) were run with each assay. Reactions were initiated with enzyme, incubated at 37°C for 10 min, and stopped with 0.5 ml of 5% TCA. Amylopectin (7.5 mg) was added to the amylopectin blanks and 0.5 ml from each reaction was assayed for Pi as described above. One unit of activity is defined as that amount of enzyme which liberates 1 μmol Pi/min.

Sucrose Synthase and Invertase. Sucrose synthase cleavage activity was assayed as previously described (24). Reaction mixtures (1 ml) contained 0.25 M sucrose, 10 mM UDP, 40 mM Tris-phosphate (pH 6.6), 10 mM NaF, 5 mM 2-mercaptoethanol, and diluted enzyme extract containing 0.1 mg protein or less. Relative invertase activity was simultaneously determined in reactions lacking UDP. The fructose liberated was determined by the arsenomolybdate method (1). One unit of activity for either enzyme is defined as that amount of enzyme which liberates 1 μmol of fructose/h.

UDPglucose Pyrophosphorylase. The buffer used to extract and dialyze UDPglucose pyrophosphorylase consisted of 50 mM Hepes (pH 7.5) containing 1 mM EDTA, 2 mM GSH, and 20% sucrose. The rate of α-D-glucose-1-P pyrophosphorolysis from UDPglucose was measured spectrophotometrically in the presence of excess phosphoglucomutase, glucose-6-P dehydrogenase, and NADP. The assay procedure was similar to that previously described (25). One unit of activity is defined as that amount of enzyme which catalyzes the formation of 1 μmol of α-D-glucose-1-P/min.

Sucrose-6-P Synthase. The buffer used to extract sucrose-6-P synthase consisted of a solution of 10% $NaHSO_3$ and 50 mM Tris-phosphate buffer (pH 7.5, 1:4 v/v) containing 10 mM GSH and 1 mM EDTA. Extracts were dialyzed against the Tris-phosphate buffer containing 10 mM $NaHSO_3$. Reaction mixtures (0.25 ml) contained 100 mM Tris-phosphate (pH 7.3), 20 mM fructose-6-P, 10 mM UDPglucose, 50 mM NaF, and diluted enzyme containing between 0.02 and 0.2 unit of enzyme. Boiled enzyme blanks and sucrose standards (0.5 μmol) were run with each assay. After incubation at 37°C for 1 h, 0.25 ml of 5 N NaOH was added to each reaction which was then placed into a boiling water bath for 10 min to destroy unreacted fructose-6-P. The concentration of sucrose-6-P formed was determined by the thiobarbituric acid method (18). One unit of activity is defined as that amount of enzyme which catalyzes the formation of 1 μmol of sucrose-6-P/h.

Acid Phosphatase. Acid phosphatase activity was extracted with 0.1 M sodium acetate buffer (pH 5.0) and assayed spectrophotometrically by continuously monitoring (A_{400}) the rate of liberation of PNP[7] from *p*-nitrophenyl phosphate substrate. Enzyme extract (0.15 ml, diluted 1 to 10), *p*-nitrophenyl phosphate substrate (1.85 ml, 20 mM in acetate buffer), and buffer (1.0 ml, pH 5.0, 2 mM sodium acetate) were incubated and the rate of PNP formation was calculated using a standard curve which provided an ϵ_{mM} of 17.424 1/mmol·cm. One unit of enzyme activity catalyzed the liberation of 1 μmol of PNP/min.

Amylase. Amylase was extracted with 0.1 M sodium acetate buffer (pH 4.5, 100% w/v) and assayed according to the procedure of Chrispeels and Varner (2). Fresh starch substrate was prepared daily using 250 mg (nonsolubilized) potato starch, 600

[7] Abbreviation: PNP, *p*-nitrophenol.

mg of KH_2PO_4, and 200 μmol of $CaCl_2$ in a final volume of 100 ml. The starch suspension was placed into a boiling water bath for 8 min, centrifuged at 8000*g* for 10 min, and the supernatant used as the starch substrate solution. Aliquots of the amylase extracts (diluted to 1 ml with buffer) were combined with 1 ml of starch substrate and incubated at 37°C for 12 min. Reactions were stopped with 1.0 ml of iodine reagent. One ml of 30% H_2O_2 was added to ensure against excessive iodine reduction. The addition of 5 ml of water to each tube was followed by thorough mixing and the absorption was measured at 620 nm. The initial absorption of the diluted starch solution without amylase action was 1.35. One unit of activity is defined as that amount of enzyme necessary to catalyze a change in absorption of 1.0 under the experimental conditions used.

Electron Microscopy. Samples of potato tubers were fixed and dehydrated by a modification of a procedure described previously (16). One-mm cubes excised from healthy and translucent pith-parenchyma tissue were submerged in vials containing a solution of 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. All vials were placed under vacuum at room temperature for 3 h with periodic mild agitation. The fixed material was washed with four changes of the same buffer and transferred to a solution of 1% OsO_4 in buffer. Osmification was continued for 18 h at 4°C. Cubes were washed twice with phosphate buffer. The fixed tissue was dehydrated in graded concentrations of ethanol (*i.e.* 30, 50, 70% with 1% uranyl nitrate [overnight], 85 and 95%, twice with absolute). The cubes were further dehydrated with two changes of propylene oxide and then infiltrated with equal parts of propylene oxide and low-viscosity resin for 2 h under vacuum with periodic mild agitation (27). All samples were then embedded in 100% resin and polymerized for 12 to 16 h at 70°C. Ultrathin sections were mounted on parlodion-filmed nickel grids and poststained with lead citrate and iodine (30). Transmission electron microscopy was performed on a Philips EM-300 electron microscope operated at 60 kv.

RESULTS AND DISCUSSION

Alterations in Tissue Solids, Starch, Sugars, and Pi. Total solids content decreased from 18.8% in normal pith from healthy tubers to 12.3% in the translucent pith from defective tubers (Table I). Starch decreased from 14.3 to 6.2% on a fresh weight basis in the same tissue areas. Free sucrose and glucose concentrations increased 7- and 4-fold, respectively, in the translucent tissue compared to the normal pith cells. Pi also was elevated approximately 50% (27.1–40.3 mM) in the defective translucent area. Nontranslucent tissue taken from the defective tubers appeared normal visually but chemical changes were already apparent. Solids, starch, sugars, and Pi were slightly altered from those seen in the healthy tubers, suggesting that a shift in metabolism was already in progress.

Electron Microscopy of Amyloplasts. The starch grains of potato tubers have been shown to be surrounded by a double membrane typical of a plastid throughout its development (16) and after 1 to 9 months of storage (9, 29) at 2° to 4°C. The amyloplast membrane has been shown to be intact when the sugar content exceeded 2% on a fresh weight basis (9). It has been suggested that the amyloplast membrane may be important in regulating the flux of certain metabolites between the amyloplast stroma and the cytoplasm (9). In 'senescent' tubers which have broken dormancy and reach free sugar levels as high as 3%, the layers of the double membrane had been observed to separate and break away from the granule. This evidence of gross physical disintegration likely represents irreversible damage to the membrane causing it to lose its ability to regulate the flow of metabolites (9). Such decompartmentalization could result in the starch granule becoming accessible to degradative enzyme systems in the cell. It was anticipated that such irreversible membrane deterioration might have occurred in the translucent regions of the defective tubers resulting in the premature sweetening as documented in Table I. However, examination of the amyloplast membrane from the Kennebec tubers which had been stored at 10° ± 1°C for 8 months revealed that it was essentially intact and continuous around the starch granules observed in both the normal and translucent tissue (Fig. 1). Although the membrane appeared physically intact, this did not preclude alterations in fine structure, composition, or permeability.

Variation in Enzyme Activity between Normal and Translucent Tissue. Table II shows that there were no significant differences in the total units of UDPglucose pyrophosphorylase, invertase, and α-amylase activities between normal and translucent tuber areas ($P > 0.05$, *t* test). Sucrose synthase, which has been shown to be low in mature tubers (24), was negligible in all tissue areas. ADPglucose pyrophosphorylase was also not detected in significant amounts as this enzyme proved to be unstable when extracted from tissue that had been frozen in liquid N_2. The total activity of acid phosphatase was only slightly elevated in translucent tissue but its increase appeared significant ($P < 0.05$, *t* test). Although the total units of most enzymes listed in Table II were similar between normal pith and translucent pith areas, their specific activities were nearly doubled in the defective tissue. This increase in specific activities reflected a reduced concentration of soluble protein in the translucent pith. Total phosphorylase (Table III) and sucrose-6-P synthase (Table IV) activities were 5.4- and 3.8-fold greater, respectively, in the translucent tissue compared to that determined in healthy tubers. Their specific activities were also significantly elevated 3- to 4-fold.

Schneider *et al.* (21) previously noted, using immunofluorescence techniques, that potato phosphorylase was adsorbed onto the amyloplast during tuber development, and after 16 months of storage the particle bound enzyme had disappeared and cytoplasmic phosphorylase was detected. They provided evidence that cytoplasmic phosphorylase was a converted molecule formed by proteolytic cleavage and that senescence was accompanied by a change in the ultrastructure of the amyloplast membrane. Since only soluble phosphorylase was measured in this study, its 5.4-fold increase in apparent activity in the trans-

Table I. *Comparison of Starch, Sugars, and Pi between Healthy and Defective Kennebec Tubers*

Tissue Area and Tuber Type	Solids	Starch	Sucrose	Glucose	Pi
	% (g/100 g fresh tissue)			*mM*	
Healthy tuber					
Normal pith	18.8	14.3 ± 0.4[a] (5)[b]	4.1 ± 0.5 (13)	3.3 ± 0.9 (13)	27.1 ± 0.5 (6)
Defective tuber					
Nontranslucent pith	17.4	11.8 ± 0.2 (3)	5.6 ± 0.5 (7)	8.2 ± 0.5 (7)	30.0 ± 3.0 (6)
Translucent pith	12.3	6.2 ± 0.1 (3)	28.7 ± 2.0 (7)	13.7 ± 1.0 (7)	40.3 ± 4.0 (6)

[a] Mean ± SD. [b] Numbers in parentheses, number of experiments.

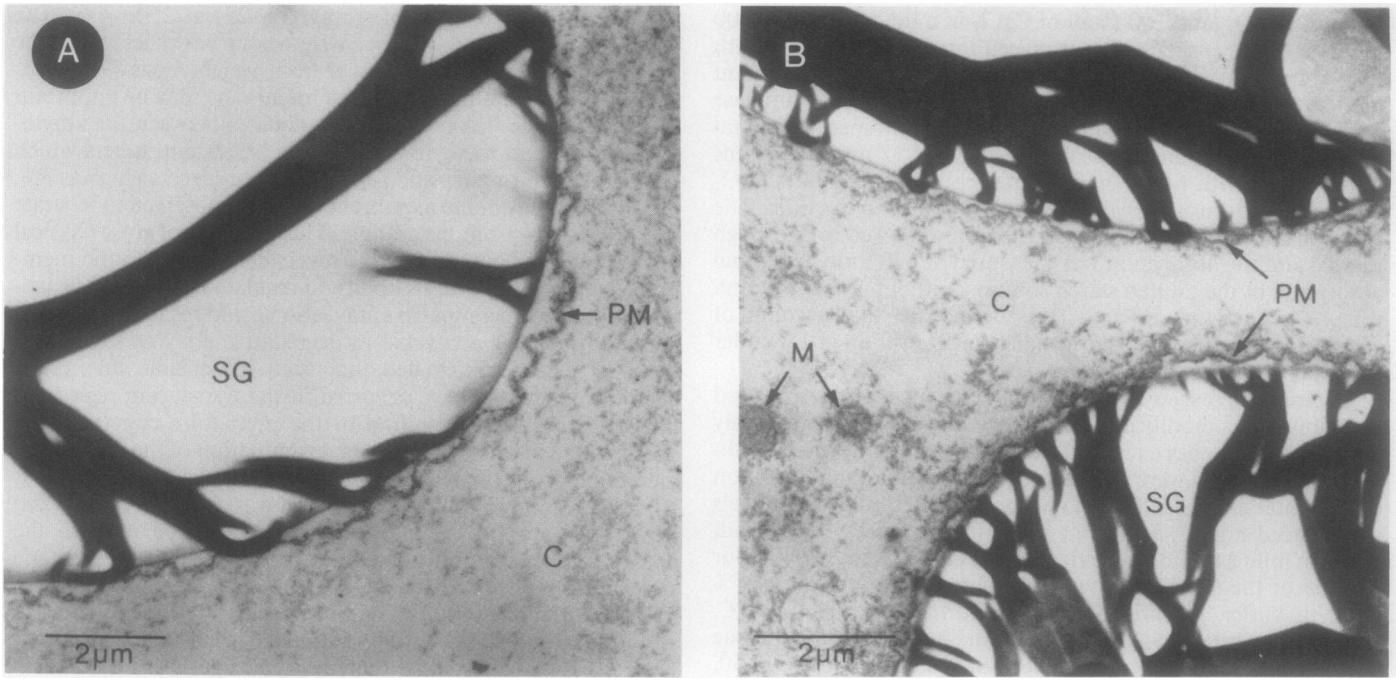

FIG. 1. Electron micrographs of starch granules (SG) in the cytoplasm (C) of differentiated parenchymal cells of Kennebec (cv) potatoes. Tubers were taken from storage after 8 months at 10° ± 1°C. Samples are representative of healthy, nonsweetened tissue (A) and translucent tissue (B) selected from defective tubers. Mitochondria (M) are evident along with starch granules surrounded by an intact amyloplast or plastid membrane (PM). Tissue preparation is described in "Materials and Methods."

Table II. *Comparative Enzyme Activities between Normal and Translucent Kennebec Tubers*

Enzyme	Tissue	Activity		Change in Total Activity Compared to Normal Tissue
		Total/10 g	Specific	
		units[a]	*units/mg protein × 10*	*-fold*
UDPglucose pyrophosphorylase	Normal	147 ± 19[b]	11.7 ± 0.1	
	Translucent	174 ± 25	18.3 ± 0.3	1.2
Sucrose synthase	Normal	0	0	
	Translucent	5.0 ± 0.4	0.5 ± 0.1	
Invertase	Normal	43 ± 3	3.5 ± 0.2	
	Translucent	49 ± 4	5.2 ± 0.5	1.1
α-Amylase	Normal	13.6 ± 2.5	2.3 ± 0.4	
	Translucent	11.3 ± 2.5	4.5 ± 0.9	0.9
Acid phosphatase	Normal	1.2 ± 0.2[c]	0.1 ± 0.02[d]	
	Translucent	1.5 ± 0.2[c]	0.2 ± 0.02[d]	1.3

[a] ± Units of activity for individual enzymes are indicated in the "Materials and Methods." [b] Mean ± SD. [c] Significant difference ($P \leq 0.05$). [d] Significant difference ($P \leq 0.10$).

Table III. *Phosphorylase Activity Compared between Healthy and Defective Kennebec Tubers*

Activity values are means ± SE of three determinations

Tissue Area and Tuber Type	Activity[a]		Change in Total Activity Compared to Normal Tissue
	Total/10 g	Specific	
	units (μmol Pi/min)	*units/mg protein × 10*	*-fold*
Healthy tuber			
Normal pith	6.4 ± 0.5[b]	0.83 ± 0.04[b,c]	
Defective tuber			
Nontranslucent pith	6.0 ± 1.8[c]	0.45 ± 0.14[b,d]	0.9
Translucent pith	34.5 ± 3.4[b,c]	3.02 ± 0.13[c,d]	5.4

[a] Identical superscripts (b, c, d) within columns indicate values are significantly different ($P \leq 0.05$, *t* test).

Table IV. *Sucrose-6-P Synthase Activity Compared between Healthy and Defective Kennebec Tubers*
Activity values are means ± SE of three determinations.

Tissue Area and Tuber Type	Activity[a]		Change in Total Activity Compared to Normal Tissue
	Total/10 g	Specific	
	units (μmol Sucrose-6-P/h)	*units/mg protein × 10*	*-fold*
Healthy tuber			
Normal pith	4.85 ± 1.14[b]	0.24 ± 0.05[b]	
Defective tuber			
Nontranslucent pith	8.02 ± 1.28[c]	0.36 ± 0.08[c]	1.7
Translucent pith	18.24 ± 1.35[b,c]	0.81 ± 0.10[b,c]	3.8

[a] Identical superscripts (b, c) within columns indicate activity values are significantly different ($P \leq 0.05$, *t* test).

lucent tissue might be due, in part, to an increased conversion and/or an increased solubilization of the intact molecule from the amyloplast membrane.

Sucrose-6-P synthase has been suggested to play an important role in regulating assimilate or sucrose biosynthesis from starch in plant leaves (20). The observation that this enzyme was increased 3.8-fold in the translucent tissue (Table IV) where sucrose and glucose content was elevated 7- and 4-fold (Table I), respectively, is suggestive that sucrose-6-P synthase may also play an important role in carbon partitioning in nonphotosynthetic potato tubers.

Significance of Pi Variation between Normal and Translucent Tissue. Recent data with nonphotosynthetic tissues has suggested that amyloplasts from maize endosperm (11), soybean (12), wheat grain (10), and potatoes (9) may be similar to leaf chloroplasts in the transfer of phosphorylated intermediates across their plastid membrane. In spinach, the main products of photosynthesis (*i.e.* 3-P-glycerate and dihydroxyacetone-P) are transported across the inner membrane in exchange for Pi via a phosphate translocator protein (4). The location of certain enzymes within the amyloplast, as noted in the hypothetical scheme seen in Figure 2, are inferred from their location in plant chloroplasts (*i.e.* particularly those enzymes involved in the reversible conversion of triose-P to Glc-1-P). Certain similarities between enzyme content and general metabolism might be anticipated since both organelles develop from proplastids and under certain conditions are interconvertible (15). The increased levels of Pi found in the translucent tissue (Table I) might be due, in part, to a slightly increased nonspecific acid phosphatase action on ester-P intermediates (Table II) and/or by a decreased rate of mitochondrial oxidative phosphorylation. Owings *et al.* (17) had previously shown that moisture stressed tubers produced increased levels of reducing sugars accompanied with a loss of respiratory ability. Elevated levels of Pi in the amyloplast stroma could inhibit the flow of carbon to starch via ADPglucose pyrophosphorylase (Fig. 2, reaction 4) (26) while serving as a substrate for starch phosphorylase (Fig. 2, reaction 6). We do not know the cellular localization of this increased Pi concentration in the sweetened tissue or how the levels of any organic-P components were changed. However, the net effect of elevated Pi in the translucent tuber tissue coupled with increased phosphorylase and sucrose-6-P synthase (Fig. 2, reaction 7) activities, could lead to an increased concentration of cytoplasmic precursors of sucrose and reducing sugar according to the theoretical scheme seen in Figure 2.

Nature of Translucent Sweetening. The accumulation of sugars in the translucent areas occurred at the expense of starch (Table I). Translucent sweetening appeared to be irreversible in nature similar to changes that occur during senescence (*i.e.* breaking of

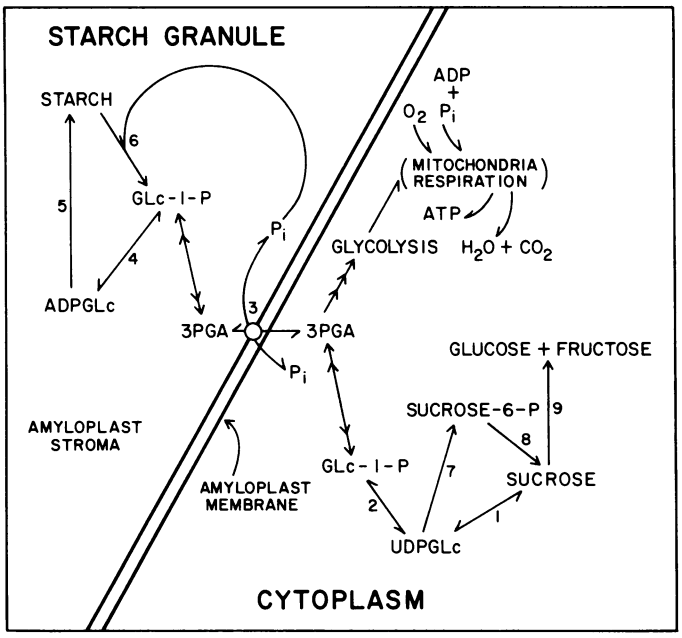

FIG. 2. Tentative scheme showing an interdependence of starch-sugar formation for triose phosphates in potato cells. Enzymic steps indicated are: (1), sucrose synthase; (2), UDPglucose pyrophosphorylase; (3), triose-P, inorganic phosphate translocator protein; (4), ADPglucose pyrophosphorylase; (5), starch synthase; (6), phosphorylase; (7), sucrose-6-P synthase; (8), sucrose-6-P phosphatase, and (9), invertase.

internal dormancy). This is in contrast to sweetening induced by anaerobiosis or cold stress which can be relieved to a degree depending upon the cultiver, time in storage, and the duration of the reconditioning period (3, 9). Events leading to this defect are still unknown but are suspected to be related to moisture and heat stress observed during the growing season. It has been suggested that changes in the intracellular compartmentalization of Pi regulated by phytohormone levels, membrane permeability, and metabolic processes could be of physiological importance in influencing carbon partitioning in potato tubers (26). Previously, elevated Pi levels had been shown to be associated with increased sugar levels in moisture (23) and cold stressed (8) potatoes. This study also indicates a possible coarse regulation of certain enzyme levels which may be indirectly related to an initial stress stimulus. Although amyloplast membranes appeared physically intact (Fig. 1) in the sweetened tissue, it was possible that changes in the composition, fine structure, and function of cellular membranes (*i.e.* amyloplast, mitochondria, vacuolar, etc.) may have been

induced by the stress environment. Membrane alterations might contribute to the premature sweetening and lead to the eventual cellular deterioration noted in these translucent areas.

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