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Light Regulation of Sink Metabolism in Tomato Fruit[1]

II. Carbohydrate Metabolizing Enzymes

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ABSTRACT

Effects of light on carbohydrate levels and certain carbon metabolizing enzyme activities were studied during the early development of tomato (*Lycopersicon esculentum*) fruit. Sucrose levels were low and continued to decline during development and were unaffected by light. Starch was significantly greater in light. Invertase activity was similar in both light- and dark-grown fruit. Sucrose synthase activity was much lower than invertase and showed a slight decrease in light-grown fruit between days 21 and 28. Light-grown fruit also had higher ADP glucose pyrophosphorylase activity than dark-grown fruit, which was correlated with higher starch levels. The rapidly decreasing activity of ADP glucose pyrophosphorylase during early fruit development in the dark in conjunction with reduced starch levels and rates of accumulation indicates that ADP glucose pyrophosphorylase is crucial for carbon import and storage in tomato. The differential stimulation of ADP glucose pyrophosphorylase activity from light- and dark-grown tissue by 3-phosphoglycerate suggests that this enzyme may be allosterically altered by light.

Tomato (*Lycopersicon esculentum*) quality and yield are mainly dependent upon sugar import and accumulation in the fruit. The composition of stored carbohydrate in tomato is associated with certain key enzymes responsible for sucrose metabolism (12). Invertase and sucrose synthase, catalyzing the breakdown of sucrose, have been correlated with reducing sugar levels and rate of carbon translocation into tomato fruit (19, 24). Furthermore, sucrose synthase has also been related to the rate of conversion of sucrose to starch (14). ADPG[2] pyrophosphorylase controls the rate limiting step of starch synthesis and is regulated by the ratio of 3-PGA and Pi concentrations in both photosynthetic and nonphotosynthetic tissues (17, 18).

Because hexoses are not preferentially taken up into tomato fruit tissue slices in comparison to sucrose, and because sugar uptake does not appear to be energy dependent (4, 10), it has been suggested that sugar accumulation may be driven by the subsequent intracellular metabolism of the translocated sugar. Based on the notion that translocation is inversely related to sucrose levels, sucrose hydrolysis was tentatively suggested as the limiting step of assimilate import (24). However, the subsequent starch accumulation rate has also been demonstrated to be correlated with an increase in the sucrose movement (6). In developing tomato fruit, starch transiently accumulates early in development (14–28 d after anthesis) with soluble sugars gradually increasing as starch levels decrease later (28–35 d after anthesis) (5). Peak levels of starch early in development are positively correlated to the final levels of soluble sugars in tomato fruit. Therefore, both hexose and starch accumulation may be very important for sucrose import and fruit growth (8).

In a previous study (7), we demonstrated that light stimulation of tomato fruit growth was due to mechanisms other than photosynthesis and that light had effects on carbohydrate accumulation in tomato fruit. Therefore, it is of interest to focus on the influence of light on carbohydrate accumulation and on the activity of certain carbohydrate metabolizing enzymes during early fruit growth.

MATERIALS AND METHODS

Enzyme Assays

Tomato (*Lycopersicon esculentum*) fruit were grown in M&S medium with 4% sucrose in the dark or in the light (PPFD 50 μmol m^{-2} s^{-1}, 16 h light/8 h dark, 25°C), as described previously (7). Tomato fruit between 7 and 35 d after anthesis were harvested and frozen in liquid nitrogen. Three grams fresh tissue of whole fruit was ground into powder in additional liquid nitrogen in 10 mL homogenization buffer. As described by Robinson *et al.* (19), the homogenization buffer contained 50 mM Hepes-KOH (pH 8.3), 2 mM EDTA, 2 mM EGTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, and 2 mM DTT. The homogenization solution was centrifuged at 20,000 rpm for 15 min at 4°C. After centrifugation, the supernatant was used for both sucrose and enzyme assays, and the pellet was used for determining starch levels.

Excellulose GF-5 columns (from Pierce) were used in the desalting process. The column, which displays stop flow characteristics, has an exclusion limit of 5000 mol wt. The column was equilibrated with 50 mM Hepes-KOH (pH 8.3). One milliliter of the supernatant was layered on the column. The column was eluted with 50 mM Hepes-KOH (pH 8.3) and the protein fraction was used for all the enzyme assays.

Acid Invertase

Acid invertase activity was determined as described by Manning and Maw (13). Extracts (0.2 mL) were incubated at

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[2] Abbreviations: ADPG, ADP glucose; M&S medium, Murashige and Skoog medium; PGA, phosphoglycerate.

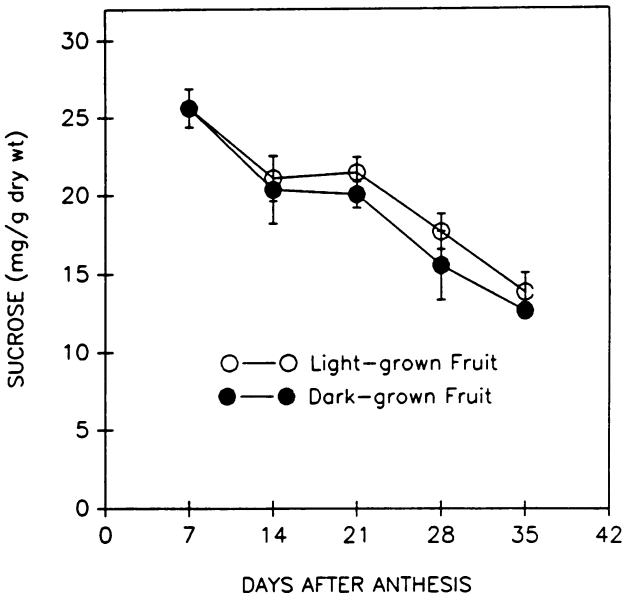

Figure 1. Changes in sucrose levels in light- and dark-grown tomato fruit during fruit development. Five days after anthesis, small tomato fruit were harvested from the greenhouse and cultured in M&S medium in the dark or in the light (PPFD 50 μmol m^{-2} s^{-1}, 16 h light/8 h dark, 25°C). Sucrose was determined weekly.

30°C with 50 mM sucrose in 200 mM acetate buffer (pH 4.5) for 20 min. Boiled enzyme was used as the control. The reaction was terminated by boiling the enzyme mixture and reducing sugars determined (21).

Sucrose Synthase

Sucrose synthase activity was measured as UDP-dependent sucrose cleavage as described by Chourey and Nelson (2). The reaction mixture, consisting of 0.2 mL enzyme extract, 50 mM sucrose, and 15 mM UDP in 200 mM Tris-HCl (pH 6.0), was incubated at 30°C for 30 min. The reaction mixture without UDP constituted the control. Reactions were terminated by boiling for 1 min. Fructose produced by UDP-dependent sucrose hydrolysis was measured as described previously (7).

ADPG Pyrophosphorylase

ADPG pyrophosphorylase activity was measured with a modification of the Sowokinos (22) method as described below. The reaction mixture contained 80 mM glycylglycine-NaOH (pH 8.0), 10 mM $MgCl_2$, 10 mM NaF, 0.1 mM glucose-1,6-bisphosphate, 1 mM pyrophosphate, 2 mM ADPG, 0.025 mM 3-PGA (unless otherwise noted), and 0.2 mL enzyme extract in a total volume of 1 mL. The reaction was initiated by adding pyrophosphate. After a 10 min incubation at 30°C, glucose-phosphate production was terminated by boiling for 1 min. The mixture was centrifuged at 2000 rpm for 5 min. The supernatant was used for determining the amount of glucose-phosphate produced. Each complete assay reaction in a total volume of 2 mL (pH 8.0) contained 0.8 mL supernatant, 1.5 mM NAD, 5 units of phosphoglucomutase (Sigma Chemical Co.), and 1 unit of glucose-6-phosphate dehydrogenase (Sigma Chemical Co.). The amount of glucose-phosphate was determined at 340 nm (recovery of glucose-1-P was 95–100%).

Sugar Assays

Sucrose and starch levels were determined as described previously (7).

RESULTS

Effects of Light on Sugar Accumulation in Tomato Fruit

In the previous paper (7), we demonstrated the effect of light on sugar accumulation in tomato fruit approximately 21 d old. In this study, we observed the effect of light on sugar accumulation during the course of tomato fruit development. The sucrose level decreased during fruit development (Fig. 1). However, no significant differences in the sucrose content were found between light- and dark-grown fruit. In contrast, starch accumulation was significantly different between light- and dark-grown fruit (Fig. 2). Even though light- and dark-grown fruit displayed a similar pattern of starch accumulation, with starch levels increasing and reaching a peak between days 21 and 28 after anthesis, dark-grown fruit had only slightly increased starch levels during the course of fruit development and light-grown fruit accumulated twice as much as dark-grown fruit 21 d after anthesis. These results suggest that light effects on tomato fruit growth and sink strength may be due to an expansion of a sink for carbon, possibly through stimulation of starch synthesis during early fruit development.

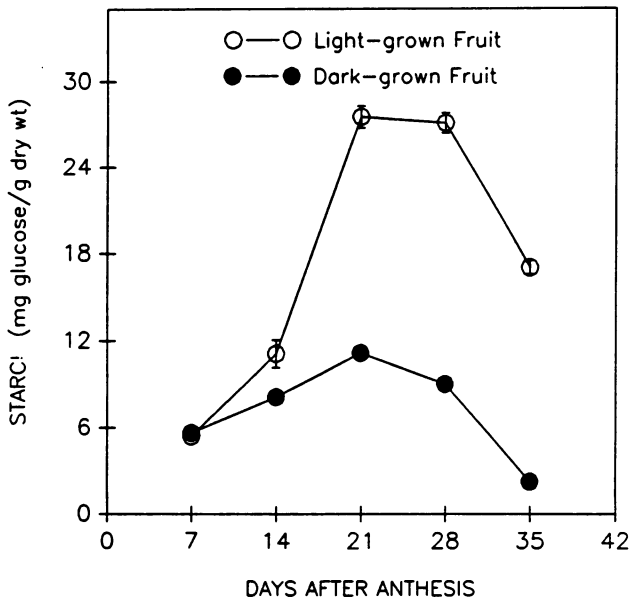

Figure 2. Changes in starch levels in light- and dark-grown tomato fruit during fruit development. Five days after anthesis, small tomato fruit were harvested from the greenhouse and cultured in M&S medium in the dark or in the light (PPFD 50 μmol m^{-2} s^{-1}, 16 h light/8 h dark, 25°C). Starch was determined weekly.

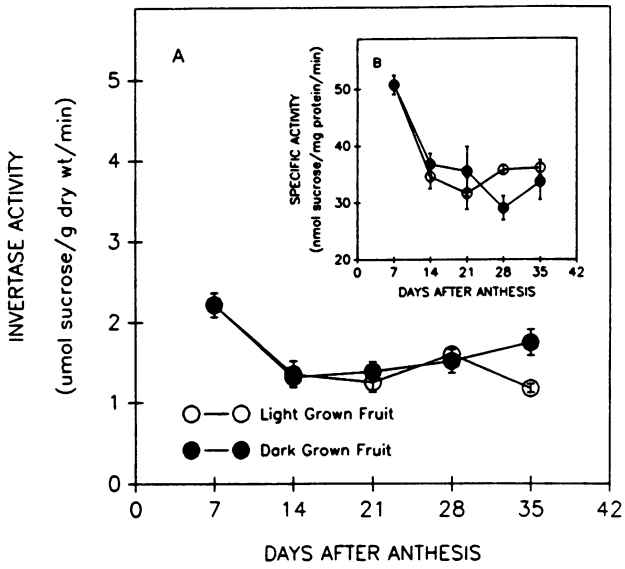

Figure 3. Changes in invertase activity (A) and specific activity (B) in light- and dark-grown tomato fruit during fruit development. Five days after anthesis, small tomato fruit were harvested from the greenhouse and cultured in the dark or in the light (PPFD 50 μmol m^{-2} s^{-1}, 16 h light/8 h dark, 25°C).

Light/Dark Effects on Enzyme Activity

The composition of stored carbohydrate has been associated with the key enzymes responsible for sucrose metabolism (12). Because hydrolysis of sucrose arriving in a sink is the initial step in sucrose metabolism, light/dark effects on invertase and sucrose synthase were examined (Figs. 3 and 4). Except for a decrease in invertase activity from 7 to 14 d after anthesis (Fig. 3), the invertase activity remained constant during fruit development. No significant differences in invertase activity and specific activity were found between light- and dark-grown fruit. Interestingly, dark-grown fruit had slightly higher sucrose synthase activity than light-grown fruit between days 21 and 28 (Fig. 4). However, invertase was much more active than sucrose synthase during fruit development in both light- and dark-grown fruit. Sucrose synthase activity remained low during development in both light- and dark-grown fruit. These results are consistent with the findings of Johnson *et al.* (10).

Light-grown fruit not only had higher starch content but also had higher ADPG pyrophosphorylase activity during fruit development (Fig. 5A). Furthermore, the specific activity of this enzyme in light-grown fruit was higher than that in dark-grown fruit and increased 14 d after anthesis (Fig. 5B). The specific activity in dark-grown fruit, however, remained low and constant during fruit development. Because ADPG pyrophosphorylase activity has been found to be correlated with the transient starch accumulation during tomato fruit development (19), and differences in starch accumulation have been noted between light- and dark-grown fruit (Fig. 2), light/dark effects on ADPG pyrophosphorylase are not unexpected.

As has been shown, all plant ADPG pyrophosphorylases studied so far are stimulated by 3-PGA (18); likewise, ADPG pyrophosphorylase activity in light-grown fruit was also stimulated by 3-PGA (Fig. 6A). In the light-grown fruit, half the maximum stimulation of this enzyme was obtained at 0.025 mM 3-PGA, with maximum stimulation at 0.10 mM 3-PGA (Fig. 6B). In dark-grown fruit, however, only a slight stimulation (≈20%) of ADPG pyrophosphorylase activity was seen over different concentrations of 3-PGA (Fig. 6A and B). However, when the enzyme activity was measured without 3-PGA, no significant differences in activity were seen between light- and dark-grown fruit (Fig. 6A). These results suggest that there are different allosteric properties of ADPG pyrophosphorylase in light- and dark-grown fruit, and starch synthesis in tomato fruit may be regulated by the 3-PGA concentration *in vivo* and/or by the ratio of 3-PGA and Pi concentrations as reported in other tissues (18, 23).

DISCUSSION

In a previous study (7), we demonstrated that light may play a more direct role in sink metabolism other than through photosynthesis. Further study shows that light-grown fruit can take up 30% more sucrose from the same source (7) and accumulate almost twice as much starch and hexose as dark-grown fruit. However, sucrose levels decrease during fruit development and show no difference between light- and dark-grown fruit (Fig. 1). Therefore, carbohydrate accumulation may be driven by the subsequent metabolism of sucrose. Storage of hexose in the vacuole and starch accumulation may decrease soluble sugar levels in the cytoplasm. Therefore, starch accumulation may not only function as a storage sink for carbon, but also create or maintain a certain osmotic potential and cell turgor. These changes may facilitate sugar

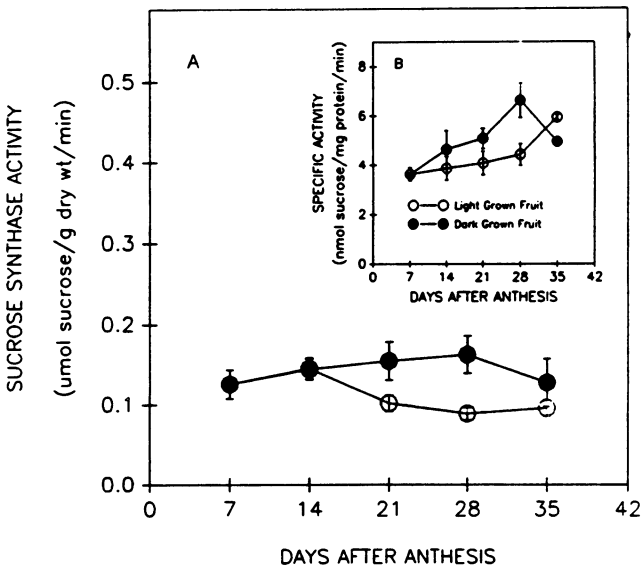

Figure 4. Changes in sucrose synthase activity (A) and specific activity (B) in light- and dark-grown tomato fruit during fruit development. Five days after anthesis, small tomato fruit were harvested from the greenhouse and cultured in M&S medium in the dark or in the light (PPFD 50 μmol m^{-2} s^{-1}, 16 h light/8 h dark, 25°C). The enzyme activity was measured in the direction of UDP-dependent sucrose hydrolysis.

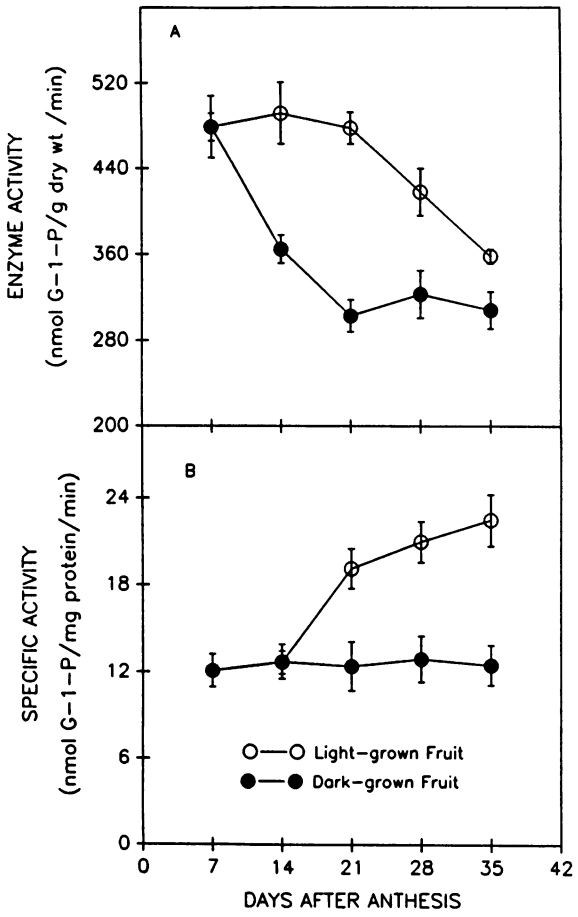

Figure 5. Changes in ADPG pyrophosphorylase activity (A) and specific activity (B) in light- and dark-grown tomato fruit during fruit development. Five days after anthesis, small tomato fruit were harvested from the greenhouse and cultured in M&S medium in the dark or in the light (PPFD 50 μmol m^{-2} s^{-1}, 16 h light/8 h dark, 25°C). The enzyme was measured in the pyrophosphorylase direction.

import into the fruit cells, as found in leaf discs of *Phaseolus coccinius* (3), in the soybean ovule (26), and in storage-root tissue of red beet (15).

Based on the inverse relationship between sucrose levels and the import rate, it has been suggested that a diffusion along a sucrose concentration gradient is probably the driving force for unloading and translocation in the fruit (9). This sucrose gradient may be maintained by the metabolic conversion of sucrose to starch for storage in the plastid or to hexose for storage in the vacuole. A question arises as to which process is the limiting step for carbon import and fruit growth. Because hydrolysis of sucrose arriving in the sink is the initial step of sucrose metabolism, invertase and sucrose synthase, catalyzing the breakdown of sucrose, are expected to play a major role (20, 24). However, no differences in invertase activity were found between light- and dark-grown fruit (Fig. 4). Invertase activity remains high and constant in light- and dark-grown fruit during the rapid growing period. Johnson *et al.* (10) reported similar findings in that invertase activity remains constant during early tomato development and increases about sixfold at ripening. According to Johnson *et al.* (10), during the rapid growth period, invertase activity is well in excess of that required for sucrose hydrolysis to maintain the unloading process. In addition, fruit at the proximal position of the fruit truss grow bigger than those at the distal position, but no differences in invertase activity were found in the developing fruit at any of these locations (10). Therefore, hydrolysis of sucrose may not be a limiting step for sucrose import and carbon metabolism. Sucrose synthase activity is much lower than invertase activity during fruit development (Fig. 5). This is consistent with the finding by Johnson *et al.* (10), who reported that a small amount of sucrose synthase activity is detected only during a limited period of the fruit growth. Although sucrose synthase has been associated with soluble sugar levels and sucrose-starch conversion (2), the low activity of sucrose synthase suggests a minor role of this enzyme in sucrose hydrolysis in tomato fruit. In contrast, Robinson *et al.* (19) suggested that sucrose synthase activity in tomato fruit was positively correlated with starch levels, sugar accumulation, and sugar import. However, they also found that only at about 20 d after anthesis was there a peak of sucrose synthase activity, which decreased immediately after that. Through the analysis of sugar concentration in the apoplast of tomato fruit, Damon *et al.* (4) found that glucose and fructose concentrations in the apoplast were present in a ratio of approximately 1:1. Sucrose concentrations in the apoplast were much lower than hexose concentrations throughout development. These low sucrose concentrations indicate the hydrolysis of sucrose in the apoplast by an

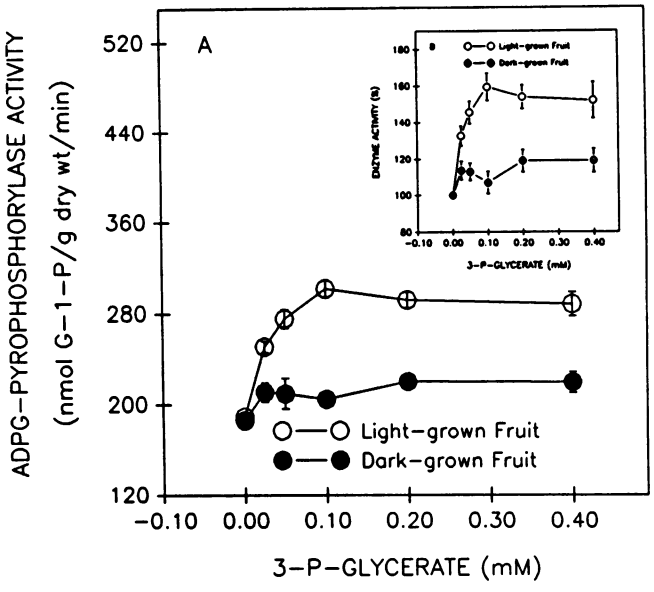

Figure 6. Effects of 3-PGA concentration on ADPG pyrophosphorylase activity in light- and dark-grown tomato fruit (A). The percentage of stimulation by 3-PGA was determined by comparing the enzyme activity with and without 3-PGA (B). Five days after anthesis, small tomato fruit were harvested from the greenhouse and cultured in M&S medium in the dark or in the light (PPFD 50 μmol m^{-2} s^{-1}, 16 h light/8 h dark, 25°C). After 21 d of culture, ADPG pyrophosphorylase activity in light- and dark-grown fruit was measured in the pyrophosphorylase direction at different 3-PGA concentrations.

extracellular invertase. It has been suggested that sucrose synthase may provide the means for utilizing sucrose that escapes extracellular hydrolysis by invertase and is taken up directly by the fruit cells (4). However, a greater uptake rate of glucose and a greater conversion rate of glucose than that of sucrose into starch in fruit slices suggest that glucose is the predominant sugar taken up by fruit cells *in vivo* (10). In addition, it is noted that glucose, a product of sucrose hydrolysis, inhibits sucrose synthase activity (25). Therefore, the localization and characterization of sucrose synthase and invertase may be crucial for understanding their function *in vivo*.

It is of interest to note that dark-grown fruit had slightly higher sucrose synthase activity than light-grown fruit between days 21 and 28. As has been shown in light- and dark-grown seedlings of maize (1), the differences in the enzyme activity may be due to unequal expression of the sucrose synthase gene in light and dark. Based on the fact that there are higher hexose levels in light-grown fruit than in dark-grown fruit, the question is asked whether hexose alters the sucrose synthase gene expression as well as inhibits the enzyme activity in tomato fruit. The possibility cannot be excluded that higher sucrose synthase activity in dark-grown fruit is to compensate for the lower ADPG pyrophosphorylase activity to produce precursors such as UDP glucose and ADPG for starch synthesis.

A positive correlation between the rate of starch accumulation and the rate of fruit growth was found in light- and dark-grown fruit. This suggests that starch accumulation in the early development of tomato fruit may be important for carbon import and sink metabolism. As has been shown by Robinson *et al.* (19), starch biosynthetic capacity is determined by levels of ADPG pyrophosphorylase rather than by starch degradative capacity in the fruit. Although light-grown fruit had higher ADPG pyrophosphorylase activity than dark-grown fruit in the presence of the activator, 3-PGA, no significant differences in the enzyme activity were found between light- and dark-grown fruit when the enzyme activity was measured without 3-PGA. This indicates that there are different regulatory properties of this enzyme in light- and dark-grown fruit. It has been shown that there are different allosteric properties of the enzyme in the bundle sheath and mesophyll cells in maize (23), and our results also indicate that the allosteric properties of this enzyme in tomato fruit favor starch synthesis in light-grown fruit while restricting it in dark-grown fruit. However, because we did not measure 3-PGA, it is possible that levels of 3-PGA in light- and dark-grown tissue differ in such a way that the *in vivo* enzyme activities are similar. Nevertheless, a nonphotosynthetic light requirement for starch synthesis has also been demonstrated in leaf tissues. While floating discs of cotton leaf in sucrose solutions in a CO_2-free atmosphere, Phillis and Mason (16) observed that starch was produced in weak light, but not in the dark. They concluded that light may affect starch synthesis indirectly by accelerating sucrose uptake. In this study, however, we demonstrated that light can modify ADPG pyrophosphorylase, although the mechanism of the light effect is unknown. It is not clear whether the difference was due to differential light stimulation of the synthesis of certain isozymes and/or through modification of the enzyme. Because Western immunoblot analysis has suggested the presence of different isozymes in carrot leaves (11), the question arises as to whether there are different isozymes in light- and dark-grown fruit. Further study on gene expression of this enzyme in light- and dark-grown tomato fruit is required.

These results suggest that sucrose hydrolysis by invertase is important but may not be the limiting step of carbon import and sink metabolism. However, ADPG pyrophosphorylase is crucial for carbon storage by controlling starch synthesis. Both hexose and starch accumulation in the early development of tomato fruit may efficiently reduce the cytoplasmic sucrose level and/or maintain a particular osmotic potential to ensure a continued sucrose gradient for further sucrose import into the fruit. Therefore, light stimulation of fruit growth and sink strength may be due to an expansion of an additional sink for carbon through a stimulation of starch synthesis during early fruit development, and ADPG pyrophosphorylase activity may be an indication of sink activity during the early developmental stages in tomato fruit.

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