Phytochemistry, Vol. 25, No. 5, pp. 1073–1076, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00

CONTROL OF THE HEXOSE CONTENT OF POTATO TUBERS

SUSAN MORRELL* and TOM AP REES

Botany School, University of Cambridge, Downing Street, Cambridge CB2 3EA, U.K.

(*Received* 12 *August* 1985)

Key Word Index – *Solanum tuberosum*; Solanaceae; potato; tuber; hexose content; [^{14}C]sucrose; enzymes.

Abstract—The amounts of glucose and fructose in a range of harvested tubers of *Solanum tuberosum* were compared with the labelling of these hexoses by [U-^{14}C]sucrose supplied to the tubers. Hexose content varied. Fructose was more heavily labelled than glucose. There was no correlation between the amounts of glucose and fructose in the tuber and their labelling. The maximum catalytic activities of α-glucan phosphorylase, acid invertase, alkaline invertase, sucrose synthase, α-amylase and β-amylase in tubers stored for 17 weeks at 5° and at 10° were estimated. The values showed no clear correlation with hexose content, but provided sound evidence that starch breakdown was phosphorolytic. It is suggested that the amounts of glucose and fructose in mature harvested tubers may be determined more by the partitioning of the translocated sucrose during the development of the tubers than by the metabolism of the harvested tuber.

INTRODUCTION

The amounts of glucose and fructose in harvested mature potato tubers vary appreciably according to the variety [1], and the conditions of growth [2, 3]. Despite their important roles in metabolism, and in determining tuber quality, little is known of the mechanisms that control the hexose content of the tubers. The aim of the work described in this paper was to investigate the extent to which the hexose content of the tuber was determined by the metabolism of the mature harvested tuber as opposed to the metabolism that occurred during the partitioning of the translocated sucrose delivered to the developing tuber. Two approaches were used. First, we compared the amounts of glucose and fructose in a range of mature tubers with the ability of the tubers to metabolize [U-^{14}C]sucrose to these hexoses. Sucrose is the principal form in which carbon is delivered to the tuber, and almost certainly an intermediate in the formation of hexose from starch [4]. Thus it is likely that sucrose is the immediate precursor of most, if not all, the hexose in the tuber. Consequently, if the hexose content of the tuber is determined primarily by the tuber's immediate metabolism, then a close correlation would be expected between the amounts of glucose and fructose present in the tuber, and the extent to which the [^{14}C]sucrose was converted to [^{14}C]glucose and [^{14}C]fructose. Our second approach was to estimate the maximum catalytic activities of enzymes likely to contribute to hexose formation and see if these corresponded to the amounts of hexose in the tubers.

RESULTS AND DISCUSSION

Metabolism of [U-^{14}C]*sucrose*

We supplied labelled sucrose to the tubers by removing a minute core of tissue with a 50-μl microcapillary pipette and then filling the hole with [U-^{14}C]sucrose. After a timed interval, a core of tissue, 1.2 cm in diameter and concentric with the original hole, was removed with a cork borer, killed and analysed. The efficacy of this procedure has been demonstrated [5, 6]. For each core of tissue, we determined both the detailed distribution of label, and the amounts of glucose and fructose present. Thus comparison of the two types of information was always made on the same sample of tissue. We compared three varieties of tubers, and for two of the varieties we used two different batches of tubers that had been grown under different conditions (Table 1).

Interpretation of our data requires evidence that neither the exogenous [^{14}C]sucrose nor the endogenous sucrose broke down appreciably during the killing and analysis of the tissue samples. To authenticate our measurements of hexose, we prepared duplicate samples of tissue, extracted one in the usual way and the other in boiling ethanol that contained measured amounts of sucrose, glucose and fructose that were comparable to those normally extracted from the sample of tissue. Comparison of the amounts of sugars found in the two extracts showed that no more than 10% of the hexoses was lost during analysis, and that no more than 12% of the sucrose was converted to hexoses. In addition, we incubated 1 ml 0.5 mM [U-^{14}C]sucrose in 0.02 M KH_2PO_4, pH 5.2, for 5 hr and then took this solution through the complete procedure for killing and analysis. The estimates of the ^{14}C recovered as [^{14}C]sucrose after this test were 97, 97, 101 and 118% of that taken at the start. We suggest that the data in Table 1 are not seriously affected by artefactual breakdown of either endogenous sucrose or the [^{14}C]sucrose, and that the estimates of hexose were made without significant loss.

Our measurements of hexose (Table 1) demonstrate two points. First, there was appreciable variation in hexose content, not only between varieties and between tubers of the same variety grown under different conditions, but even between tubers of the same variety grown under the same conditions. Second, in many tubers

*Present address: Department of Biochemistry, University of Georgia, Athens, GA 30602, U.S.A.

Table 1. Comparison of hexose content with labelling of hexoses by [U-^{14}C]sucrose supplied to potato tubers for 5 hr

Variety	Batch no.	Tuber no.	Hexose content (μmol/g fr. wt) Fructose	Glucose	Percentage of metabolized ^{14}C recovered in Fructose	Glucose	^{14}C metabolized (dpm × 10^{-4})
Wilja	I	1	43	47	29	17	188
		2	28	32	37	23	252
		3	39	45	27	22	223
Record	I	1	15	26	7	5	250
		2	20	30	13	7	551
	II	1	14	15	22	18	212
		2	22	28	38	18	397
		3	10	15	40	22	386
Maris Piper	I	1	47	48	36	29	391
		2	43	43	45	14	485
		3	45	48	51	23	406
	II	1	28	26	26	11	202
		2	29	27	41	33	400
		3	42	48	44	30	563

there was more glucose than fructose. Variation in hexose content has been found by others [1, 2, 7] and, considering the important role of hexoses in metabolism, is not surprising. Almost any substantial physiological change or stress is likely to alter the amounts of such important substrates. Thus, it seems unlikely that any single factor, or simple combination of factors, is responsible for determining the hexose content of the tubers.

The tubers also differed in the extent to which they metabolized [^{14}C]sucrose (Table 1). In order to permit comparison between tubers, the ^{14}C per fraction obtained by analysis is expressed as a percentage of the total ^{14}C metabolized by that tuber. ^{14}C metabolized is the sum of the ^{14}C recovered as $^{14}CO_2$ plus that in water-insoluble and water-soluble substances minus that remaining as [^{14}C]sucrose. After a 5-hr pulse (Table 1), the general distribution of label was broadly similar in all tubers and is exemplified by the following data from the three Wilja tubers. The percentages of metabolized ^{14}C (means ± s.e) recovered per fraction for these tubers were: CO_2, 5 ± 5; water-insoluble material, 9 ± 2; basic and acidic components of the water-soluble material, 17 ± 2, 17 ± 5, respectively; glucose, 21 ± 3; fructose, 31 ± 5. Loss of ^{14}C during the analyses was always less than 10% of the total ^{14}C metabolized.

Table 1 shows the extent to which metabolized [^{14}C]sucrose was converted to its constituent hexoses. In most tubers the hexoses were the most heavily labelled compounds. In all tubers fructose was more heavily labelled than glucose and in many tubers this difference was quite marked. The data in Table 1 show that there was no obvious relationship between the amounts of glucose and fructose present, and the labelling of these compounds by [U-^{14}C]sucrose. If glucose content is plotted against percentage of metabolized ^{14}C recovered as [^{14}C]glucose for each sample described in Table 1, r is found to be 0.30. When a similar plot is made for fructose and [^{14}C]fructose, r is 0.52.

We investigated whether the labelling of the hexoses varied with time by determining the distribution of ^{14}C after a 2-hr pulse, and a 2-hr pulse followed by an 18-hr chase (Table 2). The labelling of the hexoses after this shorter pulse was comparable to that found after the 5-hr pulse (Table 1). After the chase there were differences in the labelling pattern. The total ^{14}C recovered in hexoses had declined and the decline was greater for fructose than

Table 2. Labelling of hexoses after supplying [U-^{14}C]sucrose to potato tubers for a 2-hr pulse, and a 2-hr pulse followed by an 18-hr chase with sucrose

Tuber no.	[Treatment]	Percentage of metabolized ^{14}C recovered in Fructose	Glucose	^{14}C metabolized (dpm × 10^{-4})
1	Pulse	46	37	327
2	Pulse	48	36	427
3	Pulse and chase	12	11	209
4	Pulse and chase	13	13	251
5	Pulse and chase	25	23	246
6	Pulse and chase	18	18	169

glucose. However, even at the end of the chase the labelling pattern differed from the relative amounts of hexose present.

Enzyme activities

We compared hexose content and enzyme activities in tubers kept at 5° and in tubers kept at 10° (Table 3). We investigated the reliability of our enzyme assays. For each enzyme we optimized the pH and the concentration of each component of the reaction mixture, and showed that activity was linearly related to time and amount of extract. We did recovery or recombination experiments to see if activity was lost during extraction and assay. In the former we prepared duplicate samples, extracted one as usual and the other in buffer that contained a measured amount, comparable to that present in the sample, of pure enzyme. The difference in the activites found in the two extracts is given as a percentage of the added activity to give a measure of the recovery of the pure enzyme. The following values were obtained; acid invertase, 108 %; α-glucan phosphorylase, 81 %; β-amylase, 81 %. For recombination experiments we prepared three samples, one of potato, one of another tissue, and one that was a mixture of equal weights of potato and the other tissue. The activity in the mixture is expressed as a percentage of the value predicted from the measurements made on the separate components of the mixture. The tissues used and the values obtained were: alkaline invertase, spadix of *Arum maculatum*, 95 %; α-amylase, leaves of *Pisum sativum*, 108 %; cotyledons of germinating seeds of *Pisum sativum*, 118 %. The assay for sucrose synthase has already been authenticated [4]. We suggest that the values in Table 3 reflect the maximum catalytic activities of the tubers. These values do not reveal any marked capacity of the tubers to form hexose, since the activities of both invertases and sucrose synthase were low and neither amylase could be detected. There is no obvious relationship between the enzyme activities and the contents of glucose and fructose.

We draw three principal conclusions from the results presented in this paper. First, our measurements of enzyme activity, and the lack of any positive correlation between hexose content and the conversion of [^{14}C]sucrose to hexoses agree in suggesting that the hexose content of the mature tuber is not normally determined primarily by the metabolism of the harvested tuber but by the metabolism that occurs during the development of the tuber. This is not to say that storage conditions do not affect hexose content, they clearly do [4], but to emphasize the importance of the initial partitioning of sucrose on arrival in the tuber in determining the final hexose content of the tuber.

Our second conclusion is that starch breakdown in isolated tubers is almost certainly phosphorolytic rather than hydrolytic. This is shown by the high activities of α-glucan phosphorylase and the virtual absence of both amylases (Table 3), and by the evidence that starch is the source of sugars in isolated tubers [8]. The final conclusion is that the fact that [^{14}C]sucrose labelled fructose more than glucose strongly suggests that the contribution of sucrose synthase to sucrose breakdown is appreciable in relation to that of the invertases.

EXPERIMENTAL

Materials. [U-^{14}C]Sucrose was from the Radiochemical Centre, Amersham; enzymes and substrates were from Boehringer. Tubers of *Solanum tuberosum* L. cv. Record, Maris Piper, Wilja and Pentland Dell were grown locally. Different batches of the same variety came from different growers in the region. Unless we say otherwise, the tubers were stored at 8° in the dark immediately after harvest and used within 8 weeks.

Labelling experiments. These were done as in ref. [6] except that 50 μl 0.5 mM [U-^{14}C]sucrose (300 Ci/mol) in 0.02 M KH_2PO_4, pH 5.2, was used for pulses, and 50 μl 0.5 mM sucrose in 0.02 M KH_2PO_4, pH 5.2, for chases. The methods used in the killing and analyses of the samples are described in ref. [6]. The labelling of the following fractions was determined: CO_2; water-insoluble substances; acidic, basic and neutral components of the water-soluble substances; glucose, fructose and sucrose. ^{14}C was

Table 3. Enzyme activities and sugar contents of potato tubers stored for 17 weeks at 5° and at 10°

	Tubers stored at 5°(I)	Tubers stored at 10°(II)	Fisher's *P* values I vs. II
Enzyme activities (nmol/min/g fr. wt)*			
α-Glucan phosphorylase	688 ± 19	406 ± 31	< 0.001
Acid invertase	76 ± 7	93 ± 9	N.S.
Alkaline invertase	46 ± 4	28 ± 3	< 0.01
Sucrose synthase	22 ± 5	37 ± 7	N.S.
α-Amylase	nd	nd	–
β-Amylase	nd	nd	—
Sugar content (μmol/g fr. wt)†			
Fructose	11.9 ± 1.2	5.9 ± 0.4	< 0.001
Glucose	9.7 ± 1.2	6.0 ± 1.1	< 0.05
Sucrose	6.3 ± 0.5	7.5 ± 0.5	N.S.

*Values are means ± s.e. from six tubers except that for sucrose synthase $n = 4$.

†Values are means ± s.e. from at least three extracts from each of five different tubers.

n.d.: not determined.

measured by liquid scintillation spectrometry: aqueous samples were mixed with Beckman Ready-Solv™ EP (Beckman RIIC Ltd., High Wycombe, U.K.); insoluble material was treated with NCS tissue solubilizer (Amersham Corporation, Arlington Heights, U.S.A.) and counted as in ref. [9]. For measurement of the sugar content of the tubers, portions of the water-soluble fraction of the above samples or of comparable samples killed and extracted in the same way, were assayed for glucose and fructose as in ref. [10]. Sucrose was measured as the increase in glucose observed after treatment of the water-soluble fraction with invertase.

Enzyme assays. Samples of tissue, 0.5–1.0 g fr. wt, were homogenized, first with a pestle and mortar and then with an all-glass homogenizer, in 3–5 vols extraction medium with insoluble polyvinylpyrrolidone (250 mg/g fr.wt). Extraction was at 4° and extracts were kept at 1–3° until assayed at 25°. Procedures were as follows: invertase (EC 3.2.1.26), extracted in 0.2 M Na_2HPO_4–0.1 M citric acid, pH 8.0, homogenate centrifuged at 32 000 *g* for 30 min, supernatant dialysed for 24 hr against 10 × diluted extraction medium and assayed as in ref. [11] except that glucose was measured as in ref. [10] and the reaction mixture was 150 μl extract plus 150 μl 0.2 M Na_2HPO_4–0.1 M citric acid (pH 5.0 or 7.5) that contained 3.75 mg sucrose. α-Glucan phosphorylase (EC 2.4.1.1), extracted in 100 mM glycylglycine, pH 7.4, 20 mM EDTA, 20 mM cysteine·HCl, 20 mM diethyldithiocarbamate, homogenate centrifuged at 1000 *g* for 5 min and supernatant assayed as in ref. [12] in a reaction mixture (3 ml) that contained 20 mM Na_2HPO_4–KH_2PO_4 buffer, pH 7, 0.425 mM NAD, 5 mM $MgCl_2$, 4.1 μM glucose 1,6-bisphosphate, 3.75 mg amylopectin, 4 units phosphoglucomutase (EC 2.7.5.1), 1.4 units glucose-6-phosphate dehydrogenase (EC 1.1.1.49, NAD-dependent from *Leuconostoc*). α-Amylase (EC 3.2.1.1), extracted in 12.6 mM calcium acetate, pH 6, extract kept at 70° for 20 min, cooled to 4°, centrifuged at 34 000 *g* for 15 min, supernatant assayed as in ref. [13], activity calculated as in ref. [14]. β-Amylase (EC 3.2.1.2), extracted in 50 mM NaOAc, pH 6, 5 mM EDTA, 5 mM 2-mercaptoethanol, homogenate centrifuged at 34 000 *g* for 15 min, supernatant dialysed against 10 × diluted extraction medium followed by assay as in ref. [15] in a reaction mixture, 1 ml, that contained 50 mM NaOAc, pH 5.6, 5 mM EDTA, 5 mM 2-mercaptoethanol and 7.5 mg soluble starch. Sucrose synthase (EC 3.4.1.13), extracted in 100 mM Tris–HCl, pH 7.6, 20 mM EDTA, 20 mM cysteine–HCl, 20 mM diethyldithiocarbamate, homogenate centrifuged at 100 000 *g* for 30 min, supernatant dialysed against 10 × diluted extraction buffer before assay as in ref. [4] in a reaction mixture, 100 μl, that contained 33.3 mM Tris–HCl, pH 8.5, 13.3 mM UDPglucose, 50 mM sucrose, 30 mM [U-^{14}C]fructose (16.7 μCi/mol).

Acknowledgements—We thank Mr. E. J. Allen, Department of Applied Biology, University of Cambridge, and Dr. M. Storey, Potato Marketing Board, for potatoes. S.M. thanks the Potato Marketing Board for a postgraduate studentship.

REFERENCES

1. Miller, R. A., Harrington, J. D. and Kuhn C. D. (1975) *Am. Potato J.* **52**, 379.
2. Burton, W. G. and Wilson, A. R. (1970) *Potato Res.* **13**, 269.
3. Gray, D. and Hughes, J. C. (1978) in *The Potato Crop* (Harris, P. M., ed.) p. 504. Chapman & Hall, London.
4. Pollock, C. J. and ap Rees, T. (1975) *Phytochemistry* **14**, 613.
5. Pollock, C. J. and ap Rees, T. (1975) *Phytochemistry* **14**, 1903.
6. Dixon, W. L. and ap Rees, T. (1980) *Phytochemistry* **19**, 1653.
7. Nelson, D. C. and Shaw, R. (1976) *Am. Potato J.* **53**, 15.
8. Isherwood, F. A. (1973) *Phytochemistry* **12**, 2579.
9. Wong, W.-J. L. and ap Rees, T. (1971) *Biochim. Biophys. Acta* **252**, 296.
10. Bergmeyer, H. U. (ed.) (1974) in *Methods in Enzymatic Analysis*, 2nd edn, p. 1304. Verlag-Chemie, Weinheim.
11. Ricardo, C. P. P. and ap Rees, T. (1970) *Phytochemistry* **9**, 239.
12. Lowry, H., Schultz, D. W. and Passonneau, J. V. (1964) *J. Biol. Chem.* **239**, 1947.
13. Briggs, D. E., (1967) *J. Inst. Brewing* **73**, 361.
14. Duffus, J. H. (1969) *J. Inst. Brewing* **75**, 252.
15. Swain, R. R. and Dekker, E. E. (1966) *Biochim. Biophys. Acta* **122**, 87.