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THE ACCUMULATION OF SUGARS IN POTATO TUBERS AT LOW TEMPERATURE AND SOME ASSOCIATED ENZYMATIC ACTIVITIES

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Abstract—A comparison was made of the activities of several enzymes involved in sugar transformations in potatoes stored both at low temperature and at room temperature. Whole tubers were used, and a technique comparing halves of the same potato was developed. The potato halves and the whole tubers showed similar patterns except the halves went through the pattern more rapidly. The enzymes studied were phosphohexose isomerase, glucose-6-phosphate dehydrogenase, aldolase, and invertase. Within 4 days sucrose accumulated in the potato halves at low temperature followed by increases in reducing sugars. The results could not be explained by a simple lowering of respiration rate at low temperatures. A probable sequence of events appears to be that low temperature induces in the tuber (1) a temporary decrease in aldolase activity, (2) an increase in sucrose, (3) increased invertase activity, (4) accumulation of reducing sugars, followed by (5) a lowering of the activity of phosphohexose isomerase.

INTRODUCTION

SINCE the observation by Müller-Thurgau[1] that potatoes kept at temperatures between 0° and 6° accumulated sugars with a corresponding loss of starch, a number of explanations have been advanced for this phenomenon. A major effort has been an attempt to correlate this finding with the activity of the enzymes involved in starch–sugar interconversions. A comparison of the activity of phosphorylase, phosphatase, and β-amylase in cold-stored potatoes and potatoes at room temperature was made by Arreguin-Lozano and Bonner.[2] Similar studies of the enzymes UDP-glucose: fructose transglucosidase[3] and invertase[4] have been made. So far no significant difference has been found in comparisons of the activity of these enzymes in extracts prepared from cold-stored potatoes and those kept at room temperature (23–25°).

The work described in this report is a study of four enzymes involved in sugar transformations. The enzymes are phosphohexose isomerase (EC 5.3.1.9) glucose-6-P dehydrogenase (EC 1.1.1.49), aldolase (EC 4.1.2.7), and invertase (EC 3.2.1.26). Phosphohexose isomerase catalyses the isomerization of glucose-6-P to fructose-6-P, and it is very likely to be involved in the conversion of the glucosyl units of starch into the fructosyl unit of sucrose.[2,5] Machlachlan and Porter[6] have stated that 30 per cent of the glucose in tobacco leaf discs is metabolized by the pentose pathway. Glucose-6-P is a key intermediate in both this pathway

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and in the glycolytic sequence which starts with starch. Glucose-6-P dehydrogenase has been studied in the present work since this could be the enzyme responsible for shifting glycolytic intermediates into the pentose pathway. Aldolase was studied because of its role in the breakdown of hexose to trioses and thus would be important in determining the hexose concentration. Invertase was examined because of its obvious relationship to the conversion of sucrose to free hexoses.

RESULTS

Whole Potato Experiments

Phosphohexose isomerase. The demonstration of this enzyme in the crude potato extract was not easy to achieve. A phosphatase was present which degraded the glucose-6-P added

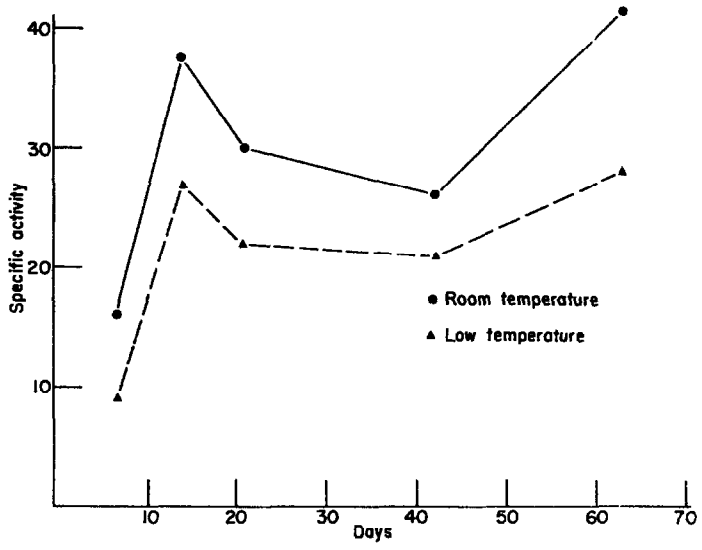

FIG. 1. COMPARISON OF THE ACTIVITY OF PHOSPHOHEXOSE ISOMERASE IN WHOLE POTATO TUBERS STORED AT LOW (2°) AND ROOM TEMPERATURE (23–25°).

The specific activity is defined as the mg fructose produced mg protein/min.

as substrate even in the presence of inhibitors such as NaF or EDTA. Increasing the amount of substrate and saturating both the phosphatase and the isomerase overcame this difficulty. Under these conditions the rate of the reaction was directly proportional to the amount of potato extract added. The specific activity of the isomerase in extracts from low (2°) and room temperature (23–25°) potatoes was compared over a period of 9 weeks (Fig. 1). Even though there was a large variation in activities between individual potatoes, within 7 days there was a decrease in activity in the cold-stored potatoes of 25–35 per cent compared to that in potatoes at room temperature. No further decrease was observed for the rest of the 9 weeks.

Glucose-6-P dehydrogenase. Under the conditions of the enzyme assay used, the oxidation of glucose-6-P was proportional to the volume of enzyme extract added. Comparisons of the specific activity of the enzyme in extracts from potatoes stored at the two temperatures showed no conclusive differences. However, a decrease in specific activity occurred with time at both storage temperatures. After 2 or 3 weeks at room temperature the potatoes sprouted, and at

this time the dehydrogenase activity dropped suddenly to between 15 and 30 per cent of the pre-sprouting activity.

Aldolase. The aldolase activity was directly proportional to the volume of extract used under the conditions of the enzyme assay. The weekly sampling showed that no change of aldolase activity could be detected at either storage temperature.

Sugar analysis. Analysis of the sugars in the extract was carried out qualitatively by paper chromatography and quantitatively by the Saffer–Somogyi micromethod.[7a] As reported previously [2,7b] sucrose was the most prevalent disaccharide and glucose and fructose were found as free hexoses. After 1 week in the cold there was an increase in the amount of sugars. Sucrose had already accumulated at this time (Table 1). In the following weeks there was an increase in the monosaccharides and very little change in the amount of sucrose.

TABLE 1. INCREASE IN SUGARS IN WHOLE POTATOES STORED AT 2°

	Increase over room temperature (23–25°) (mg sugar/g fresh wt.)	
Days	Reducing sugars	Sucrose
0	0	0
7	0·9	4·1
18	5·8	5·3
25	14·7	5·2

Experiments with Halved Potatoes

All of the work with whole potatoes described previously suffered from the variability that exists between individual potatoes. Unless one obtains statistical samples from a sufficient number of potatoes at each storage temperature at each interval of time, it is difficult to compare the two temperatures on a quantitative basis. In an effort to overcome this difficulty, a procedure was used in which the potatoes were halved and one half stored in the cold and the other at room temperature. Comparisons were made at intervals using the halves from the same potato. This at least eliminated any differences due solely to variations between individual potatoes.

Halved potatoes were prepared as described in the Experimental section. No changes in the appearance of the potato were observed during the first week. The respiration of the halved potatoes was compared to that of whole potatoes by use of a Claypool–Keefer instrument.[8] In two experiments the rate of respiration of the potato halves was 16·4 and 22·6 compared with, respectively, 9·8 and 7·5 mg CO_2/kg/hr for the whole potato at 23–25°. Since Laties[9] has shown that changes in respiration and starch disappearance are restricted to the region within 1 mm from the cut surface, the portion of the boring within 5 mm of the cut surface was discarded before preparing extracts for enzymatic or sugar analysis.

Phosphohexose isomerase. No difference in the specific activity between the two halves at the two storage temperatures was detected until the seventh day. At this time a large

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decrease in specific activity in the cold-stored half was observed relative to the half at room temperature. The results are summarized in Table 2.

TABLE 2. COMPARISON OF PHOSPHOHEXOSE ISOMERASE ACTIVITY IN POTATO HALVES STORED AT DIFFERENT TEMPERATURES

Days	Specific activity (mg fructose/mg protein/min)	
	2°	23–25
0	32	32
2	28	25
4	27	27
7	20	38

Glucose-6-P dehydrogenase. The specific activity of this enzyme decreased in both halves (Fig. 2). Within 1 week the activity at both temperatures had been lowered by 65 per cent.

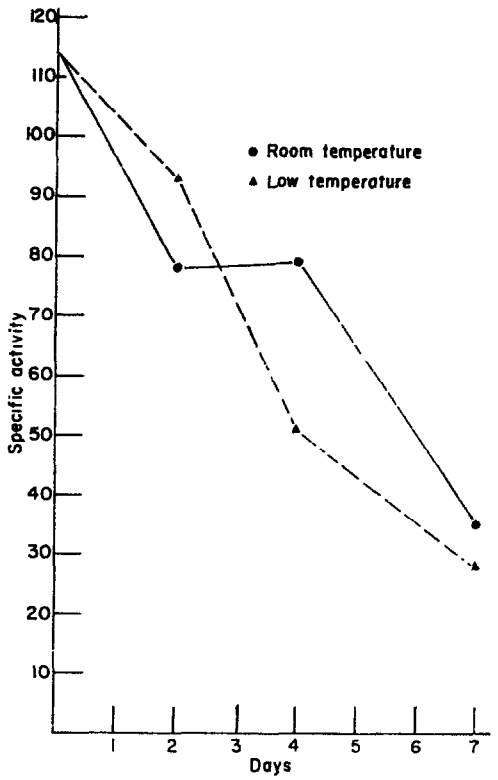

FIG. 2. COMPARISON OF GLUCOSE-6-P DEHYDROGENASE IN POTATO HALVES STORED AT DIFFERENT TEMPERATURES.

One unit of activity is defined as that which will cause an increase in absorptivity of 0·001/min at 340 mμ. The specific activity is given as units/mg protein.

Aldolase. This enzyme decreased in activity almost immediately in the halves at low temperature reaching a reduction of 50 per cent in activity after 4 days in storage. The activity then returned to its normal value by the seventh day (Fig. 3).

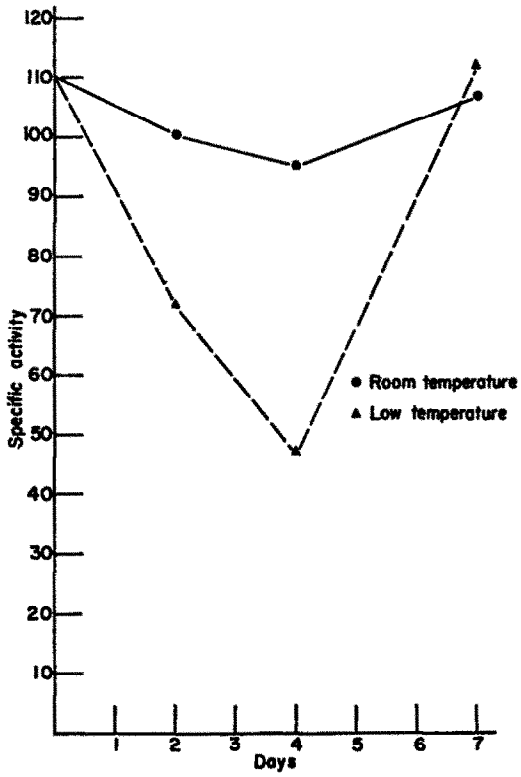

FIG. 3. ALDOLASE ACTIVITY AS A FUNCTION OF STORAGE TEMPERATURE IN POTATO HALVES. The specific activity is the number of S–L units/mg protein. An S–L unit is equivalent to 1/22·4 μmoles of fructose-1,6-P split per hour at 37°.

Sugar accumulation and analysis. The amount of sugar accumulated in the halved potatoes reached higher levels than in the whole potatoes and also attained these levels more

TABLE 3. INCREASE IN SUGARS IN HALVES OF POTATOES STORED AT 2°

Days	Increase over room temperature (23–25°) (mg sugar/g fresh wt.)	
	Reducing sugars	Sucrose
0	0	0
1	−0·3	0·3
3	−0·2	0·5
4	0	4·5
5	0·5	5·6
7	3·8	7·3

rapidly. The results for the halved potatoes are shown in Table 3 (compare with Table 1). In either case (intact or halved) sucrose accumulation preceded the increase in reducing sugars. This is in accord with previous observations made on whole potatoes, and verified in the studies on whole potatoes reported in this work. An explanation of this relationship was sought by comparing the invertase activities of the potatoes at the two storage temperatures. Previous studies[4] had reported that cold-stored potatoes of the White Rose variety had invertase activity after 1 year in storage, but activity was absent from the freshly harvested tubers. Our fresh potatoes had some invertase activity, but there was a large increase on the fourth day of cold-storage in the halved samples, and on the seventh day in the whole tubers stored at low temperature (Fig. 4).

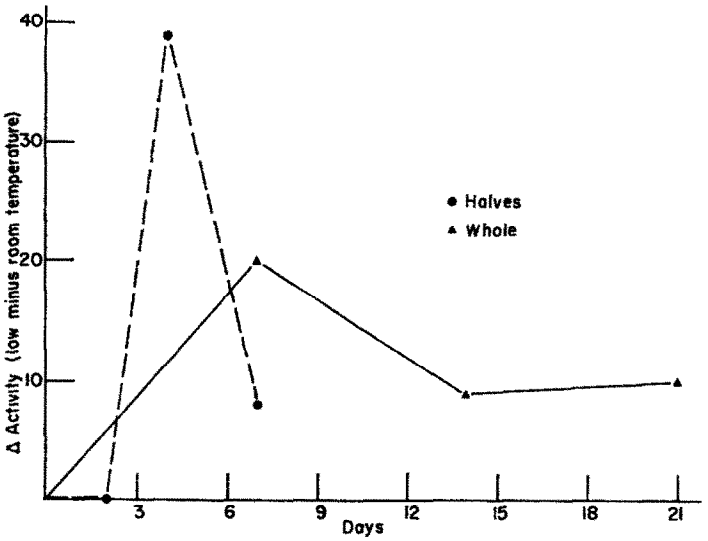

FIG. 4. THE ACTIVITY OF INVERTASE IN POTATO TISSUE KEPT AT LOW AND ROOM TEMPERATURES.
The results are given as the difference in activity (mg reducing sugars mg protein) between the comparable samples at the different storage temperatures.

DISCUSSION

While the results obtained in this study do not conclusively demonstrate a specific enzyme as the causal factor in sugar accumulation at low temperature, they do suggest certain possibilities and eliminate others. Of the enzymes examined in this work phosphohexose isomerase and invertase did undergo changes in activity on cold storage. The former decreased after a week, and the latter increased after 4 days. A very suggestive correlation exists between the appearance of sucrose and increase in invertase activity. In the whole potatoes, sucrose had appeared after 7 days, and invertase also had increased at this time. In halved potatoes the appearance of sucrose at low temperatures was hastened, so that at the fourth day there was a large increase of sucrose. Concomitantly, a large increase of invertase activity was also found at this time. A reasonable inference could be made that invertase synthesis is induced by the presence of sucrose. This would explain the relationship observed in both the halves and the whole potatoes, viz. sucrose accumulation being followed by reducing sugar increases.

In general the potato halves followed the same pattern as the whole tubers. However, the rate at which the pattern was produced was greatly accelerated in the halved potatoes. After 4 days the halves had accumulated sucrose, increased invertase activity, and decreased phosphohexose isomerase activity. The same results were found in the whole tubers after 7 days.

An interesting difference in aldolase activity was found in the halved potatoes. The half at low temperature decreased in activity for the first 4 days to a level of about 50 per cent of that in the room temperature half. The activity then increased so that after 7 days there was no difference in activity between the two halves. This decrease was not noted in the whole potatoes; however, the first assay was made only after 7 days, and this depression of aldolase activity may have been overlooked.

The respiration studies with the halved potatoes eliminate the possibility that the increased sugar accumulation is related to the slowing down of respiration at low temperatures. The maximum rate of respiration in the halved potatoes was 22·6 mg CO_2 per kg tissue/hr at room temperature. In 4 days 2250 mg CO_2/kg would be evolved. This is equivalent to the complete oxidation of 1500 mg of glucose. If respiration at low temperature were completely absent, this should be the amount of glucose accumulated if decreased respiration were the only factor involved. Actually 3400 mg/kg were accumulated after 4 days at low temperature, and this simple explanation must be discarded.

The results discussed above allow us to formulate a probable sequence of events. Low temperature induces, (1) a temporary decrease in aldolase activity, (2) sucrose accumulation, (3) increase in invertase activity, (4) increased monosaccharide concentration, followed by (5) a decrease in the activity of phosphohexose isomerase.

The relationship between (2), (3) and (4) permit a reasonably straightforward explanation of the observed appearance of sucrose followed by monosaccharides at low temperature storage. As to whether the temporary decrease in aldolase activity actually triggers the increase in sucrose, and if so, how the decrease in temperature leads to the lowering of the aldolase activity, are questions which must be left to future studies.

EXPERIMENTAL

Whole Potatoes

Freshly harvested dormant White Rose potatoes were used. Comparisons with whole potatoes were made between lots stored at 2° and at room temperature (23–25°). The potatoes were stored in the dark in either case. Samples were taken weekly for enzymic or other analysis. The usual length of an experiment was 3 weeks, however, in one case the duration was for 9 weeks.

Halved Potatoes

Freshly harvested White Rose potatoes were halved longitudinally. The cut surface of each potato was dipped for a very short time in liquid wax at 60° ± 5°. This was repeated three times. The potato halves were then brought to room temperature, after which one half of each potato was stored in the cold and the other at room temperature. Samples were taken at 2-day intervals for at least 1 week.

Preparation of Enzyme Extracts

A number of samples were taken from each potato by means of a cork-borer; the outermost edges for a distance of 5–7 mm were discarded. The total sample from each potato,

7–10 g, was homogenized for 2 min in the cold in 25 ml of 0·01 M $NaHCO_3$. The homogenate was centrifuged for 30 min at 25,000 *g* in a refrigerated centrifuge. The supernatant solution from the above centrifugation was divided into 1 ml portions and stored at −10°. These were assayed as desired for aldolase activity. Other enzymes were assayed after dialysis of the above solution against 0·02 M $NaHCO_3$ in the cold for 12–24 hr. After dialysis the enzyme solution was divided into aliquots and stored as above. After analysis for enzyme activity, the remainder of an aliquot was discarded.

Enzyme Assays

Phosphohexose isomerase. The substrate was added in 0·4 ml of 0·2 M tris-buffer, pH 8·8, containing 8·3 μmoles glucose-6-P. The frozen stock enzyme was thawed and diluted 1:10 with cold 0·01 M $NaHCO_3$. An aliquot of the diluted enzyme (0·2–0·4 ml) was added and then distilled water to a final volume of 2 ml. The reaction mixture was incubated at room temperature for 15 min, and then terminated by the addition of 7 ml of 30% HCl. A control reaction mixture to which HCl was added at zero time was used as a blank. The reaction mixtures were assayed by the method of Hers.[10]

Glucose-6-P dehydrogenase. The assay was done spectrophotometrically as described by DeMoss.[11]

Aldolase. The activity of this enzyme was assayed by the procedure of Beck.[12]

Invertase. The reaction mixture was made up, incubated, and the reaction terminated as described by Schwimmer *et al.*[4] The addition of the anion exchange resin was omitted in our procedure, since a zero time control and dialyzed enzyme extracts were used. The colorimetric method of Nelson[13] was utilized to assay for reducing sugars.

Identification of sugars. The sugars were extracted from potato tissue by boiling 80% ethanol. The qualitative identification was made by ascending paper chromatography with Whatman No. 1 paper. The solvent systems used were ethyl acetate–pyridine–water (12:5:4); and *n*-butanol–water–acetic acid (52:35:13). In the latter solvent mixture additional acetic acid was added dropwise, as necessary, until a single-phase solution was obtained.

Protein determination. The protein concentration was measured spectrophotometrically.[14]

Materials

The sodium salts of D-fructose-1,6-P and NADP were purchased from Sigma Chemical Co. Na glucose-6-P was obtained from Calbiochem.

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