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MECHANISM OF STARCH–SUGAR INTERCONVERSION IN *SOLANUM TUBEROSUM*

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; tuber; mechanism of starch-sugar interconversion; phosphate esters; amyloplast; membranes.

Abstract—The changes in glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, 2-phospho-glycerate, phosphoenol-pyruvate, pyruvate, adenosine mono-, di- and tri-phosphates, NAD and NADH, sugars and respiration of mature potato tubers (variety King Edward) caused by transfer from +10° to +2° and back to +10° were followed throughout 4–8 weeks of storage. The results obtained showed a characteristic two phase pattern. In the case of the transfer from +10° to +2° a number of the phosphate esters showed wide individual variations in concentration during the first phase but only slow changes during the second phase when most of the phosphate esters tended to follow a common pattern. In the first phase the sugar concentration remained roughly constant, but in the second a considerable increase in both sucrose and respiration occurred. In the case of potatoes transferred from +2° to +10° the two phase character of the results was not so marked. In the case of potatoes transferred from +10° to +2° the changes in the phosphate esters in the first phase did not appear to be related to the conversion of starch to sucrose which only occurred to a significant extent in the second phase. Electron micrographs of potato tubers which had been stored at +2° for 38 days (sugar content 2·4%) showed that the starch grains were still enclosed in a double membrane (amyloplast membrane). Analysis of starch grains prepared by a non-aqueous method from potato tubers stored at +10° and +2° indicated that a large part of the K, Na, Cl, citrate and glucose-6-phosphate was inside the amyloplast but that the sugar (storage at +2°) was outside; sweetening therefore involved the transport of metabolites through the amyloplast membrane. Comparison with other treatments (anaerobiosis, cyanide, ethylene chlorhydrin) which cause sweetening suggested that the regulation of the starch-sugar interconversion was effected at the amyloplast membrane and possibly involved electron transfer. In the case of potatoes which sweetened due to senescence, electron micrographs showed that the amyloplast membranes were disintegrating.

INTRODUCTION

In an earlier paper [1], it was shown that the main changes in the carbohydrates of mature and immature King Edward potatoes during the dormant period (January–March) as a result of a change in the temperature of storage (from +10° to +2° and back to +10°) concerned only starch and the sugars. The increased respiration which accompanied any change in temperature was related quantitatively to the formation of sucrose from starch (change from +10° to +2°) and of starch from sugar (+2° to +10°). The ATP equivalent of the extra CO_2 output in each case was of the same order as that predicted on the basis of known biochemical pathways linking starch to sucrose and sugars to starch. The calculation was made on the basis of certain assumptions about the basal respiration of the tubers and about the proportion of the respiration which is coupled to phosphorylation but, even so, the fact that the ATP equivalents were so close to prediction suggests that only a small amount of high energy phosphate compounds were expended in the actual transport of intermediates across the membranes in the cell. Starch grains are enclosed in membrane limited structures (amyloplasts) during the maturation of the tuber [2]. These are analogous to chloroplasts except that they are specialized plastids containing no chlorophyll. If the membranes remain during storage at +2° then it follows that, at some stage in the interconversion of the carbohydrates, unless all the sugar remains in the amyloplasts, an intermediate must be transported across the amyloplast membrane. It is possible that one of the steps in the biochemical pathways linking starch and sugar is effected at the membrane and forms part of the transport system and may be coupled to, or accompany, electron transport across the membranes [3]. Earlier workers [2], however, had been unable to demonstrate the existence of continuous membranes round the starch grains after storage of the potatoes at low temperature and had suggested that "sweetening" was due to the starch grain being open to attack by degradative enzymes as a result of a break in the membrane. It was important therefore to establish whether continuous membranes remain round the starch grains after storage of the potatoes at low temperature before attempting to outline a mechanism for the sweetening process.

In the present study those phosphate compounds likely to be involved in the biochemical pathways between starch and the sugars were measured in samples similar to those used for the analysis of starch and sugars in the physiological experiments described previously [1],

because the samples had been taken at intervals of 3–10 days to span conveniently the period of "sweetening" (28 days at +2°) and "conditioning" (28 days at +10°) only long term overall changes were therefore recorded. The aim was to obtain a picture of the changes which occur when the temperature of storage is altered and especially the steps in the pathways which may regulate the interconversion of the carbohydrates. Particular attention was given to the role that the transport of intermediates across the amyloplast membrane might play in the interconversion.

A knowledge of the mechanism of the interconversion has a wider implication than merely the changes in the potato because the ability to convert starch, inulin and similar high molecular weight polysaccharides to oligosaccharides under chilling conditions is widely distributed in plants and possibly plays some part in tolerance to low temperatures.

RESULTS

Changes in the phosphate esters in potatoes stored at +2° and +10°

The analyses were made on samples from the experiment on mature potatoes described in an earlier paper [1]. The potatoes were lifted on 6th October 1970, and the experiment started October 29th, 1970, when the potatoes were placed in a room at +10°. These potatoes were then transferred from +10° to +2° on 23rd November, 1970, and after 28 days at +2°, half of the potatoes were transferred from +2° to +10° on 21st December, 1970, the other half remaining at +2° for a further 28 days (total period at +2° 56 days). The potatoes which had been transferred after 28 days at +2° to +10° remained at this temperature for 28 days. The experiment finished on the 18th January, 1971. The material from this experiment was chosen because mature potatoes at this time of the season are in a dormant state and this means that processes such as the turnover of protein or organic acids are likely to be small and relatively constant. It follows from this that the observed changes in phosphate esters and associated glycolytic intermediates will probably be mainly linked with the comparatively large changes in starch and sugars which occur as a result of a change in the temperature of storage. The results for glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate and pyruvic acid are shown in Figs. 1a and 1b. Similar analyses made on samples from comparable experiments in 1971–72 and 1972–73 showed the same patterns of change. In the present study it is

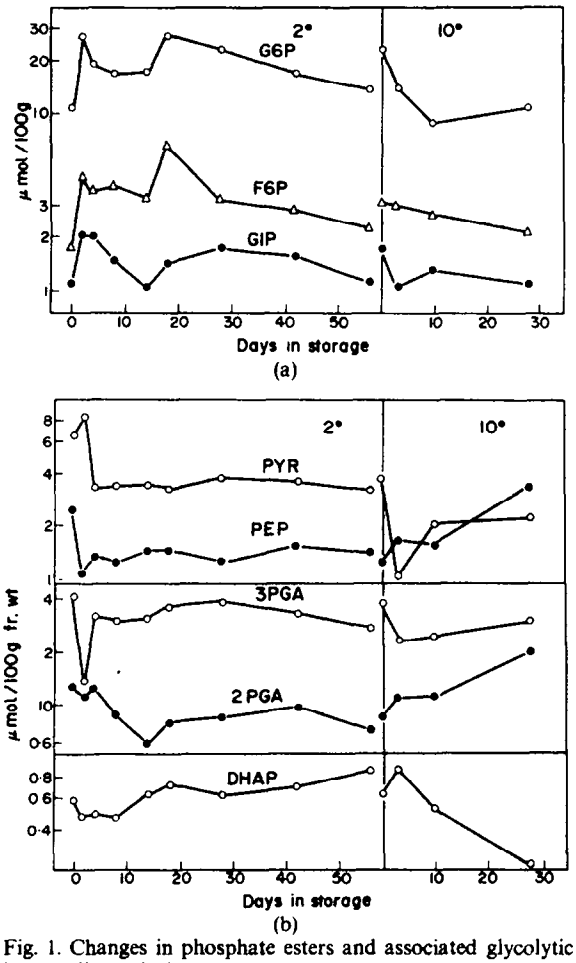

Fig. 1. Changes in phosphate esters and associated glycolytic intermediates during storage at +2° and +10°. In order to show relativities between the various compounds, concentrations have been plotted on a logarithmic scale. (a) Glucose-1-phosphate, glucose-6-phosphate and fructose-6-phosphate; (b) dihydroxyacetone phosphate, 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate and pyruvic acid.

Table 1. Composition of starch grains prepared by a non-aqueous method from potatoes (var. King Edward) stored at +10° and +2° (14 days. Total sugar 1·5%)

Solute determined	Composition (calculated for whole tuber) mg/100g fresh weight 10°	2°
P	7·5 (18)*	5·6
Na	10·5 (60)	27
K	212 (256)	180
Cl	29 (50)	27
Citrate	128 (200)	90
Malate	25·5 (45)	26·5
Glucose	55	69
Fructose	50 } 0·1% (0·3%)	52 } 0·14% (1·5%)
Sucrose	11	23
Glucose-6-phosphate	1·4 (3·4)	1·5 (4·7)

* Figures in parentheses are analyses on the whole tuber.

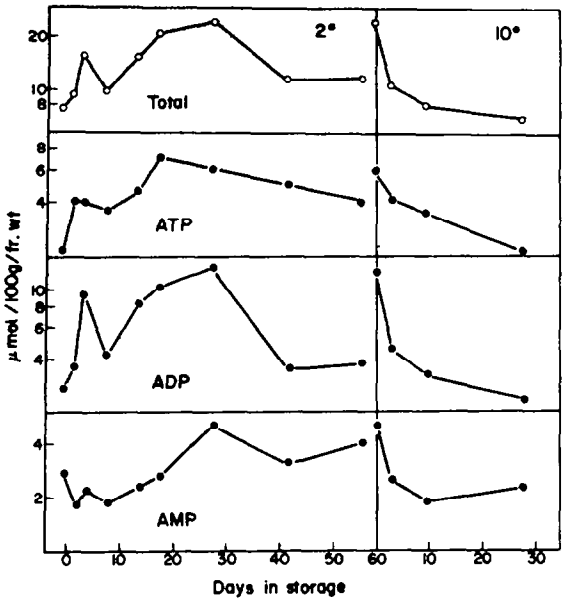

Fig. 2. Changes in AMP, ADP and ATP and total adenylate during storage at +2° and +10°. The concentrations have been plotted on a logarithmic scale as in Fig. 1.

the pattern of change which is of importance. The figures given in Figs. 1–4 and also in Table 1 are the mean of duplicate estimations. The coefficient of variation of individual results (8 samples) for glucose-6-phosphate, glucose-1-phosphate, phosphoenolpyruvate, 3-phosphoglycerate, 2-phosphoglycerate, pyruvic acid and fructose-6-phosphate was 19, 41, 23, 12, 26, 48 and 37% respectively. It is to be noted that the results plotted in Fig. 1a and 1b and 2 were on a logarithmic scale. The figures for adenosine monophosphate, diphosphate and triphosphate are shown in Fig. 2 and the corresponding changes in respiration and in individual sugars are given in Fig. 3. Measurements of the ratio NADH/NAD + NADH throughout storage at +2° and subsequently at +10° after 28 days at 2° are shown in Fig. 4.

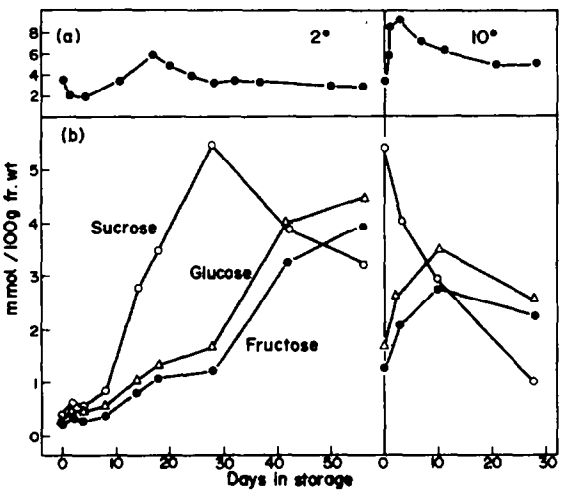

Fig. 3. Effect on CO_2 production and sugar content of exposure to different temperatures (+2° and +10°) (a) CO_2 production; (b) sugar content.

A comparative study of these results suggests that the reactions in the tissue occur in two phases. This is particularly noticeable when potatoes are transferred from +10° to +2°. In the first phase which lasts up to 4 days, the respiration remains substantially constant at a value about half that at +10° and the concentration of the sugars changes very little (Fig. 3). The remarkable feature about this phase is the rapid change in the concentration of certain phosphate esters which is apparently unrelated to respiration. The glucose-6-phosphate reaches a maximum 2–3 times the initial value and then declines and the 3-phosphoglycerate a minimum about one third of the initial (Fig. 1a, b). The second phase extends from 4 days onwards and is characterized by a considerable increase in sucrose concentration and while the sucrose is increasing, by a large increase in respiration (Fig. 3). The corresponding changes in the concentration of the various phosphate esters follow a standard pattern. A gradual increase up to about 25 days and then a slow decline. In the case of a transfer from +2° to +10° the picture is not so clear but the results still suggest that the reactions occur in two phases.

Leakage of electrolytes from slices of potatoes stored at +2°

It has been reported that slices cut from potato tubers stored for two months at 0° show several fold greater leakage of salts into 0·25M sucrose at a temperature of 15° than those from potato tubers stored at 12·8° [4,5] and that the "leakiness" of the tissue after storage at 0° is possibly linked with a change in the membranes and that this is involved in the increase in the sugar content. However, no indication was given as to whether the potatoes after storage at 0° were showing signs of senescence when the membranes might be expected to be particularly fragile and susceptible to the kind of osmotic shock that slicing and placing in 0·25M sucrose causes. The loss of salts would then be caused by a break in the membranes due to the high content of sugar in the cells as a result of prolonged storage at low temperature. It was of interest therefore to ascertain whether this was true in the present experiments which used potatoes at the beginning of the dormant period when the effects of senescent changes would be negligible. A temperature of +2° was chosen instead of 15° for measurements of salt leakage as being less likely to cause a physiological reaction with potato tissue previously

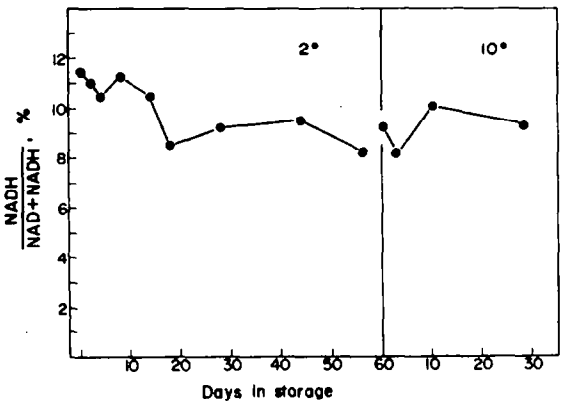

Fig. 4. Changes in the ratio NADH/NADH + NADH during storage at +2° and +10°.

Fig. 5. (a) Electron micrograph of amyloplasts from a potato tuber (*Solanum tuberosum*, var. King Edward) which had been stored at +2° for 38 days. Fixation: osmium followed by aqueous uranyl acetate; embedding Epon; staining uranyl acetate and lead acetate. Membranes are present round each starch grain and these are clearly double membranes as shown in b). (c) Electron micrograph of an amyloplast from a potato tuber (*Solanum tuberosum*, var. Home Guard) which had been stored at 20° until senescent (sugar content 3%). Fixation: glutaraldehyde and osmium followed by aq uranyl acetate; embedding Epon; staining uranyl acetate and lead acetate. The membrane is still present round the starch grain but is showing evidence of disintegration; the layers apparently peeling away from each other.

stored at +2°. The leakage of salts was followed by measurements of the electrical conductivity of the 0·25M sucrose solution. The movement of electrolytes out of the slices occurred in 2 phases, an initial rapid phase (up to 3 hr) followed over the next 12 hr by a much slower rate. The rate of leakage was not markedly faster at 10° than at +2° so it seemed unlikely that there was active secretion of electrolytes from the cells. Assuming that the increase in conductivity after the initial period of rapid change (3 hr) was a measure of the leakage of electrolytes, the change in conductivity after varying periods of storage at +2° was compared with the extent to which the potatoes sweetened. It was found that though the potatoes sweetened in a characteristic manner (0·3–1·5% total sugar after 41 days at 2°), the extent to which electrolytes leaked from the slices did not change. These potatoes (storage experiments November–December 1974) were at the beginning of their dormant period and therefore should show no possibility of "senescence". Experiments on potatoes late in the season (May–June 1975) showed an increased leakage of electrolytes when the observations were carried out at room temperature but not at +2°. This could be due to the membranes being more fragile as a result of the onset of "senescence" and rupturing when exposed to a rapid change in temperature (+2° to +20°).

Effect of storage on the structure of the membranes

The changes in the morphology of the starch grains and particularly the structure of their membranes was followed by electron microscopy. Interest centred on whether the amyloplast membrane remained intact even when the potatoes had been stored at +2° until they had reached optimum sugar content. Fig. 5 shows that even after 38 days at +2°, the starch grains still retain their membranes. It was noticed in preliminary work on the embedding and sectioning of potato specimens that the amyloplast membranes were fragile and easily destroyed during the fixing procedures. The method described in the experimental section using osmic acid at +37° was very successful in revealing the presence of the membranes, though there was a suggestion that the apparent gap between the edge of the starch grain and the membrane was increased. Other methods using glutaraldehyde as fixing agent in the presence of sucrose, followed by osmic acid at room temperature, gave electron micrographs in which the gap was smaller. Samples were taken at varying distances from the skin (2, 5, 8, 12 and 15 mm) along the shortest axis of the potato (20 mm). All showed that the starch grains were enclosed in amyloplast membranes. It was important that fixation in osmic acid should be complete (as judged by the blackening of the tissue) before proceeding to the next stage. Sections cut at different distances from the outside of the potato did not blacken at the same rate, probably because the rate at which the osmium penetrated varied. In some sections, a starch grain was found in which the membrane was apparently damaged or not continuous. This could be the result of the cold treatment rather than the embedding technique and might be related to the fact that on returning the potato tubers to +10°, the respiration remains at a higher level suggesting that the system has changed.

The fragility of the amyloplast membranes was particularly noticeable in the case of "senescent" potatoes and it was difficult to decide whether the membranes were still intact in the original potato but much weaker, or whether they had actually broken. An electron micrograph is shown in Fig. 5 for Home Guard potatoes which had been stored at +20° until they were senescent (June 7th, 1973). Home Guard potatoes were chosen because they are classified as a first early potato and by June at this temperature were definitely senescent containing, depending on the sample, from 1·5 to 3% total sugar.

Solutes present in starch grains prepared by a non-aqueous method from stored potatoes

In an attempt to measure the concentration of solutes in the amyloplasts, a non-aqueous isolation technique was tried based on the method used for chloroplasts [6]. Examination of the isolated material under the microscope showed that the starch grains with the remains of the amyloplast also included a great deal of adhering cytoplasmic material. This was unsatisfactory because such material would contain solutes from the cytoplasm as well as those from the amyloplast. To obtain a less equivocal indication of the solutes actually in the amyloplast the method was modified so that only starch grains were obtained without any adhering material either from the amyloplast or the cytoplasm. Starch grains are very porous and in contact with liquid water contain about 38% water. It is to be expected that in the amyloplast the aqueous phase inside the starch grains will be in equilibrium with the fluid outside in the amyloplast. Analyses of starch grains from freeze-dried material will give therefore an indication of the composition of the fluid inside the amyloplasts. Some preliminary figures for starch grains prepared from potatoes stored at +10° and from potatoes stored at +2° for 14 days are given in Table 1. The figures have been calculated for the whole potato on the assumption that starch represents 16% of the weight at +10° and 14·5% at 2°.

The results show that a large part of the P, K, Na, Cl, citrate, malate and glucose-6-phosphate are contained within the amyloplast, but that when sweetening occurs (at +2°) sugar accumulates outside the amyloplast.

DISCUSSION

The carbohydrate interchange in stored potato tubers involves two separate but related phenomena. The first occurs as a result of a change in temperature. Dormant potatoes stored at temperatures between 10–20° usually contain only a small amount of sugar but if transferred to low temperatures (+2°) they rapidly sweeten after a lag period of a few days. If the sweetened potatoes are then transferred to higher temperatures (10–20°), they lose sugar ("conditioning") and revert almost to their original state. The second phenomenon occurs when potatoes have been stored for a long time at any temperature but is most obvious at temperatures between 10° and 20°, the main characteristic being that the potatoes sweeten (initially the process is very slow) but that this sweetening, unlike that produced by exposure to low temperature, is irreversible. The contribution of either phenomenon to sweetening depends on a number of factors such as variety, temperature of storage, the time in store and the previous physiological history of the tubers. A number of experiments on low temperature sweetening described by previous workers [7,8] in which changes in sugars and phosphate esters were measured almost certainly involve some element of "senescent"

sweetening. This will complicate any interpretation of the results. In the present study the experiments have been carried out on mature dormant tubers of a variety that does not readily show "senescent" sweetening and at a time in the season when "senescent" sweetening was negligible. The changes observed were therefore due almost entirely to the change in temperature of storage.

Changes in membranes

In the mature potato tuber, the starch grains are surrounded by a double membrane typical of a plastid and this membrane remains intact even when the potatoes have been stored at +2° for 38 days and the sugar content has risen to 2·5% (Fig. 5a). Storage at low temperature may affect fine details of the structure or of the chemical composition but the general appearance under the electron microscope is unchanged. By analogy with the properties of chloroplast membranes it is likely that the properties of the amyloplast membranes will be important in regulating the flow of metabolites between the starch grain in the amyloplast and the cytoplasm outside. Even in potatoes which have been stored at +20° until they are clearly senescent (sugar content 3%), long past the dormant period referred to above, it is found that most of the starch grains are enclosed by a membrane but that this membrane (Fig. 5c), in contrast to those observed for dormant potatoes, is very delicate and shows evidence of disintegration, the layers of the double membrane separating and breaking away. The implication is that in the case of "senescent" tubers the membranes have been damaged irreversibly and can no longer function to control the flow of metabolites.

Changes in phosphate esters

In an earlier paper [1] it has been suggested that the steady state concentrations of sugar and starch at +10° represent a balance between the synthesis of starch and the synthesis of sucrose, in a system in which some of the starch is continuously being broken down and resynthesized. A change in temperature by affecting one of the regulatory enzymes in the pathways linking starch and sucrose disturbs this steady state. Based on this idea one approach to the problem as to why potato tubers sweeten at low temperature has been to examine the properties of certain key enzymes such as ADP glucose pyrophosphorylase [9] in the synthesis of starch, phosphorylase [10] in the breakdown of starch and sucrose phosphate synthetase [11] in the synthesis of sucrose. However, no unequivocal evidence can be advanced that any of these are mainly responsible for the phenomenon though they may contribute. By analogy with the work on the enzyme from *Vicia faba* cotyledons [12], the concentration of citrate could govern the activity of the sucrose phosphate synthetase but the figures in Table 1 suggest that citrate is largely contained in the amyloplasts and remains there even when the sugar in the rest of the cell (see later) has risen to 1·5% which makes it unlikely that citrate is primarily controlling the formation of sucrose from starch. A more comprehensive approach to the problem depends on an analysis of the changes in the phosphate ester intermediates. The relation between the glycolytic intermediates and respiration is particularly interesting because the respiration falls sharply when the temperature is first reduced and only rises again when sweetening begins.

Interpretation of the changes in the concentration of the various phosphate esters is limited by a number of factors, the main effect of which is to obscure all changes except those of very general character, viz. the measured concentration represents the mean concentration over the whole tuber and it is unlikely that all the cells will react to a change in temperature in the same manner; the cells at the centre of the tuber will not reach ambient temperature until 6–8 hr after those on the outside and short term changes are likely to be missed because the samples were taken at intervals of 3–10 days. Since the phosphate esters are distributed between various organelles in the cell, a change in the measured amount may represent a large relative change in concentration in a particular organelle, but this will be difficult to assess. However, the results shown in Fig. 1a and 1b do indicate that the concentration of the phosphate esters follows certain characteristic periodic patterns which appear significant. These are described as occurring in two phases. The first phase covers a period of several days and during this period a number of phosphate esters show considerable individual variations in concentration. In the second phase most of the phosphate esters follow the same general pattern of change. The same two phases were observed with the adenylates; the ratio of ADP/ATP/AMP changed markedly during the first few days but subsequently the ratios did not change appreciably. The total adenylate fraction showed changes at +2° parallel with the behaviour of the hexose phosphates rising to a maximum after 28 days and then declining.

Comparison between hexose phosphates

Since it is assumed that the first stage in the interconversion of starch to sucrose is the production of glucose-1-phosphate it was of interest to compare changes in the relative proportions of glucose-1-phosphate, glucose-6-phosphate and fructose-6-phosphate during storage at +2° when "sweetening" occurs and subsequently at +10° when the sugar is reconverted to starch. Examination of the results shown in Fig. 1a indicates that the relative proportions remained largely unchanged throughout storage at either temperature or during changes in temperature, which suggests that phosphoglucomutase and glucose phosphate isomerase were in sufficiently high concentration to maintain the hexose phosphates near equilibrium throughout the period when the temperature is changing. During the comparatively long term changes that were considered in the present experiments there was no significant change in the levels of any of the hexose phosphates that would indicate which one was primarily affected by the change in temperature.

Relation between glycolytic intermediates, respiration and carbohydrate interconversion

A comparison between the results in Fig. 1a and 1b with the corresponding changes in sugar and respiration in Fig. 3 indicated that the relationship between the various glycolytic intermediates showed no marked changes in the second phase when the respiration was rising after 5–8 days at +2° and sucrose was being formed. The same was largely true in the reverse situation when potatoes which had been stored at +2° for 28 days were transferred to +10°, for in the second phase after 3–5 days at +10° when the respiration was falling, the rela-

tive proportions of the various intermediates showed no marked changes. A more detailed analysis using the cross-over theorem of Chance [13] as applied to the glycolytic sequence to determine reactions which might be regulating the rate of respiration and also affecting sucrose synthesis (at +2°) since glucose-1-phosphate and frustose-6-phosphate are precursors of sucrose, indicated that no major cross overs were present in the second phase. This was also true for the results described by Barker [8,14]. It was noticed, however, that the pyruvic acid concentration tended to change in the opposite direction to the respiration, particularly in the first phase, which suggested that regulation of the respiration might involve pyruvic dehydrogenase [15]. Pyruvate dehydrogenase is a key enzyme linking the glycolytic pathway to the mitochondrial enzymes which finally convert the carbohydrate to CO_2. It has been shown to be very sensitive to the ratio NADH/NAD + NADH and in the present experiments it was found that during storage at +2° and +10°, the ratio (Fig. 4) showed a link with the respiration, falling to a minimum at the same time as the respiration reached a maximum (16 days at +2° or 3 days at +10°).

In considering the formation of sucrose perhaps the most significant fact about the study described above is the negative conclusion that the factor controlling the formation of sucrose appears to be outside the glycolytic sequence.

Sucrose synthesis probably occurs outside the amyloplasts. Comparative analyses of starch granules (Table 1) prepared by a non-aqueous method from potatoes which had been stored at +10°, and at +2° until sweet, indicated that the sucrose was not found to any extent inside the amyloplast. This is in line with the observation that sucrose synthesis in pea shoots and spinach leaves occurs mainly in the cytoplasm not the chloroplasts [16]. Since sucrose is formed from starch during low temperature storage, it is clear that an intermediate in the pathway between the two must be transferred through the amyloplast membrane. At +10° the membrane controls the movement of intermediates so that only a low concentration of sugars is present in the cytoplasm, and this state appears to persist during storage at +2° until the end of the first phase despite considerable variations in the concentrations of individual phosphate esters. In the second phase all the intermediates tend to change in a roughly parallel fashion. In the case of chloroplasts, the suggestion has been made [17] that the transport of glycolytic intermediates produced by photosynthesis across the chloroplast membrane involves these compounds in an electron transport system and if the same is true of amyloplasts, then the change at the end of the first phase may well involve a radical change in the properties of the membrane so that the electron transport is affected and concomitantly with it the movement of the various glycolytic intermediates.

The unknown factor involved in controlling the formation of sucrose would, on this basis, be the amyloplast membrane.

It is known that a variety of treatments (anaerobiosis, cyanide [18], γ-radiation, ethylene chlorhydrin, storage in atmospheres containing 20% CO_2, 20% O_2 at 7·5° or 5° [19], and high and low temperature storage) will cause potatoes to sweeten and most of them could well affect the electron transport properties of membranes. Even when electron transport is largely inhibited (anaerobiosis or treatment with cyanide) starch still appears to be broken down, appearing as lactic acid or sucrose respectively. In the case of anaerobiosis calculations made from the data of Barker & Mapson [20] show that the lactic acid (900 μmol/100g fr. wt) could not have been produced by the loss of the sucrose originally present (maximum 100 μmol/100g) but must have been derived from metabolites transferred across the amyloplast membrane from starch. Most of the rise in sucrose and hexoses that occurs after the potatoes are transferred back to air (900 and 500 μmol/100g respectively) must also have come from starch, which means that the amyloplast membranes are permeable to metabolites derived from starch for 2–4 days after the end of anaerobiosis. It has also been found that if potatoes are stored for a long period in air at 1°C, anaerobiosis followed by a return to air at +1° does not cause any increase in sucrose. The implication is that anaerobiosis and low temperature are causing similar changes to the membranes and because of this their effects on the sweetening of potatoes are not additive. Ethylene chlorhydrin induces sweetening and an increase in CO_2 output with potatoes stored at +12·8° but has little effect on those stored at 0° [21]. Ethylene chlorhydrin is similar to many anaesthetics which are known to cause a change in lipid structure and to affect lipid bound enzymes [22]. It therefore seems possible that ethylene chlorhydrin is affecting enzymes in the amyloplast membranes and because the effects of ethylene chlorhydrin and low temperature are similar and non-additive, that the membranes are also affected when potatoes are subjected to prolonged storage at low temperature. These treatments may also be affecting the membranes of the mitochondria because it has been shown that ether [22] affects the lipid-bound ATPase of the mitochondria of leaves and raises the level of the endogenous ATP in the leaves, and one effect of storage at low temperature is to raise the level of all adenylates in the potato tuber (the level of ATP rises more than three fold, cf. Fig. 2), particularly in the second phase.

In view of the variety of treatments which cause potatoes to sweeten, it was of interest to examine the energy changes involved. The heat of formation of starch (solid, 2 anhydrohexose units) −221·3 kg Cals. and sucrose (solid, one mol, 2 anhydrohexose units) −217·0 kg Cals plus the heat required for solution in water −0·92 kg Cals, indicates that the tendency would be for starch to be converted into sucrose.

In an earlier paper [1] on low temperature sweetening it was shown that the ATP equivalent of the extra CO_2 output when "sweetening" occurs was of the same order as that predicted on the basis of known biochemical pathways linking starch and sugars. The fact that the ATP equivalent of the extra CO_2 was so close to that predicted suggests that only a small amount of high energy phosphate compounds were expended in the actual transport of metabolites across the membranes in the cell. This conclusion strictly only applies when rapid sweetening is occurring. Under constant storage conditions at +10° the level of sugar in equilibrium with the starch in the amyloplasts is low and the process of the transfer of metabolites across the membranes may be different. The main feature of the process of "sweetening" is that it does not require extra energy for the transport of metabolites and under these conditions the membrane acts as a passive barrier. The tendency for starch to be

converted into sucrose causes sucrose to accumulate outside the amyloplast.

Mechanism of sweetening

The evidence described above suggests that movement of metabolites through the membranes of the amyloplast may represent a balance between electron transport activated influx and a passive efflux [3]. The balance could be easily influenced by raising or lowering the temperature or by any process which interfered with the electron transport activated influx (cyanide, anaerobiosis). The passive efflux is mediated either by specific proteins or perhaps by electron transport carriers in forms which are not coupled to electron transport (e.g. after treatment with cyanide [3]) and which allow a non-energy dependent transport of the phosphate ester intermediates across the membrane.

The effect of temperature on the maximum accumulation of sugar in King Edward potatoes [23] is particularly interesting for it illustrates the way the balance between influx and efflux of metabolites can be changed. After long storage at $-1°$, $+1°$ and $+3°$ the maximum accumulation of sugar was 6·7, 3·5 and 1% respectively. In this case the fall in temperature reduces the influx relative to the efflux; the lower the temperature the greater the effect on the influx. It has also been observed [24] that the sucrose content of potatoes stored for a few days at non-lethal temperatures (33°) above 20° rises rapidly (to over 1%) and that if these potatoes are subsequently returned to 20° the sugar content falls. In this case the rate of efflux relative to influx increases rapidly with temperature and sugar accumulates.

Lag phase

While a number of the treatments which cause sweetening appear to effect an immediate response, other treatments such as low temperature and possibly anaerobiosis exhibit a lag phase. This is difficult to explain. It could be due to the structure of the membrane changing after 5–8 days at low temperature and then showing the characteristics of a chilling-sensitive plant [25] or it could be that the behaviour of the enzymes in the amyloplast membranes is controlled by plant hormones and that these change during the 5–8 days of the lag period.

Working hypothesis

The steady state levels of starch and sugars at a variety of temperatures represent equilibrium states in which there is a balance between influx and efflux of certain intermediates in the pathways linking these carbohydrates to and from the amyloplasts. This balance can be influenced by raising or lowering the temperature but in the case of low temperature storage the process shows a lag phase before sweetening occurs. Any interference with the electron coupled influx causes accumulation of intermediates outside the amyloplast membrane which in the presence of oxygen are manifested as sucrose but in nitrogen appear as lactic acid. Poisons which act on the electron transport system cause immediate sweetening.

EXPERIMENTAL

Respiration and estimation of sugars. The methods used were the same as those described previously [1].

Phosphate esters. Glucose-6-phosphate, glucose-1-phosphate, fructose-6-phosphate and ATP were estimated as described previously [26]. Dihydroxy acetone phosphate, 3-phosphoglycerate, 2-phosphoglycerate, pyruvate and phosphoenol pyruvate were estimated [27] in an extract prepared as described for the estimation of the sugars, which had been treated [28] with charcoal to remove the phenols and then the extract (equivalent to 30g potato; 16 ml pH 7) passed through a column (7 × 1 cm diameter) of Chelex 100 (200–400 mesh) chelating resin in the ammonium form. The column was washed with 10 bed vol H_2O and the combined extract and washings concentrated (10 ml). The Chelex column removed heavy metals which interfered with the fluorimetric enzymatic analyses. The ADP and AMP were estimated in an extract prepared as described for the estimation of the sugars which was purified by passing through a column (30 × 1·5 cm diam.) of G25 Sephadex gel. The extract (pH 7, 10 ml) was added to the gel in triethanolamine chloride buffer 0·04M, pH 7·5 and then eluted with the same buffer. Those fractions having a peak at 260 nm were combined and the adenylates estimated spectrophotometrically [29]. The NAD and NADH were estimated in a separate extract [30].

Leakage of electrolytes from potato slices. Potato discs were prepared by cutting transverse slices (0·5 mm thick) from potato tubers (10 tubers, 2 slices from the centre of each) and discs cut with a cork borer (12 mm diam) from each. Immediately after cutting, the discs (about 1·6g) were washed in 0·25M sucrose for 0·5 min, dried by blotting on filter paper and immersed in 0·25M sucrose (30 ml) at $+2°$ with gentle agitation. Salt leakages was determined by measuring the conductance of the solutions surrounding the discs using a Philips Conductivity Bridge PR 950J.

Electron microscopy. In early expts the potato specimen was fixed [31] for 1–2 hr in 2% glutaraldehyde in 0·05M sodium cacodylate buffer, pH 7·2 containing 0·2M sucrose. It was then post fixed in 1% OsO_4 in Palades buffer solution for 2 hr at 37° followed by aq 0·5% uranyl acetate for 1 hr, dehydrated and embedded in Epon [32]. Sections were cut on an LKB Ultratome III and stained with uranyl acetate [33] and lead acetate [34] and examined on an EM 6B AEI electron microscope. In later experiments the specimens were directly fixed in the hot OsO_4 solution with no pre-treatment with glutaraldehyde.

Non-aqueous method for isolation of starch grains. Potato tubers were rapidly sliced using a clean carpenters steel plane (Record No. 04) and the slices (0·5 mm thick) dropped immediately into liquid N_2. These slices (500 g) whilst still frozen were dried at $-20°$ *in vacuo* over P_2O_5 (250 g) for about 8 weeks. At the end of this time the dry slices were similar to domestic potato crisps in texture and could be easily powdered.

The dried potato slices (5g) were crushed to a fine powder using a pestle and mortar and the powder brushed through a 300 mesh sieve. The sieved powder was lightly rubbed between 2 circular mild steel plates (10·2 cm diam, top plate weighed 807 g and the rubbing surfaces were ground flat) using no extra pressure. The object was to roll the starch grains between the plates as if they were ball bearings until all the adhering cytoplasmic material had been removed. Frequent microscopic examinations were made of the powder and the process stopped when practically all the starch grains were free of adhering material. The powder was then passed through a sieve with a pore size of 38 μm (Endicotts (Test Sieves) Ltd., London) and finally one with a pore size of 30 μm. The choice of which residue is fractionated further by flotation depends on an examination of the residues under the microscope to determine which has less cytoplasmic material present. A final separation of the starch grains from other material was made by layering a suspension of the residue in chloroform on to chloroform containing 1% of polymethyl methacrylate to give it a suitable viscosity (25 ml in a 25 ml stoppered measuring cylinder) and allowing it to stand overnight. Samples (5 ml) were withdrawn successively, centri-

fuged and the precipitate of starch grains examined microscopically. Those in the penultimate sample (from the top) contained starch grains substantially free from other materials. For the analysis of glucose-6-phosphate, sugars, malic and citric acids [29] the starch grains (200 mg) were extracted with aq 5% $HClO_4$ (5 ml) at room temp. for 0·5 hr with shaking, the mixture neutralized with N.KOH and then made up to volume (10 ml) with H_2O and centrifuged. K and Na were measured in an aqueous extract of the grains by absorption flame photometry; phosphate and chloride [35] were also measured in the same extract.

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