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# ACTIVITIES OF ENZYMES OF SUGAR METABOLISM IN COLD-STORED TUBERS OF *SOLANUM TUBEROSUM*

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**Key Word Index**—*Solanum tuberosum*; Solanaceae; cold-stored tubers; sugar accumulation; activities and temperature coefficients of enzymes.

**Abstract**—Storage of tubers of *Solanum tuberosum* at 10° or 2° for 15 days did not alter significantly the maximum catalytic activities of sucrose phosphate synthetase, sucrose synthetase, glucose-6-phosphate dehydrogenase, aldolase, and glyceraldehydephosphate dehydrogenase. The temperature coefficients of phosphofructokinase, glyceraldehydephosphate dehydrogenase, and pyruvate kinase from the tubers were shown to be higher between 2° and 10° than between 10° and 25°. The rate of sugar accumulation at 2° exceeded the activity of sucrose synthetase but was less than that of sucrose phosphate synthetase. It is suggested that sucrose accumulation at 2° is catalysed by sucrose phosphate synthetase, is not due to changes in the maximum catalytic activities of any of the above enzymes, but may be due, in part, to the susceptibility of key glycolytic enzymes to cold.

## INTRODUCTION

Storage of potato tubers at 0–6° causes sucrose and reducing sugar to accumulate. We do not know the pathway or the mechanism of this accumulation, although its importance and general characteristics are well documented [1]. The aim of the work in this paper was to compare the rates of sugar accumulation with the maximum catalytic activities of key enzymes of sugar metabolism in tubers stored at 10°, the usual storage temperature, and in tubers stored at 2°. Such information could indicate the pathway and the mechanism of sweetening. In particular it could reveal the relative roles of fine and coarse control of metabolism. We define maximum catalytic activity of an enzyme, as did Scrutton and Utter [2], as the maximum capacity of a tissue to catalyse a given reaction. We measured sucrose phosphate synthetase (E.C.2.4.1.14) and sucrose synthetase (E.C.2.4.1.13) because both could regulate sucrose synthesis and the latter could control sucrose breakdown. We measured glucose-6-phosphate dehydrogenase (E.C.1.1.1.49), aldolase (E.C.4.1.2.7), and glyceraldehydephosphate dehydrogenase (E.C.1.2.1.12) because changes in the activities of enzymes of carbohydrate oxidation could alter the concentration of hexose phosphates. This in turn could influence sucrose synthesis. High activities of polyphenolase make it difficult to measure maximum catalytic activities of enzymes in potatoes. Thus we paid particular attention to the possibility of losses of activity during extraction of the tissue. In order to relate the enzyme activities to the rates of sugar accumulation we made both measurements on tissue from the same tuber. We also determined the temperature coefficients of the enzymes so that we could calculate the maximum catalytic activities of the enzymes at the different storage temperatures. There are reports of the effect of cold-storage on the activities of the above enzymes [3, 4]. The points that we have made above are not covered by these reports.

## RESULTS

Figure 1 shows the course and extent of the sweetening that occurred in our experiments. Storage at 10° had no detectable effect on the sugar

content of the tubers. At 2° a 5-day lag was followed by a period of 10 days in which the sugar content increased almost linearly. The rate of sugar accumulation during the linear phase can be calculated and the mean value ± S.E. from eight experiments was 5·9 ± 0·48 μmol anhydrohexose/g fr. wt per day. Most of the accumulated sugar was sucrose; chromatographic analyses indicated that the remainder consisted of equal amounts of glucose and fructose.

From Fig. 1 it is clear that any changes in enzyme activities that could have led to sweetening must have occurred within the first 15 days of storage. Thus, we measured enzyme activities prior to and after 8 and 15 days storage. We checked the reliability of our assays for each enzyme in respect of unstored tubers, of tubers that had been stored at 2° for 15 days, and of tubers that had been stored at 10° for 15 days. Firstly, we varied the components of the reaction mixtures so as to give optimum rates. Secondly, we checked whether activity was lost during the preparation of the extracts. For glucose-6-phosphate and glyceraldehydephosphate dehydrogenases, and aldolase, we prepared duplicate samples of tissue. We extracted one in buffer, and the other in buffer that contained known amounts of commercially purified preparations of the enzymes. Comparison of the activities recovered from the two samples revealed the extent to which the added enzymes were inactivated during extraction of the tissue. In no instance was more than 10% of the added enzyme lost. When this technique was used for phosphofructokinase (E.C.2.7.1.11) at least 70% of the added enzyme was lost regardless of whatever precautions we took. Thus, although we subsequently managed to obtain a preparation of phosphofructokinase from potatoes, we were unable to measure its maximum catalytic activity in crude extracts. For sucrose phosphate and sucrose synthetases we compared their activities in extracts of three samples. One sample was from an unstored tuber, the second was from a tuber that had been stored at 2° for 15 days, and the third consisted of a mixture of equal amounts of tissue from the unstored and stored tubers used to prepare the first two samples. For both enzymes the activity recovered from the mixed sample was within 5% of that predicted from the measurements made on the first two samples. Estimates of the activities of the different enzymes during storage at 2° and at 10° are given in Table 1. The protein content of the extracts did not alter during storage at either 2° or 10° and the mean value ± S.E. of measurements made on 35 different extracts was 10·5 ± 0·5 mg protein/g fr. wt. We emphasize two points. Firstly,

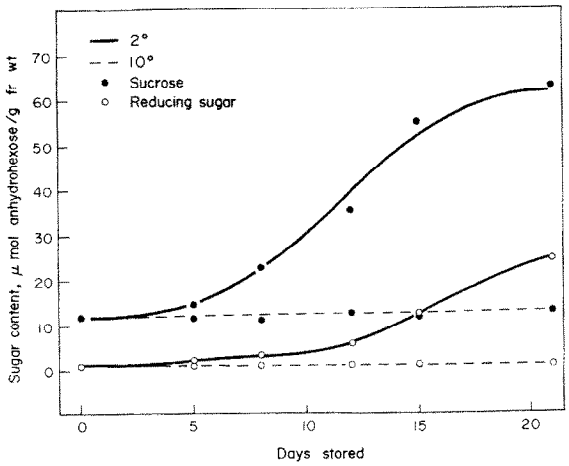


Fig. 1. Sugar content of potato tubers during storage at 2° or 10°. Each point is the mean of values from eight different tubers. The standard errors were all within ± 5% of the means.

Table 1. Enzyme activities of potato tubers after storage at 2° or 10°

| Days stored | Storage temp. (°) | Enzyme activity* (nmol product/g fr. wt per min) | | | | |
|---|---|---|---|---|---|---|
| | | Sucrose phosphate synthetase | Sucrose synthetase | Glucose-6-phosphate dehydrogenase | Aldolase | Glyceraldehydephosphate dehydrogenase |
| 0 | — | 115 ± 5 | 7·5 ± 0·5 | 370 ± 14 | 224 ± 12 | 453 ± 40 |
| 8 | 2 | 130 ± 28 | 8·9 ± 1·2 | 376 ± 41 | 202 ± 12 | 512 ± 27 |
| 15 | 2 | 125 ± 17 | 7·5 ± 0·4 | 459 ± 57 | 212 ± 16 | 605 ± 39 |
| 8 | 10 | 135 ± 20 | 7·5 ± 0·8 | 366 ± 42 | 216 ± 12 | 452 ± 37 |
| 15 | 10 | 125 ± 18 | 8·7 ± 0·9 | 453 ± 37 | 258 ± 20 | 597 ± 27 |

* Values are means ± s.e. of activities of three different extracts for sucrose phosphate and sucrose synthetases, and of four different extracts for the other enzymes.

Table 2. Temperature coefficients of potato enzymes at saturating concentrations of substrates

| Enzyme | Temperature coefficient* $Q_{10}(10–25°)$ | Temperature coefficient* $Q_{10}(2–10°)$ | Fisher's $\tilde{P}$ value† $Q_{10}(10–25°)$ vs $Q_{10}(2–10°)$ |
|---|---|---|---|
| Sucrose phosphate synthetase | 1·92 ± 0·01 | 2·25 ± 0·24 | N.S. |
| Sucrose synthetase | 1·86 ± 0·03 | 1·96 ± 0·04 | N.S. |
| Glucose-6-phosphate dehydrogenase | 1·97 ± 0·01 | 2·49 ± 0·23 | N.S. |
| Aldolase | 2·35 ± 0·08 | 2·65 ± 0·52 | N.S. |
| Glyceraldehydephosphate dehydrogenase | 2·38 ± 0·03 | 5·10 ± 0·33 | <0·001 |

* Values are means ± s.e. of measurements made with extracts of five different unstored tubers. Similar results were obtained for 5 tubers that had been stored at 2° for 15 days.
† Fisher's $\tilde{P}$ values were calculated by Student's $t$ test; N.S., not significant ($\tilde{P} > 0·05$).

the activity of sucrose synthetase was low, both in absolute terms and in relation to the activities of the other enzymes. Secondly, storage at either 2° or 10° for 15 days had no detectable effect on the activity of any of the enzymes.

We determined the temperature coefficients of the enzymes over the ranges 10–25° and 2–10° (Table 2). These measurements were made under optimum conditions. Thus we calculate that at 2° the maximum catalytic activities of sucrose phosphate synthetase, sucrose synthetase, glucose-6-phosphate dehydrogenase, aldolase and glyceraldehydephosphate dehydrogenase were, respectively, 71, 5, 101, 20 and 26 μmol anhydrohexose metabolized/g fr. wt per day. Except for glyceraldehydephosphate dehydrogenase, the temperature coefficient of each enzyme at 10–25° was similar to that at 2–10°. However, the $Q_{10}$ for glyceraldehydephosphate dehydrogenase at 2–10°, the temperatures at which sweetening occurs, was appreciably higher than that at 10–25°. This effect of temperature on the activity of the extracted enzyme was readily reversible. We investigated whether the effect could be observed at lower concentrations of substrates that approached those likely to occur *in vivo*. We also extended our observations so as to include two other key glycolytic enzymes, phosphofructokinase and pyruvate kinase (E.C.2.7.1.41). We estimated the concentration of substrates *in vivo* from Barker's [5] data and from the evidence that about 5% of plant storage tissue is cytoplasm [6]. We then reduced the concentrations of substrates in the assay mixtures as near to these estimates as was practicable. Our results (Table 3) show that at these lower concentrations of substrates the temperature coefficients at 2–10° of all three enzymes were significantly higher than at 10–25°.

## DISCUSSION

We think that we have obtained reliable estimates of the maximal catalytic activities of the different enzymes. We have established that the assays were carried out under optimum conditions and we have provided evidence that there were no significant losses during extraction. The work of Bird *et al.* with pea shoots has led them to suggest that our assay for sucrose phosphate synthetase involves inhibitory concentrations of fluoride, EDTA and Tris, as well as insufficient $MgCl_2$ [7]. Bird *et al.* assayed the enzyme by measuring sucrose production in the presence of high activity of endogenous sucrose phosphate phosphatase. We do not think that this assay, when used with crude extracts, is specific enough to sustain the above criticisms. Until it can be shown that the assay used for sucrose is equally effective with sucrose phosphate, we cannot eliminate the possibility that the

Table 3. Temperature coefficients of glycolytic enzymes of potato at physiological concentrations of substrates

| Enzyme | Temperature coefficient* $Q_{10}(10–25°)$ | Temperature coefficient* $Q_{10}(2–10°)$ | Fisher's $\tilde{P}$ value† $Q_{10}(10–25°)$ vs $Q_{10}(2–10°)$ |
|---|---|---|---|
| Phosphofructokinase | 1·47 ± 0·05 | 3·33 ± 0·12 | <0·001 |
| Glyceraldehydephosphate dehydrogenase | 2·48 ± 0·06 | 4·96 ± 0·38 | <0·002 |
| Pyruvate kinase | 1·45 ± 0·05 | 2·38 ± 0·12 | <0·001 |

* Values are means ± s.e. of measurements made with extracts of five different unstored tubers.
† Fisher's $\tilde{P}$ values calculated as in Table 2.

effects found by Bird *et al.* were due to changes in the activity of sucrose phosphate phosphatase and not that of the synthetase. It is known that the phosphatase is inhibited by both fluoride and EDTA, and is stimulated by $MgCl_2$ [8]. In our assays we found that variation in the concentrations and omission of sodium fluoride, EDTA, and Tris all reduced the detectable activity of sucrose phosphate synthetase. Inclusion of $MgCl_2$ in the reaction mixtures also reduced activity. We suggest that our assay gives the best available estimate of the activity of sucrose phosphate synthetase in potatoes; our values are considerably higher than any others reported for the same tissue [4].

We think that our estimate of the rate of sugar accumulation at 2° represents the minimum rate of sucrose synthesis in the cold. Most of the accumulated sugar was sucrose and the rest consisted of equal amounts of glucose and fructose. This last point, the time course shown in Fig. 1 and in Isherwood's [1] experiments, and the presence of invertase in cold-stored potatoes [9], suggest that the accumulated hexose was formed from sucrose. Our estimates of the minimum rate of sucrose synthesis and of the maximum catalytic activities of sucrose phosphate and sucrose synthetases at 2° are, respectively, 5·9, 71, and 5 μmol anhydrohexose metabolized/g fr. wt per day. These results strongly suggest that sucrose synthesis during sweetening is catalysed by sucrose phosphate synthetase and not by sucrose synthetase.

Our measurements of the enzyme activities during storage provide sound evidence that storage at 2° does not alter the amounts of the enzymes present during the period in which sweetening is most marked. We suggest that variation in the maximum catalytic activities of the enzymes listed in Table 1 is not the cause of sweetening. It seems likely that sweetening is due to fine and not to coarse control. Our measurements of the temperature coefficients of the three glycolytic enzymes suggest one way in which lowering the temperature could exercise fine control over the metabolism of potatoes. We have shown that the activities of these three enzymes can be reduced disproportionately by lowering the temperature to that at which sweetening occurs. Cold-lability of phosphofructokinase does not appear to have been reported previously but the phenomenon has been demonstrated for glyceraldehydephosphate dehydrogenase from rabbit muscle [10], and for pyruvate kinase from yeast [11]. Cold-lability of certain regulatory enzymes could result in alterations in the relative activities of different metabolic pathways when the temperature is lowered. On general grounds it can be argued that phosphofructokinase and pyruvate kinase, certainly, and glyceraldehydephosphate dehydrogenase, possibly, contribute to the regulation of glycolysis in potatoes [12]. Thus, lowering the temperature to 2–8° could inhibit glycolysis immediately and cause hexose phosphates to accumulate. Such an accumulation would be expected to stimulate sucrose synthesis, particularly as it is known that sucrose phosphate synthetase shows a sigmoidal saturation curve for fructose-6-phosphate [13]. Measurements of glycolytic intermediates in cold-stored potatoes support the above hypothesis. There is evidence that one of the early results of transferring potatoes from 10° to 1° is an increase in the contents of fructose-6-phosphate, glucose-6-phosphate, glucose-1-phosphate, and dihydroxyacetone phosphate [5, 14]. Conversely, Walsh and Rowan [15] have provided evidence that there is a rapid decline in the contents of fructose-6-phosphate and glucose-6-phosphate when tubers are transferred from 1° to 18°. We stress that the effects of cold on potato metabolism are likely to be so complex that it is improbable that there is any single cause of sweetening. We merely suggest that cold-lability of the above glycolytic enzymes may be one of the causes.

## EXPERIMENTAL

*Material.* Tubers of *Solanum tuberosum* L. var. Record were obtained from Walkers Crisps Limited and were kept in the dark at 10° until used. For each experiment replicate samples of tubers were prepared and either used at once (unstored tubers) or stored in the dark at 2° or 10° (stored tubers). All the experiments were done with mature tubers within 9 months of harvest.

*Extraction of enzymes.* The following applies to all of the enzymes except phosphofructokinase. Samples of 5–6 g fr. wt of tuber were extracted in 1·5 vol extraction medium. Complete cell breakage was achieved by using a pestle and mortar and then a Ten-Broek homogenizer. The extraction medium that we used for sucrose phosphate and sucrose synthetases has been described [16]. For the other enzymes we used the same medium except that we substituted 100 mM glycylglycine-NaOH buffer (pH 7·5) for the Tris HCl buffer. The homogenates were centrifuged at 35000 *g* for 15 min. Samples of the supernatant were assayed directly for the glyceraldehydephosphate dehydrogenase and after desalting with Sephadex G-25 (coarse) for all the other enzymes. The above crude extracts

showed no phosphofructokinase activity. We obtained an active preparation of this enzyme as follows. We homogenized 500 g fr. wt of tuber in 250 ml 100 mM imidazole–HCl buffer (pH 7·6) that contained EDTA (20 mM), cysteine-HCl (20 mM) and sodium diethyl-dithiocarbamate (20 mM). The homogenate was filtered through cheesecloth and centrifuged at 20000 *g* for 15 min. Phosphofructokinase was obtained from the supernatant by the procedure of Kelly and Turner [17].

*Assay of enzymes.* Sucrose phosphate and sucrose synthetases were assayed as described previously [16] except that for the former the reaction mixture contained 4·0 μmol UDPG and 6·0 μmol [U-$^{14}$C]-fructose-6-phosphate (0·08 μCi). Glucose-6-phosphate dehydrogenase was assayed as described previously [18] except that the reaction mixture contained 0·67 μmol $NADP^+$ and 12·5 μmol glucose-6-phosphate at pH 8·0. Aldolase and glyceraldehydephosphate dehydrogenase were assayed according to Wu and Racker [19]. For aldolase the reaction mixture was altered to contain 150 μmol glycylglycine–NaOH at pH 7·5 and 15 μg triosephosphate isomerase/glycerolphosphate dehydrogenase mixture. For measurements of the maximum catalytic activity of glyceraldehydephosphate dehydrogenase the reaction mixture contained: 300 μmol glycylglycine–NaOH at pH 7·5, 30 μmol EDTA, 90 μmol sodium arsenate, 3·6 μmol $NAD^+$, 6·0 μmol fructose-1,6-diphosphate, and 0·2 units of aldolase in a volume of 3·0 ml. For the measurements of the temperature coefficients of this enzyme reported in Table 3, the concentration of fructose-1,6-diphosphate was reduced to 0·07 mM and that of $NAD^+$ to 0·6 mM. Phosphofructokinase was assayed according to Scott *et al.* [20] except that the concentration of ATP was reduced to 0·33 mM and that of fructose-6-phosphate to 0·5 mM. Pyruvate kinase was assayed by measuring NADH oxidation in the reaction mixture that contained: 75 μmol glycylglycine–NaOH, pH 7·5, 12·0 μmol $MgSO_4$, 825 μmol KCl, 1·38 μmol ADP, 12 μmol phosphoenolpyruvate, 0·45 μmol NADH, 150 μmol NaF, and 1·5 units of lactate dehydrogenase in a volume of 3·0 ml. The reported activities of pyruvate kinase were shown to be dependent upon the presence of ADP. Measurements of maximum catalytic activities were made at 25°. Temperature coefficients were determined from measurements made at 25, 10 and 2°.

*General methods.* Sugars were extracted with boiling 80% (v/v) aq. EtOH and measured as described earlier [21]. Previously described methods [18] were used for the assay of protein and $^{14}$C.

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