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Regulation of partitioning between sucrose and starch in wheat leaves

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Introduction

Triose phosphates produced by the reactions of photosynthesis are used for the biosynthesis of storage compounds such as sucrose and starch, and also in essential respiration associated with the provision of carbon skeletons for N-assimilation. Tight co-ordination of fluxes through these pathways are essential for maximising rates of end product synthesis and for maintaining optimal rates of photosynthesis. In starch storing plants, two reactions are thought to be crucial in co-ordinating the rate of cytosolic sucrose synthesis and the rate of photosynthesis. These are catalysed by the enzymes SPS and the cytosolic FBPase. The former synthesises sucrose phosphate and is regulated both by multiple site phosphorylation and by allosteric effectors (Huber and Huber, 1996). FBPase catalyses the conversion of FBP to F6P, and is regulated by the concentrations of substrate, product (Pi), the competitive inhibitor F26BP, and AMP (Stitt et al., 1987). The amount of F26BP is dependent on two enzymes that synthesise (PFKII) and degrade (F26BPase) the metabolite, and the activities of these are regulated by the concentrations of intermediates in the pathway of sucrose synthesis (F6P, triose phosphates, PGA, and Pi).

Detailed studies in spinach have demonstrated that F26BP is involved in co-ordinating the rate of sucrose synthesis with the rate of photosynthesis, particularly at low light (Neuhaus et al., 1990). Direct evidence for this role for F26BP, particularly during the early part of the day, has come from the analysis of genetically engineered tobacco (Scott *et al.*, 1995; 2000) and *Kalanchoe daigremontiana* (Truesdale et al., 1999). F26BP has also been shown to be significant in regulating the partitioning of carbon between sucrose and starch in the leaves of spinach (Neuhaus et al., 1990), *Clarkia xantiana* (Neuhaus et al., 1989), tobacco (Scott *et al.*, 1995; 2000) and *K.daigremontiana* (Truesdale et al., 1999). Unfortunately these detailed studies have all been performed on species that predominantly use starch for the transitory storage of carbon in their leaves. In contrast, the evidence from plants that store predominantly sucrose in their leaves, such as cereals, is very limited. In a study of a range of crop species, including barley, Sicher et al. (1986; 1987) found no correlations between the amount of F26BP and the relative rates of accumulation of sucrose and starch. In a previous study in wheat (Trevanion 2000), although the amounts of F26BP were lower during the day than the night, neither the amount of the metabolite, nor the ratio of sucrose to starch accumulated in the leaf varied during the day. These observations suggest that a role for F26BP in regulating carbohydrate partitioning in plants may not be universal. The work presented here further examines the role of F26BP in wheat by identifying correlations, or the lack thereof, between the amounts of F26BP and the rates of synthesis of sucrose and starch measured by incorporation of label from $^{14}CO_2$. Experiments were carried out under three sets of conditions (i) altering the time of day (ii) altering [CO_2], (iii) altering light intensity. The data suggest that F26BP is not involved in regulating the partitioning of fixed carbon between

sucrose and starch in wheat leaves, although it may co-ordinate rates of sucrose synthesis and rates of photosynthesis under certain conditions.

Materials and methods

All experiments used the third fully expanded leaves of the spring wheat cultivar Bob White grown in a controlled environment cabinet (16h day). Photosynthesis rates of leaves of intact plants were measured using an IRGA (ADC Mark 3, ADC, Hoddesdon, UK) attached to a six chamber open-circuit gas-exchange with automatic data handling. For the radiolabelling experiments, $^{14}CO_2$ was added to the gas stream and fed for five minutes to leaves with steady state rates of photosynthesis, under the exact conditions of $[CO_2]$ and light intensity at which photosynthesis had been measured. Samples were freeze clamped ground to a fine powder in $N_{2(l)}$ and divided into two. One of these was extracted and assayed for F26BP as described by Trevanion (2000). The other was extracted in 80% (v/v) ethanol, heated at 70°C, and centrifuged to separate the soluble and insoluble fractions. The soluble components were further separated into neutral, acidic and basic fractions by ion exchange chromatography using 1 mL Dowex-50 (H^+) and Dowex-1 (Cl^-) columns. The insoluble pellet was thoroughly washed with 50mM Na acetate (pH 4.8) and digested with α-amylase and amyloglucosidase (2d at 37°C) to release the label in starch. Radioactivity was measured by liquid scintillation counting. Chlorophyll was measured in 80 % (v/v) acetone as described by Porra et al. (1989).

Results

Measurement of rates of sucrose and starch synthesis

Calculating rates of sucrose and starch synthesis from measurements of the accumulation of ^{14}C in these compounds is accurate only when the latter are linear with time, i.e. when the pools of intermediates between CO_2 and sucrose/starch have become saturated with ^{14}C. Time courses of the incorporation into sucrose and starch under a range of light intensities and $[CO_2]$ (results not shown) demonstrated that there were lags in achieving a steady rate of label accumulating in these fractions, and these were therefore accounted for when calculating the rates of synthesis described below. Recovery of ^{14}C in sucrose, starch, acidic and basic fractions was 85 ± 11 %.

Diurnal changes in F26BP, sucrose and starch synthesis

Plants were grown at three different light intensities (150, 300 or 500 μmol m^{-2} s^{-1}) until the third leaf was fully expanded, and were sampled either one hour after the beginning or one hour before the end of the photoperiod. The amounts of F26BP and rates of sucrose and starch synthesis are shown in Table 1. Although there were about 3.5-fold increases in sucrose content in the leaves over the course of the day (results not shown), there was no suppression of photosynthesis and no feedback inhibition of sucrose synthesis; if anything rates of the latter were higher at the end than at the beginning of the day. In all three sets of plants rates of starch synthesis were greater at the end of the day, and consequently there were significant decreases in the ratio of sucrose:starch synthesis. However these increases in the partitioning of CO_2 into starch were not accompanied by increases in the amounts of F26BP.

Table 1. Diurnal variation in rates of sucrose and starch synthesis and amounts of F26BP. Results are mean ± s.e.m. for three samples.

growth light time of		photosynthesis	synthesis			F26BP
($\mu mol.m^{-2}.s^{-1}$)	day	($\mu mol.min^{-1}.mg^{-1}chl$)	($dpm \times 10^{-3}.mg^{-1}chl.min^{-1}$)			($pmol.mg^{-1}chl$)
			sucrose	starch	sucrose : starch	
150	Beginning	10.1 ± 0.5	795 ± 113	50.3 ± 12.1	**16.5 ± 1.5**	**105 ± 21**
	End	10.8 ± 2.5	979 ± 50	144 ± 10.8	**6.8 ± 0.3**	**115 ± 11**
300	Beginning	18.7 ± 2.0	1190 ± 115	97 ± 11	**12.3 ± 0.8**	**153 ± 44**
	End	19.8 ± 1.1	2040 ± 152	264 ± 26	**7.7 ± 1.0**	**88 ± 15**
500	Beginning	29.9 ± 1.6	1960 ± 324	105 ± 21	**19 ± 0.9**	**204 ± 19**
	End	25.1 ± 3.3	1590 ± 197	252 ± 29	**6.3 ± 0.1**	**240 ± 10**

Effect of altering [CO_2] on F26BP, sucrose and starch synthesis.
Plants were grown at 150 $\mu mol.m^{-2}.s^{-1}$, third fully expanded leaves of intact plants were placed in leaf chambers and then exposed to different [CO_2] under saturating light ($1000\mu mol.m^{-2}.s^{-1}$). $^{14}CO_2$ was fed to the leaves (during the last 3 h of the photoperiod) and rates of synthesis of sucrose and starch measured (Fig. 1). Altering the Ci had a dramatic effect on the amounts of F26BP, with a 26-fold decrease in amounts between the lowest and the highest Ci values (Fig. 1C). Although rates of synthesis of sucrose (Fig. 1D) and starch (Fig. 1E) generally correlated well with rates of photosynthesis (Fig. 1A), at high Ci there was a large decrease in the rate of starch synthesis. However over the wide range of CO_2 concentrations used there was no consistent change in the ratio of sucrose : starch synthesis (Fig. 1F).

Effect of Altering Light Intensity
Plants were grown at 150 $\mu mol.m^{-2}.s^{-1}$, third fully expanded leaves of intact plants placed in leaf chambers and exposed to different light intensities with ambient [CO_2] ($340\mu L.L^{-1}$). $^{14}CO_2$ was fed to the leaves (during the last 3 h of the photoperiod) and rates of synthesis of sucrose and starch measured (Fig. 2). There was an increase in F26BP when light intensity was decreased below the growth conditions (150 $\mu E.m^{-2}.s^{-1}$) (Fig. 2C), but the amount of the metabolite did not alter at higher light intensities. The rate of sucrose synthesis (Fig. 2D) generally correlated well with the rate of photosynthesis. Although rates of starch synthesis also increased more or less proportionally with light intensity (Fig. 2E), there was a large stimulation of starch synthesis at the very highest light intensity used. Over the wide range of light intensities used, there was a large decrease in ratio of sucrose : starch synthesis with increasing light. (Fig. 2F).

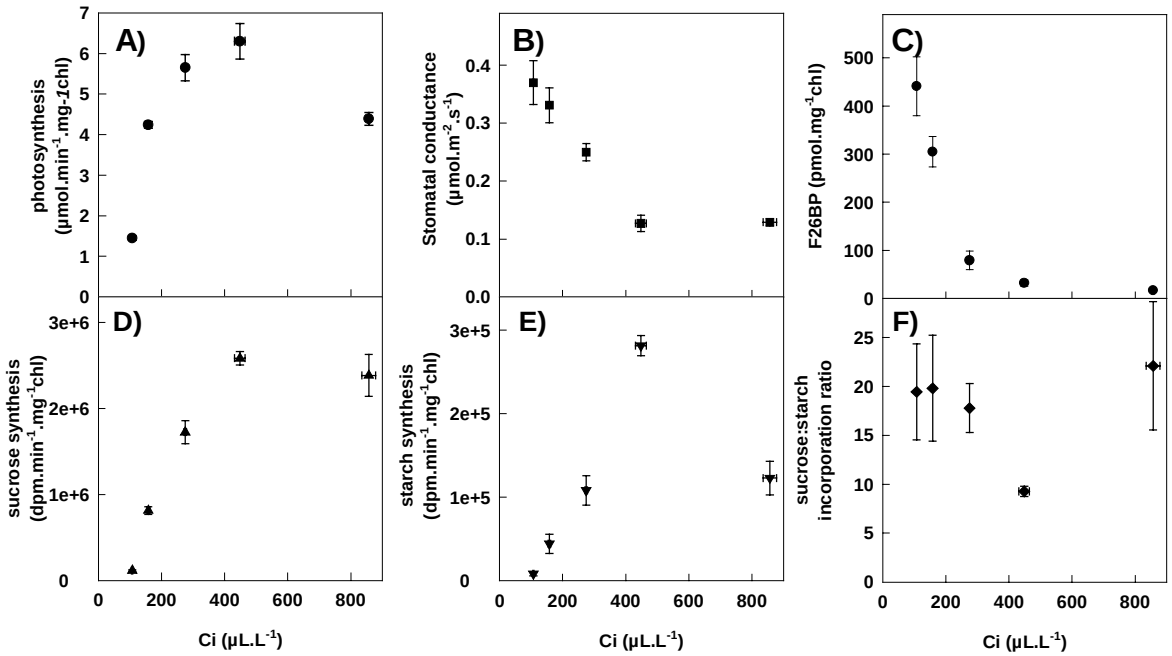

Fig. 1. Response of F26BP, sucrose and starch synthesis to changes in [CO_2]
Effect of altering light intensity on F26BP, sucrose and starch synthesis.

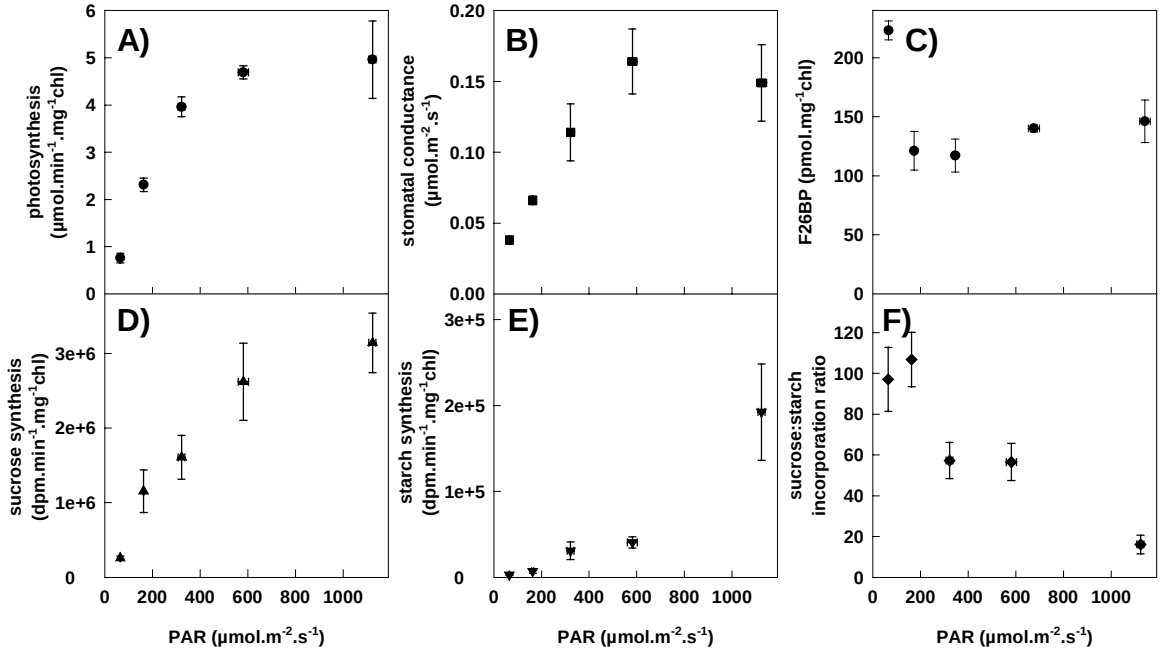

Fig. 2 Response of F26BP, sucrose and starch synthesis to changes in light intensity

Discussion

Regulation of sucrose synthesis by F26BP

The results obtained are consistent with a role for F26BP in co-ordinating rates of sucrose synthesis and photosynthesis in wheat leaves. (i) At low light an increase in the light intensity

causes an increase in the rate of sucrose synthesis and a decrease in F26BP. (ii) A decrease in Ci, such as would be encountered by as plant under water stress, results in a fall in the rate of sucrose synthesis and a large increase in the amount of F26BP. These conclusions are entirely consistent with the proposed role for F26BP in co-ordinating photosynthesis and sucrose synthesis in spinach leaves (Neuhaus et al., 1990)

Regulation of carbohydrate partitioning by F26BP

In contrast, the results do not identify any correlations between F26BP and carbohydrate partitioning, and suggest that under the three different experimental conditions studied, F26BP does not regulate partitioning between sucrose and starch in wheat leaves. (i) Partitioning into starch is greater at the end than the beginning of the photoperiod, but F26BP does not change. (ii) Reducing the [CO_2] causes a 26-fold increase in the amount of F26BP, but this has no effect on the partitioning between sucrose and starch. (iii) Although altering the light intensity does cause changes in the amount F26BP and the partitioning between sucrose and starch, these are not correlated with each other.

This conclusion contrasts with that in spinach (Neuhaus et al., 1990), but the full significance of this observation cannot be judged until measurements of the metabolites that, at least in spinach, also have a role in the complex regulatory network (triose-P, PGA and F6P) have been completed. Nevertheless it is still interesting to speculate as to why wheat and spinach appear to differ in this respect. First, the evidence for F26BP regulating carbohydrate partitioning in spinach is from experiments where starch synthesis is stimulated in response to the inhibition of sucrose synthesis. However as there is apparently no feedback inhibition of sucrose synthesis in wheat, then even though starch synthesis can stimulated, for example at the end of the day, we might well expect the regulatory mechanisms to differ from those seen in spinach. Second, the difference between spinach and wheat may represent a more general one, in which F26BP regulates partitioning in plants that store predominantly starch but not in those that store sucrose. Unfortunately, due to the absence of data from a larger number of different species this issue cannot yet be addressed.

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