Distribution of Amylases within Sweet Potato (*Ipomoea batatas* L.) Root Tissue

Vital Hagenimana,† Louis-P. Vézina,‡ and Ronald E. Simard*,†

Département de Sciences et Technologie des Aliments, Université Laval, Ste-Foy, Québec G1K 7P4, Canada, and Station de Recherche, Agriculture Canada, 2560 Boulevard Hochelaga, Ste-Foy, Québec G1V 2J3, Canada

To understand the effect of various processing techniques on starch hydrolysis in the sweet potato, α-amylase, β-amylase, and starch phosphorylase were localized inside the roots by a combination of immunological detection and activity measurements. Antibodies raised against α-amylase, β-amylase, and starch phosphorylase were used for the detection of immobilized proteins from tissue prints. Distribution of α-amylase, β-amylase, and starch phosphorylase was also assessed by measuring the activity in the outer and inner portion of the root following mechanical separation. α-Amylase tissue-print immunoblots show that this enzyme is strongly localized in the laticifers and in the cambium layers, and very little in storage parenchyma tissues. The tissue prints with anti-β-amylase demonstrate that this enzyme is ubiquitously distributed throughout the root. Starch phosphorylase immunoblots show that it is concentrated in the anomalous cambium and in the vascular cambium. Furthermore, sectional distribution in all three enzyme activities confirmed results obtained by immunoblotting. The tissue printing technique was simpler, quicker, and usually more precise in screening for the presence of amylases within the root than the activity measurement. The different distributions, particularly that of thermostable α-amylase in the outer tissues of the root, may be responsible for rapid starch hydrolysis on the surface during lye peeling, or the discoloration of the cambial and laticifers zones during 6-min lye peel treatment of sweet potato roots.

INTRODUCTION

Endogenous amylases play an important role in the storage and processing of sweet potato roots (Deobald et al., 1968; Hoover, 1967; Walter et al., 1975). These enzymes cause starch breakdown during cooking, and their control results in the best combination of saccharification and physical properties in the preparation of processed products. Hoover (1967) reported an enzyme-activation technique for producing sweet potato flakes from starchy and freshly harvested roots without adding extraneous saccharifying enzymes. He showed that in sweet potato puree, starch conversion was directly related to amylase concentration and that starch was not converted to maltose or dextrins until the temperature was above that for starch gelatinization. Gelatinization occurs during heating of the puree, allowing the starch to become more susceptible to attack by endogenous enzymes present in the roots. β-Amylase is unable to attack ungelatinized starch, and α-amylase is the only hydrolase capable of attacking native starch granules (Dunn, 1974; Halmer, 1985). Sweet potato starch gelatinizes at 73–75 °C (Takeda et al., 1986), and β-amylase and starch phosphorylase, which can also intervene in gelatinized starch hydrolysis, are denatured at that temperature range (Nakayama and Kono, 1958; Chang et al., 1987).

Walter et al. (1976), while studying the effect of peeling method on the starch conversion process, found that significant starch hydrolysis occurred during lye peeling. The lye peeling process is a typical and critical step in the commercial processing of the sweet potato and involves soaking the intact sweet potatoes in a 10% NaOH solution at 103 °C for about 5–6 min, followed by washing in water. Very little maltose is formed in hand-peeled puree which is steam injected at 103 °C, thus eliminating heat alone as the causative agent of starch hydrolysis. Chang-Rupp and Schwartz (1988), using high-performance size-exclusion chromatography, found that the profile of starch polysaccharides from the centers of lye-peeled sweet potatoes, where little or no heat penetration occurred, resembled the profile of those from hand-peeled sweet potatoes. This observation suggests that the principal hydrolyzing enzymes are probably located in the outer layer removed in hand peeling. However, Ikemiya and Deobald (1966) reported that the sweet potato α-amylase, which alone is active at 73–75 °C, is distributed throughout the inner tissues of the sweet potato root, with a minor concentration in the outer layer and skin portion of the root.

It seems imperative to localize with precision the most important starch hydrolyzing enzymes in root tissue, since their utilization in processing involves heating roots in any form to 60–80 °C, to gelatinize the starch fraction, and to facilitate amylase activity.

Recently, it has been shown that tissue prints on nitrocellulose membranes reveal surprising amounts of anatomical detail (Cassab and Varner, 1987; Ye and Varner, 1991). The tissue sections are pressed onto nitrocellulose paper to make an imprint that can be used for immunological studies. This technique can be used to study the localization of proteins, as long as they transfer to nitrocellulose during the printing process. del Campillo et al. (1990) used this technique to demonstrate areas of cellulase localization within *Phaseolus vulgaris* explants.

Since many amylases are water-soluble proteins (Fischer and Stein, 1960), we have attempted in this study to examine the anatomical distribution of sweet potato α-amylase, β-amylase, and starch phosphorylase in the root by tissue-print immunoblots. These results were compared with the more crude determinatioin of activity in section.

MATERIALS AND METHODS

Plant Material. Vines of Porto Rico, Regal, White Delight, and Jewel sweet potatoes were a kind gift from Professor William

† Université Laval.
‡ Agriculture Canada.

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Table I. Purification of α-Amylase

purification steps	total volume, mL	activity, units/mL	protein, mg/mL	specific activity (units/mg of protein)	total activity, units	yield, %	purification
crude extract	400	0.296	1.247	0.237	118.40	100	1
20–80% ammonium sulfate precipitation	40	2.598	4.169	0.623	103.92	87.8	2.62
affinity chromatography (pooled fractions)	7.5	6.627	0.067	98.910	49.70	42.0	417.4
Sepharose 4B (pooled fractions)	15	2.825	0.018	156.944	42.38	35.8	662.1

Table II. Purification Steps for β-Amylase

purification steps	total volume, mL	activity, units/mL	protein, mg/mL	specific activity, (units/mg of protein)	total activity, units	yield, %	purification
crude extract	350	506.7	3.447	147.01	177345	100	1
20–80% $(NH_4)_2SO_4$ precipitation	35	3811.6	10.601	359.55	133406	75.2	2.44
Sephadex G-50	50	2383.0	5.513	432.20	119150	67.2	2.93
affinity chromatography (pooled fractions)	8	545.9	0.570	957.71	4367	2.4	6.51
Sepharose 4B (pooled fractions)	10	822.9	0.230	3577.80	8229	4.6	24.33

M. Walter from North Carolina State University. They were grown in a greenhouse for 160 days. Sweet potato roots 1 or 2 in. long of each variety were maintained under ambient air conditions, washed, and used 3 days after harvest.

Antibody Production. Sweet potato α- and β-amylases were purified as follows:

Enzyme Extraction. Sweet potato roots (Regal variety) were thoroughly washed in water and sliced. A 150-g amount of sliced sweet potato was homogenized in a Waring blender for 1 min with 450 mL of cold 20 mM sodium phosphate buffer (pH 6.0), containing 0.3% NaCl (w/v), 0.2% $CaCl_2$ (w/v), and 0.005 M β-mercaptoethanol, and then filtered through four layers of cheesecloth. This extract was centrifuged for 10 min at 13200*g*. The supernatant was removed and kept on ice. All subsequent manipulations were performed at 4 °C.

Ammonium Sulfate Precipitation. Ammonium sulfate was slowly added to the crude extract with constant stirring to 20% saturation, and the extract centrifuged for 20 min at 13200*g*. After discarding the precipitate, the ammonium sulfate concentration of the supernatant was slowly increased to 80% saturation. After centrifugation at 13200*g* for 20 min, the precipitate was collected, dissolved in 35 mL of sodium phosphate (pH 6.0), and centrifuged again for 15 min at 15000*g* to remove insolubles. The supernatant was desalted by a passage through a Sephadex G-50 column equilibrated with 50 mM sodium acetate, pH 5.0 (β-amylase purification buffer), or with 50 mM sodium acetate (pH 6.0), 1 mM $CaCl_2$, and 0.04% NaCl (α-amylase purification buffer).

Affinity Chromatography. For β-amylase purification, the Sephadex G-50 column extract was applied to a cyclohexaamylose–Sepharose affinity chromatography column prepared as described by Vretblad (1974). The loaded column was washed with β-amylase buffer until the effluent was protein free. Bound protein was eluted with the β-amylase buffer containing 10 mg/mL cyclohexaamylose. Cyclohexaamylose was then removed by applying the enzyme solution through a Sepharose 4B column after concentration with a centriprep-30 (Amicon Canada Ltd., Oakville, Canada).

α-Amylase was purified by affinity chromatography on cycloheptaamylose–Sepharose as described by Silvanovich and Hill (1976), using a column equilibrated with α-amylase buffer. Eluted α-amylase was concentrated and the cycloheptaamylose removed on a Sepharose 4B column as described above.

Protein Quantification. Total proteins were quantified by the method of Bradford (1976), with BSA as standard.

α-Amylase Assay. α-Amylase activity was assayed as described by Hall et al. (1970), with a procedure based on hydrolysis of amylose azure (20 mg/mL). The amylose azure suspension was prepared in 20 mM sodium phosphate (pH 6.0), 2 mM $CaCl_2$, and 0.005 M β-mercaptoethanol. Assays were conducted at 40 °C for 10 min, and the reaction was stopped by addition of 1.2 mL of 18% acetic acid (v/v). One unit (U) of activity was defined as the amount of enzyme liberating a quantity of colored, soluble material corresponding to 2.5 absorbance units at 595 nm from 54 mg of amylose azure under defined assay conditions.

β-Amylase Assay. β-Amylase activity was assayed as described by McCleary and Codd (1989). Aliquots (0.2 mL) of the extracts (or dilutions in 100 mM sodium malate, pH 5.5) were incubated with 0.2 mL of substrate mixture (*p*-nitrophenyl maltopentaoside, 5 mM; α-glucosidase (20 U), in distilled water), in the presence of Na_2–EDTA (1 mM), BSA (1 mg/mL), and NaN_3 (3 mM) at 40 °C for 10 min. The reaction was terminated and color developed by adding 1% (w/v) Trizma base (3.0 mL), and the absorbance was measured at 410 nm. One unit (U) of enzyme was defined as the amount of enzyme releasing 1 mmol of *p*-nitrophenol per minute under defined assay conditions.

Gel Electrophoresis. SDS–PAGE was performed as described by Laemmli (1970) with a 12.5% separating gel and a 4.65% stacking gel.

Antibody Production. Rabbits were injected subcutaneously at multiple sites. The two first injections were made at 4-week intervals with Freund's complete adjuvant. One additional injection was made 6 weeks later with incomplete adjuvant. Final sera were collected 15 weeks after initial injections. Potato starch phosphorylase antibody was kindly provided by Dr. N. Brisson, Université de Montréal (Brisson et al., 1989).

Tissue Prints. Nitrocellulose sheets (Bio-Rad) were first soaked in a small volume of phosphate buffer saline (PBS) (pH 7.4) and air-dried. Cross sectioning was always carried out through the root, and fresh cut sections were blotted onto the nitrocellulose paper for 20 min at 4 °C. Nitrocellulose tissue prints were immediately washed three times in 75 mL of a solution containing 5% skim milk powder (Carnation), 0.01% Antifoam A (Sigma, No. A-5758), and 0.001% merthiolate (Sigma, No. T-5125) in PBS (pH 7.4) to block protein-binding sites. Prints were incubated for 1 h at 37 °C with 100 mL of each antiserum (anti-β-amylase, anti-α-amylase, and anti-starch phosphorylase). Nitrocellulose tissue prints were also incubated in blotting solution with nonimmune serum as blanks. Each nitrocellulose print was then washed three times with 75 mL of PBS and again three times with 75 mL of blotting solution. Prints were then incubated with 75 mL of rabbit peroxidase-conjugated swine anti-rabbit (Dimension Laboratories Inc., Mississauga, Canada) solution for 4 h at room temperature and washed with Tris buffer saline (TBS) (pH 7.5). Finally, peroxidase activity was revealed by incubation in 10 mL of 0.6% (w/v) of 4-chloro-1-naphthol in methanol with 50 mL of 0.08% (v/v) H_2O_2 in TBS, at room temperature. The reaction was stopped after 30 s by washing the prints in 200 mL of distilled water.

Sectional Distribution of Starch Hydrolyzing Enzyme Activity. Roots (ca. 300 g) were hand-peeled (mean amount removed represented 22%); the peeled outer layer contained all of the cortical region (about 0.3 cm), which included the periderm, laticifer, and cambium. The inner portion was the remaining tissue. The appropriate sections were combined from all the roots, chopped, and homogenized in a Waring blender in the presence of 50 mM sodium citrate (pH 6.9), containing 2 mM DTT and 0.1 mM EDTA. Sweet potato α- and β-amylase activities were determined as described above in each fraction. Starch phosphorylase activity (synthetic) was measured as described by Chang and Su (1986), with soluble starch as primer. The reaction mixture contained 0.05 mL of 20 mM glucose-1-phosphate (Sigma, No. G-6875), 0.15 mL of 0.3% (w/v) soluble starch and 0.2 mL of sweet potato extract, in a total volume of 0.5 mL. Incubation was at 40 °C for 5 min in 40 mM Mes-NaOH buffer (pH 5.9), and the reaction stopped by adding 0.5

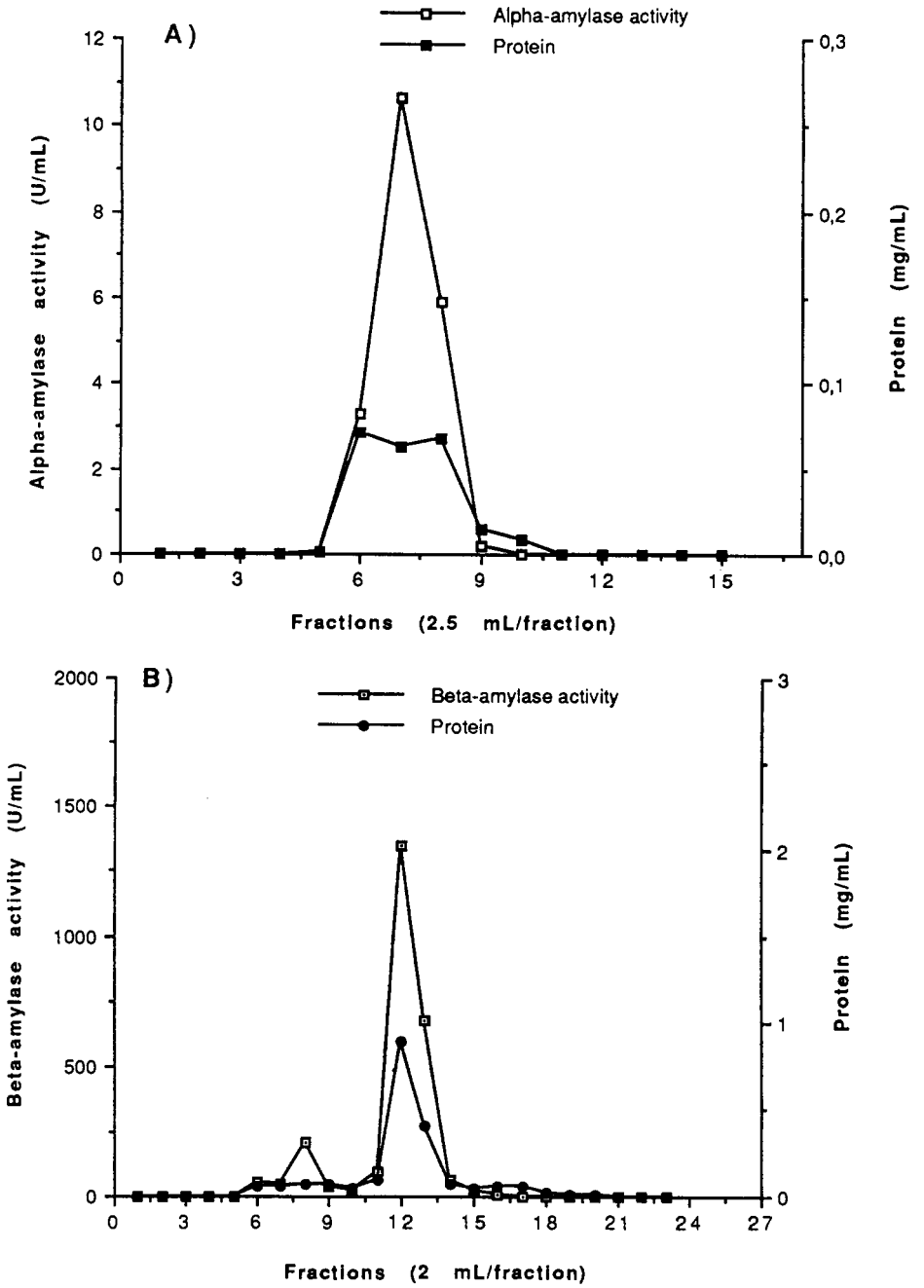

Figure 1. Elution profile after affinity chromatography.

mL of TCA (5%). A 0.5-mL sample of this mixture was withdrawn, clarified by centrifugation, and used for determination of inorganic phosphorus by the method of Fiske and Subbarow (1925).

RESULTS

Amylase Purification. Cycloamylose affinity chromatography (CAC) was the most effective step in the purification of sweet potato amylases, resulting in a 417-fold and 6.5-fold purification of α- and β-amylases, respectively. The recovery of amylase activity after CAC, as a percentage of total activity, was 42% for α-amylase (Table I) and only 2.4% for β-amylase (Table II). One peak was observed in the elution of α- or β-amylase (Figure 1). The most active fraction had a specific activity of 168.5 units/mg of protein for α-amylase or 1529 units/mg of protein for β-amylase. However, these measured activities were inaccurate due to the presence of cycloamyloses, known inhibitors of amylases, in eluates (Nomura et al., 1986).

Cycloamyloses were removed from eluted samples by Sepharose 4B gel filtration. Gel filtration of sweet potato amylases also resulted in significant increases in enzyme purity. SDS–PAGE analysis of the two amylases preparations from CAC column showed two contaminating peptides (data not shown) which were eliminated by gel filtration.

Ultimately sweet potato α-amylase was purified 662-fold while β-amylase was purified 24-fold. The high degree of purity attained is evident from SDS–PAGE (Figure 2), which showed a single band for the final preparations of α-amylase and β-amylase. These preparations were examined with respect to their ability to hydrolyze a variety of specific substrates (Table III). α-Amylase preparation was able to hydrolyze amylose azure which is specific to α-amylase (Doehlert and Duke, 1983), while the ability to hydrolyze *p*-nitrophenyl maltopentaoside is specific to β-amylase (McCleary and Codd, 1989). In addition, none of these purified amylase preparations hydrolyzed *p*-ni-

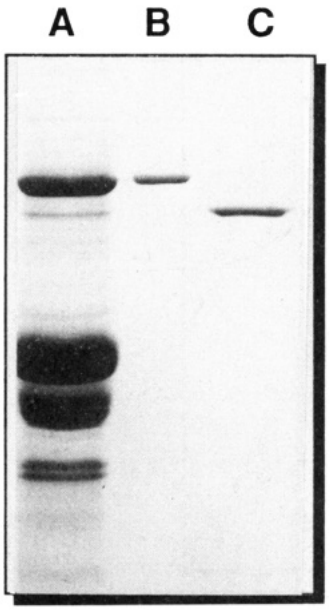

Figure 2. SDS–PAGE analysis of sweet potato amylase preparation. Lane A, α-amylase preparation (5 μg); lane B, β-amylase preparation (5 μg); lane C, 20–80% ammonium sulfate precipitate (20 μg).

Table III. Sweet Potato Amylase Substrate Specificity

substrate	α-amylase	β-amylase
amylose azure	+	0
boiled sweet potato starch[a]	+	+
p-nitrophenyl glycoside	0	0
p-nitrophenyl maltopentaoside	0	+
soluble potato starch	+	+
sweet potato starch granules[b]	0	0

[a] Starch granules were maintained at 100 °C for 3 min before assaying with sweet potato amylase preparations. [b] Incubated for 1 h at 40 °C. Combination of α- and β-amylase liberated any reducing sugar.

Table IV. Effect of Temperature and Nature of Starch on Sweet Potato Amylases

	boiled sweet potato starch[a]		sweet potato starch granule[b]	
temp, °C	α-amylase, μg of glucose equiv	β-amylase, μg of glucose equiv	α-amylase, μg of glucose equiv	β-amylase, μg of glucose equiv
40	13.2	15.8	0	0
50	30.9	28.9	0	0
60	59.3	21.6	0	0
70	63.7	10.0	0	0
80	12.4	1.6	0	0

[a] Purified sweet potato amylases (or dilutions) were maintained with 1% starch mixture (w/v) for 15 min, pH 6.0, and liberated amount of reducing sugars determined as described by Bernfeld (1955). [b] Incubated for 1 h.

trophenyl glycoside (Table III), indicating that these enzymes do not belong to the α-glucosidase group.

We also found that purified amylases were unable to hydrolyze native sweet potato starch granules after 1 h of incubation at different temperatures (Table IV). However, boiled starch granules were hydrolyzed readily at rates similar to those found when soluble starch served as substrate. Buléon et al. (1989) reported that the high degradability of gelatinized starch is due to the increase in its water absorption capacity, which results in enhanced accessibility to enzymes.

Sectional Distribution of Amylolytic Enzyme Activity. (*a*) *α-Amylase.* The α-amylase tissue-print immunoblots (Figure 3)show that this enzyme is strongly

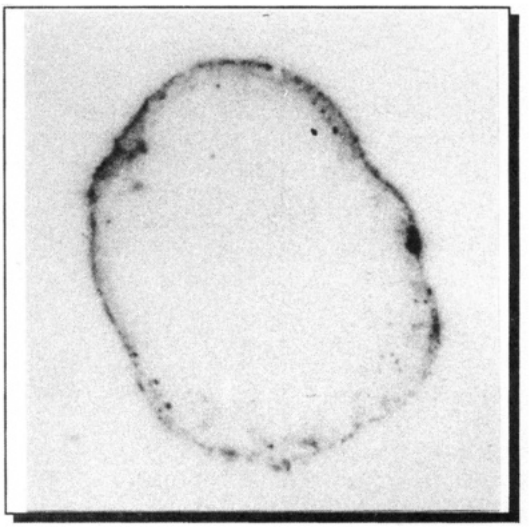

Figure 3. Localization by tissue print immunoblot of α-amylase protein in the outer layer from a freehand cross section of Regal sweet potato root.

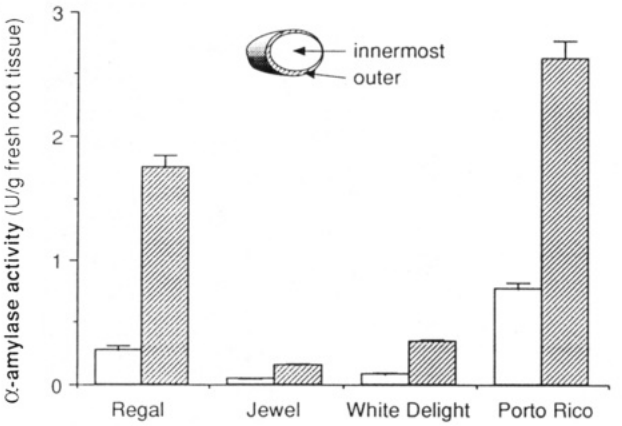

Figure 4. Sectional distribution of α-amylase activity in four sweet potato varieties. Lines on each bar represent standard deviations of the data. Data are for three repetitions.

localized in the periderm, the vascular cambium, and the anomalous cambium. Our results disagree with those obtained by Ikemiya and Deobald (1966), who reported that α-amylase is distributed uniformly throughout the inner tissues, with the outer layer and skin being low in that enzyme. There is a good correlation between the α-amylase activity determined for inner or outer tissues of the roots and the tissue-print immunoblots (Figures 3 and 4). The highest level of α-amylase activity was found in the outer tissue of the root for all cultivars (Figure 4). The α-amylase activity in the outer tissue was 6 times that of the inner tissue in Regal, 3 times in Jewel and Porto Rico, and 4 times in White Delight. Porto Rico showed the highest α-amylase activity and Jewel the lowest.

(*b*) *β-Amylase.* Tissue immunoblots with anti-β-amylase show that this enzyme is abundant and well distributed within sweet potato root tissue (Figure 5), in agreement with the report of Nakamura (1991). Contrary to α-amylase activity, β-amylase activity was significantly ($P < 0.01$) higher in the inner than in the outer tissues of the roots (Figure 6), with the exception of White Delight, where no difference was observed between the outer and the inner tissues. β-Amylase activity in the inner tissue was found to be 1.5–2.5 times higher than in the outer tissues. Porto Rico had the highest β-amylase activity, and Jewel the lowest.

(*c*) *Starch Phosphorylase.* Nitrocellulose tissue print immunoblotted with starch phosphorylase antibody show

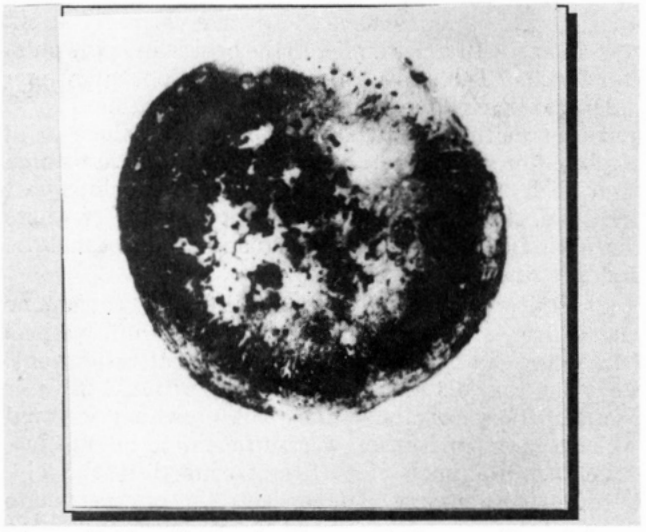

Figure 5. Localization by tissue-print immunoblot of β-amylase protein in all tissues from a freehand cross section of Regal sweet potato root.

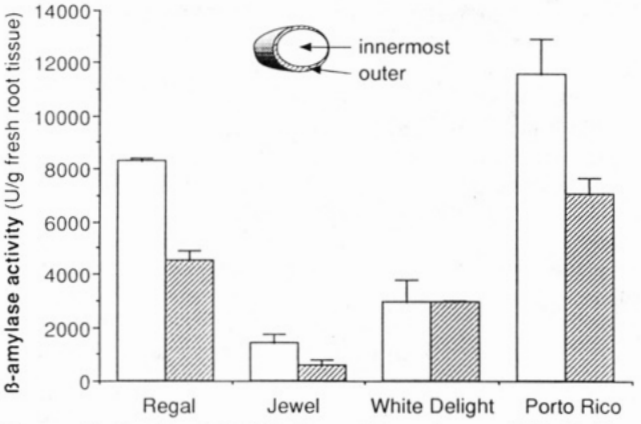

Figure 6. Sectional distribution of β-amylase activity in four sweet potato varieties. Lines on each bar represent standard deviations of the data. Data are for three repetitions.

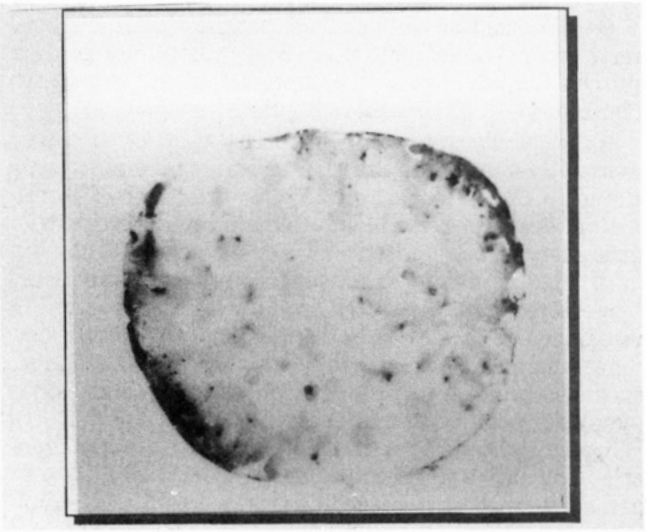

Figure 7. Localization by tissue-print immunoblot of starch phosphorylase protein from a freehand cross section of Regal sweet potato root.

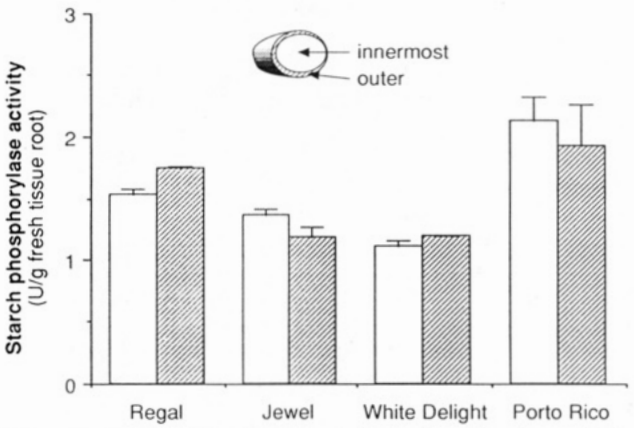

Figure 8. Sectional distribution of starch phosphorylase activity in four sweet potato varieties. Lines on each bar represent standard deviations of the data. Data are for three repetitions.

that starch phosphorylase (Figure 7) is concentrated in anomalous cambium and in the vascular cambium. Starch phosphorylase activity in the inner tissues was not statistically different from that of the outer tissues, and in general, its activity was low in all cultivars compared to other amylases (Figure 8). Porto Rico had the highest activity while White Delite had the lowest. There is no apparent correlation between the starch phosphorylase tissue-print immunoblots and the activity determined. Starch phosphorylase tissue prints show a lot of protein in the root while the measured activity seems low. The advantage of the tissue printing method is that it shows exactly where the enzyme is localized, even if not active, while the activity measurement measures only the active protein and does not show its localization.

DISCUSSION

In this paper, we have studied the occurrence and distribution of α-amylase, β-amylase, and starch phosphorylase in sweet potato roots by detection on nitrocellulose tissue prints using antibodies raised against each enzyme. The specific antibodies distinguished each hydrolytic enzyme on tissue prints from a large number of other proteins and from brown polyphenolic compounds.

According to Walter and Schadels' (1982) description of the sweet potato root tissues, we have shown with tissue-print immunoblotting (Figure 3) that α-amylase accumulates in the area of the tissue located between the periderm and cambium, in lacticifer, in cambium and in xylem elements, but little in storage parenchyma tissue.

Our results from the distribution of β-amylase show that this enzyme is abundant throughout the root. These results agree with those reported by Okamoto and Akazawa (1979) and with those of Laurière et al. (1986), who found that β-amylase was uniformly associated with the periphery of starch granules in the starchy endosperm cells of rice and barley seeds. We also showed that β-amylase activity is higher in inner than in outer tissues of the root, in agreement with the report of Ikemiya and Deobald (1966). However, the tissue prints with anti-starch phosphorylase show that this enzyme is located predominantly in the vascular cambium and in the anomalous cambium. The lack of correlation between the starch phosphorylase activity determined and the tissue-print immunoblots could have a double explanation: first, phosphorylase was found to be activated by germination (Abbott and Matheson, 1972) and thus could have been inactive in certain root sections; second, β-amylase, which is widespread throughout the root (Figure 6), has been reported to noncompetitively inhibit starch phsophorylase by actually combining with it (Porter, 1950; Pan et al., 1988).

Chang et al. (1987) reported that sweet potato starch phosphorylase as well as its noncompetitive inhibitor β-amylase was localized in the amyloplasts of the roots. It

is possible that at cellular level both are located in the same compartment, but that some cells do not contain starch phosphorylase. That would plead for starch phosphorylase to be a more regulated enzyme.

Some comparisons can be made between sweet potato roots and cereal seeds. Starch in seeds is characteristically stored in the endosperm, which, at the onset of germination, does not contain suitable enzymatic breakdown machinery. The required enzymes are either not present in a predominantly inactive form or are effectively sequestered from the starch granules. Reserve starch in cereal seeds is thought to be degraded only hydrolytically. Since maltose and especially glucose are the almost exclusive breakdown products, phosphorolysis does not play any significant role (Ashford and Gubler, 1984). Whereas α-amylase is the only hydrolase capable of attacking native starch granule (Manners, 1985), it is not present in resting cereal seeds, with the possible exception of some activity packaged in lytic bodies in maize and *Sorghum* endosperm (Adams et al., 1975). It must be synthesized by living tissues and secreted into endosperm upon germination. The scutellum or aleurones layers are thought to synthesize and secrete it during germination (Halmer, 1985).

In sweet potato roots, the starchy parenchyma tissue is poor in α-amylase, while β-amylase is abundant throughout the root, where it has an intense inhibition on starch phoshorylase (Pan et al., 1988) so that, as in cereal seeds, phosphorolysis does not seem to play any significant role in starch breakdown. In connection with secretion of α-amylase, the presence of α-amylase concentrated in cambium and the laticifers layers (Figure 3), make us believe that there is some physiological similarity between those layers and cereal scutella or aleurone layers. However, further investigations on α-amylase synthesis and its evolution in sweet potato root starch parenchyma during germination are needed to confirm that.

Our results, particularly with α-amylase, are of interest for another two practical reasons:

(1) The lye peeling treatment is a commercial process where the intact sweet potatoes remain in contact with a 10% NaOH solution at 103–140 °C for about 5–6 min to slough off their peel. When lye-peeled sweet potatoes are immediately pureed, and maltose and dextrins instantaneously analyzed, compared to those of hand-peeled, it has been found that their maltose and dextrins are very elevated. It was thought that lye peeling was responsible for the high maltose and dextrin levels, or that the unheated interior of the root rapidly attacked the gelatinized starch from outer tissue.

We think that the concentration of thermostable α-amylase in the outer tissues of the root (Figure 4) is mainly responsible of rapid starch hydrolysis during lye peeling and elevates the content of maltose and dextrins.

It is known that β-amylase has no action on partially digested starch granules and has very little effect on the rate of starch digestion by α-amylase (Dunn, 1974), although the synergistic hydrolysis of granules could occur when β-amylase is combined with α-amylase (Maeda et al., 1978). In sweet potatoes, endogeneous β-amylase levels do not directly affect the amount of maltose produced during baking. Although the relationship between α-amylase activity and maltose or dextrin formation in baked sweet potato roots is complex, α-amylase is related to starch conversion (Walter et al., 1975).

Furthermore, calcium is required for activity and thermal stability of α-amylase (Fincher, 1989; Vallee et al., 1959), and Makki et al. (1986), using two Egyptian sweet potato varieties, have shown that calcium concentration was 6–10 times higher in the peel than in the pulp. Our results (Table IV) showed that the optimum temperature range of sweet potato α-amylase is around 70 °C and that neither β-amylase nor α-amylase alone or in combination could hydrolyze native sweet potato starch after a 1-h reaction time. Thus, during lye peeling, heat and alkali gelatinize starch of the root outer layers, where thermostable α-amylase is located and produces maltose and dextrins.

(2) Walter and Schadel (1982) reported that among several lye peeling treatments, only the 6-min lye peel formed the cambial and the laticifers discoloration zone, and that this discoloration occurred within 1 h after peeling. They concluded that this browning occurred because heat penetration was sufficient to disrupt laticifer organization, but insufficient to inactivate the polyphenol oxidase enzyme. The concentration of sweet potato thermostable α-amylase around the cambium and in the laticifers as observed in the current study supports that view to some extent.

The discoloration or darkening may be attributed to the reaction between polyphenol oxidase and *o*-dihydroxyphenols, as was well demonstrated in sweet potato roots by Walter and Schadel (1982), or to a Maillard type reaction between reducing sugars and amino acids.

It is known that the normal end products of α-amylase from starch are reducing sugars, especially maltose, maltotriose, and glucose, together with branched oligosaccharide α-dextrins (Manners, 1974). These end products have been associated equally with the degree of heat treatments above or near the gelatinzation temperature of sweet potato starch and with the amount of α-amylolytic activity in the puree (Deobald et al., 1969). Interaction of reducing sugars and amino compounds in browning reactions was described by Habib and Brown (1956) and by Hodge (1953). Otherwise, Woodroof (1988) reported that the phenolase enzyme can be inactivated at approximately 200 °F (93 °C) and that the browning phenomenon is somewhat different with sweet potato in that browning occurs primarily in the cambial area, located about $^1/_8$ in. (0.32 cm) beneath the surface of the root. Comparing those reports with our results, we concluded that after 6-min lye peeling of the sweet potato, the gelatinized starch is hydrolyzed by the outermost thermostable α-amylase of the root into reducing sugars that subsequently form brown products with amino acids in Maillard type reaction.

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Registry No. NaOH, 1310-73-2; α-amylase, 9000-90-2; β-amylase, 9000-91-3; starch phosphorylase, 9035-74-9.