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Regulation of Fructan Metabolism in Leaves of Barley (*Hordeum vulgare* L. cv Gerbel)

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ABSTRACT

Excised primary leaf blades of barley (*Hordeum vulgare* L. cv Gerbel) rapidly synthesized large quantities of fructan in the light and, upon transfer to the dark, they rapidly degraded it again. In the course of such a light/dark cycle the activities of sucrose-sucrose-fructosyltransferase (SST), fructan hydrolase, and invertase were measured in cell-free extracts of the blades. SST activity increased 20-fold within 24 hours in the light and disappeared again upon transfer to the dark during a similar period of time. Cycloheximide inhibited the increase of SST activity in the light indicating *de novo* synthesis. The loss of SST activity in the dark, however, was unaffected by cycloheximide. No SST activity appeared in the light if photosynthesis was inhibited by lowering the CO_2 concentration in the atmosphere. However, SST activity and fructan synthesis were induced even in the dark and at a low CO_2 concentration when the leaf blades were immersed in a solution of sucrose. Several other sugars, maltose and fructose in particular, had the same effect. Trehalose induced SST activity but no fructan synthesis occurred. The activities of fructan hydrolase and invertase changed little during the light/dark cycle. It is suggested that the control of SST activity in conjunction with the supply of photosynthates plays a key role in the regulation of fructan metabolism.

Fructan, a polyfructosylsucrose of varying molecular size, is the main carbohydrate reserve for intermediate and long-term storage in vegetative organs of perennial forage grasses and cereals, particularly in temperate and frigid climate zones (1, 7, 11, 15). Whereas the diurnal difference between production, export, and consumption of photosynthates in the leaves is largely balanced by storage and mobilization of sucrose and starch (2, 16), fructan seems to be the reserve used preferentially for balancing seasonal differences due to changes in plant development and weather. Large quantities of fructan are usually stored in leaves and stems of grasses during autumn and winter. They are mobilized again during initial growth in early spring, during regrowth after mowing, and during the grain filling period (3, 10, 12, 17). As a general rule fructan is accumulated when the conditions allow photosynthesis but are restricting growth or transport; fructans are depleted under opposite conditions.

Fructan can be produced in enormous amounts which may exceed 70% of the dry weight of the leaves (13, 18). Fructan and the fructan metabolizing enzymes were found to be located in the vacuoles of barley mesophyll cells (18, 19). The same localization was found in the cells of the storage parenchyma of the tubers of Jerusalem artichokes (5).

In spite of the central role of fructan metabolism in carbon partitioning in economically important grasses such as wheat and barley, very little work has been done on the enzymology and regulation of fructan synthesis and degradation (8, 11, 15). With the excised cereal leaf blades we had a system at hand in which fructan synthesis could be induced very rapidly (18). It was tempting to use this system to elucidate further the regulation of fructan metabolism in grasses. In this report evidence is presented that the variation of the activity of SST,[1] probably regulated itself by the sucrose level in the cells, plays a central role.

MATERIALS AND METHODS

Plants and Growth Conditions. Seeds of barley (*Hordeum vulgare* L. cv Gerbel) were soaked in slightly running tap water for 24 h and sown in 12 cm pots on commercially available soil on a peat, clay basis (about 150 seeds per pot). The pots were placed in a growth cabinet with a daylength of 12 h and a temperature regime of 25/10°C. The RH was 70% and the photon flux density about 500 to 600 μmol photons$\cdot m^{-2}s^{-1}$. The seedlings were watered daily and grown for 10 to 12 d.

Conditions Inducing Accumulation and Depletion of Fructan. Primary leaf blades of barley were excised and the lowest parts of the blades were immersed in water. Fructan synthesis was induced by continuous illumination of the blades with a 400 W HPLR-lamp (Philips) from a distance of 60 cm (500–600 μmol photons$\cdot m^{-2}s^{-1}$) and with a heat filter between. Thereafter degradation of the fructan formed could be induced by transferring the blades to the dark. The whole treatment was carried out at 22°C.

To examine the effect of protein synthesis on changes in enzyme activities and fructan synthesis, leaf blades were immersed in a solution of 5 μg/ml CHI instead of water and illuminated as described above. The inhibitor was thus taken up in the transpiration stream. The effect of CHI during depletion of fructan was studied in leaf blades standing in 5 μg/ml CHI during the dark period only.

Induction of Fructan Synthesis by Exogenous Sugars. Leaf blades were immersed in 0.5 M solutions of sucrose or other sugars and kept in a desiccator containing solid KOH (5 g$\cdot L^{-1}$ volume) to induce opening of the stomata and transpiration favoring sugar uptake in the dark. The desiccator was kept at 27°C in the dark for up to 24 h. All sugars used were supplied by Fluka AG, Buchs SG, Switzerland.

Extraction of Nonstructural Carbohydrates. Primary leaf blades were cut in pieces and immediately dropped in boiling 25% ethanol. They were extracted twice under reflux for 5 min (2 × 20 ml/g fresh weight). Both extracts were combined and brought to dryness under reduced pressure at 40°C. The residue

[1] Abbreviations: SST, sucrose-sucrose-fructosyltransferase; CHI, cycloheximide; DP, degree of polymerization.

was dissolved in water (1 ml/g initial fresh weight) and incubated for 10 min with the carbonate form of Amberlite MB-3 (Fluka AG, Buchs SG, Switzerland). After centrifugation at 2000g for 5 min the supernatant was ready for carbohydrate measurements.

Analysis of Nonstructural Carbohydrates. Fructose, sucrose, fructosylsucrose (trisaccharide), and the fructans of DP>3 were separated by silica gel TLC-foils, cut out, extracted, and quantified as described earlier (18). Glucose was determined with the glucose oxidase test from Boehringer, Mannheim, GFR. The content of starch was analyzed as reported earlier (18). All measurements were made at least in duplicate.

Extraction and Assays of Fructan Enzymes. The leaf blades were homogenized in a mortar with 1 ml/g fresh weight 20 mM citrate-P-buffer, pH 5.7, at 4°C. The homogenate was centrifuged at 2000g for 10 min and the supernatant was desalted by passage through a Bio-Gel P-10 column (18) or by dialysis against 2 mM citrate-P-buffer, pH 5.7, at 4°C for 16 h. These treatments efficiently removed sugars and, if present in the extracts of the leaf blades, also the fructan. The activities of SST and invertase were determined in the same assay mixture containing 0.1 ml enzyme sample (with approximately 340 μg of protein), 0.1 M sucrose, 20 mM citrate-P-buffer, pH 5.7, 1 mg/ml BSA (final volume: 0.2 ml). After incubation at 29°C for 1 to 4 h the reaction was stopped by incubation in a boiling water bath for 3 min, followed by centrifugation at 12,000g for 2 min. The amount of trisaccharide present in the supernatant was taken as a measure for the activity of SST and the amount of fructose released as a measure for the activity of invertase.

The activity of fructan hydrolase was measured by mixing 0.1 ml enzyme sample with 0.1 ml 50 mM citrate-P-buffer, pH 5.2, containing 2 mg fructan extracted from barley leaves as described by Wagner *et al.* (19). After incubation at 34°C for 1 to 8 h the reaction was stopped as described above. Fructose released was taken as the measure for the activity of fructan hydrolase. Zero time controls were run for all enzyme assays. The determinations were carried out in duplicate or triplicate.

RESULTS

Accumulation and Depletion of Fructan in Excised Leaf Blades during a Light/Dark Cycle. Continuous illumination of excised leaf blades for 24 h induces the accumulation of nonstructural carbohydrates (Fig. 1). The contents of sucrose and starch increase rapidly from the beginning and reach a plateau after about 16 h. In contrast, the content of fructan starts to increase only after a lag of about 8 h. After 16 h, however, fructan is the sole nonstructural carbohydrate the content of which continues to increase. It has been shown previously that the increase continues upon prolonged illumination until fructan accounts for more than 70% of the dry weight of the leaves (18). The trisaccharide behaved as would be expected of an intermediate between sucrose and fructan. Upon transfer to the dark the content of sucrose declines rapidly, whereas the content of fructan at first increases further and falls only after a lag of about 8 h. Apparently sucrose and the trissaccharide are still transformed to fructan at the beginning of the dark period. Sucrose appears to be utilized as a short-term reservoir of carbohydrate, whereas fructan has properties of a long-term storage compound. It was tempting to see whether the activities of the enzymes of fructan metabolism reflect the marked changes observed in the fructan contents during the light/dark cycle.

Indeed the activity of SST, forming 1 molecule of trisaccharide from 2 molecules of sucrose, increases about 20-fold during the light period (Fig. 2). Simultaneously the activity of fructan hydrolase drops slightly but remains at an appreciably high level (Fig. 2). Invertase activity is hardly affected by the light/dark treatment.

During the degradation of fructan in the dark, the activity of fructan hydrolase increases slowly to its initial level. Most remarkably, however, the activity of SST drops quickly within 24 h and was practically zero after 2 to 3 d (Fig. 2).

Role of Protein Synthesis and Photosynthesis in the Control of Fructan Metabolism. The large increase of SST activity during the light period is totally inhibited by CHI, indicating that protein synthesis is involved (Table I). Accordingly the fructan content of the leaves does not increase in the presence of CHI. On the other hand, the loss of SST activity and the fructan depletion during the dark period was unaffected in the presence of CHI.

Photosynthesis was inhibited by lowering the CO_2 concentration in the atmosphere. This treatment prevents both the increase of SST activity and the accumulation of fructan during the light period (Table II). Thus, induction of SST activity is dependent on photosynthesis or, possibly at least on the presence of photosynthates in excess to the actual demand of the cells. This question was studied further as follows.

Effect of Exogenously Applied Sucrose or Other Sugars on Fructan Metabolism. The results described above suggest that SST activity and fructan synthesis could be induced also by exogenously applied sucrose, being both the substrate of SST and the main initial product of photosynthesis rapidly transferred to the vacuoles (9), *i.e.* the site of fructan synthesis (18). Indeed, leaf blades standing in a solution of sucrose accumulated large amounts of fructan after a lag phase of about 8 h, even in the dark and at low concentrations of CO_2 (Fig. 3). The activity of SST increased about 10-fold within 24 h after a lag phase of only

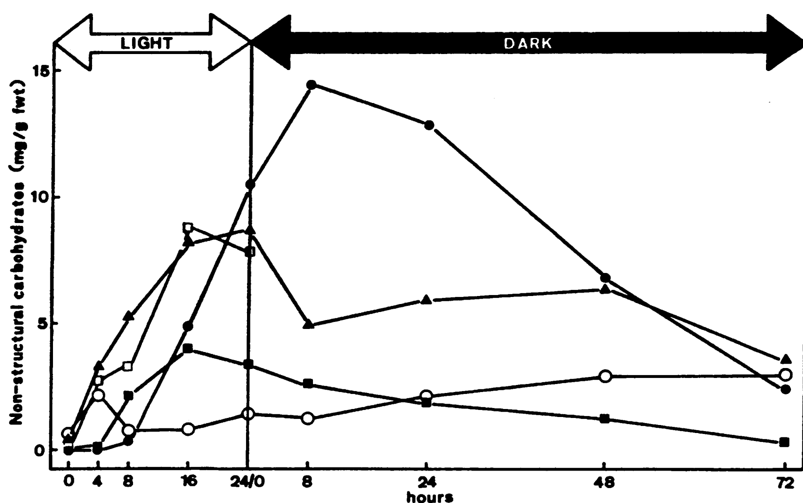

FIG. 1. Changes of the amounts (mg/g fresh weight of blades) of nonstructural carbohydrates in excised primary leaf blades of *H. vulgare* during a light/dark cycle. (○) Fructose, (▲) sucrose, (■) fructosylsucrose (trisaccharide), (●) fructan (DP>3), (□) starch.

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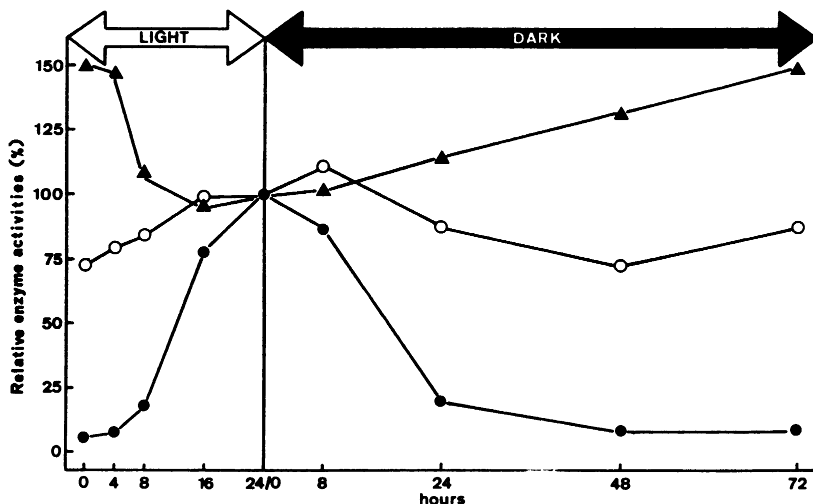

FIG. 2. Changes of the enzyme activities in excised primary leaf blades of *H. vulgare* during a light/dark cycle. (●) SST, (○) invertase, (▲) fructan hydrolase. (Activities after 24 h of light = 100%; for absolute values, see Table I.)

Table I. *Changes of Fructan Content and Enzyme Activities in Excised Primary Leaf Blades of H. vulgare*

The leaf blades were standing in 5 μg/ml CHI or water during a light period (24 h) followed by a dark period (24 h).

		After Excision	After 24 h Light		After 24 h Light/24 h Dark	
			In H_2O	In CHI	In H_2O	In CHI[a]
Fructan (DP>2)	(mg/g fresh weight)	0.07	12.58	0.93	1.10	1.36
SST	(nkat/ml)	0.09	0.82	0.02	0.14	0.22
Invertase	(nkat/ml)	2.19	2.82	1.73	1.77	1.73
Fructan hydrolase	(nkat/ml)	0.77	0.65		0.69	0.54

[a] During dark period only.

Table II. *Effect of an Inhibition of Photosynthesis on the Changes of the Activity of SST and the Content of Fructan and Sugars in Excised Primary Leaf Blades of H. vulgare during a Light Period*

The blades were exposed to a reduced CO_2 concentration in the atmosphere (low CO_2) (see "Material and Methods").

		Excised Blades	Excised Blades after 24 h Light	
			Control	Low CO_2
Fructose	(mg/g fresh weight)	0.17	1.53	0.03
Glucose	(mg/g fresh weight)	0.58	4.93	0.01
Sucrose	(mg/g fresh weight)	0.25	8.85	0.11
Fructan (DP>2)	(mg/g fresh weight)	0.07	12.58	0.03
SST-activity	(nkat/ml)	0.05	1.02	0.05

4 h (Fig. 3). This shows that photosynthetically produced endogenous sucrose can be replaced by exogenous sucrose to induce SST activity and fructan synthesis. A surplus of sucrose obviously is sufficient. Similar effects of exogenously applied sucrose on fructan synthesis have recently been found in *Lolium temulentum* L. (8).

Employing the system described above a number of other sugars were tested to see if sucrose is essential for the induction of SST. It appears from results compiled in Table III that fructose, maltose, melezitose, maltotriose, raffinose, and trehalose induce SST activity nearly as efficiently as sucrose. Interestingly trehalose, the analogous disaccharide of sucrose which occurs in fungi, induced SST without being transformed to fructan. Various other sugars or sugar analogs tested were found to have no effect on the level of SST activity, *e.g.* mannose, galactose, sorbose, arabinose, xylose, palatinose, turanose, mannitol, fructose-6-P, α-methylglucoside, 2-deoxyglucose, and sucrose-octoacetate (data not shown). Sucrose in combination with CHI did not induce SST activity (Table III), ruling out the possibility that the effect of CHI shown in Table I is due to the inhibition of sucrose accumulation rather than to the inhibition of cytoplasmatic protein synthesis.

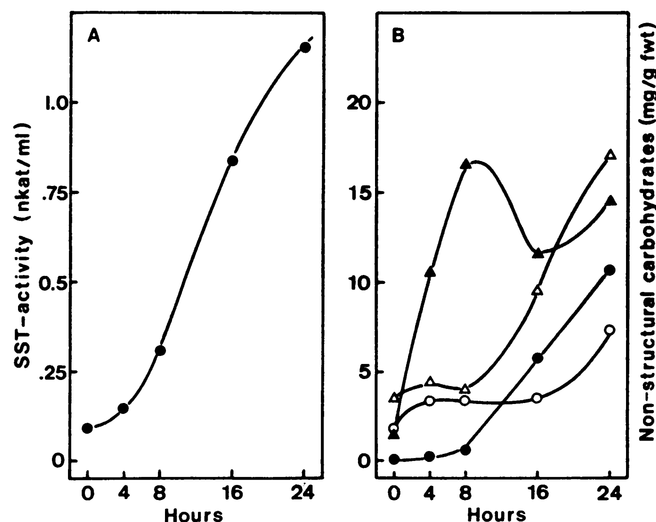

FIG. 3. Changes of SST activity (A) and of the amounts of nonstructural carbohydrates (B) in excised primary leaf blades of *H.vulgare* standing in a solution of 0.5 M sucrose in the dark. (○) Fructose, (△) glucose, (▲) sucrose, (●) fructan (DP>2).

DISCUSSION

The results indicate that the regulation of fructan metabolism in the leaves of barley is closely connected with a regulation of

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Table III. *Fructan Content and SST Activity in Excised Primary Leaf Blades of H. vulgare*

The leaf blades were standing in 0.5 M solutions of different sugars for 16 h in the dark.

Sugar	SST Activity	Fructan Content
	nkat/ml	*mg/g fresh weight*
None	0.08 ± 0.01	0.01
Sucrose	0.64 ± 0.17	1.48
Maltose	0.99 ± 0.19	2.07
Melezitose	0.87 ± 0.19	—[a]
Maltotriose	0.50 ± 0.07	0.38
Trehalose	0.48 ± 0.08	0.01
Fructose	0.47 ± 0.21	1.08
Raffinose	0.42 ± 0.07	—[a]
Cellobiose	0.33 ± 0.08	0.43
Lactose	0.31 ± 0.07	0.13
Lactulose	0.23 ± 0.10	—[a]
Glucose	0.22 ± 0.15	0.31
Melibiose	0.11 ± 0.05	0.11
Sucrose + 5 μg/ml CHI	0.08 ± 0.01	0.01

[a] Determination of fructan content impossible because of interference with the sugar tested.

the activity of SST, a possible key enzyme of fructan synthesis. Accumulation of fructan was found to be strictly preceded by an increase in the activity of SST (Fig. 3). If this increase was inhibited by CHI, no fructan was synthesized (Table I). This finding is in agreement with previous reports showing that inhibition of cytoplasmatic protein synthesis also inhibits the accumulation of fructan in certain Asteraceae (4, 14).

Besides the induction of SST activity a surplus of photosynthates, most probably of sucrose, is necessary to allow accumulation of fructan in the leaves (Table II). Photosynthates are not only the substrates for fructan synthesis but they appear to be also effectors for the induction of SST activity as the experiment with exogenously supplied sucrose demonstrates. Curiously, not only sucrose but also a number of other sugars induce SST activity in the leaves, whereby, as the result with trehalose shows, it is not necessary that the sugar can actually be transformed to fructan (Table III). It will be an interesting task to study the mechanism of SST induction by these sugars.

Another question concerns the control of fructan degradation. The activity of fructan hydrolase changed only little and slowly upon transfer of the leaves to the dark which induced the degradation of fructan (Fig. 2). A further difficulty in the understanding of the control of fructan hydrolysis emerges from the recent finding that the hydrolase is located in the vacuoles (19), *i.e.* the same subcellular compartment in which fructan synthesis and accumulation takes place (18). The question arises, therefore, whether the depletion of fructan in the dark is initiated by a decrease of the rate of synthesis only, caused by inactivation of SST, in the presence of an unchanged rate of degradation. This possibility could be tested by measuring turnover rates of fructan. In any case, it will be interesting to see whether or how the cells prevent a futile cycle of continuous fructan synthesis and degradation. It may be asked also how SST, a vacuolar enzyme shown to be quite stable *in vitro* (unpublished results), can suddenly lose its activity so rapidly. It is feasible that SST is unstable in the absence of its substrate, sucrose, and is consequently digested by the vacuolar proteinases (6). Another question concerns the possible regulatory role of oligosaccharide synthetic activity (8). A clear answer to this question must probably await successful purification and characterization of all the enzymes involved in fructan metabolism. These and many other fundamental problems of fructan metabolism in cereals are unsolved. This is surprising since the vacuolar fructan pool plays such a prominent role on the partitioning of photosynthates in cereals.

LITERATURE CITED

1. ARCHBOLD KH 1940 Fructosans in the monocotyledones. A review. New Phytol 39: 185–219
2. BORLAND AM, JF FARRAR 1985 Diel patterns of carbohydrate metabolism in leaf blades and leaf sheaths of *Poa annua* L. and *Poa jemtlandica* (Almq.) Richt. New Phytol 100: 519–531
3. BLACKLOW WM, B DARBYSHIRE, P PHELOUNG 1984 Fructans polymerised and depolymerised in the internodes of winter wheat as grain filling progressed. Plant Sci Lett 36: 213–218
4. CHANDOKAR KR, FW COLLINS 1972 De novo synthesis of fructooligosaccharides in leaf disks of certain Asteraceae. Can J Bot 50: 295–303
5. FREHNER M, F KELLER, A WIEMKEN 1984 Localization of fructan metabolism in the vacuoles isolated from protoplasts of Jerusalem artichoke tubers (*Helianthus tuberosus* L.). J Plant Physiol 116: 197–208
6. HECK U, E MARTINOIA, P MATILE 1981 Subcellular localization of acid proteinase in barley mesophyll protoplasts. Planta 151: 198–200
7. HEGNAUER R 1962–1973 Chemotaxonomie der Pflanzen, Vol 1–6. Birkhäuser Verlag, Basel
8. HOUSLEY TL, CJ POLLOCK 1985 Photosynthesis and carbohydrate metabolism in detached leaves of *Lolium temulentum* L. New Phytol 99: 499–507
9. KAISER G, E MARTINOIA, A WIEMKEN 1982 Rapid appearance of photosynthetic products in the vacuoles isolated from barley mesophyll protoplasts by a new fast method. Z Pflanzenphysiol 107: 103–113
10. KÜHBAUCH W 1978 Die Nichtstrukturkohlehydrate in Gräsern des gemässigten Klimabereiches, ihre Variationsmöglichkeiten und mikrobielle Verwertung. Landwirtsch Forsch 31: 251–268
11. MEIER H, JSG REID 1982 Reserve polysaccharides other than starch in higher plants. *In* FA Loewus, W Tanner, eds, Plant Carbohydrates, Encyclopedia of Plant Physiol, New Series, Vol 13A. Springer-Verlag, Heidelberg, pp 418–471
12. POLLOCK CJ 1979 Pathway of fructosan synthesis in leaf bases of *Dactylis glomerata*. Phytochemistry 18: 777–779
13. POLLOCK CJ 1984 Sucrose accumulation and the initiation of fructan biosynthesis in *Lolium temulentum* L. New Phytol 96: 527–534
14. PONTIS HG 1966 Observations on the de novo synthesis of fructosans in vivo. Arch Biochem Biophys 116: 416–424
15. PONTIS HG, E DELCAMPILLO 1985 Fructans. *In* PM Dey, RA Dixon, eds, Biochemistry of Storage Carbohydrates in Green Plants. Academic Press, New York, pp 205–227
16. SICHER RC, DF KREMER, WG HARRIS 1984 Diurnal carbohydrate metabolism of barley primary leaves. Plant Physiol 76: 165–169
17. SMITH D 1972 Carbohydrate reserves in grasses. *In* VB Younger, CM McKell, eds, The Biology and Utilization of Grasses. Physiological Ecology. Academic Press, New York, pp 318–333
18. WAGNER W, F KELLER, A WIEMKEN 1983 Fructan metabolism in cereals: induction in leaves and compartmentation in protoplasts and vacuoles. Z Pflanzenphysiol 112: 359–372
19. WAGNER W, A WIEMKEN 1986 Properties and subcellular localizaton of fructan hydrolase in the leaves of barley (*Hordeum vulgare* L. cv Gerbel). J Plant Physiol. In press

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