Phytochemistry, 1980. Vol. 19, pp 1653–1656. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0801-1653 $02.00/0

CARBOHYDRATE METABOLISM DURING COLD-INDUCED SWEETENING OF POTATO TUBERS

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(*Received* 18 *December* 1979)

Key Word Index– *Solanum tuberosum*; Solanaceae; potato; tuber; cold; sweetening; glucose-[^{14}C]; pulse and chase.

Abstract– The aim of this work was to discover the effects of lowering the temperature from 25° to 2° on the metabolism of glucose-[U-^{14}C] by tubers of *Solanum tuberosum*. Isotope was applied to tubers via a 50-μl hole made with a capillary pipette. Tubers were incubated for 2 hr, the pulse; then the glucose-[U-^{14}C] was replaced with glucose, and incubation was continued for 18 hr, the chase. The detailed distribution of ^{14}C was determined at the end of the pulse and at the end of the chase at 2°, and compared with those found at 25°. Lowering the temperature reduced the proportion of metabolized ^{14}C that entered the respiratory pathways. At 2°, but not at 25°, hexose phosphates were the most heavily labelled fraction after the pulse; during the chase at 2° much of this label was metabolized to sucrose. We conclude that lowering the temperature preferentially restricts glycolysis and diverts hexose phosphates to sucrose. We suggest that this is an important cause of cold-inducing sweetening of the tubers and is due to cold-lability of key glycolytic enzymes.

INTRODUCTION

Studies of the mechanism of cold-induced sweetening of potato tubers indicate that their phosphofructokinase and pyruvate kinase are more sensitive to cold than are other enzymes involved in the metabolism of hexose phosphates [1]. Phosphofructokinase plays a dominant role in regulating entry of glucose 6-phosphate into glycolysis in the tuber. In addition, there is evidence that pyruvate kinase controls exit from glycolysis and contributes to the regulation of the concentrations of glycolytic intermediates, many of which are effectors of phosphofructokinase [2]. Thus lowering the temperature could restrict glycolysis in potatoes to a greater extent than other reactions that consume hexose 6-phosphates, and this could lead to enhanced synthesis of sucrose through increased availability of hexose phosphates. The effect of lowering the temperature from 25° to 2° on the short-term metabolism of glucose-[^{14}C] by potato tubers is to cause an accumulation of label in hexose phosphates [3]. If the above theory of sweetening is correct, then lowering the temperature should divert hexose phosphates from entering the respiratory pathways and cause these intermediates to be converted to sucrose. In particular, label that accumulates in hexose phosphates in a short pulse from glucose-[^{14}C] should be metabolized to sucrose in a chase following the removal of non-metabolized glucose-[^{14}C]. The aim of the work described in this paper was to test the above predictions by determining the detailed distribution of label, in tubers supplied with glucose-[U-^{14}C], at the end of the pulse and the end of a chase at 2°, and comparing them with those found at 25°.

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RESULTS AND DISCUSSION

It is not practicable to feed glucose-[^{14}C] to intact tubers. Slices of tubers are easily fed, but we avoided this because slicing alters both the rate and direction of carbohydrate metabolism [4]. We labelled tubers with the minimum of wounding by removing a minute cylinder of tissue with a 50-μl capillary pipette and placing in the hole 50 μl glucose-[^{14}C] which was replaced with glucose for the chase. At the end of the pulse or chase we removed a core of tissue (4 × 1 cm) that was concentric with the hole that had contained the glucose-[^{14}C], and analysed the distribution of ^{14}C amongst the components of this core of tissue. Comparable experiments in which we put the dye methylene blue in the hole instead of glucose-[^{14}C] showed that material diffused radially at least 0.5 cm from the hole during the pulse. We suggest that the wounding that did occur during our procedure did not seriously affect our results. First, the wounded cells formed only a very small proportion of the labelled cells that we analysed as the wound effects in potato tuber are restricted to the two to three layers of cells next to the cut surface [5]. Second, previous studies [3] have shown that the initial products of glucose-[U-^{14}C] metabolism are the same in tubers, labelled as in the present experiments, and in potato tissue cultures, labelled without any wounding. Finally, our method gave extremely consistent results despite variation in the age and origin of the tubers to which we applied it.

The proportion of the added glucose-[^{14}C] that was metabolized by the core of tissue varied considerably with the temperature, from 20 to 53%, and to some degree with the tubers. Each pulse and each chase was carried out on a separate tuber. In order to compare tubers and treatments, the ^{14}C recovered per cell fraction from a particular pulse or chase is expressed as a percentage of the amount of glucose-[^{14}C] metabolized in that pulse or chase. We estimated the ^{14}C metabolized as follows. We measured

the labelling of sucrose, glucose, and fructose in the extracts. We assumed that any labelled fructose had been derived from sucrose-[^{14}C] formed from glucose-[U-^{14}C], and that such sucrose-[^{14}C] had been equally labelled in the glucosyl and fructosyl moieties. These assumptions are based on: the lack of hexose monophosphatases in plant tissues in general, the presence of invertase in potato tubers [6], the kinetics of accumulation of sucrose and hexoses during sweetening [7], and the evidence that the hexose 6-phosphates in potatoes are in equilibrium [2]. Accordingly, the ^{14}C found as fructose-[^{14}C] was doubled to give ^{14}C converted to hexose via sucrose. The value obtained, together with the ^{14}C present as sucrose-[^{14}C], was added to the sum of the ^{14}C recovered as $^{14}CO_2$, in the water-insoluble substances, and in the acidic and basic components of the water-soluble substances, to give total ^{14}C metabolized.

The procedure that we used to replace glucose-[U-^{14}C] with glucose at the end of the pulse could have removed labelled insoluble material from damaged cells and, or, labelled soluble material that had leaked out of the cells surrounding the hole. We analysed the material taken from the hole at the end of the pulse. The water-soluble fraction was highly radioactive but the only labelled compound that we would detect in it was glucose-[^{14}C]. We found no labelled compound in the water-insoluble fraction. We conclude that we did not remove any significantly labelled product of metabolism at the end of the pulse.

The results of our analyses for pulse and chase at 2°, and at 25°, are given in Tables 1 and 2, respectively. We emphasize that each treatment was applied to an appreciable number of different tubers, that there was close agreement between replicates, and that the fractionations were achieved without serious loss of labelled compounds. The only significant loss occurred during the fractionation of the insoluble substances from the pulse at 2°, but even this amounted to no more than 10% of the total ^{14}C metabolized. This loss is attributed to the difficulty of achieving quantitative recovery of all of the monomers from the complex mixture of polymers present in the water-insoluble fraction.

The distribution of ^{14}C at 2° (Table 1) is discussed first. The labelling at the end of the pulse is similar to that reported previously [3] except that our more complete analyses, that now include CO_2 and the components of the insoluble fraction, permit more definitive conclusions. Two points are stressed. First, there is clear proof of accumulation of label in the hexose phosphate fraction: this was the most heavily labelled fraction and it contained 39% of the metabolized ^{14}C. Second, the proportion of metabolized label that entered the respiratory pathways was demonstrably low. This proportion was calculated by summing the contributions to CO_2, organic acids, amino acids and protein. The value obtained, 22%, is much lower than the contribution to hexose phosphates.

During the subsequent chase at 2° there were dramatic changes in the distribution of ^{14}C. Most pertinent to our argument is the fall in the labelling of the hexose phosphates. The fate of the labelled hexose phosphates present at the end of the pulse may be established by consideration of the detailed distribution of ^{14}C. Extremely little of the hexose phosphates labelled in the pulse can have entered the respiratory pathways in the chase. The labelling of the products of these pathways, detailed above, amounted to only 19% of the metabolized label at the end of the chase, and thus showed no increase over the value obtained at the end of the pulse. The labelled hexose phosphates did not accumulate as insoluble material during the chase as the proportion of metabolized ^{14}C in this fraction fell from 32 to 7.5% during the chase. The only substances to show an increase in labelling during the chase that could account for the fall in the labelling of the hexose phosphates were sucrose and hexose. This shows that much of the labelled hexose phosphates were converted to free sugars during the chase.

The above conclusion is confirmed by the fact that the large increase in labelling of the free sugars during the chase, which amounted to 55% of the ^{14}C metabolized,

Table 1. Distribution of ^{14}C after metabolism of glucose-[U-^{14}C] in 2-hr pulse and in 18-hr chase by potato tubers at 2°

Fraction	Percentage* of metabolized ^{14}C recovered per fraction at end of		Fisher's P values for comparison of pulse vs chase
	Pulse	Chase	
CO_2	0.2 ± 0.02 (3)	1.1 ± 0.2 (3)	<0.01
Water-soluble substances	65.4 ± 1.3	96.7 ± 2.0	<0.001
Hexose phosphates	38.5 ± 2.0	10.5 ± 0.7	<0.001
Organic acids	3.3 ± 0.5	4.3 ± 0.4	n.s.
Amino acids	10.1 ± 1.1	12.6 ± 0.7	n.s.
Sucrose	10.7 ± 0.3	57.3 ± 1.2	<0.001
Hexose	2.8 ± 0.2	11.4 ± 1.0	<0.01
Water-insoluble substances	32.0 ± 0.7	7.5 ± 0.8	<0.001
Protein	8.4 ± 0.7	1.0 ± 0.05	<0.001
Starch	13.0 ± 0.4	2.8 ± 0.3	<0.001
Non-starch polymeric glucose	0.2 ± 0.1 (3)	1.1 ± 0.1 (3)	n.s.
Polymeric galactose	0 (3)	0.1 ± 0.2 (3)	
Total ^{14}C metabolized (dpm × 10^{-6} per core of tissue)	3.59 ± 0.12 (7)	4.45 ± 0.44 (5)	n.s.

* Values are means ± s.e. of estimates from five, unless shown otherwise in parentheses, different tubers.

Table 2. Distribution of ^{14}C after metabolism of glucose-[U-^{14}C] in 2-hr pulse and in 18-hr chase by potato tubers at 25°

Fraction	Percentage* of metabolized ^{14}C recovered per fraction at end of		Fisher's P values for comparison of pulse vs chase
	Pulse	Chase	
CO_2	0.6 ± 0.06	10.5 ± 0.4	<0.001
Water-soluble substances	69.8 ± 0.8 (5)	59.8 ± 0.7 (5)	<0.001
Hexose phosphates	9.7 ± 0.4	3.6 ± 0.3	<0.001
Organic acids	5.0 ± 0.2	7.8 ± 0.2	n.s.
Amino acids	24.1 ± 1.0	8.4 ± 0.5	<0.001
Sucrose	28.2 ± 1.0	34.7 ± 0.5	<0.001
Hexose	2.8 ± 0.1	5.3 ± 0.5	<0.01
Water-insoluble substances	28.1 ± 1.1 (5)	38.3 ± 0.9 (5)	<0.001
Protein	5.7 ± 0.9 (5)	12.4 ± 0.9 (5)	<0.001
Starch	13.1 ± 0.6 (5)	23.6 ± 2.0 (5)	<0.001
Non-starch polymeric glucose	3.6 ± 0.5	1.9 ± 0.8	n.s.
Polymeric galactose	4.5 ± 1.2	2.0 ± 0.4	<0.05
Total ^{14}C metabolized (dpm × 10^{-6} per core of tissue)	7.1 ± 0.07	9.69 ± 1.12	n.s.

* Values are means ± s.e. of estimates from three, unless shown otherwise in parentheses, different tubers.

cannot be accounted for unless it is accepted that the hexose phosphates were metabolized to free sugars. As potatoes are not gluconeogenic [8], no ^{14}C can have moved into sugars from organic acids, amino acids, protein or CO_2. However, hexose phosphates could have contributed 28% of the metabolized label to sugars during the chase. A further 10% must have been due to the sharp fall in the labelling of starch. The remaining 17% probably had two origins. One is polysaccharide. It is probable that most of the label lost during the fractionation of the insoluble substances was in starch. Isherwood [7] has provided evidence that starch is the only significant source of carbon for sucrose synthesis during cold-induced sweetening. Analyses of the insoluble fraction from another series of pulses at 2° with tubers from a different crop gave recoveries of 90% of the insoluble ^{14}C in starch and protein. Thus the contribution of labelled starch to sucrose during the chase in the experiments reported in Table 1 was probably near 20% of the metabolized label. The second possible source of labelled sucrose in the chase is unmetabolized glucose-[U-^{14}C] that was not removed at the end of the pulse. Quantitative removal of all the unmetabolized glucose-[^{14}C] from the free space of the tuber is unlikely when only 50-μl rinses of the hole could be made. Thus some glucose-(U-^{14}C] could have remained at the end of the pulse and could have been converted to sucrose-[^{14}C] during the chase. The amounts converted must have been relatively small as we were unable to demonstrate any significant difference between the total ^{14}C metabolized at the end of the pulse and that at the end of the chase (Table 1).

Comparison of the data at 2° with those at 25° reveals the features that were associated with metabolism in the cold. Both the predictions made in the introduction were fulfilled. First, at 25° the percentage of metabolized label recovered in products of respiration (CO_2, organic acids, amino acids and protein) was considerably greater than at 2° for both pulse and chase. At 2° the percentages for pulse and chase were 22 and 19, respectively; at 25° they were 35 and 39. Thus lowering the temperature did restrict the proportion of metabolized hexose that entered the respiratory pathways. Second, the inverse relationship between the labelling of the hexose phosphates and free sugars in the pulse and chase was clearly associated with lowering the temperature. At 25° there was no marked accumulation of label in hexose phosphates at the end of the pulse, and the decline during the chase was no more than 6.1% of the label metabolized. This behaviour of the hexose phosphates at 25° correlates closely with the fact that at this temperature there was only a small increase in the labelling of free sugars during the chase.

The general distribution of label at 25° is that expected from our present knowledge of intermediary metabolism and requires no further comment. However two additional effects of cold are noted. First, at 2° starch labelled in the pulse was rapidly broken down in the chase. The data of Isherwood [7] show that this almost certainly represents a net breakdown of starch. This effect of cold could make an important contribution to sweetening provided that the products of starch breakdown were not respired. The effect could be a contributory primary cause of sweetening resulting from a direct response of amylolytic enzymes to cold. However no such response has been demonstrated and it is just as likely that the effect is secondary in that it results from changes in effectors of amylolytic enzymes caused by the response of some other enzymes, e.g. those of glycolysis, to cold. This point cannot be resolved until we know the pathway of starch breakdown during sweetening and the effects of cold on the regulatory enzymes. The final effect of cold on potato metabolism to be noted from our work is that lowering the temperature led to rapid breakdown of protein formed in the pulse. The data of Fitzpatrick and Porter [9] suggest that this may reflect some net breakdown of protein, although increased turnover of protein may have made a substantial contribution to the rapid movement of label out of protein in the cold.

We conclude that when potato tubers, that are capable of sweetening, are transferred from 25° to 2° carbohydrate metabolism is not only reduced in rate but is also

redirected. A particularly marked aspect of this redirection is a reduction in the proportion of hexose 6-phosphates that enter the respiratory pathways and a diversion of these compounds to sucrose. Such a diversion is precisely what would be expected if the enzymes that control glycolysis were preferentially sensitive to cold. We suggest that cold-lability of phosphofructokinase in particular, and possibly also pyruvate kinase, is responsible for diverting hexose phosphates to sucrose and is thus an important cause of sweetening in potatoes.

EXPERIMENTAL

Materials. Glucose-[U-^{14}C] was from the Radiochemical Centre, Amersham. Tubers of *Solanum tuberosum* L. cv Record were from Walkers Crisps Ltd and were kept in the dark at 10° until used. All expts were made with small (60 × 30 cm) uniformly shaped tubers within 4 months of harvest.

Pulse and chase. For each expt two carefully matched tubers were transferred from the store to the experimental temp., 2° or 25°. After 6 hr, when the tubers had equilibrated to the new temp. [10], a 50-μl capillary pipette was used to remove a core of tissue (1 × 100 mm) from each tuber. The core was taken through the centre of the tuber parallel to the longitudinal axis. We then put into the resulting hole in the tuber 50 μl 0.5 mM glucose-[U-^{14}C] (325.2 μCi/μmol) in 0.02 M KH_2PO_4 (pH 5.2), and sealed the hole with vaseline. Each tuber was then placed in a 250-ml beaker that was closed with a rubber bung and incubated in the dark at the appropriate temp. A stream of air, previously equilibrated to the experimental temp., was passed through the beaker and around the tuber throughout the incubation. After 2 hr the tubers were removed from the beakers. For the pulse a plug of tissue (4 × 1 cm), concentric with the hole that contained the glucose-[U-^{14}C], was rapidly removed with a cork borer and killed at once with boiling 80% aq. EtOH. For the chase an empty 50-μl capillary pipette was carefully passed through the hole that contained the glucose-[U-^{14}C] to remove the latter. This process was repeated 5 ×. Then 50 μl 0.5 mM glucose in 0.02 M KH_2PO_4, pH 5.2, was put in the hole which was again sealed with vaseline. The tuber was returned to the beaker and incubated as described above for a further 18 hr. At the end of the chase a plug of tissue was removed and killed as described for the pulse. The above procedure was varied to measure $^{14}CO_2$ production. The stream of air that emerged from the beaker in which the tuber was incubated was bubbled through 20% (w/v) KOH: the resulting $K_2^{14}CO_3$ was counted directly. Labelled bicarbonate retained in the tuber was estimated by chopping up the complete tuber and killing in a closed vessel with 80% aq. EtOH acidified with glacial HOAc. The $^{14}CO_2$ evolved was trapped and counted as above. The values obtained by the two procedures were summed to give the total $^{14}CO_2$ production.

Analyses. The cores of tissue were extracted by boiling for 10 min in, successively, 80%, 60%, 20% aq. EtOH, H_2O, and 80% aq. EtOH. The extracts were combined and reduced to 3–4 ml at 28° to give the H_2O-soluble substances that were divided into their acidic, basic, and neutral components by ion-exchange chromatography as in ref. [11]. Further fractionation was by PC as in ref. [3] which also describes how the hexose phosphate fraction was obtained and characterized. ^{14}C in the H_2O-insoluble substances was determined after combustion to $^{14}CO_2$ as in ref. [12]. The labelling of protein was determined by hydrolysing samples of the H_2O-insoluble material as in ref. [13] and then isolating the amino acids from the hydrolysate by ion-exchange chromatography as above. The label in polysaccharide was measured by treating portions of the H_2O-insoluble material with α-amylase and amyloglucosidase as in ref. [14]. The label found in the supernatant is ascribed to starch. The ppt. was then hydrolysed with H_2SO_4 and individual sugars were isolated by PC as in ref. [15]. The label recovered from this hydrolysate as glucose-[^{14}C] is ascribed to non-starch polymeric glucose, and that as galactose-[^{14}C] to polymeric galactose. In all instances ^{14}C was eluted from paper chromatograms before being counted. ^{14}C was measured by scintillation spectrometry as in ref. [16]. Fisher's *P* values were calculated by Student's *t*-test. Values of 0.05 or less are considered significant. Values greater than 0.05 are given as n.s. (not significant).

Acknowledgements—We thank Walkers Crisps for their gifts of potatoes. W.L.D. thanks the Potato Marketing Board for a postgraduate studentship.

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