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EFFECT OF COLD ON GLUCOSE METABOLISM BY CALLUS AND TUBERS OF *SOLANUM TUBEROSUM*

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Abstract—This work was done in order to discover the immediate effects of low temperature on glucose metabolism by tissue of *Solanum tuberosum*. [U-^{14}C]-Glucose was supplied to tubers, and to callus derived from tubers, for 3 hr at 2 and 25°. The detailed distribution of label showed that lowering the temperature of both callus and tuber tissue to 2° caused a striking increase in the percentage of the metabolized label that was recovered in glucose-6-phosphate, fructose-6-phosphate, and glucose-1-phosphate. It is suggested that these results, together with the cold-lability of glycolytic enzymes, indicate that lowering the temperature of potato tissue reduces glycolysis in relation to the activities of other reactions involving hexose phosphates.

INTRODUCTION

Potato tubers accumulate sugars when they are stored at 0–6°. In a study of this sweetening, we found that the temperature coefficients of the key glycolytic enzymes, phosphofructokinase, glyceraldehydephosphate dehydrogenase, and pyruvate kinase, were higher between 2 and 10° than between 10 and 25° [1]. Thus it is possible that one of the responses of potato tubers to low temperatures is an immediate reduction in glycolysis relative to the activities of other reactions involving hexose phosphates. The aim of the present work was to test the above hypothesis by determining the immediate effect of cold on the metabolism of glucose by potato tissue. We did this by measuring the detailed distribution of ^{14}C after supplying [U-^{14}C]-glucose at 2 and 25° to callus cultures and to tubers of the variety of potato which we used in our previous experiments. We chose callus tissue as our main experimental material for two reasons. Firstly, we have shown that transfer of such cultures from 25 to 2° results in a sweetening that has the same general physiological characteristics as that found in intact tubers [2]. Secondly, callus can be cooled much more rapidly and can be fed [^{14}C]-glucose under more carefully controlled conditions than is possible for intact tubers.

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RESULTS AND DISCUSSION

We determined the effects of cold on glucose metabolism by callus tissue as follows. For each experiment we prepared duplicate samples of callus. One sample was kept at 2° for 15 min and then incubated in [U-^{14}C]-glucose for 3 hr at 2°. The other sample was kept at 25° for 15 min and then incubated in [U-^{14}C]-glucose for 3 hr at 25°. At the end of the 3-hr incubations both samples were killed and fractionated to reveal the detailed distribution of ^{14}C (Table 1). Lowering the temperature severely reduced both the rate of respiration and the uptake of [^{14}C]-glucose. In order to compare the distribution of ^{14}C at the two temperatures, we have expressed the ^{14}C recovered in the different fractions of each sample as percentages of the total ^{14}C metabolized by that sample. This latter figure is the amount of label respired as $^{14}CO_2$ plus that recovered from the callus minus any contribution made by [^{14}C]-glucose. Thus for each sample the label recovered

Table 1. Effect of temperature on metabolism of [U-^{14}C]-glucose by potato callus

Measurement*	Tissue incubated at 25°	Tissue incubated at 2°	Fisher's $\tilde{P}$ values† (25° vs 2°)
CO_2 production (μl/g fr. wt/hr)	40·0	8·6	
^{14}C supplied to tissue (μCi)	1·0	5·0	
% of supplied ^{14}C metabolized	16·5 ± 1·9	2·1 ± 0·1	<0·001
% of metabolized ^{14}C recovered in			
CO_2	21·5 ± 1·4	3·5 ± 0·4	<0·001
Acidic components of H_2O-soluble material	29·9 ± 2·2	65·3 ± 2·9	<0·01
Basic components of H_2O-soluble material	11·1 ± 2·1	7·2 ± 1·4	n.s.
Fructose	5·0 ± 1·3	4·8 ± 1·3	n.s.
Sucrose	19·5 ± 1·3	13·0 ± 1·8	n.s.
Material insoluble in water	12·3 ± 1·3	5·4 ± 1·7	n.s.

* Values are means ± s.e. of measurements from four samples.
† Fisher's $\tilde{P}$ values were calculated by Student's t-test; n.s., not significant ($\tilde{P} > 0{\cdot}05$).

in the fractions that contained CO_2, sucrose, fructose, the acidic and basic components of the water-soluble material, and the water-insoluble material have been summed to give total ^{14}C metabolized. The distribution of label in the callus tissue incubated at 25° (Table 1) is that expected for non-photosynthetic tissues of higher plants [3,4]. The distribution found at 2° shows two important differences from that found at 25°. Firstly, lowering the temperature greatly reduced the percentage of the metabolized [^{14}C]-glucose that was converted to $^{14}CO_2$. Secondly, the percentage of the metabolized [^{14}C]-glucose recovered as acidic components of the water-soluble material was increased spectacularly by lowering the temperature.

We investigated whether these effects of cold on glucose metabolism could be found in potato tubers or whether they were peculiarities of callus tissue. The extensive changes that occur when a tuber is cut [5] precluded the use of disks or other small pieces of tuber tissue as experimental material. Therefore we adopted the following procedure for supplying [^{14}C]-glucose to tubers. For each experiment we chose two comparable tubers. We incubated one at 2° and the other at 25° for 6 hr so that each tuber attained its ambient temperature throughout [6]. We then used a 50 μl capillary pipette to remove a core of tissue (1 × 100 mm) from each tuber. Each core was taken through the centre of the tuber along the longitudinal axis. Next we placed [U-^{14}C]-glucose in the holes made in the tubers by the removal of the cores. The tubers were then incubated at 2 or 25° for 3 hr. In comparable experiments, in which we placed methylene blue rather than [^{14}C]-glucose in the tubers, we found that the dye did not spread radially more than 1 cm from the hole in the tuber. Thus at the end of the 3-hr incubation we used a cork borer to remove from each tuber a plug of tissue (2 × 10 cm) that was concentric with the hole that contained the [^{14}C]-glucose.

The distribution of ^{14}C in the different fractions of the plugs of tuber tissue is given in Table 2. We found that the above feeding technique gave

Table 2. Effect of temperature on metabolism of [U-^{14}C]-glucose by potato tubers

Measurement	Tubers incubated at 25°	Tubers incubated at 2°
Metabolized ^{14}C recovered from tubers* (dpm × 10^{-6})	1·61	0·8
% metabolized ^{14}C in tubers found in		
Acidic components of H_2O-soluble material	13·0	34·5
Basic components of H_2O-soluble material	23·6	12·3
Fructose	6·4	2·0
Sucrose	31·6	12·4
Material insoluble in H_2O	18·5	25·2

* This value is the sum of the ^{14}C recovered in the cell fractions listed in the table. All measurements are means of data from triplicate tubers.

reproducible results but did not permit accurate measurement of $^{14}CO_2$ production because variable amounts of the $^{14}CO_2$ were retained in the tissue. Thus we have expressed the ^{14}C per fraction as percentages of the metabolized ^{14}C recovered from the plugs of tissue as compounds other than $^{14}CO_2$. This value is the sum of the ^{14}C recovered in the fractions that contained sucrose, fructose, the acidic and basic components of the water-soluble material, and the water-insoluble material. The data in Table 2 do not differ greatly from those in Table 1. The essential feature of the results in Table 2 is that they show that lowering the temperature increased the percentage of metabolized [^{14}C]-glucose which was recovered as soluble acidic compounds.

The effect of cold on the labelling of the acidic fractions of the water-soluble substances prompted us to determine the detailed distribution of ^{14}C within these fractions. Analysis by PC showed that [^{14}C]-malate, [^{14}C]-succinate, and [^{14}C]-citrate between them accounted for 40–45% of the label in the acidic fractions of both callus and tuber when the [^{14}C]-glucose was supplied at 25°. When callus and tubers were fed at 2° the above acids accounted for only 8 and 19%, respectively, of the ^{14}C in the acidic fractions. The ^{14}C that was not accounted for as [^{14}C]-malate, [^{14}C]-succinate, and [^{14}C]-citrate was recovered quantitatively from four spots close to the origin of the chromatograms. This was true for the acidic fractions from callus and tuber fed at both 2 and 25°. The closeness of these spots to each other and to the origin did not permit either their separation or their positive identification.

The positions of the above spots, in relation to those of markers, strongly suggested that they were due to sugar phosphates. This view was confirmed in respect of the acidic fractions from callus and from tubers fed at 2 and at 25°. In each instance the area of the chromatogram that contained the four spots was eluted to give a fraction referred to as sugar phosphates. Firstly, we showed that in all instances incubation of the sugar phosphate fraction with acid phosphatase released at least 80% of the ^{14}C in the sugar phosphate fraction as [^{14}C]-glucose and [^{14}C]-fructose. Secondly, two-dimensional chromatography, followed by autoradiography, of the water-soluble substances from callus and tubers fed at 2 and at 25° showed heavy labelling in the areas that corresponded to the positions of glucose-6-phosphate, glucose-1-phosphate, fructose-6-phosphate, and 3-phosphoglycerate. Finally, we measured the extent to which [^{14}C]-glucose-6-phosphate, [^{14}C]-glucose-1-phosphate, and [^{14}C]-fructose-6-phosphate contributed to the labelling of the sugar phosphate fractions. We did this by incubating portions of the sugar phosphate fractions with enzymes that would convert the above compounds to 6-phosphogluconate which we then isolated and counted. Thus to measure the label present as [^{14}C]-glucose-6-phosphate we incubated some of each sugar phosphate fraction with NADP and glucose-6-phosphate dehydrogenase. Then we isolated and counted the resulting 6-phosphogluconate. For [^{14}C]-fructose-6-phosphate we added glucosephosphate isomerase to the above reaction mixture, and for [^{14}C]-glucose-1-phosphate we added phosphoglucomutase. We established that this procedure quantitatively converted known amounts of glucose-6-phosphate, glucose-1-phosphate, and fructose-6-phosphate to 6-phosphogluconate. We found that these three

Table 3. Distribution of ^{14}C in acidic components of extracts of callus and tubers supplied with [U-^{14}C]-glucose at 25 and 2°

Component of acidic fraction	% ^{14}C metabolized by callus recovered per component		% metabolized ^{14}C recovered from tuber per component	
	25°	2°	25°	2°
All the acidic components of water-soluble material	29·9	65·3	13	34·5
Malate	7·7	3·4		
Succinate	4·8	0·7	5·2	6·7
Citrate	1·1	1·1		
Glucose-1-phosphate	4·5	17·4	2·1	5·8
Glucose-6-phosphate	3·7	16·3	1·2	4·7
Fructose-6-phosphate	3·7	11·1	2·0	8·0

hexose phosphates together accounted for 70–80% of the ^{14}C in the sugar phosphate fractions from callus and from tubers incubated at 2 and at 25°. In Table 3 the ^{14}C found in these compounds is expressed as percentages of the metabolized ^{14}C. The results show that lowering the temperature of both callus and tubers to 2° caused a striking increase in the proportion of metabolized ^{14}C which was recovered as hexose phosphates.

The results presented in this paper are consistent with the view that lowering the temperature of potato tissue to 2° leads to an inhibition of carbohydrate oxidation relative to other reactions involving hexose phosphates. The close agreement between the present studies *in vivo* and our previous investigation of the properties of glycolytic enzymes *in vitro* [1] suggests that cold-lability of key glycolytic enzymes causes potato tissue to respond immediately to a reduction in temperature to 2° by decreasing the relative activity of glycolysis. Although our work indicates an important response of potato tissue to cold, further investigation of the relationship between hexose phosphates and the synthesis and accumulation of sucrose in whole tubers will be necessary before we can decide whether this response contributes to sweetening.

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. Only mature tubers within 9 months of harvest were used. Callus cultures were obtained from such tubers and were grown in the dark at 25° on the solidified medium of Ingram and Robertson [7] as described previously [2]. Cultures were harvested after 8 weeks growth, pooled, washed 4× (each time with 20 vol. 0·02 M KH_2PO_4, pH 5·2), blotted dry and sampled by wt.

Metabolism of [U-^{14}C]-*glucose.* Replicate samples of 3·5 g fr. wt of callus tissue were suspended in 10 ml 0·5 mM glucose in 0·02 M KH_2PO_4 (pH 5·2) and incubated at the appropriate temp. for 15 min. Then we added 5 μCi [U-^{14}C]-glucose (250 μCi/μmol) to samples at 2° and 1 μCi to samples at 25°. Incubations were carried out in 250-ml Erlenmeyer flasks fitted with a centre well that contained 10% KOH for the collection of respired $^{14}CO_2$. After incubation in the [^{14}C]-glucose for 3 hr the samples were washed free of labelled soln by 3 successive rinses, each of 10 ml 0·5 mM glucose in 0·02 M KH_2PO_4 (pH 5·2) at the incubation temp. The tissue was then killed with boiling 80% (v/v) aq. EtOH. Tubers were labelled with [^{14}C]-glucose in 0·02 M KH_2PO_4, pH 5·2, as described under Results. 50 μl of 0·5 mM [U-^{14}C]-glucose, containing 1 μCi ^{14}C, were added to each tuber. The plugs of tuber were killed as described above. Callus and tuber tissue were extracted and fractionated in the same way. Both tissues were extracted successively with boiling 80% EtOH, boiling 20% EtOH, and boiling H_2O. The residue is called the water-insoluble material. Extracts were combined, adjusted to pH 7·0, reduced almost to dryness *in vacuo* at 28°, and made up to volume with H_2O to give the H_2O-soluble material. This was separated into acidic, basic, and neutral components by ion-exchange chromatography as described previously [4]. Sugars were isolated from the neutral fractions by PC in EtOAc–C_6H_5N–H_2O (8:2:1). 95–98% of the ^{14}C present in the neutral fraction of all extracts was accounted for as [^{14}C]-glucose, [^{14}C]-fructose, and [^{14}C]-sucrose. The acidic components of the water-soluble substances were fractionated by PC in *n*-BuOH–HCO_2H–H_2O (4:1:5). Sugar phosphate fractions were treated with phosphatase by incubation for 12 hr at 25° with 10 units purified acid phosphatase (Boehringer, Mannheim) in 20 mM AcOH–NaOH buffer at pH 5·5. Sugars were isolated from the reaction mixture as described above. 2-D PC and autoradiography were carried out as described by Bassham and Calvin [8]. For conversion of hexose phosphates to 6-phosphogluconate, portions of the sugar phosphate fractions were incubated at 25° for 3 hr in a reaction mixture that contained in a final vol. of 100 μl: glycylglycine at pH 7·4 (20 mM), $MgCl_2$ (20 mM), $NADP^+$ (0·5 mM), glucose-6-phosphate (1·5 mM) and 10 μg of purified preparations of each of the appropriate enzymes. At the end of incubation the reaction mixture was applied as a 2 cm streak to Whatman No. 1 paper and 6-phosphogluconate isolated by electrophoresis for 40 min at 3·5 kV in HOAc–C_6H_5N–H_2O (1:10:89) at pH 3·5.

^{14}C was assayed as described previously [9]. CO_2 production of callus tissue was measured manometrically by Warburg's direct method at 25°.

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