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TURNOVER OF STARCH AND SUCROSE IN ROOTS OF *PISUM SATIVUM*

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Key Word Index—*Pisum sativum*; Leguminosae; pea; roots; starch; sucrose; turnover.

Abstract—Six-day-old seedlings of *Pisum sativum* were incubated for 5 hr with their roots in [^{14}C]glucose, the pulse, and then transferred to glucose for 24 hr, the chase. In the chase the ^{14}C present in starch fell by 25% of that present at the end of the pulse, and the amount of starch fell by 45%. The corresponding figures for sucrose were 90 and 33%. It is suggested that there was appreciable turnover of sucrose but not of starch.

INTRODUCTION

Two mechanisms whereby plants regulate their content of the storage carbohydrates, starch and sucrose, may be envisaged. The first is independent control of both synthesis and breakdown. The second is control of either synthesis or breakdown against a constant rate of breakdown or synthesis, respectively. An example of the latter is the suggestion that in leaves starch degradation may continue without any appreciable regulation and that starch content may be determined by control of synthesis [1]. Mechanisms of this second type imply appreciable turnover of the storage carbohydrates; that is, concomitant synthesis and breakdown.

We know little about the turnover of starch and sucrose, particularly in the non-photosynthetic cells of plants. Recently we [2] failed to detect significant turnover of starch in pea leaves, and argued that evidence for such turnover in leaves as a whole was sparse. Starch in tobacco callus, labelled in a pulse, lost ^{14}C in a subsequent chase [3], during which there may have been net breakdown of starch [4]. Studies of the turnover of sucrose are largely dominated by the evidence of a sucrose cycle in sugar cane [5].

The aim of the work reported in the present paper was to investigate whether starch and sucrose turn over in a growing non-photosynthetic tissue, the pea root. Our approach was to label starch and sucrose with [^{14}C]glucose in a pulse and then observe whether label was lost from these compounds in a subsequent chase in glucose. We also measured changes in the absolute amounts of starch and sucrose during the pulse and chase.

RESULTS AND DISCUSSION

The experiments were done with minimum disturbance to the roots. In each experiment 15 pea seedlings were supported with their roots in [U-^{14}C]glucose. After 5 hr five seedlings were removed and the apical 5 cm of their roots excised and killed, the pulse. The remaining seedlings were transferred to glucose and at various times during the chase, samples of the apical 5 cm of five roots were killed and analysed. We emphasize that label is attributed to starch only after it has been shown to be present in [^{14}C]glucose produced by digestion of the ethanol-insoluble material with amyloglucosidase and α-amylase. We checked that re-digestion of the insoluble material with these enzymes did not release further labelled glucose. To measure the label in sucrose the ethanol-soluble fraction was divided into its acidic, basic and neutral components by ion-exchange chromatography and sucrose was then isolated from the neutral components by paper chromatography. Minimum recoveries of ^{14}C after these two forms of chromatography were 86 and 95%, respectively. Thus we argue that our measurements of label were not seriously affected by losses during the analyses.

The behaviour of starch during the pulse and chase in three separate experiments is shown in Table 1. The weight of the samples varied slightly so values are given per g fresh weight. There was also some variation in the initial content of starch. To take this into account data are given both in absolute units and as percentages of the values found, in each experiment, at the end of the pulse. The pulse resulted in appreciable labelling of starch. There was a small decline in this labelling during the chase: P for comparison of labelling at the beginning and end of the chase is <0.05. The percentage decline in the labelling of starch was almost identical in the three different experiments. The key feature of our data for starch is that even during the longest chase the decline in labelling was small: never more than 28% of that present at the end of the pulse. These data strongly suggest considerable metabolic stability of the starch formed during the pulse.

Our measurements of starch content show a decline during the chase that ranged from 40 to 60% of that present at the end of the pulse. We attribute this net breakdown of starch to its use to support growth of the roots at a stage when the seedlings were changing from dependence on a depleting seed reserve to photosynthesis. The fact that there was a net decrease in starch

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Table 1. Labelling of starch in apical 5 cm of pea roots after pulse in [U-^{14}C]glucose and chase in glucose

Treatment	Experiment	Fresh wt. of sample (mg)			^{14}C in starch (dpm × 10^{-5}/g fr.wt)			Starch content (μg/g fr.wt)		
		I	II	III	I	II	III	I	II	III
Pulse, 5 hr		385	441	440	5.78	4.65	5.57	172	95	118.7
Chase, 2 hr		366	—	—	5.11	—	—	128	—	—
4 hr		—	472	509	—	3.89	4.85	—	68.1	54.0
24 hr		413	458	572	4.28	3.32	4.15	90	59.2	48.6
					As % of that at end of pulse					
Chase, 2 hr					88	—	—	75	—	—
4 hr					—	84	87	—	72	46
24 hr					74	72	75	52	62	41

during the chase means that loss of label may be attributed to this net decrease and is not necessarily due to turnover. In each sample that we analysed the decline in starch was somewhat greater than the decrease in labelling. Without knowing more about the way in which starch grains are made and degraded, it is difficult to explain this observation. Two points may be relevant. One is that the root is a complex system and the starch may be heterogeneous so that some of the starch broken down in the chase was not labelled during the pulse. The other is that the synthesis of labelled starch may not have stopped immediately at the end of the pulse as rinsing the roots would not necessarily remove labelled glucose from the cytosol. Regardless of the above, it is clear that there was only a small drop in the labelling of starch during the chase and that this may be attributed to net breakdown of starch. Thus our results provide no convincing evidence for rapid turnover of starch in the roots of these pea seedlings. The situation in pea roots is comparable to that in pea leaves [2]. Whilst metabolic stability of starch might be expected in perennating organs and seeds, it is important to note that it also appears to be true of rapidly growing non-photosynthetic tissues; whether this is a general feature of such tissues is not clear.

The behaviour of sucrose was followed in the same samples used for the study of starch and the results (Table 2) are expressed in the same way. As with starch, there was appreciable labelling of sucrose at the end of the pulse. In contrast to starch, however, there was a dramatic and extensive loss of label from sucrose during the chase. This loss was roughly proportional to the length of the chase and in two of the three experiments reduced the labelling to 6% of that at the end of the pulse. There was some decline in the amount of sucrose present in the roots but this was quite insufficient to account for the rapid loss of label. This strongly suggests that there is appreciable turnover of sucrose in pea roots, a conclusion indicated by earlier but less extensive studies [5]. This turnover may be due to hydrolysis of vacuolar sucrose by acid invertase, movement of the resulting hexoses into the cytosol, resynthesis of sucrose and transport back into the vacuole. The fact that we detected rapid turnover of sucrose in the same sample that did not reveal rapid turnover of starch suggests that our method was capable of detecting turnover of starch if it had occurred. Our results as a whole provide experimental backing for the view that in plants sucrose is a much more readily available substrate than starch. A similar conclusion has been drawn recently from quite different studies on sycamore cells [6].

EXPERIMENTAL

Material. [U-^{14}C]glucose was from the Radiochemical Centre, Amersham. Seedlings of *Pisum sativum* L. cv Kelvedon Wonder were grown as in ref. [7] except that the plants were grown in aerated distilled H_2O, instead of 0.2 mM $CaCl_2$, for 72 hr so as to give 6-day-old seedlings.

Methods. For each experiment we chose 15 closely comparable seedlings and supported them on a plastic grid over a 50 ml beaker so that their roots were in 52 ml 0.3 mM [U-^{14}C]glucose (0.96 Ci/mol) in 0.02 M KH_2PO_4, pH 5.2. The solution was constantly aerated, and incubation was in the light at 25°. After 5 hr the seedlings were taken out of the [^{14}C]glucose and the roots were given 3 successive 2-min rinses, each with 50 ml distilled H_2O. Then the apical 5 cm were cut off the roots of five plants to give the pulse sample that was killed immediately in boiling 80% (v/v) EtOH. The remaining seedlings were transferred to a 50 ml beaker that contained 52 ml 0.3 mM glucose in 0.02 M KH_2PO_4, pH 5.2 and otherwise were incubated as described for the pulse. At intervals during the incubation in glucose samples of 5 seedlings were taken and the apical 5 cm were excised and killed to give the chase samples.

Each sample was extracted, 2 × before and 3 × after homogenization, with 20 ml lots of boiling 80% (v/v) EtOH. The extracts were combined, reduced to 5 ml *in vacuo* at 28° and then the resulting suspension was centrifuged at 32 000 *g* for 15 min. The supernatant was the soluble fraction; the sediment was added to the material insoluble in 80% EtOH to give the insoluble fraction. The latter was dried, dispersed in H_2O and incubated with amyloglucosidase and α-amylase; the glucose released was isolated by P.C and counted to give the label in starch as in ref. [8]. To measure the amount of starch, portions of the enzymic digest were centrifuged at 2500 *g* for 10 min and glucose in the supernatant was measured as in ref. [9] in a reaction mixture that contained in 2.96 ml: 0.25 M TEA buffer, pH 7.5, 2.5 mM $MgSO_4$, 1.1 mM ATP, 0.8 mM NADP, 2.8 units hexokinase and 2.8 units glucose-6-phosphate dehydrogenase. ^{14}C in sucrose was measured after the sucrose had been isolated from the EtOH-soluble fraction by ion exchange and paper chromatography as in ref. [10]. The amount of sucrose present was determined by incubating a portion of the EtOH-soluble fraction for 2 hr at 37° with 30 units yeast invertase (BDH) and then measuring the increase in glucose as described above. ^{14}C was measured as in ref. [10].

Table 2. Labelling of sucrose in apical 5 cm of pea roots after pulse in [U-^{14}C]glucose and chase in glucose

Treatment	Experiment	Fresh wt of sample (mg)			^{14}C in sucrose (dpm × 10^{-5}/g fr. wt)			Sucrose content (μg/g fr. wt)		
		I	II	III	I	II	III	I	II	III
Pulse, 1 hr		385	441	440	2.47	1.30	2.72	17.9	13.37	15.16
Chase, 2 hr		366	—	—	2.35	—	—	19.62	—	—
4 hr		—	472	509	—	0.81	1.65	—	11.02	12.70
24 hr		413	458	572	0.14	0.23	0.15	8.56	10.83	9.79
					As % of that at end of pulse					
Chase, 2 hr					96	—	—	109	—	—
4 hr					—	63	61	—	82	84
24 hr					6	18	6	48	81	65

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