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ORIGINAL ARTICLE

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Regulation of sucrose and starch synthesis in wheat (*Triticum aestivum* L.) leaves: role of fructose 2,6-bisphosphate

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Abstract Fructose 2,6-bisphosphate (F26BP) is a competitive inhibitor of the cytosolic fructose 1,6-bisphosphatase (cytFBPase, EC 3.1.3.11). In spinach (*Spinacia oleracea* L.) leaves it is a significant component of the complex regulatory network that co-ordinates rates of photosynthesis, sucrose synthesis and starch synthesis. However the role of F26BP has only been studied in plants that predominantly store starch in their leaves and its role in other species is not clear. This paper examines the significance of F26BP in the regulation of photosynthetic carbon metabolism in the intact leaves of wheat (*Triticum aestivum* L.), a plant that accumulates predominantly sucrose. The approach taken was to vary rates of photosynthesis and then correlate measurements of F26BP and a range of other metabolites with rates of carbohydrate synthesis obtained from $^{14}CO_2$-feeding experiments performed under physiological conditions. It was found that: (i) Amounts of 3-phosphoglycerate and fructose-6-phosphate are correlated with the amount of F26BP. (ii) F26BP is involved in inhibiting cytFBPase at low light and low CO_2, but other factors, for example triose-phosphate, must also be involved. (iii) Amounts of both F26BP and substrate are involved in co-ordinating rates of photosynthesis and sucrose synthesis, but the relative importance of these depends on the conditions. (iv) Amounts of F26BP do not correlate with the partitioning of fixed carbon between sucrose and starch. Together these data suggest that the amount of F26BP in wheat is regulated by mechanisms similar to those in spinach, and that the metabolite is one of the factors involved in co-ordinating sucrose synthesis and photosynthesis. However F26BP does not appear to be involved in regulating the partitioning of fixed carbon between sucrose and starch in wheat under the experimental conditions examined.

Keywords Fructose 2,6-bisphosphate · Photosynthesis · Starch · Sucrose · *Triticum* (starch, sucrose)

Abbreviations Ci: leaf internal CO_2 concentration · DHAP: dihydroxyacetone phosphate · FBP: fructose 1,6-bisphosphate · cytFBPase: cytosolic fructose 1,6-bisphosphatase (EC 3.1.3.11) · F26BP: fructose 2,6-bisphosphate · F26BPase: fructose 2,6-bisphosphatase (EC 3.1.3.46) · F6P: fructose 6-phosphate · G6P: glucose 6-phosphate · PAR: photosynthetically active radiation · PFKII: frucose-6-phosphate 2-kinase (EC 2.7.1.105) · PGA: 3-phosphoglycerate · Pi: inorganic phosphate · SPS: sucrose phosphate synthase (EC 2.4.1.14) · triose-P: triose-phosphate

Introduction

The role of fructose 2,6-bisphosphate (F26BP) in photosynthetic carbon metabolism has been extensively studies in plants such as spinach that store predominantly starch in their leaves. One role for the metabolite in these plants is in co-ordinating rates of sucrose synthesis and photosynthesis (reviewed in Stitt et al. 1987; Stitt 1990a). Under low light and low CO_2, cytosolic fructose 1,6-bisphosphatase (cytFBPase, EC 3.1.3.11) is inhibited predominantly by a combination of high amounts of F26BP and Pi, and low amounts of substrate (≈0.25 mM; Gerhardt et al. 1987). The activity of sucrose phosphate synthase (SPS, EC 2.4.1.14) is inhibited by low hexose-phosphate, high Pi and by phosphorylation (Stitt et al. 1987). When photosynthesis is stimulated by increases in light or CO_2, cytFBPase is activated by both an increase in the amount of substrate and a fall in F26BP. The activation of cytFBPase leads to an increase in hexose-phosphate, which, together with the decrease in Pi, activates SPS, leading to an increase in the rate of sucrose synthesis. This

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role for F26BP has been quantified in spinach (Neuhaus et al. 1990) and another starch-storing plant *Clarkia xantiana* (Neuhaus et al. 1989). In both plants, F26BP is significant in regulating sucrose synthesis at low light only; at high light any excess of triose-phosphate (triose-P) will be converted to sucrose, independent of the amount of F26BP (or the activation state of SPS). In tobacco plants that have been genetically manipulated to have altered amounts of the metabolite, F26BP has a flux response coefficient of –0.48 for sucrose synthesis (Scott et al. 1995, 2000) during the early part of the day, but less influence during the later part of the photoperiod. F26BP is also significant in regulating the rate of sucrose synthesis during the early part of the day in the crassulacean acid metabolism plant (CAM) plant *Kalanchöe daigremontiana* (Truesdale et al. 1999). Recent analysis of transgenic *Arabidopsis thaliana* plants (Drabourg et al. 2001) demonstrates that a decrease in F26BP to less than 50% of the wild-type amount is required to affect rates of sucrose synthesis at the end of the photoperiod.

A second role for F26BP in starch-storing plants is in regulating the partitioning of fixed carbon between sucrose and starch (reviewed in Stitt et al. 1987, Stitt 1990a). In spinach, an increase in F26BP accompanies the switch from sucrose accumulation early in the photoperiod, to starch accumulation later in the photoperiod (Stitt et al. 1983a). Sucrose synthesis is inhibited under these conditions by a decrease in the activity of SPS (Stitt et al. 1988), resulting in an increase in the concentration of phosphorylated intermediates including fructose 6-phosphate (F6P), and a decrease in Pi. Being an inhibitor of the bifunctional fructose 2,6-bisphosphatase (F26BPase, EC 3.1.3.46; Larondelle et al. 1989) and an activator of frucose-6-phosphate 2-kinase (PFKII, EC 2.7.1.105; Larondelle et al. 1986), the enzymes that degrade and synthesise F26BP, respectively, the rise in F6P leads to an increase in the concentration of this metabolite. The increase in F26BP inhibits cytFBPase, leading to a slight rise in triose-P, which, together with the fall in Pi, restricts export of triose-Ps from the chloroplast. This leads to an increase in the stromal 3-phosphoglycerate (PGA):Pi ratio, activation of adenosine diphosphoglucose pyrophosphorylase (EC 2.7.7.27; Preiss 1980), and a stimulation of starch synthesis. As is the case for its role in regulating sucrose synthesis, F26BP is more significant in regulating partitioning at low light than at high light in spinach (Neuhaus et al. 1990) and *C. xantiana* (Neuhaus et al. 1989), and at the beginning rather than the end of the day in tobacco (flux response coefficient = 0.8; Scott et al. 1995, 2000). Partitioning in transgenic *A. thaliana* with 10% of wild-type levels of F26BP is altered at both the beginning and the end of the photoperiod (Drabourg et al. 2001).

This understanding of the role of F26BP in co-ordinating rates of photosynthesis, sucrose and starch synthesis has arisen from studies on a limited number of plant species, all of which predominantly use starch for the transitory storage of carbon in their leaves. F26BP from plant tissues is often difficult to extract (Scott and Kruger 1994) and accurately assay (Stitt 1990b; Trevanion 2000), and there has been very little examination of the role of this metabolite in species, such as wheat, that predominantly store sucrose in their leaves (Lawlor et al. 1987; Trevanion 2000). The available evidence for a role for F26BP in regulating rates of carbohydrate synthesis in these types of plants is limited and equivocal, and can be summarised as follows. (i) In the only reported study of cytFBPase from wheat, Stitt et al. (1982) used a selective assay to measure the properties of cytFBPase in a cytosol-enriched fraction prepared from protoplasts. This demonstrated that wheat cytFBPase has similar affinities for fructose 1,6-bisphosphate (FBP), AMP, Pi and F26BP to the spinach enzyme. However, studies of the purified enzyme are required to determine the properties in detail. (ii) Although changes in the amounts of hexose-phosphates and uridine diphosphoglucose in wheat protoplasts under different experimental conditions are broadly consistent with sucrose synthesis being inhibited by the inactivation of cytFBPase and SPS (Stitt et al. 1983b), F26BP was not measured in this study. (iii) Barley leaves treated with vanadate show inverse correlations between the amounts of F26BP and both sucrose synthesis and the ratio of sucrose:starch synthesis, but the treatment also induced reciprocal changes in the activation state of SPS (Brauer and Stitt 1990). (iv) In barley there is no correlation between the amount of F26BP and the relative rates of accumulation of sucrose and starch (Sicher et al. 1984 , 1986). (v) There is no correlation between amounts of F26BP and the partitioning between sucrose and starch in *Lolium temulentum* (Pollock et al. 1995).

A previous study developed reliable extraction and assay methods for the F26BP from wheat leaves of a range of ages, and demonstrated that although the amounts of the metabolite are lower during the day than the night, neither the amounts of F26BP, nor the ratio of sucrose to starch accumulated in the leaf, changed during the day (Trevanion 2000). The work presented here examines the regulation of photosynthetic carbohydrate metabolism in the intact mature source leaves of wheat, with particular emphasis on the role of F26BP. The questions addressed are: What regulates the amount of F26BP? How is the rate of sucrose synthesis inhibited at low rates of photosynthesis? What activates sucrose synthesis in response to increases in photosynthesis? How is the partitioning of fixed carbon between sucrose and starch regulated?

Materials and methods

Plant material

The spring wheat (*Triticum aestivum* L.) cultivar Bob White was grown in a mixture of 70% compost/30% perlite in a controlled-environment cabinet. The plants were grown under a 16-h day with a day-time temperature of 18 °C and a night-time temperature of 15 °C. Relative humidity was 70% during the day and 80% during the night. Photosynthetically active radiation was 150, 300 or 500 μmol photons m^{-2} s^{-1}. Plants were grown for up to 4 weeks until the third leaves were fully expanded, and all measurements

were made on the middle sections (6 cm long) of these youngest source leaves.

Measurement of rates of photosynthesis

Rates of photosynthesis were measured with an infra-red gas analyser (Mark 3; ADC, Hoddesdon, UK) attached to a six-chamber open-circuit gas-exchanger with automatic data handling. Leaves from intact plants were used in all experiments and the temperature within the leaf chambers was 20 °C. Light intensity was varied by placing layers of neutral filter material between the light source and the leaf chambers. The CO_2 concentration of the air stream was regulated using a gas blender (Signal Instruments Co., Croydon, UK) to mix nitrogen, CO_2 (both $^{12}CO_2$ and $^{14}CO_2$) and oxygen from gas cylinders. The humidity of the air both before and after passage over the leaf was monitored using a Humitech humidity sensor (Vaisala, Helsinki, Finland) and leaf temperature was monitored using a thermocouple. Leaf internal CO_2 concentration (Ci) and stomatal conductance were calculated as described by von Caemmerer and Farquar (1981).

$^{14}CO_2$-feeding experiments

Unless specifically stated otherwise, all experiments used plants grown at 150 μmol photons m^{-2} s^{-1}, and were performed during the last 4 h of the photoperiod. Rates of photosynthesis were initially measured for about 1 h under ambient conditions (growth light intensity, CO_2 concentration about 390 μl l^{-1}) until a steady state was obtained. If required, the conditions (light intensity and/or CO_2 concentration) were altered and rates of photosynthesis measured for another 1 h until the leaf had again reached a steady state. $^{14}CO_2$ was then added to the gas stream and routinely fed to leaves for 5 min. In all cases $^{14}CO_2$ was fed to the leaves under the exact conditions of CO_2 concentration and light intensity at which the leaf had been pre-treated. After feeding, samples were immediately freeze-clamped and stored in liquid nitrogen prior to extraction and analysis. $^{14}CO_2$ was prepared in either air or nitrogen, and rates of synthesis of different components calculated knowing the specific activities of the $^{14}CO_2$ used.

Extraction and measurement of ^{14}C-labelled fractions

The frozen leaf sample (up to 3 cm^2) was ground to a powder using a pestle and mortar pre-cooled with liquid nitrogen. About half was transferred to a second mortar containing 360 μl of ice-cold 80% (v/v) ethanol, and homogenised thoroughly. Aliquots were removed for the measurement of chlorophyll and total label metabolised, the latter being mixed with scintillation liquid (Ultima gold; Packard, Meriden, Conn., USA) and counted (Model 2500 TR; Packard). The extract was then transferred to an eppendorf tube, placed in heating block at 70 °C for 20 min, and cooled on ice. The sample was centrifuged (10 min, 4 °C, 14,000 *g*), the supernatant removed and the pellet washed five times with 360 μl of 80% (v/v) ethanol. The washes were combined with the original supernatant, dried under vacuum and the soluble fraction redissolved in 360 μl of 80% (v/v) ethanol. The pellet was washed in 720 μl of 50 mM Na-acetate (pH 4.8), centrifuged (10 min, 4 °C, 14,000 *g*) and resuspended in a further 360 μl of 50 mM Na-acetate (pH 4.8). Both the supernatant and insoluble pellet were stored at –40 °C for up to a week before further analysis. Recovery of metabolised label in the soluble and insoluble fractions was 85 ± 11%.

The soluble fraction was separated into neutral, acidic and basic components by ion-exchange chromatography. An aliquot (up to 200 μl) of the soluble fraction was added to a 1-ml column of Dowex-50 (H^+), which in turn dripped onto a 1-ml column of Dowex-1 (Cl^-). The columns were washed with four 1-ml aliquots of water, and the resulting effluent containing the neutral fraction collected. The basic fraction was eluted from the Dowex-50 with four 1-ml aliquots of 5 M NH_4OH, and the acidic fraction was eluted from the Dowex-1 with four 1-ml aliquots of 2 M HCl. The fractions were dried under vacuum, resuspended in up to 1 ml of water, and aliquots counted. Recovery of label during ion exchange was 100 ± 11%.

Incorporation of $^{14}CO_2$ into starch was calculated by measuring the amount of label released after enzymatic digestion of the insoluble pellet (Haissig and Dickson 1979). Aliquots (up to 100 μl) of the resuspended pellet were incubated with 5 IU α-amylase (EC 3.2.1.1; from pig pancreas; Roche Diagnostics, Lewes, UK) and 5 IU amyloglucosidase (EC 3.2.1.3; from *Aspergillus niger*; Sigma, Poole, UK) for 2 days at 37 °C with occasional gentle mixing. After digestion, the mixture was centrifuged (10 min, 4 °C, 14,000 *g*) and the supernatant removed. The pellet was washed with two volumes of 50 mM Na-acetate (pH 4.8), centrifuged (10 min, 4°C, 14,000 *g*), the wash combined with the original supernatant and counted. The remaining insoluble material was resuspended in 50 mM Na-acetate (pH 4.8) and counted. For each sample a control with no addition of enzymes was performed. 79 ± 1.9% (mean ± SE, $n = 82$) of label in the insoluble pellet was released during starch digestion, and further incubation did not release more label.

Extraction and assay of metabolites

Sucrose and starch were measured in the 80% (v/v) ethanol extracts prepared from the labelled samples prepared above. Amounts of sucrose and the glucose released by the digestion of the starch were determined as described by Jones et al. (1977) using a Spectromax 340 plate reader. The remainder of each labelled sample was extracted in 50 mM KOH and used for the assay of F26BP as described by Trevanion (2000). Amounts of F26BP after altering either light intensity or CO_2 concentration were at steady state.

For the measurement of phosphorylated intermediates, leaf tissue was extracted in 1 M $HClO_4$ as described by Leegood (1992). Amounts of triose-P, PGA, glucose 6-phosphate (G6P) and F6P were measured using standard techniques (Lowry and Passonneau 1972) on an SLM-Aminco DW2000 dual-wavelength spectrophotometer with 410 nm as the reference wavelength.

Thin-layer chromatography

Aliquots of the neutral fractions were analysed by thin-layer chromatography (TLC) on Silica Gel 60 plates with the solvent being ethyl acetate/isopropanol/water (65:22.75:12.25, by vol.; Lewis and Smith 1969). Markers were ^{14}C-labelled sugars (sucrose, glucose and fructose) and label was detected by autoradiography. At least 90% of the label in the soluble fractions was in sucrose, the remainder was in glucose and fructose and there was none in fructan.

Measurement of chlorophyll

Aliquots of extract were immediately neutralised with an equal volume of 1 M Tris-HCl (pH 8.0) and stored in the dark on ice for up to 1 h. Chlorophyll was measured in 80% (v/v) acetone as described by Porra et al. (1989).

Statistical analysis

The statistical significance of the results was assessed by applying student *t*-tests to the data. Differences between results are described as being significant where $P \leq 0.05$, and not significant where $P > 0.05$.

Results

Calculation of rates of incorporation of label into soluble and insoluble fractions

Calculating rates of synthesis using measurements of the incorporation of ^{14}C will be accurate only when rates of

accumulation are linear with time, i.e. when the pools of intermediates between CO_2 and end products have become saturated with ^{14}C. To examine this, time courses of the fate of metabolised label were made over 7 min, using four sets of plants under environments representing the extremes of the conditions used in subsequent experiments – low and high Ci (70 and 670 μl l^{-1}), and low and high light (80 and 1,200 μmol photons m^{-2} s^{-1}; results not shown). There were essentially no lags in incorporation of label into the acidic and basic fractions under any of the conditions (results not shown). Under all conditions the kinetics of the accumulation of label into starch and sucrose were identical, and, except at low Ci, when label appeared in these compounds immediately, there was about a 2.5-min lag before label accumulated in these fractions. Rates of synthesis in the experiments described below were calculated taking these lags into account.

Since the labelling experiments used leaves from intact plants, even though the duration was short there was the potential for export of [^{14}C]sucrose from the leaves during the course of the experiments. This would lead to underestimation of the rates of sucrose synthesis and the ratio of sucrose:starch synthesis. Analysis of leaves with low rates of photosynthesis (between 0.5 and 1.5 μmol min^{-1} mg^{-1} Chl) showed that 92 ± 5% (mean ± SE) of the label assimilated by photosynthesis was recovered in the 80% ethanol extract, and for leaves with high rates of photosynthesis (between 4 and 6 μmol min^{-1} mg^{-1} Chl) the recovery was 84 ± 5%. Export of [^{14}C]sucrose from the leaves during the feeding experiment was therefore judged as negligible, and was not considered in the results described below.

Diurnal changes in amounts of metabolites and metabolism of $^{14}CO_2$ in plants grown at different light intensities

Changes in the fate of metabolised $^{14}CO_2$, and amounts of metabolites (sucrose, starch, triose-P, PGA, G6P, F6P, and F26BP) were examined at the beginning and the end of the photoperiod in leaves of plants grown at either 150, 300, or 500 μmol photons m^{-2} s^{-1}. Feeding experiments were performed under ambient CO_2 concentration (390 μl l^{-1}) at light intensities identical to those used during growth. Comparisons were made between metabolism 1 h after the beginning, and 1 h before the end of the photoperiod. Rates of photosynthesis and metabolism of $^{14}CO_2$ are shown in Table 1. The results from all three sets of plants were very similar. There was no significant inhibition of photosynthesis at the end of the day, and rates of incorporation of label into the soluble fractions (neutral, acidic and basic) did not vary much between the beginning and the end of the day. However rates of starch synthesis were about 2.5- to 3-fold higher, and consequently the ratios of sucrose:starch synthesis were significantly lower, at the end than at the beginning of the day.

Amounts of metabolites under these conditions are shown in Table 2. Whilst the sucrose content of leaves increased over the course of the experiment by about 2.5-fold in all sets of plants, the starch content increased by 2.6-, 4-, and 5-fold in the plants grown at 150, 300 and 500 μmol photons m^{-2} s^{-1}, respectively. Consequently, the ratios of sucrose:starch content were lower at the end than at the beginning of the day, particularly in the high-light-grown plants. There were no significant changes in the amounts of either individual (triose-P, PGA, hexose-phosphates, F26BP) or total phosphorylated intermediates, or the ratios of PGA:triose-P or G6P:F6P, between the beginning and the end of the day in plants grown under any light intensity.

The consequences of altering the light intensity per se are also shown in Tables 1 and 2. Chlorophyll per leaf area, rates of photosynthesis, amounts of some phosphorylated intermediates (total, triose-P and PGA), and rates of incorporation of label into soluble and insoluble fractions were greater in plants grown and measured at higher light intensities. However, amounts of hexose-phosphates and the ratios of sucrose:starch synthesis were not significantly affected, either at the beginning or the end of the photoperiod. There were no significant differences in F26BP content between plants grown at 150 and 300 μmol photons m^{-2} s^{-1}, but there was an increase in plants grown at 500 μmol photons m^{-2} s^{-1}.

Table 1. Diurnal variation in rates of synthesis of sucrose, starch, acidics and basics. Wheat (*Triticum aestivum*) plants were grown under three different light intensities until the third leaf was fully expanded. Intact leaves were then placed in leaf chambers connected to an infra-red gas analyser and photosynthesis rates measured for 1 h under ambient conditions. The leaf was then given a 5-min pulse of $^{14}CO_2$, freeze-clamped and stored in liquid nitrogen. Measurements were made 1 h after the beginning and 1 h before the end of the light period. Samples were extracted in 80% (v/v) ethanol and the incorporation of label into sucrose, starch, acidic and basic fractions measured. Results are mean ± SE for at least three samples

Growth light (μmol m^{-2} s^{-1})	Chlorophyll (mg m^{-2})	Time of day	Photosynthesis (μmol min^{-1} mg^{-1} Chl)	Synthesis (μmol C min^{-1} mg^{-1} Chl)				Sucrose:starch
				Sucrose	Starch	Acidics	Basics	
150	260 ± 14	Beginning	2.58 ± 0.27	1.22 ± 0.06	0.08 ± 0.01	0.17 ± 0.01	0.36 ± 0.02	16.5 ± 1.5
		End	2.34 ± 0.21	1.37 ± 0.04	0.20 ± 0.01	0.12 ± 0.01	0.34 ± 0.02	6.8 ± 0.3
300	292 ± 12	Beginning	4.31 ± 0.57	1.87 ± 0.13	0.15 ± 0.02	0.27 ± 0.03	0.54 ± 0.09	12.3 ± 0.8
		End	3.97 ± 0.13	2.88 ± 0.14	0.36 ± 0.02	0.26 ± 0.06	0.60 ± 0.01	7.7 ± 1.0
500	336 ± 19	Beginning	4.40 ± 0.41	2.90 ± 0.34	0.16 ± 0.02	0.35 ± 0.03	0.59 ± 0.06	19 ± 0.9
		End	4.34 ± 0.34	2.41 ± 0.36	0.38 ± 0.05	0.30 ± 0.02	0.60 ± 0.09	6.3 ± 0.1

Table 2. Diurnal variation in amounts of metabolites. Wheat plants were grown under three different light intensities until the third leaf was fully expanded. Intact leaves were then placed in leaf chambers connected to an infra-red gas analyser and photosynthesis rates measured for 1 h under ambient conditions. The leaf was then freeze-clamped and stored in liquid nitrogen. Measurements were made 1 h after the beginning and 1 h before the end of the light period. Samples were extracted in 80% (v/v) ethanol for the measurement of sucrose and starch, 1 M perchloric acid for measurement of triose-P, PGA, G6P and F6P, or 50 mM KOH for the measurement of F26BP. Results are mean ± SE for three samples

Growth light (μmol m^{-2} s^{-1})	Time of day	Amount (μmol mg^{-1} Chl)		Amount (nmol mg^{-1} Chl)					Ratio		F26BP (pmol mg^{-1} Chl)
		Sucrose	Starch	Triose-P	PGA	G6P	F6P	Total	PGA: triose-P	G6P:F6P	
150	Beginning	5.6 ± 0.9	1.4 ± 0.2	65 ± 7	427 ± 44	144 ± 20	73 ± 6	709 ± 50	6.8 ± 1.2	2.0 ± 0.1	105 ± 21
	End	13 ± 1.3	3.6 ± 0.2	50 ± 12	346 ± 51	90 ± 9	54 ± 7	539 ± 65	7.9 ± 2.0	1.7 ± 0.1	115 ± 11
300	Beginning	6.8 ± 0.9	1.3 ± 0.1	80 ± 9	512 ± 60	140 ± 35	124 ± 13	856 ± 103	6.6 ± 1.2	1.1 ± 0.2	153 ± 44
	End	24 ± 4.2	5.2 ± 1.2	67 ± 2	488 ± 73	135 ± 23	90 ± 8	780 ± 83	7.3 ± 0.9	1.5 ± 0.1	88 ± 15
500	Beginning	14 ± 0.5	2.8 ± 0.4	132 ± 5	617 ± 38	148 ± 8	100 ± 6	998 ± 48	4.7 ± 0.4	1.5 ± 0.1	204 ± 19
	End	36 ± 4.3	14 ± 1.9	174 ± 25	638 ± 7	128 ± 11	99 ± 12	1,040 ± 44	3.9 ± 0.6	1.3 ± 0.0	240 ± 10

Effect of altering CO_2 on amounts of metabolites and metabolism of $^{14}CO_2$

Correlations between the fate of metabolised $^{14}CO_2$, amounts of phosphorylated intermediates and F26BP were examined in response to altering CO_2 concentration. All experiments used a saturating light intensity of about 1,000 μmol photons m^{-2} s^{-1}. The rate of photosynthesis was inhibited at Ci values above about 450 μl l^{-1} (Fig. 1a). Although rates of synthesis of sucrose (Fig. 1b) and starch (Fig. 1c) generally correlated well with rates of photosynthesis, there was a large increase in the rate of starch synthesis at a Ci of 450 μl l^{-1} and a decrease at higher Ci. Although the ratio of sucrose:starch synthesis was slightly lower at a Ci of 450 μl l^{-1}, no consistent trend was observed over the wide range of concentrations used (Fig. 1d). Rates of incorporation of label into the acidic

Fig. 1a–f. Effect of short-term changes in CO_2 concentration on photosynthetic carbon metabolism in wheat (*Triticum aestivum*) leaves. Fully expanded third leaves from 4-week-old plants (grown at 150 μmol photons m^{-2} s^{-1}) were placed in leaf chambers with an ambient CO_2 concentration (390 μl l^{-1}) and high light (1,200 μmol photons m^{-2} s^{-1}) for about 1 h. The CO_2 concentration in the chamber was then altered, and, after photosynthesis had reached a steady state (after about 1 h) the leaves were given a 5-min pulse of $^{14}CO_2$. The samples were freeze-clamped, extracted in 80% (v/v) ethanol, and the distribution of ^{14}C measured. **a** Photosynthesis. **b** Sucrose synthesis. **c** Starch synthesis. **d** Ratio of sucrose to starch synthesis. **e** Rate of incorporation of label into acidic compounds. **f** Rate of incorporation of label into basic compounds. All measurements were made during the last 4 h of the photoperiod and values are mean ± SE for five samples. Rates of synthesis were normalised for differences in rate of photosynthesis between leaves

fraction correlated with rates of photosynthesis (Fig. 1e) whereas incorporation of label into the basic fraction was much less sensitive to changes in Ci (Fig. 1f).

As shown in Fig. 2a, the sum of the phosphorylated intermediates measured decreased from about 890 to 330 nmol mg^{-1} Chl with reducing CO_2. The amount of PGA decreased 4.5-fold as Ci was lowered from 570 to 70 µl l^{-1} (Fig. 2b), but since the amount of triose-P was largely unaffected (Fig. 2c) the ratio of PGA:triose-P decreased from 5.4 at high Ci to 1.3 at low Ci (Fig. 2d). Amounts of G6P (Fig. 2e) and F6P (Fig. 2f) fell by about 2-fold over the range of concentrations used and there was a slight increase in the ratio of G6P:F6P (from 0.9 to 1.2) as Ci was reduced (Fig. 2g). The ratio of PGA:F6P did not change much with Ci (Fig. 2h). Figure 3 shows the relationship between amounts of F26BP and Ci. This demonstrates a dramatic effect of CO_2 concentration on the amount of F26BP, with a 26-fold decrease in amounts between the lowest and the highest Ci values obtained.

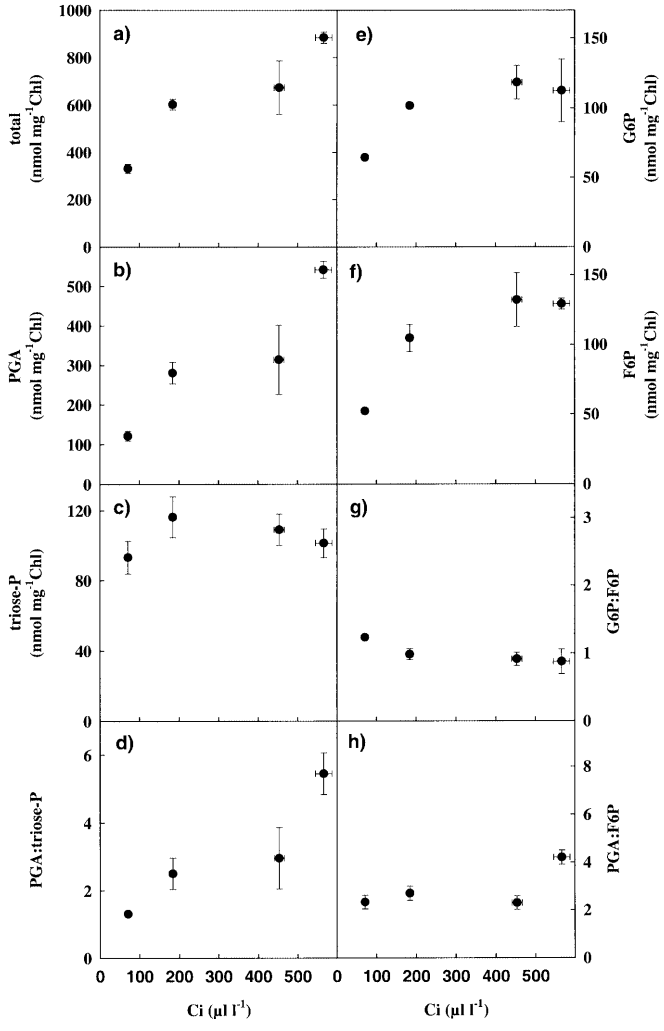

Fig. 2a–h. Effect of altering CO_2 concentration on amounts of intermediates of sugar synthesis. Fully expanded third leaves from 4-week-old wheat plants (grown at 150 µmol photons m^{-2} s^{-1}) were placed in leaf chambers with ambient CO_2 concentration (390 µl l^{-1}) and high light (1,200 µmol photons m^{-2} s^{-1}) for about 1 h. The CO_2 concentration in the chamber was then altered, and, after photosynthesis had reached a steady state (after about 1 h) the samples were freeze-clamped and stored in liquid nitrogen. Samples were extracted in 1 M perchloric acid and amounts of metabolites measured. **a** Total. **b** PGA. **c** Triose-P. **d** Ratio of PGA to triose-P. **e** G6P. **f** F6P. **g** Ratio of G6P to F6P. **h** Ratio of PGA:F6P. All measurements were made during the last 4 h of the photoperiod and values are mean ± SE for three samples

Effects of changes in light intensity on amounts of metabolites and metabolism of $^{14}CO_2$

Correlations between the fate of metabolised $^{14}CO_2$, amounts of phosphorylated intermediates and F26BP were also examined under a range of light intensities. All experiments used a CO_2 concentration in the chamber of 390 µl l^{-1}. The rate of photosynthesis increased with light intensity, becoming saturated at about 600 µmol photons m^{-2} s^{-1} (Fig. 4a) and rates of sucrose synthesis were correlated with rates of photosynthesis (Fig. 4b). However, rates of starch synthesis were very low at light intensities below 600 µmol photons m^{-2} s^{-1}, did not increase proportionally with the rate of photosynthesis over this range, and were greatly stimulated at the highest light used (Fig. 4c). Consequently the ratio of sucrose:starch synthesis decreased as light intensity increased (Fig. 4d). Rates of incorporation of label into the acidic (Fig. 4e) and basic (Fig. 4f) fractions were correlated with rates of photosynthesis.

As shown in Fig. 5a the sum of phosphorylated intermediates measured decreased from about 880 to 340 nmol mg^{-1} Chl as light was reduced. The amounts of PGA and triose-P each decreased by about 2.5-fold as the light was lowered from 1,200 to 80 µmol photons m^{-2} s^{-1} (Fig. 5b, c), and consequently the ratio of PGA:triose-P was almost constant over the range of light intensities used (Fig. 5d). Amounts of G6P fell by less than 2-fold (Fig. 5e) but amounts of F6P fell by nearly 5-fold (Fig. 5f).

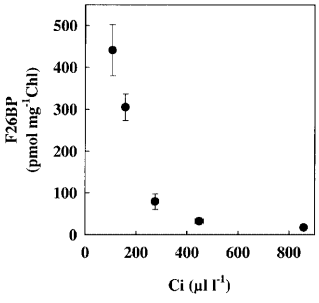

Fig. 3. Effect of altering CO_2 concentration on amounts of F26BP. Fully expanded third leaves from 4-week-old wheat plants (grown at 150 µmol photons m^{-2} s^{-1}) were placed in leaf chambers with an ambient CO_2 concentration (390 µl l^{-1}) and high light (1,200 µmol photons m^{-2} s^{-1}) for about 1 h. The CO_2 concentration in the chamber was then altered, and, after photosynthesis had reached a steady state (after about 1 h) the samples were freeze-clamped and stored in liquid nitrogen. Samples were extracted in 50 mM KOH and amounts of F26BP assayed. All measurements were made during the last 4 h of the photoperiod and values are mean ± SE for at least three samples

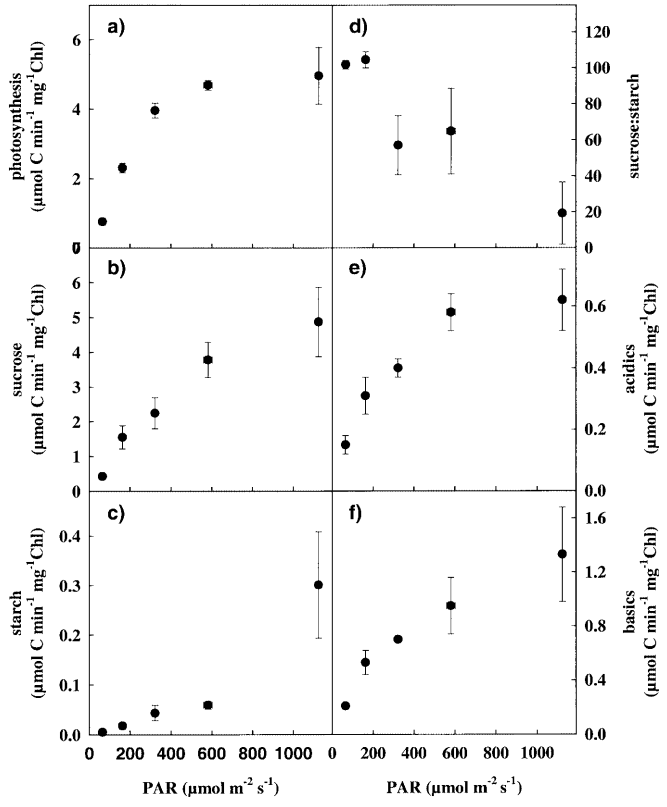

Fig. 4a–f. Effect of short-term changes in light intensity on photosynthetic carbon metabolism in wheat leaves. Fully expanded third leaves from 4-week-old wheat plants (grown at 150 µmol photons m^{-2} s^{-1}) were placed in leaf chambers with an ambient concentration of CO_2 (390 µl l^{-1}) and growth light intensity. Photosynthesis was measured for about 1 h, light intensity then altered, and, after photosynthesis had reached a steady state (after about 1 h) the leaves were given a 5-min pulse of $^{14}CO_2$. The samples were freeze-clamped, extracted in 80% (v/v) ethanol, and the distribution of ^{14}C measured. **a** Photosynthesis. **b** Sucrose synthesis. **c** Starch synthesis. **d** Ratio of sucrose to starch synthesis. **e** Rate of incorporation of label into acidic compounds. **f** Rate of incorporation of label into basic compounds. All measurements were made during the last 4 h of the photoperiod and values are mean ± SE for five samples. Rates of synthesis were normalised for differences in rate of photosynthesis between leaves

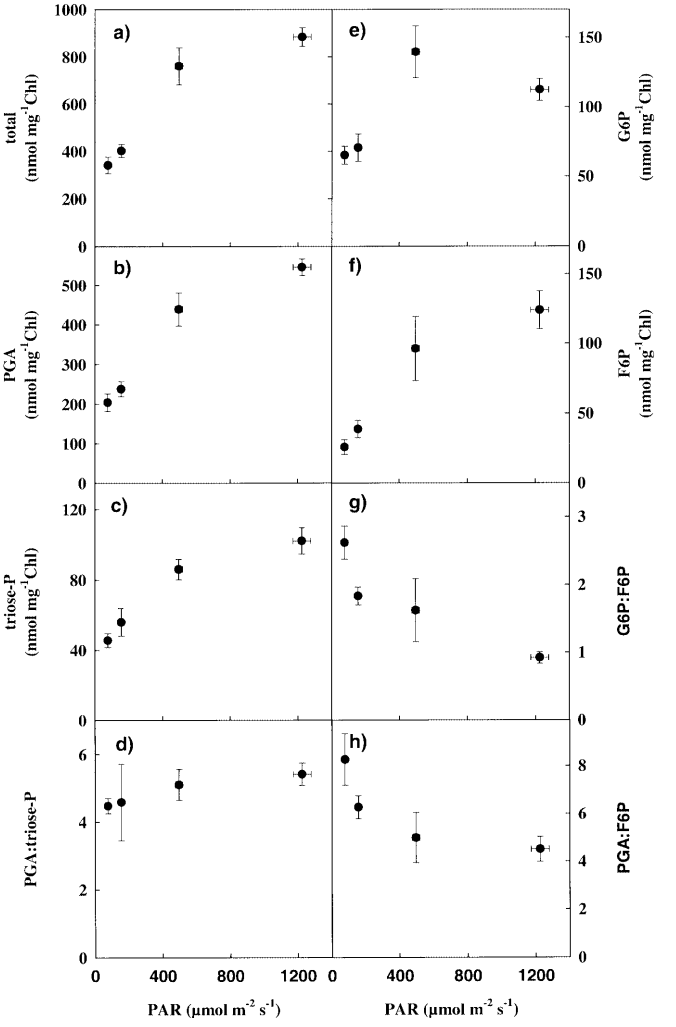

Fig. 5a–h. Effect of light intensity on amounts of intermediates of sugar synthesis. Fully expanded third leaves from 4-week-old wheat plants (grown at 150 µmol photons m^{-2} s^{-1}) were placed in leaf chambers with an ambient concentration of CO_2 (390 µl l^{-1}) and growth light intensity. Photosynthesis was measured for about 1 h, light intensity then altered, and, after photosynthesis had reached a steady state (after about 1 h) the samples were freeze-clamped and stored in liquid nitrogen. Samples were extracted in 1 M perchloric acid and amounts of metabolites measured. **a** Total. **b** PGA. **c** Triose-P. **d** Ratio of PGA to triose-P. **e** G6P. **f** F6P. **g** Ratio of G6P to F6P. **h** Ratio of PGA:F6P. All measurements were made during the last 4 h of the photoperiod and values are mean ± SE for three samples

Consequently, there was an increase in the ratio of G6P:F6P from 0.9 to 2.6 as light was reduced (Fig. 5g). There was also a large increase in the ratio of PGA:F6P (from 4.5 to 8.3) at low light (Fig. 5h). Amounts of F26BP were almost completely insensitive to changes in light, there being an increase at the lowest intensity only (Fig. 6).

Discussion

On the approach

The experiments reported in this paper were designed to study the relationships between amounts of phosphorylated intermediates, F26BP and the metabolism of $^{14}CO_2$ in the source leaves of wheat. The feeding experiments reported here differ from previous studies in this area, which have generally have fed $^{14}CO_2$ to leaf discs in an O_2 electrode under saturating concentrations of CO_2 (e.g. Neuhaus et al. 1989, 1990). Although the CO_2 concentration in an O_2 electrode can be varied by altering either pH or buffer composition (e.g. Stitt et al. 1984), due to the diffusion pathways present the absolute CO_2 concentration in the electrode cannot be calculated. In contrast, the approach taken in the study presented here, where a gas blender has been used to control the concentration of $^{14}CO_2$ fed to leaves in leaf chambers, allows the feeding of $^{14}CO_2$ to intact leaves under physiological conditions.

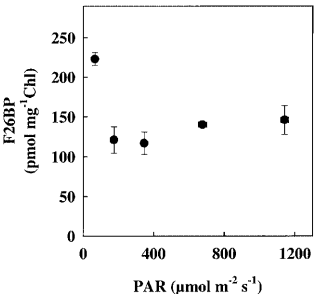

Fig. 6. Effect of altering light intensity on amounts of F26BP. Fully expanded third leaves from 4-week-old wheat plants (grown at 150 μmol photons m^{-2} s^{-1}) were placed in leaf chambers with an ambient concentration of CO_2 (390 μl l^{-1}) and growth light intensity. Photosynthesis was measured for about 1 h, light intensity then altered, and, after photosynthesis had reached a steady state (after about 1 h) the samples were freeze-clamped and stored in liquid nitrogen. Samples were extracted in 50 mM KOH and amounts of F26BP assayed. All measurements were made during the last 4 h of the photoperiod and values are mean ± SE for at least three samples

Regulation of F26BP content in wheat leaves

The amount of F26BP is dependent on the activities of enzymes that synthesise and degrade the metabolite, PFKII and F26BPase respectively. The activities of these enzymes are regulated by the amounts of the intermediates of sucrose synthesis and this network of interactions is integral to our understanding of the role of F26BP in spinach leaves (reviewed in Stitt et al. 1987). The mechanisms regulating F26BP content in wheat must therefore be understood if the role of the metabolite in regulating photosynthetic carbon metabolism in this plant is to be understood. Measurements of total amounts of triose-P, PGA, F6P and Pi in wheat leaves under different experimental conditions cannot be used to make exact predictions as to the steady-state concentration of F26BP – this would require both a knowledge of the subcellular distribution of the metabolites and a kinetic analysis of PFKII and F26BPase from wheat leaves. However, they can provide some insight into the important factors regulating the concentration of this effector.

Decreasing CO_2 has a much greater effect than decreasing light on the amount of F26BP in wheat leaves (Figs. 3, 6). Interestingly, low CO_2 also leads to a greater increase in the amount of F26BP than does low light in barley primary leaves, but only at the beginning of the photoperiod (Sicher and Bunce 1987). The amounts of other metabolites measured are similar to those in spinach leaves (Gerhardt et al. 1987) and fully expanded wheat leaves (Kobza and Edwards 1987; Savitch et al. 1997), but are greater than those measured in wheat protoplasts (Stitt et al. 1983b).

Pi is a powerful inhibitor of the spinach leaf monofunctional F26BPase (MacDonald et al. 1989) and an activator of PFKII (Cséke and Buchanan 1983). The concentration of Pi in the cytosol could not be measured directly due to the large amounts of Pi localised in the vacuole, but assuming that vacuolar Pi does not rapidly cross the tonoplast (Foyer et al. 1982), indirect indications can be obtained from measurements of the total amounts of phosphorylated intermediates. Low CO_2 (Fig. 2a) and low light (Fig. 5a) induce similar decreases in the sum of all the phosphorylated intermediates measured, suggesting similar increases in the amounts of Pi under these conditions. The amounts of ribulose 1,5-bisphosphate (RuBP) were not measured, but since these are likely to decrease more in response to changes in light than to CO_2 (Stitt et al. 1983b), cytosolic Pi may actually be higher under low light than under low CO_2.

Since F26BP increases far more under low CO_2 than low light, it is clear that factors other than Pi must be contributing to the regulation of F26BP content in wheat leaves. First, PGA is an inhibitor of the spinach leaf PFKII (Cséke and Buchanan 1983). In wheat leaves the decrease in PGA is greater under low CO_2 (Fig. 2b) than under low light (Fig. 5b), correlating with the observed changes in F26BP. Interestingly, the usual association between the amounts of PGA and triose-P is not present at low CO_2 – there is a large fall in the amount of the former (Fig. 2b) but no effect on the latter (Fig. 2c). It therefore seems likely that, as in spinach, PGA is has a role in regulating the amount of F26BP but triose-P has little influence (Neuhaus and Stitt 1989). Second, F6P is an inhibitor of the spinach leaf bifunctional F26BPase (Larondelle et al. 1989) and an activator of PFKII (Larondelle et al. 1986). F6P decreases less at low CO_2 (Fig. 2f) than at low light (Fig. 5f), and again this correlates with measured changes in F26BP.

These results strongly suggest that PGA and F6P are significant in regulating the amount of F26BP in wheat leaves. In addition, it appears that even though the differences in the responses of PGA and F6P under the two experimental conditions are small, they have large consequences for the amount of F26BP. Both of these conclusions are consistent with our understanding of the role of F26BP in spinach, suggesting that the mechanisms by which the amount of F26BP is regulated in may well be conserved between spinach and wheat. However, the properties of PFKII from spinach leaves, maize leaves (Soll et al. 1983) and a range of sink tissues (Stitt et al. 1985b) do vary, and detailed studies of the properties of PFKII and F26BPase from wheat leaves are required before we can fully assess if the mechanisms regulating F26BP content have been conserved between these plants with such different strategies for carbohydrate storage.

Co-ordination of sucrose synthesis and photosynthesis at low light and low CO_2

Rates of sucrose synthesis need to be inhibited at low rates of photosynthesis in order to prevent the depletion of metabolites from the chloroplast stroma, which would otherwise lead to an additional inhibition of photosynthesis. The measurements of amounts of key metabolites in intact wheat leaves under low CO_2 and

low light presented here have been used to assess the nature of the regulation occurring in this plant.

In intact wheat leaves, restricting photosynthesis by reducing CO_2 has no effect on the amount of triose-P; amounts are about 90 nmol mg^{-1} Chl at the lowest CO_2 concentration used. When photosynthesis is restricted by reducing light, triose-P is reduced, but the amounts are still considerable, 46 nmol mg^{-1} Chl. The concentrations of triose-P under these conditions can be calculated with a knowledge of the distribution of triose-P between the chloroplast and cytosol. Although this has not been measured for intact wheat leaves, the proportion of triose-P in the cytosol has been determined in spinach protoplasts (between 50 and 70%; Stitt et al. 1980), spinach leaves (between 25% at the beginning of the day and 50% at the end of the day; Gerhardt et al. 1987) and wheat protoplasts (between 70 and 80% under both high and low CO_2; Stitt et al. 1983b). Because of this variability, the concentrations of triose-P in the cytosol of intact wheat leaves have been calculated assuming that 30, 50 or 70% of the metabolite in the leaf as a whole is in this compartment. Assuming that the cytosolic volume of wheat mesophyll cells is the same as for barley (13.5 µl mg^{-1} Chl; Winter et al. 1993), triose-P in the cytosol will therefore be between 2.1 and 4.8 mM at low CO_2, and between 1 and 2.4 mM under low light. Making the further assumption that the reactions catalysed by aldolase and triose-P isomerase are close to equilibrium, the concentrations of FBP in the cytosol calculated from these estimates (Herzog et al. 1984) are between 1.1 mM and 6.1 mM at low CO_2, and between 0.3 and 1.4 mM at low light. Even the lowest of these are greatly in excess of the K_m of the uninhibited cytFBPase from wheat protoplasts (3.6 µM; Stitt et al. 1982), graphically demonstrating the effectiveness of inhibitors in restricting activity of the enzyme in vivo.

There are many potential inhibitors of cytFBPase (Stitt et al. 1985a), and given our lack of knowledge of the properties of wheat cytFBPase and the concentrations of the potential inhibitors, the relative significance of the different metabolites is difficult to assess. However, the response of partially purified wheat cytFBPase to F26BP has been characterised (Stitt et al. 1982), and we do have direct measurements of the metabolite in intact leaves. Assuming that F26BP in wheat is confined to the cytosol as it is in spinach (Stitt et al. 1983a) and oat (Steingraber et al. 1988), the estimated concentrations are approx. 33 and 16 µM under low CO_2 and low light, respectively. Since the inhibition of wheat cytFBPase by F26BP ($K_i = 0.1$ µM) is competitive with FBP, the degree of inhibition under low CO_2 and low light can be calculated. Then, making the assumption that the maximum catalytic activity of cytFBPase is equivalent to the maximum rate of sucrose synthesis (certainly an underestimation), the approximate activities of cytFBPase under low CO_2 and low light can be calculated (filled symbols Fig. 7a, b, the three lines represent the different distributions of triose-P between the cytosol and chloroplast). This figure demonstrates that the changes in F26BP and FBP that occur at low rates of photosynthesis will partially inhibit the activity of cytFBPase. However, also shown in this figure are the rates of sucrose synthesis determined from the $^{14}CO_2$-feeding experiments (open symbols). Taking these as minimum estimates of the activities of cytFBPase in vivo it is clear that F26BP cannot by itself inhibit the enzyme to the degree that occurs in vivo, and that metabolites other than F26BP must also have a role in inhibiting cytFBPase when rates of photosynthesis are low.

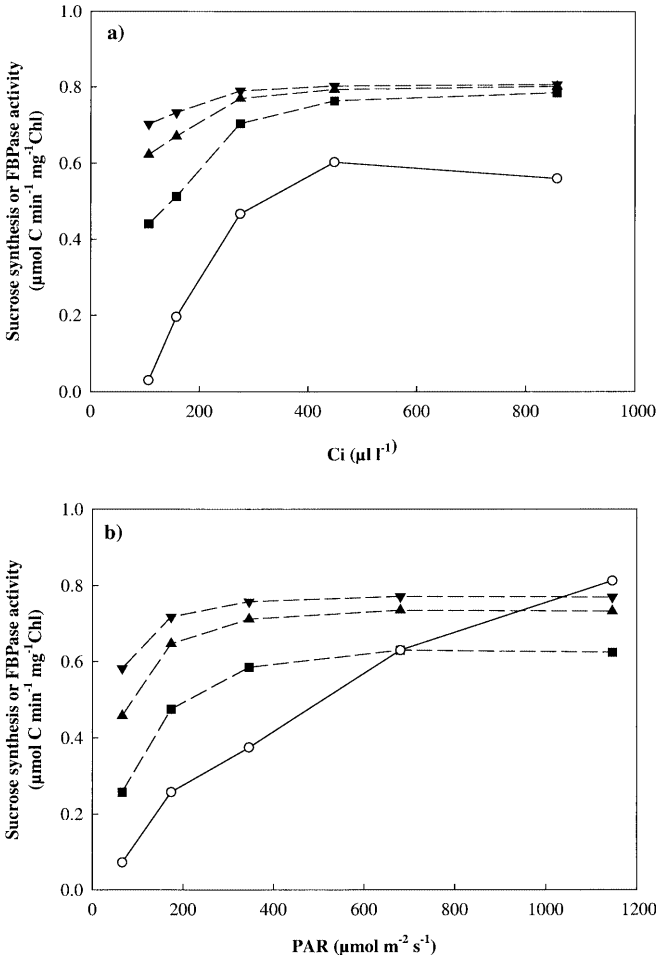

Fig. 7a, b. Comparison of simulated cytFBPase activities with rates of sucrose synthesis. Rates of sucrose synthesis (*circles*) were measured by $^{14}CO_2$ labelling under different CO_2 concentrations (**a**) and light intensities (**b**). The activity of cytFBPase under these conditions was also calculated using estimates of concentrations of FBP and F26BP, assuming that 30% (*squares*), 50% (*triangles, point up*) or 70% (*triangles, point down*) of the triose-P measured is in the cytosol

Of the other known effectors of the spinach enzyme, dihydroxyacetone phosphate (DHAP) between 0.5 and 1.5 mM inhibits non-competitively with FBP, even in the presence of F26BP (Stitt et al. 1985a). Estimates of the concentrations of DHAP in wheat leaves with low rates of photosynthesis are between 1 and 4.8 mM (see above), suggesting that the DHAP may well be significant in inhibiting cytFBPase in wheat leaves under these

conditions. Although Pi inhibits cytFBPase, acting competitively with FBP (Stitt et al. 1985a), given the high amounts of FBP it is unlikely to have significant direct effects on the activity of the enzyme in wheat. Likewise, AMP is a competitive inhibitor of spinach leaf cytFBPase and is unlikely to have direct effects on the activity of the wheat enzyme. However, the inhibition by AMP is complex, altering the requirement of the enzyme for F26BP, Mg^{2+}, and affecting the pH optimum (Stitt et al. 1985a), and it is possible that the metabolite has significant indirect effects on the activity of cytFBPase. Unfortunately, we know nothing about how the amounts of AMP or Mg^{2+}, or the pH, might change in response to alterations in photosynthesis in wheat, and the role of AMP is impossible to assess at present. Similarly, metabolites such as succinate, glutamate or UDP (Stitt et al. 1985a) may inhibit cytFBPase but their concentrations in wheat leaves are not known.

Activation of sucrose synthesis in response to increases in photosynthesis

Rates of sucrose synthesis must be activated in response to increases in photosynthesis, releasing the Pi required for the synthesis of the ATP in the chloroplast that is essential for the continuing functioning of the reductive pentose phosphate pathway. Measurements of rates of sucrose synthesis, amounts of F26BP and other key metabolites in wheat leaves have been used to assess the nature of the regulation occurring in this plant.

When photosynthesis was increased by raising CO_2 there was no effect on the amount of triose-P (Fig. 2c). This result is completely unexpected, given that the spinach model depends on an increase in substrate concentration to activate cytFBPase in response to rising rates of photosynthesis, and that a previous study in wheat protoplasts did show an increase in triose-P with rising CO_2 (Stitt et al. 1983b). There is a large decrease in the amount of F26BP (Fig. 3) and, as shown in Fig. 8a (filled symbols), this is inversely correlated with rates of sucrose synthesis. As described above, Fig. 7a compares the activities of cytFBPase calculated at specific concentrations of FBP and F26BP (filled symbols) with estimated in vivo activities of the enzyme (open symbols). Although the former are greater than the latter, the two are correlated over the range of CO_2 concentrations used. Together these data strongly suggest that F26BP is significant in regulating the activity of cytFBPase in response to changes in CO_2. Of course metabolites other than F26BP will be involved in inhibiting activity of cytFBPase in vivo, and an involvement of one or more of these in regulating activity in response to changes in CO_2 is likely.

In contrast to altering CO_2, when photosynthesis of wheat leaves is increased by altering light intensity there is a rise in the amount of triose-P (Fig. 5c) but only a limited effect on the amount of F26BP (Fig. 6). Indeed, as shown in Fig. 8a (open symbols) there is no

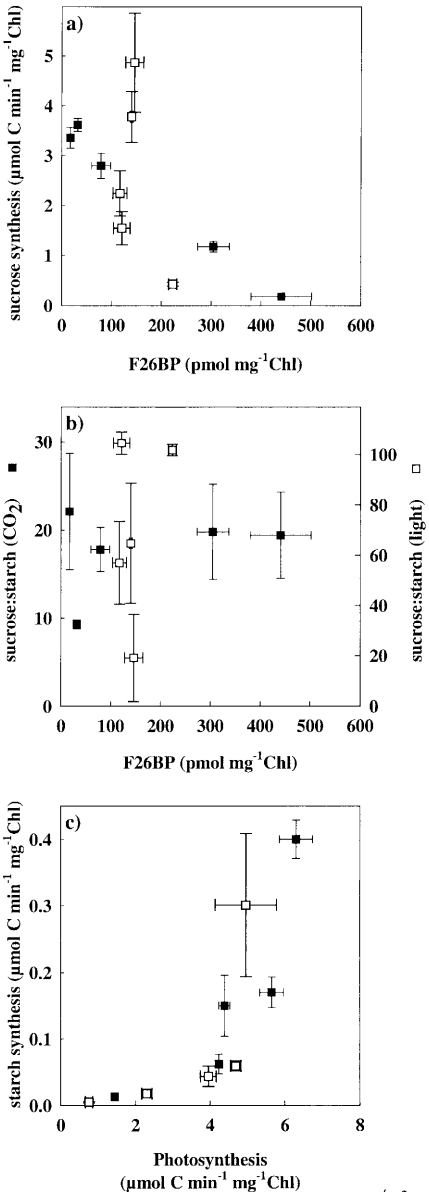

Fig. 8a–c. Relationships between F26BP, carbohydrate synthesis and photosynthesis. Data from Figs. 3, 5, 6, 8 were re-analysed to examine the relationships between F26BP and sucrose synthesis (**a**), F26BP and partitioning between sucrose and starch (**b**), photosynthesis and starch synthesis (**c**). Data were from both varying CO_2 (*filled squares*) and light (*open squares*)

correlation between amounts of F26BP and rates of sucrose synthesis under these conditions. Figure 7b shows that there is a correlation between the calculated activities of cytFBPase (filled symbols) and the estimated in vivo activities of the enzyme (open symbols) at light intensities below about 400 µmol photons m^{-2} s^{-1}. However, at higher light the changes in FBP and F26BP cannot account for alterations in the activity of cytFBPase in vivo. These data suggest that F26BP plays a minor role in regulating the activity of cytFBPase in response to altered light in wheat and that, as in spinach, changes in substrate availability are relevant only at low

light. Other inhibitors of cytFBPase must be involved, both in enhancing the effects of F26BP and substrates at low light, but also by directly inhibiting the enzyme at higher light intensities.

Regulation of carbohydrate partitioning in wheat leaves

Studies in plants that store predominantly starch have demonstrated that F26BP can be significant in co-ordinating sucrose synthesis in the cytosol with starch synthesis in the chloroplast, but that the importance of this depends on the conditions (Neuhaus et al. 1990). Measurements of rates of carbohydrate synthesis and amounts of metabolites have been used to examine the nature of the regulation occurring in wheat leaves.

In wheat plants grown at three different light intensities there are significant increases in the rate of starch synthesis at the end of the photoperiod, but no significant inhibition of the rates of sucrose synthesis (Table 1) nor increases in the amounts of triose-P, PGA, F6P, G6P or F26BP (Table 2). This suggests that in wheat plants grown under these conditions, the increase in starch synthesis that occurs at the end of the day does so in the absence of any feedback inhibition of sucrose synthesis and does not involve F26BP. However, since starch represents a minor sink for fixed carbon in wheat (the fluxes are about 6 and 14% of the rates of sucrose synthesis at the beginning and the end of the photoperiod, respectively), relatively large increases in starch synthesis could be supported by relatively small decreases in the rate of sucrose synthesis, which would of course be difficult to measure. In this context, the data do perhaps suggest that in plants grown at 500 μmol photons m^{-2} s^{-1}, which accumulate 50% more sucrose than those grown at 300 μmol photons m^{-2} s^{-1}, there may be a small decrease in the rate of sucrose synthesis and small increases in the amounts of F26BP and triose-P (but no change in F6P). Although not statistically significant, if true this would imply a possible involvement of F26BP in inhibiting cytFBPase activity and rates of sucrose synthesis in wheat plants grown at the higher light intensity. Of course, since estimated concentrations of FBP in the cytosol are high (2–12 mM, and 4–21 mM, at the beginning and end of the photoperiod, respectively), any role for F26BP in inhibiting cytFBPase during the feedback inhibition of sucrose synthesis would require the additional involvement of other inhibitors of the enzyme.

Changes in carbohydrate partitioning were also examined in response to alterations in either CO_2 concentration or light intensity. Reducing the Ci from 1,030 to 120 μl l^{-1} (with saturating light) has a dramatic effect on the amount of F26BP (Fig. 3), as observed previously for spinach (Stitt et al. 1984), and primary barley leaves (Sicher and Bunce 1987). However, the ratios of sucrose:starch synthesis (Fig. 3d) and G6P:F6P (Fig. 4g) are essentially constant under these conditions. This contrasts with soybean (Sharkey et al. 1985) and *A. thaliana* (Sun et al. 1999) where starch synthesis is favoured at high CO_2. When light intensity is altered (with ambient CO_2) there is only a small effect on F26BP (Fig. 6). This is in contrast to spinach (Stitt et al. 1984; Neuhaus et al. 1990) and primary barley leaves (Sicher and Bunce 1987) where large increases at low light were observed. However, despite the relatively constant amount of F26BP, increasing light intensity in wheat favours the partitioning of fixed carbon into starch (Fig. 4d) and reduces the ratio of G6P:F6P (Fig. 5g). A shift in partitioning in favour of starch synthesis at high light was also observed in spinach (Neuhaus et al. 1990), tomato (Galtier et al. 1995), soybean (Sharkey et al. 1985), and maize (Lunn and Hatch 1997).

The relationships between amounts of F26BP and the partitioning between sucrose and starch are summarised in Fig. 8b. This clearly shows that there was no correlation between carbohydrate partitioning and the amounts of this inhibitor of the cytFBPase when either CO_2 (filled symbols) or light intensity (open symbols) was altered. As discussed above there was also no correlation between carbohydrate partitioning and the amount of F26BP at the beginning and the end of the day. The conclusions from these studies in wheat, made under a wide range of physiologically relevant conditions, are that (i) changes in F26BP do not lead to changes in carbohydrate partitioning, and (ii) carbohydrate partitioning is altered in the absence of any change in F26BP.

Two differences between wheat and spinach, perhaps related to each other, are relevant when attempting to explain the contrasting roles of F26BP in these two plants. First, plants such as spinach have a large capacity for starch synthesis and can use this pathway to prevent phosphorylated intermediates accumulating and inhibiting photosynthesis when sucrose synthesis is inhibited. However, the capacity for starch synthesis is much lower in sucrose-storing plants such as wheat, and these species may therefore have to employ other strategies to prevent inhibition of photosynthesis (e.g. fructan synthesis, storage of photosynthate in other tissues such as internodes). Second, the evidence for F26BP in regulating carbohydrate partitioning in spinach is restricted to conditions where sucrose synthesis is inhibited by feedback inhibition. However, there is very little, if indeed any, feedback inhibition of sucrose synthesis in wheat under the conditions tested. In support of this, preliminary experiments using wheat leaves detached from the plant and kept in continuous light for 48 h, found no changes in the rates of accumulation of sucrose and starch or the amounts of F26BP (results not shown). Detailed analysis of a wide range of plants would help in examining these possibilities. Interestingly, a lack of communication between sucrose and starch metabolism is also observed in detached leaves of *Lolium temulentum*, where the addition of cycloheximide (to inhibit fructan synthesis) or mannose (to sequester Pi) lead to large changes in sucrose content but have no effect on starch accumulation (Cairns et al. 2002).

Regulation of starch synthesis in wheat

Starch synthesis in wheat is stimulated when rates of photosynthesis are raised above about 4 μmol min^{-1} mg^{-1} Chl by increasing either CO_2 or light intensity (Fig. 8c). Changes in the rates of synthesis of starch are not accompanied by measurable changes in the incorporation of label into either acidic or basic components (Table 1, Figs. 1, 4) and consequently the source of the extra carbon required for enhanced starch synthesis is as yet unknown. However, since starch synthesis represents a minor fate for assimilated carbon in wheat, reciprocal changes in the synthesis of other compounds would be difficult to measure and could be missed.

There is no correlation between the amount of F26BP and the rate of starch synthesis, and measurements of metabolites give no indication as to how this increase is bought about – for example there is no increase in PGA or apparent fall in Pi. However, the synthesis of starch must be considered as far more than a way of coping with an excess of photosynthate (Geiger et al. 2000), and there are undoubtedly mechanisms other than just the ratio of PGA:Pi (Preiss 1980) that regulate starch synthesis, for example the role of phosphoglucomutase (Neuhaus and Stitt 1990; Hattenbach and Heineke 1999). Further studies are required to examine the regulation of starch synthesis in wheat leaves.

Concluding comments

In summary, PGA and F6P are involved in regulating the concentration of F26BP in intact wheat leaves. Rates of photosynthesis and sucrose synthesis are co-ordinated by changes in the amounts of F26BP, triose-P and probably other inhibitors, and the relative importance of these varies with the conditions. However, there is no evidence for a role for F26BP in regulating carbohydrate partitioning under the wide range of physiologically relevant experimental conditions used. This may reflect a fundamental difference in how partitioning is regulated in plants with different patterns of carbohydrate storage, and/or it may be because wheat does not appear to regulate sucrose synthesis by feedback inhibition. These conclusions demonstrate that the role for F26BP elucidated from studies in spinach is not universal. Kruger and Scott (1995) suggest that the real significance of F26BP in photosynthetic cells may lie primarily in the co-ordination of chloroplastic and cytosolic metabolism, and it may be that regulation of carbohydrate partitioning in starch-storing plants is but one example of this.

A full understanding of how carbohydrate synthesis is regulated in wheat leaves requires further study. Particularly interesting is the observation that concentrations of triose-P in the cytosol are apparently much higher than in spinach, and do not alter when the rate of photosynthesis is increased due to a rise in CO_2. Since the kinetic properties of wheat cytFBPase (Stitt et al. 1982) were measured with a partially purified enzyme it is important to confirm these with a purified enzyme preparation. Similarly, it will be important to examine the properties of the purified wheat PFKII and F26BPase in detail. Other areas for future work are the role of SPS, and the subcellular and intracellular compartmentation of metabolites. Finally, a quantitative approach using transgenic plants will be required to fully assess the significance of F26BP in wheat.

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