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COLD-INDUCED FRUCTOSAN SYNTHESIS IN LEAVES OF *DACTYLIS GLOMERATA*

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Key Word Index—*Dactylis glomerata*: Gramineae; leaves; fructosan; polysaccharide; cold-induced synthesis.

Abstract—*Dactylis glomerata* accumulated fructosan more rapidly at 5° than at 15–20°. The pattern of incorporation of $^{14}CO_2$ into fructosan was determined in plants grown at 5°. During the major period of fructosan synthesis there was initial incorporation of label into mono- and disaccharides, and progressive synthesis of polymeric material occurred subsequently. Rates and levels of synthesis were much lower in leaf blades than in leaf bases. The MW distribution of the polymeric material in leaf bases differed from that in the blades and from that observed in plants which synthesize inulin.

INTRODUCTION

Fructosans are the principal storage carbohydrates of temperate grasses. The basic structure is considered to be a repeating β, 2–6 linked D-fructofuranose unit, but there is considerable variation in polymer size and detailed composition [1,2]. The largest polymers of this kind found in grasses have a chain length of some 250 fructose residues [3]. Much of the work on the biosynthesis of fructosans has been performed on the inulin series of polymers, which have a different major linkage and a shorter chain length [4]. A synthetic mechanism has been proposed for inulin based on the synthesis of 2-fructosyl sucrose and subsequent reversible transfructosylation leading to the appearance of longer chain polymers [5]. Chandorkar and Collins showed that labelling patterns of *de novo* synthesized fructosans in leaf discs of chicory fed $^{14}CO_2$ were consistent with the proposed mechanism [6]. Schlubach and Grehn [7] have proposed that a similar mechanism operates in grasses, but no kinetic evidence was presented and none of the enzymes thought to be concerned were isolated from grasses.

The aim of the present study was to investigate the *in vivo* patterns of fructosan synthesis in grasses. A Norwegian ecotype of *Dactylis glomerata* ssp. *glomerata* was used because the plant shows marked differences of growth habit and carbohydrate metabolism when grown at different temperatures [8,9]. In addition, *Dactylis* accumulates long chain fructosans which are sufficiently large to be readily separable by gel filtration. We compared the gross rates of fructosan accumulation in plants grown under two temperature regimes, and determined the patterns of incorporation of $^{14}CO_2$ into fructosan in the leaves of plants grown at low temperatures. The results were compared with those obtained by other workers in experiments on plants which synthesize inulin.

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RESULTS

We determined the changes in fructosan levels after transferring established tillers to two different temperature regimes. Harvesting commenced four weeks after transfer, by which time material growing at 5° had lost the original leaves and produced the characteristic prostrate short leaves. The results (Table 1) indicate a steady accumulation of fructosan in both leaf blades and bases of cold-grown material. This accumulation did not occur in material grown at 20/15°. Levels of fructosan were considerably higher in the leaf bases than in the leaf blades throughout this period.

Following these observations, plants were used for radioactive feeding studies 6 weeks after transfer to 5°. After 1 hr incorporation of $^{14}CO_2$ at 20° and 3 hr further incubation at 20° the plants were returned to 5° and harvested at various time intervals. Table 2 shows the distribution of radioactivity between cold-water soluble and cold-water insoluble material at different times after feeding. The initial rapid incorporation of radioactivity into the cold-water soluble fraction of the leaf blades was followed by a progressive loss of label from the blades and the appearance of some of this label in the leaf bases. Cold-water insoluble material accounted for a maximum of 17% of the radioactivity in any one

Table 1. Variation in the levels of fructosan in the leaf blades and leaf bases of *D. glomerata* grown at two temperatures

Weeks after transfer to controlled temperature	Fructosan content* (mg/g fr. wt) Leaf blades grown at 5°	Leaf blades grown at 15/20°	Leaf bases grown at 5°	Leaf bases grown at 15/20°
4	15	0	44	11
5	25	0	60	9
6	35	4	66	20
7	45	2	80	20

* All values are the mean of triplicate determinations.

Table 2. Distribution of radioactivity in extracts obtained from cold-grown *D. glomerata* supplied with $^{14}CO_2$

Time after feeding	Radioactivity per fraction* (μCi/g fr. wt) Cold-water soluble fraction from: Leaf blades	Leaf bases	Cold-water insoluble fraction from: Leaf blades	Leaf bases
4 hr	20.2	1.3	1.5	0.2
2 days	8.1	1.8	0.8	0.3
4 days	5.3	4.0	0.4	0.5
6 days	2.5	3.9	0.5	0.4

* All values are the mean of triplicate determinations.

sample and further fractionation of this material was not attempted.

Further fractionation of the cold-water soluble material was performed by gel filtration on a column of Bio-Gel P150. Figure 1 shows the patterns of distribution of fructose-containing material obtained from leaf bases and blades harvested 4 hr after feeding. These patterns remained essentially unchanged throughout the experiment. There were considerable differences in the patterns obtained from different leaf parts. Fructose, glucose, and sucrose markers appeared in fractions 9 and 10. Figures 2 and 3 show the distribution of radioactivity between the major fractions throughout the experiment. In both blades and bases there was movement of label from the low MW fractions into polymeric material, but there were marked differences in the extent and speed of this movement.

Pooled material from fractions 1–8 was hydrolysed and the distribution of radioactivity determined by PC. There were no significant differences between samples extracted from blades or bases, or between samples from material harvested at different times. Overall, 88% of the radioactivity in polymeric material was as fructose, 8% was as glucose and 4% was not identified. Thus the label incorporated into the polymeric fraction was present almost entirely as fructosan.

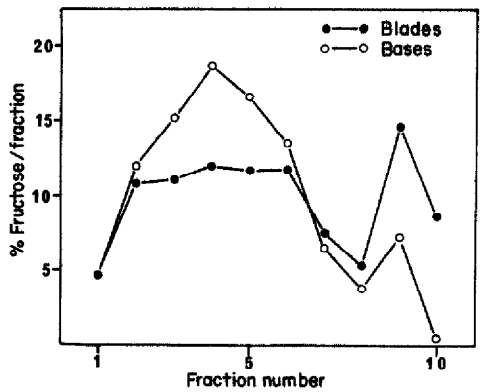

Fig. 1. The percentage of the total cold-water soluble fructose present in each of the fractions obtained after gel filtration of extracts of leaf blades and leaf bases of *D. glomerata* harvested 4 hr after feeding $^{14}CO_2$. Each point is the mean of triplicate determinations.

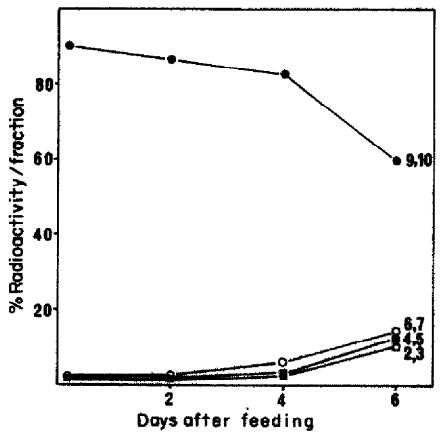

Fig. 2. The percentage of the cold-water soluble radioactivity present in fractions obtained by gel filtration of leaf blade extracts of *D. glomerata* harvested at various times after feeding $^{14}CO_2$. Each point is the sum of the values obtained for the fractions indicated and is the mean of triplicate determinations.

Fractions 9 and 10, which contained the low MW material, were combined and fractionated further by PC (Table 3). Sucrose, fructose and glucose were identified by co-chromatography with authentic materials. Oligosaccharides were measured by eluting the material between the origin and the sucrose spot, hydrolysing it and re-separating by PC. The sum of the label in glucose and fructose from this hydrolysate was taken to be the oligosaccharide component and the remainder to be radioactivity present in non-carbohydrate material. There was a steady movement of label from monosaccharides into sucrose, and subsequently into oligosaccharides, during the course of the experiment. Less material remained in the oligosaccharide fraction in the leaf bases than in the blades. Throughout the experiment recovery of material from columns and PC accounted for 95–102% of the added radioactivity and 93–99% of the added fructose.

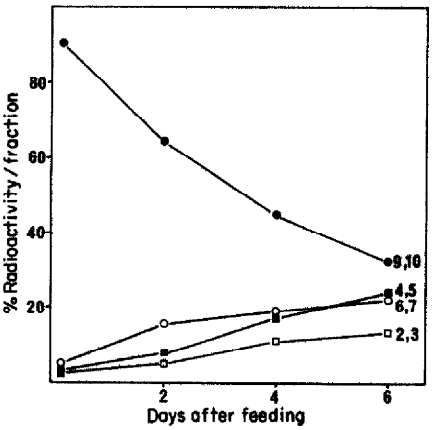

Fig. 3. The percentage of the total cold-water soluble radioactivity present in fractions obtained by gel filtration of leaf base extracts of *D. glomerata* harvested at various times after feeding $^{14}CO_2$. Each point is the sum of the values obtained for the fractions indicated and is the mean of triplicate determinations.

Table 3. Distribution of radioactivity in low MW components of the cold-water soluble fraction of cold-grown *D. glomerata* supplied with $^{14}CO_2$

Component	Leaf tissue	Time after feeding: 4 hr	2 days	4 days	6 days
		Percentage of total radioactivity in cold-water soluble fraction present in each component*			
Unseparated low MW components	blades	91	85	80	59
	bases	84	65	46	33
Glucose	blades	39	20	18	13
	bases	38	18	7	6
Fructose	blades	41	20	20	12
	bases	36	14	10	7
Sucrose	blades	1	34	5	7
	bases	1	21	4	4
Oligosaccharides	blades	1	6	25	22
	bases	3	5	18	12
Non-carbohydrate material	blades	6	3	9	4
	bases	3	7	4	4

* All values are the mean of triplicate determinations.

DISCUSSION

The cold-induced synthesis of fructosan offers advantages as a system for the study of polysaccharide metabolism in temperate grasses. Accumulation is extensive and reproducible (Table 1) and distinct from the pattern of metabolism in plants grown at higher temperatures. The prostrate habit and high net assimilation rate of the cold-grown plants facilitates feeding of $^{14}CO_2$ [8], and the high MW of the polymers formed makes separation straightforward.

The majority of the initial products of $^{14}CO_2$ fixation appeared in the low MW component of the cold-water soluble fractions, particularly those of the leaf blade (Table 2). There was rapid loss of this material from the blade and a progressive accumulation of label in the bases. In both blades and bases the label was found initially in glucose and fructose (Table 3). This implies that hydrolysis of sucrose occurred, at least in the leaf bases, prior to synthesis of fructosan. If synthesis occurs in the vacuole as proposed for inulin accumulation in *Helianthus tuberosus* [5], this hydrolysis could be linked with the transport of sugars into the vacuole. After 2 days considerable movement of label into sucrose had occurred, and subsequently most of this label was found in oligosaccharides.

The patterns of distribution and synthesis of polymeric material differed markedly between leaf bases and blades. Fig. 1 shows that each of the major polysaccharide fractions 2 to 6 from leaf blade extracts contained about the same proportion of fructose. Hence there were fewer molecules of the longer chain species than the shorter chain ones. This is similar to the distribution of fructose in the inulin oligosaccharides isolated from *Helianthus* [10], and is consistent with the proposed synthetic scheme [5]. In contrast there was a marked preponderance of fructose in fractions 3, 4 and 5 from leaf base extracts, indicating a concentration of material over a narrower range of MW. This suggests that the synthetic mechanism operating in the bases must differ from that in the blades and also from that operating in *Helianthus*.

The appearance of the label in polymeric material also showed significant differences between leaf bases and blades (Figs. 2 and 3). In the bases, label appeared in the high MW fractions by day 2 after feeding, and incorporation proceeded steadily until 66% of the label was in polysaccharide by day 6. During this time only 40% of the label had passed into polysaccharide in the leaf blades, and the majority of this movement occurred in the last 2 days. The accumulation of radioactivity in the different fractions of leaf base extracts reflected the differential distribution of fructose (Fig. 1). Although label accumulated rapidly in fractions 6 and 7, there was a progressive appearance of label in fractions 4 and 5. This contrasted with the more symmetrical distribution obtained in fractions of leaf blade extracts.

In conclusion we propose that the synthesis of fructosan in leaves of cold-grown *Dactylis* occurs most rapidly in the leaf bases. This process of sequential formation of polymers of increasing chain length leads to a distribution of molecular size which contrasts with that found in leaf blades and in plants which synthesize inulin.

EXPERIMENTAL

Material. Plants of *Dactylis glomerata* ssp *glomerata* Bc 4095 were grown from 5 cm tillers in a glasshouse. They were transferred to controlled environment chambers 3 weeks after planting. The chambers had an 8 hr photoperiod and day/night temps of 20/15° and 5/5°, respectively. Plants were harvested for assay of total fructosan from 4 weeks after transfer to controlled environments. $^{14}CO_2$ feeding experiments were conducted 6 weeks after transfer of plants to 5°. *Methods.* Whole plants were placed in boxes with Perspex lids in the light at 20° and exposed to $^{14}CO_2$ (1 mCi/12 plants). After 1 hr the remaining CO_2 was absorbed in KOH, and after a further 3 hr the plants returned to 5°. Plants were harvested at various times, the leaves separated at the ligule into blades and bases, weighed, and killed and extracted in 15 vol H_2O at 80° (3 × 3 min). The extracts were pooled, reduced in vol, cooled to 20° and cleared by centrifugation to give the cold-H_2O soluble fraction. All the remaining material was homogenized in H_2O and the resulting suspension treated as the cold-H_2O insoluble fraction. Measurements of total fructosan were made on the cold-H_2O soluble fraction. Total fructose was assayed by the resorcinol method [11], reducing sugars by the dinitrosalicylic acid method [12], and sucrose was separated by descending PC in EtOAc–C_5H_5N–H_2O (8:2:1), eluted into H_2O and assayed by the anthrone method [13]. Fructosan was taken as total fructose less 50% of the combined values for sucrose and reducing sugars. Radioactive cold-water soluble extracts were separated on columns of Bio-Gel P 150. Aliquots equivalent to 0.2 g of original material were applied and the resultant 2 ml fractions assayed for fructose as above and for radioactivity by liquid scintillation spectrometry. Portions of individual fractions were hydrolysed at 90° for 1 hr in 0.02 M HCl, and these, together with unhydrolysed portions, were separated by PC as above. Radioactivity was estimated directly from the papers by liquid scintillation spectrometry at 48% efficiency, and individual sugars were identified by co-chromatography with known compounds. Aliquots (0.2 ml) of the cold-H_2O insoluble fractions were prepared for counting by incubation with 0.5 ml of tissue solubilizer at 50° for 12 hr.

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