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Control of Photosynthetic Sucrose Synthesis by Fructose 2,6-Bisphosphate[1]

II. PARTITIONING BETWEEN SUCROSE AND STARCH

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ABSTRACT

The role of fructose 2,6 bisphosphate in partitioning of photosynthate between sucrose and starch has been studied in spinach (*Spinacia oleracea* U.S. hybrid 424). Spinach leaf material was pretreated to alter the sucrose content, so that the rate of starch synthesis could be varied. The level of fructose 2,6-bisphosphate and other metabolites was then related to the accumulation of sucrose and the rate of starch synthesis. The results show that fructose 2,6-bisphosphate is involved in a sequence of events which provide a fine control of sucrose synthesis so that more photosynthate is diverted into starch in conditions when sucrose has accumulated to high levels in the leaf tissue. (a) As sucrose levels in the leaf rise, there is an accumulation of triose phosphates and hexose phosphates, implying an inhibition of sucrose phosphate synthase and cytosolic fructose 1,6-bisphosphatase. (b) In these conditions, fructose 2,6-bisphosphate increases. (c) The increased fructose 2,6-bisphosphate can be accounted for by the increased fructose 6-phosphate in the leaf. (d) Fructose 2,6-bisphosphate inhibits the cytosolic fructose 1,6-bisphosphatase so more photosynthate is retained in the chloroplast, and converted to starch.

During photosynthesis, sucrose is exported from the leaf to the rest of the plant, but a portion of the photosynthate can be temporarily accumulated in the leaf. Some is stored as sucrose (7, 10, 20) in the vacuole (8, 15), but usually most is converted to starch in the chloroplast. Starch and sucrose are later remobilized for export or respiration. The extent to which starch is accumulated is variable, depending on the species and variety (12, 14), age (3), the conditions in which a plant has previously been growing (2), as well as the conditions in which it is photosynthesizing. Moreover, even within a single day in constant conditions, the rate of starch accumulation varies, being slow initially and increasing after several hours illumination (7, 17, 20). These observations point to a control of starch synthesis in the long term, possibly by alteration of enzyme capacities (3, 16), but also to the need for a fine control of starch synthesis.

The first irreversible reaction in the conversion of triose P to sucrose in the cytosol is catalyzed by the cytosolic FBPase,[2] and control of this enzyme will be necessary if an increasing proportion of the photosynthate is to be diverted towards starch rather than sucrose. Previously (22), it has been discussed how a restriction of sucrose synthesis might lead to an accumulation of phosphorylated intermediates in the cytosol, so that Pi decreased and triose P rose, restricting export of triose P out of the chloroplast and stimulating synthesis of starch. However, in a preceding article we have shown that the cytosolic FBPase activity actually increases in response to an increased supply of triose P (21). It would appear that additional factors must be involved in controlling the activity of the cytosolic FBPase in conditions when leaves contain high levels of sucrose, high levels of metabolites, and are carrying out rapid starch synthesis.

In earlier investigations (20), we observed that when spinach leaves were illuminated for several hours so that they contained high levels of sucrose, starch synthesis was stimulated and this was accompanied by an increase in the signal metabolite Fru 2,6-P_2. Fru 2,6-P_2 is a potent inhibitor of the cytosolic FBPase and it was suggested that this increase was involved in diverting more of photosynthate towards starch (20). This proposal has now been studied in more detail by treating spinach leaf material in various ways to alter its sucrose content, and then measuring the effect on the content of Fru 2,6-P_2, and other phosphorylated metabolites, and on starch synthesis. The results show that when sucrose accumulates in a tissue, the synthesis of sucrose from hexose P and UDP glucose is restricted. The resulting increase in Fru 6-P leads to the rise in Fru 2,6-P_2, which in turn restricts the activity of the cytosolic FBPase.

MATERIALS AND METHODS

Plant material, growth conditions, extraction and assay of Fru 2,6-P_2, hexose P, UDP glucose, triose P, PGA, Fru 1,6-P_2, starch and sucrose were as in Stitt *et al.* (20). For experiments with whole plants, the samples were taken during an uninterrupted 9-h light/15-h dark cycle. Leaves were cold girdled by a 4-cm-long coil of plastic tubing round the leaf petiole through which a water/salt mixture (−5°C) was pumped. Detached leaves were excised under water and illuminated with the cut petiole in distilled H_2O. For experiments with leaf discs, the plants were kept in the dark for 15 to 18 h and leaves were excised and stored in water-saturated air. Discs (35 mm diameter) were cut out and immediately placed in the O_2 electrode. Experiments with an O_2 electrode were carried out in saturating light (190 w m^{-2}) and saturating CO_2 supplied by a 1 M $NaHCO_3$ buffer (pH 9). The O_2 was removed by blowing air through when the concentration reached 30% and 200 μl additional $NaHCO_3$ buffer was added to ensure that the CO_2 concentration remained high during illumination of leaf discs for 1 to 2 h. To decrease the rate at

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[2] Abbreviations: FBPase, fructose 1,6-bisphosphatase; Fru 6-P, fructose 6-phosphate; Fru 2,6-P_2, fructose 2,6-bisphosphate; Glc 6-P, glucose 6-phosphate; PGA, 3-phosphoglycerate.

which O_2 was accumulated, the amount of leaf material present was limited to 0.07 to 0.1 g fresh weight during long incubations.

RESULTS AND DISCUSSION

Sucrose Content and Fructose 2,6-Bisphosphate. When spinach plants are illuminated for 9 h, there is an increase in the level of Fru 2,6-P_2, which correlates with an accumulation of sucrose in the leaf (Table I). It is plausible to suggest that increased Fru 2,6-P_2 is due, directly or indirectly, to the increase in sucrose (20). However, it cannot be excluded that Fru 2,6-P_2 changes in response to other unrelated events occurring during several hours in the light. We have therefore investigated the response of Fru 2,6-P_2 to several other treatments which alter the amount of sugar in the leaf.

In one approach, the accumulation of sucrose in leaf tissue was speeded up by illuminating leaf discs in an O_2 electrode in saturating CO_2 and a water-saturated atmosphere. No export of sucrose is possible, so within 1 h the leaf disc contains more sucrose than is found after 9 h illumination of leaves on a whole plant. Within this time, the Fru 2,6-P_2 increases 2- to 3-fold (Table I). Leaf discs were sampled after 15, 30, and 60 min, and the relation between Fru 2,6-P_2 and sucrose after these short times of illumination compared with those after 0.25, 1, 4, and 9 h illumination of whole plants (Fig. 1A). The level of Fru 2,6-P_2 depended on the sucrose content, and not the length of illumination as such.

In another series of experiments, plants were illuminated for 2 h, and the sucrose content of the leaf tissue varied by changing the rate at which sucrose was exported from the leaf (Table II). Shading the remainder of the plant to favor export of sucrose from the illuminated leaf allowed the accumulation of sucrose in the leaf to be restricted. Alternatively, by detaching a leaf, or by cooling the stem, an increased accumulation of sucrose was obtained although some export continued. Cooling did not totally inhibit phloem transport in the thick stem of spinach, and the detached leaves were placed with their stems under water to prevent loss of turgor and some export continued into the water (data not shown). In a leaf disc held in a moist air, however, all export was prevented. As shown in Table II, the Fru 2,6-P_2 increases as the sucrose content of the leaves rises, even though in these experiments the illumination time was constant. When the results of these experiments are compared with those where sucrose content in leaves was varied by altering the length of illumination it can be seen that a similar dependence is obtained between sucrose content and Fru 2,6-P_2 (Fig. 1A) levels in both cases.

In a third approach, leaf discs were preincubated with 100 mM exogenous sugar for 8 h before being illuminated for 30 min. Compared with sorbitol, the Fru 2,6-P_2 (Table III) level was higher after preincubation with sucrose or glucose. Taken together, the results from Tables I to III and Figure 1A show that the Fru 2,6-P_2 content of leaf material increases in the light when there are ample supplies of soluble carbohydrate in the leaf. It seems that in this respect, leaves are analogous to mammalian liver, where Fru 2,6-P_2 increases in response to an increased supply of glucose (11).

Fructose 2,6-Bisphosphate and Starch Synthesis. Treatments which produce an increased sucrose content in leaves also lead to increased rates of starch synthesis (Tables I–III), so that an increase in Fru 2,6-P_2 is associated with an increase in the rate of starch synthesis. This is summarized in Figure 1B for the experiments of Tables I to III. Irrespective of the treatment used to increase sucrose content, an increase in sucrose content leads to more rapid accumulation of starch, and this is associated with higher levels of Fru 2,6-P_2. It might be noted that starch synthesis is more rapid in leaf discs than in leaves for a given Fru 2,6-P_2 level, but this can be attributed to the higher rates of photosynthesis for leaf discs in saturating CO_2 and water-saturated air. In leaves, there is some variability between individual experiments, probably due to differences in plant material.

The experiment of Figure 2 investigates the relation between Fru 2,6-P_2 and starch synthesis in more detail. Leaf discs were illuminated for up to 2 h in a leaf O_2 electrode and their rate of photosynthesis was measured continuously. At intervals, samples were taken and the content of sucrose and starch measured, as well as Fru 2,6-P_2 and other metabolites. After an initial decrease in the Fru 2,6-P_2 level (Fig. 2A) which is probably due to the 20- to 30-fold increase in DHAP as discussed elsewhere (21), there is a 6-fold increase in the Fru 2,6-P_2 over the next 2 h, eventually reaching values even higher than those in the dark. This pattern resembles that in spinach levels on the plant (20) except that in this simpler model system with leaf discs even larger alterations could be found than those in whole plants. No sucrose export could occur out of the leaf discs so sucrose and starch synthesis could both be estimated from their accumulation in the leaf (Fig. 2B). During 2 h there was a 30% decrease in the rate of sucrose synthesis, and a 4-fold rise in the rate of starch synthesis. The increased starch synthesis did not fully compensate for the lower sucrose synthesis, and the rate of photosynthesis decreased by about 20%. This meant that the quotient of sucrose synthesis: starch synthesis decreased markedly as the Fru 2,6-P_2 content increased (Table IV).

In three such experiments, a similar relation was found between Fru 2,6-P_2 content and the relative rates of starch and sucrose synthesis (Fig. 3). The partitioning of photosynthate between sucrose and starch responds quite sensitively to alterations in the Fru 2,6-P_2 level. For example, doubling the Fru 2,6-P_2 from 0.1 to 0.2 nmol/mg Chl increases the percentage of

Table I. *Fructose 2,6-Bisphosphate, Metabolites, Sucrose Content, and Starch Synthesis in Intact Spinach Plants and Leaf Discs after Varying Times of Illumination*

The results are the mean ± SE of three separate experiments each having four replicate samples (whole plant) or three replicates (leaf discs). Starch synthesis was determined as the accumulation of starch in the time interval 0.25 to 1 and 5 to 8 h (whole plants), or 0.15 to 0.25 and 0.5 to 1 h (leaf discs). The plant material had previously been in the dark for 15 h (whole plant) and 18 to 20 h (leaf discs).

Material	Time in Light	Fru 2,6-P_2	Sucrose Content	Starch Synthesis	Metabolites					
					UDPGlc	Glc 6-P	Fru 6-P	DHAP	PGA	Fru 1,6-P_2
		pmol mg^{-1} Chl	*μmol hexose mg^{-1} Chl*	*μmol hexose mg^{-1} Chl h^{-1}*	*nmol mg^{-1} Chl*					
Plants	1	110 ± 18	4.9 ± 0.9	1.1 ± 0.4	24 ± 1	94 ± 6	41 ± 3	43 ± 3	181 ± 15	22 ± 2
	8	201 ± 23	11.3 ± 2.0	4.5 ± 0.7	40 ± 3	139 ± 8	53 ± 4	53 ± 3	222 ± 17	26 ± 2
Discs	0.25	99 ± 13	3.9 ± 0.4	2.7 ± 0.5	21 ± 3	123 ± 8	44 ± 2	27 ± 2	140 ± 14	11 ± 1
	1	250 ± 23	19.5 ± 1.8	6.8 ± 0.5	23 ± 3	156 ± 9	62 ± 2	40 ± 1	133 ± 8	9 ± 1

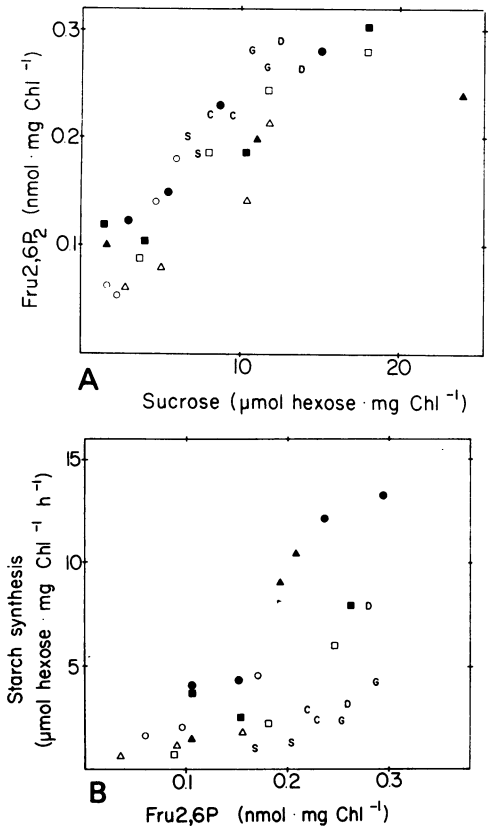

FIG. 1. Relation between sucrose, starch, and fructose 2,6-bisphosphate. The results are collected from three experiments (●, ▲, ■) with leaf discs illuminated 10, 15, 30, and 60 min; three experiments with whole plants (○, △, □) illuminated 0.25, 1, 4, and 9 h; and two experiments with 2 h illumination of detached leaves (D), cold-girdled leaves (G), a leaf on a plant where all other leaves were shaded (S), or untreated controls (C). Each point is the mean of four replicate samples. A, Sucrose and fructose 2,6-bisphosphate; B, starch and fructose 2,6-bisphosphate.

carbohydrate end product being accumulated as starch from 13 to 30%.

Changes in Metabolite Levels During Starch Synthesis. Obviously, by inhibiting the cytosolic FBPase, an increase in Fru 2,6-P_2 could be involved in diverting photosynthate towards starch synthesis in the stroma. However, it has also been suggested that accumulation of sucrose lowers its rate of synthesis, so that phosphorylated intermediates accumulate, and Pi declines (22). This would decrease export of triose P out of the chloroplast, and favor starch synthesis (22). In order to assess the interaction between regulation by Fru 2,6-P_2, and regulation by alterations in phosphorylated intermediates and Pi, we also investigated the effect of sucrose accumulation on the levels of selected metabolites in spinach leaves.

As sucrose accumulates in leaf discs and starch synthesis becomes more rapid, there is a gradual increase in the total content of UDP glucose, hexose P and DHAP, but no large changes are found for PGA or Fru 1,6-P_2 (Fig. 2, C and D). These results are typical for spinach leaf tissue where sucrose is accumulating to high levels, whether this is due to prolonged illumination of whole plants (Table I), or leaf discs (Table I), or interruption of the export of sucrose (Table II). Similar increases are also found when sugars are added exogenously (Table III). In earlier experiments (18, 20), it has also been observed that spinach leaves contain higher levels of metabolites at the end of the day than they do after only a short period of light.

The increment is most marked for glucose 6-P and fructose 6-P, varying from 27 to 67%, and is consistent but smaller for triose P (15–50%) and UDP-glucose (10–60%). Previous studies of intracellular compartmentation in leaves suggest that these four metabolites are predominantly located in the cytosol (9, 18, 19). Unless the increments occur exclusively in the chloroplast, these results provide evidence for a marked increase of the cytosolic metabolite pools in conditions when sucrose has accumulated and starch synthesis is stimulated. The level of PGA and Fru 1,6-P_2 did not show any consistent alteration in these experiments, sometimes increasing by up to 35% but sometimes decreasing by up to 10%. It might be noted that the PGA content varied far more between replicate samples than did other metabolites. Fractionation studies (9, 18, 19) show that these two metabolites are mainly located in the stroma in the light.

Regulation of Cytoplasmic FBPase and Sucrose P Synthase during Starch Accumulation. The general rise in intermediates, especially those located primarily in the cytosol, provides evidence that accumulation of sucrose in leaves indeed leads to a restriction of its further synthesis and an accumulation of phosphorylated intermediates in the cell. A decrease in Pi is also likely to accompany the accumulation of phosphorylated intermediates, as has been discussed previously (19). These results suggest that the enzymes involved in sucrose synthesis are indeed being regulated in response to accumulation of sucrose in the leaf, as there is an increase in the levels of substrates for the enzymes catalyzing the conversion of triose P to sucrose in conditions when a larger proportion of the triose P is actually being diverted away from sucrose (Tables I–III) or the absolute rate of sucrose synthesis is decreasing (Fig. 2).

A restriction of the activity of the cytosolic FBPase is indicated

Table II. *Fructose 2,6-Bisphosphate, Metabolites, Sucrose Content, and Starch Synthesis in Spinach Leaf Tissue with Varying Export of Sucrose*

The results are the mean ± SE of 8 to 16 replicates. The treatments were to shade all the other leaves on the plant (shaded), to cool the petiole of a leaf (girdled), to detach a leaf and hold its petiole under water (detached), or to remove a leaf disc and place it in H_2O-saturated air (leaf disc). All were illuminated 2 h. Starch synthesis was estimated as accumulation of starch between 0.25 and 2 h.

Treatment	Fru 2,6-P_2	Sucrose	Starch Synthesis	Metabolites				
				UDPGlc	Glc 6-P	Fru 6-P	DHAP	Fru 1,6-P_2
	pmol mg⁻¹ Chl	*μmol hexose mg⁻¹ Chl*	*μmol hexose mg⁻¹ Chl h⁻¹*	*nmol mg⁻¹ Chl*				
Shaded	170 ± 18	6.5 ± 0.9	1.1 ± 0.3	44 ± 6	103 ± 9	36 ± 2	42 ± 6	21 ± 4
None	230 ± 19	8.5 ± 0.4	2.7 ± 0.3	43 ± 3	122 ± 7	47 ± 4	53 ± 3	32 ± 2
Girdle	270 ± 21	11.4 ± 0.5	3.1 ± 0.5	46 ± 3	140 ± 5	54 ± 1	55 ± 2	36 ± 2
Detached	270 ± 29	13.5 ± 0.9	5.5 ± 1.8	42 ± 7	135 ± 6	49 ± 3	51 ± 5	29 ± 2
Discs	610 ± 22	31.0 ± 4.0	8.5 ± 1.1	50 ± 9	175 ± 9	80 ± 6	50 ± 4	14 ± 1

Table III. *Fructose 2,6-Bisphosphate and Metabolites in Spinach Leaf Discs Preincubated on Sugars*

Leaf discs were removed from spinach plants after 15 h dark, incubated on 100 mM sugar + 5 mM Mes (pH 5.5), for 4 h and then illuminated in water-saturated air for 30 min. Results are presented as mean ± SE of eight replicates, each containing four leaf discs (diameter 1 cm).

Preincubated on 100 mM Sugar	Fru 2,6-P_2	Amount					
		UDPGlc	Glc 6-P	Fru 6-P	DHAP	PGA	Fru 1,6-P_2
	pmol mg^{-1} Chl	*nmol mg^{-1} Chl*					
Sorbitol	190 ± 11	33 ± 2	104 ± 7	33 ± 2	35 ± 5	142 ± 5	35 ± 4
Glucose	380 ± 31	42 ± 1	171 ± 4	51 ± 1	41 ± 4	146 ± 25	41 ± 6

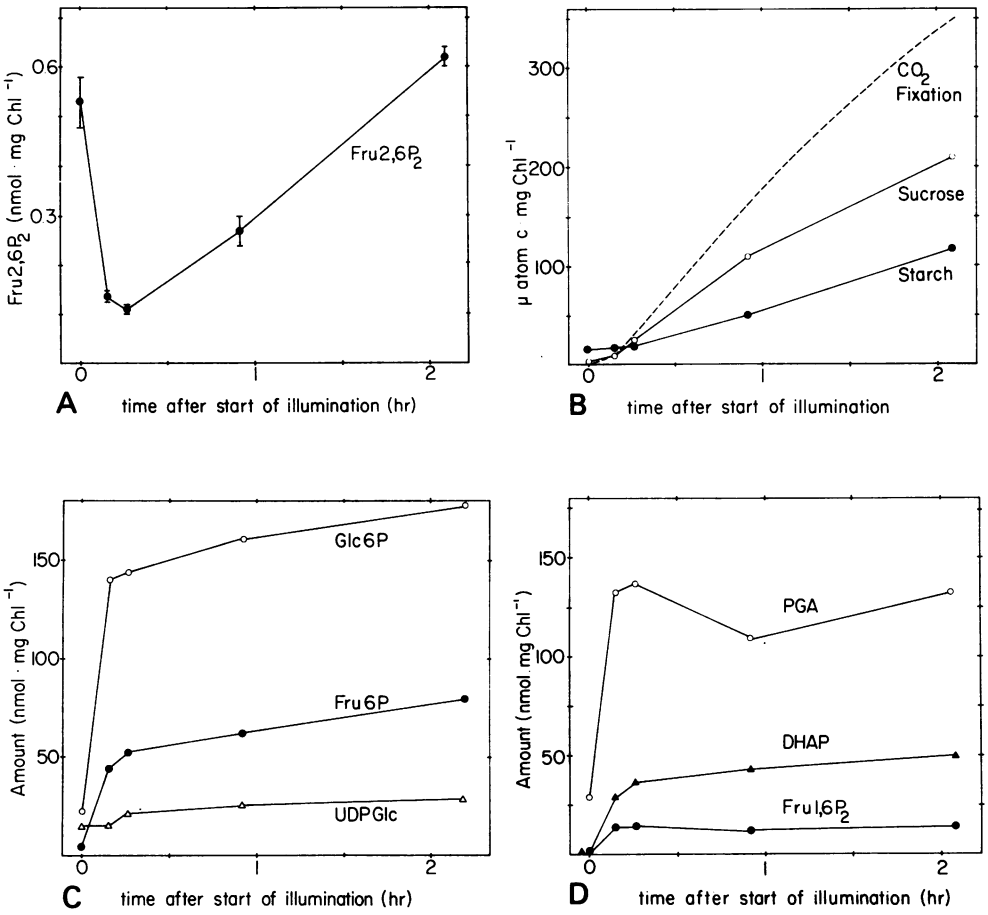

FIG. 2. Fructose 2,6-bisphosphate, sucrose, and starch synthesis, and metabolites in illuminated spinach leaf discs. A, Fructose 2,6-bisphosphate; B, O_2 evolution, sucrose, and starch; C, hexose P and UDP glucose; D, fructose 1,6-bisphosphate, 3-phosphoglycerate, and dihydroxyacetone phosphate.

by the small rise in triose P levels (Tables I to III; Fig. 2). This shows that the increased Fru 2,6-P_2 concentration indeed plays a role in controlling the rate of sucrose synthesis in these conditions. However, cytosolic FBPase is not the only rate-limiting enzyme, and our experiments also provide evidence for regulation of sucrose P synthase. As the sucrose content increases, there is an increase of hexose P and UDP glucose levels (Tables I to III), even although the rate of sucrose synthesis may decline (Fig. 3C).

These experiments do not show how sucrose P synthase is being controlled. Spinach leaf sucrose P synthase is inhibited by sucrose 6-P (1) and sucrose inhibits sucrose P synthase in leaves from some species (13), but it is not known if the concentration of sucrose or sucrose 6-P in the cytosol are ever high enough for these effects to be of importance *in vivo*.

Extractable activity of sucrose P synthase also declines in leaves when sucrose accumulates and starch synthesis increases (3, 16), but the reason for these fluctuations is not understood. It might be noted that Fru 2,6-P_2 does not appear to inhibit sucrose P synthase (Huber, personal communication). Another possibility is that the rising levels of sucrose may lead to enhanced activities of enzymes which degrade sucrose, such as sucrose synthase, or invertase.

Regulation of Fru 2,6-P_2 Concentration by Fru 6-P in Response to Accumulation of Sucrose. The question arises, whether the increase in Fru 2,6-P_2 during starch accumulation is a direct response to the rising levels of sucrose in the leaf, or if the cause is more indirect, for example as a response to the accumulation of metabolites following inhibition of sucrose P synthase. Previous studies have shown that Fru 2,6-P_2 is synthesized by Fru

Table IV. *Fructose 2,6-Bisphosphate and Partitioning between Starch and Sucrose*

Synthesis of sucrose and starch synthesis was estimated from Figure 2 as the increment between 9 and 16, 16 and 55, and 55 and 125 min.

Time as Light	Fru 2,6-P_2	Synthesis Rate		Starch Synthesis / Sucrose Synthesis
		Sucrose	Starch	
min	*pmol mg^{-1} Chl*	*μmol hexose mg^{-1} Chl·h^{-1}*		
9	135	20.9	3.7	5.6
16	107	21.6	7.8	2.8
55	262	14.2	8.5	1.6
125	611			

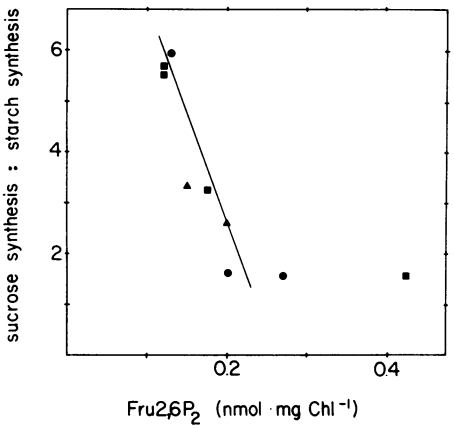

FIG. 3. Relation between fructose 2,6-bisphosphate and the partitioning of photosynthate into sucrose and starch in spinach leaf discs. The results are calculated from Figure 2 and two other similar experiments. The synthesis of starch and sucrose in a time interval is related to the mean of the fructose 2,6-bisphosphate present at the start and end of the time interval.

6-P, 2-kinase (4) and degraded by a specific Fru 2,6-bisphosphatase (5) in spinach leaves. More detailed studies, which will be presented elsewhere (Stitt, Cseke, and Buchanan, in press), show that the activity of these enzymes is not affected by sucrose at concentrations up to 100 mM but depends strongly on the Fru 6-P concentration. In model experiments with the partially purified Fru 6-P, 2-kinase and Fru 2,6-bisphosphatase, a 50% alteration in the Fru 6-P could lead to a 4-fold increase in Fru 2,6-P_2. Fru 6 P, 2-kinase is activated by Fru 6-P, having a sigmoidal dependence on the Fru 6-P concentration in the absence of Pi (4) or even in the presence of Pi when PGA or DHAP are also present. Fru 6-P also inhibits Fru 2,6-bisphosphatase (5). By having opposite effects on the synthesis and degradation of Fru 2,6-P_2, small changes of Fru 6-P can produce large alterations in the concentration of Fru 2,6-P_2.

It appears that the increased levels of hexose P which accompany accumulation of sucrose could account for the rise of Fru 2,6-P_2 in these conditions. Figure 4 summarizes the relation between sucrose content, Glc 6-P and Fru 2,6-P_2 for the experiments of Tables I to III and Figure 3. For this purpose, Glc 6-P is chosen as representative of the cytosolic hexose P rather than Fru 6-P because nonaqueous fractionation of spinach leaves suggests that about 80% of the total Glc 6-P is present in the cytosol, while the Fru 6-P is less assymmetrically distributed so that up to half may be in the stroma (Gerhardt, unpublished). The Fru 6-P in cytosol is about 25% to 30% of the Glc 6-P, as expected if the reaction catalyzed by phosphoglucose isomerase is close to equilibrium. As shown in Figure 4A, Glc 6-P increases as sucrose accumulates in a leaf in the light. When sucrose doubles or triples from 5 μmol hexose/mg Chl up to 10 or 15 μmol hexose/mg Chl, the Glc 6-P content rises by 16% and 32%, respectively. An increase in Glc 6-P also correlates positively with an increased Fru 2,6-P_2 level, as summarized in Figure 4B. As Glc 6-P rises from 70 to 150 nmol/mg Chl, the Fru 2,6-P_2 content also increases sharply, in this region a 50% increase of Glc 6-P being associated on average with a doubling of Fru 2,6-P_2. The response of the partially purified Fru 6-P, 2-kinase and Fru 2,6-bisphosphatase (see above) to Fru 6-P is more than sensitive enough to account for the increase in Fru 2,6-P_2 which is observed *in situ* as hexose P increase, although these experiments cannot exclude the possibility that other factors are also involved.

Fru 6-P, 2-kinase and Fru 2,6-bisphosphatase are also regulated by DHAP, PGA, and Pi and some of these metabolites are also varying when sucrose accumulates and may partially compensate for the rise in Fru 6-P. The level of DHAP increases as sucrose accumulates, so there is a positive correlation between DHAP and Fru 2,6-P_2 (Fig. 4C). As DHAP inhibits Fru 6-P, 2-kinase (see 21), an increase in DHAP might have been expected to lead to a decrease of the Fru 2,6-P_2 levels. Indeed, when the rate of photosynthesis is low, a clear inverse relation is found between DHAP and Fru 2,6-P_2 (21). When the rate of photosynthesis is increased by raising the light intensity or CO_2 concentration, there are 2- to 10-fold increases in the DHAP level (19, 21) which are associated with decreasing Fru 2,6-P_2 (21). In such conditions of limiting photosynthesis in leaf material with a low sucrose content, the alterations of DHAP are much larger than those of Fru 6-P, and are often accompanied by parallel changes in PGA which is another inhibitor of Fru 6-P, 2-kinase. In contrast, in conditions when spinach leaves photosynthesize rapidly and Fru 2,6-P_2 is increasing in response to high sucrose content (Tables I–III; Fig. 3), the increase in Fru 6-P plays a predominant role in controlling the Fru 2,6-P_2 concentration, so that the 15 to 40% rise in DHAP in these conditions can be more than compensated for by the larger increase (25–67%) of hexose P.

The general increase in phosphorylated intermediates in response to a high content of sucrose also implies that the Pi concentration decreases (see above). Pi is an activator of the Fru 6-P, 2-kinase (4) and inactivates the Fru 2,6-bisphosphatase (5). However, it is unlikely that this decrease in Pi has a marked effect on the Fru 2,6-P_2 level. In experiments which will be presented elsewhere (Stitt, Cseke, and Buchanan, in press), it has been shown that Fru 6-P, 2-kinase does not respond to Pi in the presence of mM concentrations of DHAP, and that Pi is only a poor inhibitor of Fru 2,6-bisphosphatase in the presence of Fru 6-P, especially when Fru 2,6-P_2 is high. Model experiments showed that an increase in Fru 6-P would overide a much larger accompanying decrease in Pi as long as DHAP is present. In the presence of 2 mM DHAP, the Fru 6-P, 2-kinase activity rises and Fru 2,6-bisphosphatase activity decreases continuously as the Fru 6-P concentration is increased, even though Pi is simultaneously decreased. The 4-fold increase of Fru 2,6-P_2 in response to a 50% rise in Fru 6-P (see above) was actually obtained in the presence of a reciprocally varying Pi concentration. It might be mentioned that this insensitivity of the Fru 2,6-P_2 content to Pi is only found in the presence of DHAP. As will be shown elsewhere, in spinach leaves in the dark when there is negligible DHAP, the Fru 2,6-P_2 level does not depend so strongly on the Fru 6-P level. Apparently in the dark in the absence of DHAP, the cytosolic Pi concentration is high enough to keep the Fru 6-P, 2-kinase active even when the Fru 6-P concentration is very low.

Contribution of Fru 2,6-P_2 to Regulation of Partitioning between Sucrose and Starch. In summary, the increase in Fru 2,6-P_2 observed when leaves contain high levels of sucrose can be best accounted for by the reciprocal stimulation of Fru 2,6-P_2

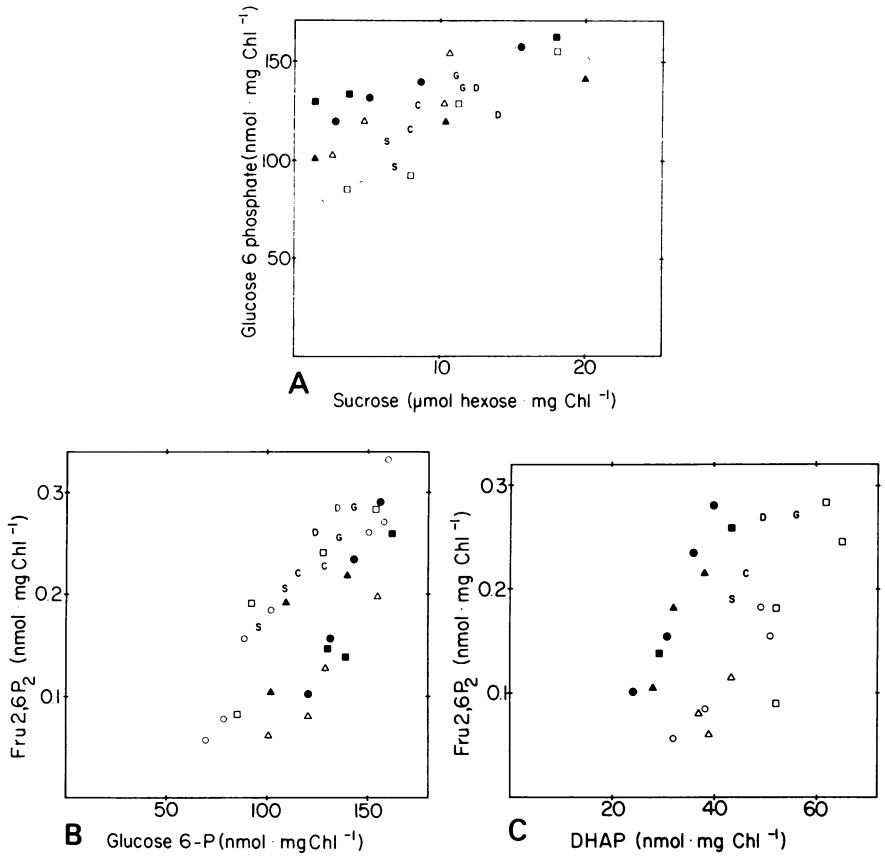

FIG. 4. Relation between sucrose, glucose 6-phosphate, fructose-2,6-bisphosphate and dihydroxyacetone P. The experiments and symbols are as in Figure 1. A, Sucrose and glucose 6-phosphate; B, glucose 6-phosphate and fructose 2,6-bisphosphate; C, dihydroxyacetone phosphate and fructose 2,6-bisphosphate.

synthesis, and inhibition of its breakdown by Fru 6-P. This Fru 6-P accumulates due to a previous inhibition of sucrose P synthase, although the details of this process need more study. The increased Fru 2,6-P_2 inhibits the cytosolic FBPase, so that triose P accumulate. Restriction of the activity of both sucrose P synthase and the cytosolic FBPase leads to an accumulation of phosphorylated intermediates in the cytosol, and probably to a lowering of the Pi. The resulting decrease of the triose P/Pi ratio will decrease the rate at which carbon can be withdrawn from the chloroplast, and Pi returned, so that the stromal Pi decreases. In these conditions, starch synthesis in the chloroplast is stimulated as the PGA/Pi ratio increases (22). In this way, Fru 2,6-P_2 interacts with the control of metabolism by Pi. In particular, by inhibiting the cytosolic FBPase in response to a relatively small change in the hexose P levels, it signals to the chloroplast that the export of triose P to the cytosol should be decreased, without this response requiring excessive alteration of the metabolite pools in the cytosol.

Control of Sucrose Synthesis by Supply and Demand. In this and the previous article (21), two opposing roles for Fru 2,6-P_2 in control of photosynthetic sucrose metabolism have been proposed. It is involved both in a feed forward control to coordinate the rate of sucrose synthesis with the ability of the chloroplast to supply triose P during photosynthesis, and is also involved in a feedback control so that photosynthate is diverted away from sucrose towards starch when the rate of synthesis of sucrose exceeds the ability of the leaf to export or store it.

During photosynthesis in limiting conditions, the rate of sucrose synthesis is stimulated as the triose P supply increases. Alterations of C_3 metabolites like DHAP and PGA serve as a signal for the availability of substrate for sucrose synthesis and inhibit the Fru 6-P, 2-kinase so that the Fru 2,6-P_2 concentration declines. This increases the activity of the cytosolic FBPase, so sucrose synthesis is stimulated. In these conditions, the large alterations of C_3 metabolites like DHAP outweigh smaller increases in C_6 metabolites. On the other hand, in conditions of rapid photosynthesis as sucrose accumulates in the leaf, an increasing level of Fru 6-P signals that the FBPase is producing C_6 metabolites faster than they are being converted to sucrose and exported. Simultaneous activation of Fru 6-P, 2-kinase and inactivation of Fru 2,6-bisphosphatase leads to an increased Fru 2,6-P_2 concentration. This restricts FBPase activity and diverts more carbon towards starch synthesis in the stroma. In both cases, the Fru 2,6-P_2 concentration is adjusted in response to alterations of the C_3 and C_6 metabolite concentrations in the cell which provide a signal for an excess or deficiency in these pools. The new Fru 2,6-P_2 concentration plays a role in modifying the activity of the cytosolic FBPase to readjust the gluconeogenetic flux to changes in the supply of carbon for sucrose synthesis, and to the demand for continued synthesis of sucrose.

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