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Sucrose-to-Starch Metabolism in Tomato Fruit Undergoing Transient Starch Accumulation[1]

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Immature green tomato (*Lycopersicon esculentum*) fruits undergo a period of transient starch accumulation characterized by developmental changes in the activities of key enzymes in the sucrose (Suc)-to-starch metabolic pathway. Activities of Suc synthase, fructokinase, ADP-glucose (Glc) pyrophosphorylase, and soluble and insoluble starch synthases decline dramatically in parallel to the decrease in starch levels in the developing fruit. Comparison of "maximal" in vitro activities of the enzymes in the Suc-to-starch pathway suggests that these same enzymes are limiting to the rate of starch accumulation. In contrast, activities of invertase, UDP-Glc pyrophosphorylase, nucleoside diphosphate kinase, phosphoglucoisomerase, and phosphoglucomutase do not exhibit dramatic decreases in activity and appear to be in excess of starch accumulation rates. Starch accumulation is spatially localized in the inner and radial pericarp and columella, whereas the outer pericarp and seed locule contain little starch. The seed locule is characterized by lower activities of Suc synthase, UDP-Glc pyrophosphorylase, phosphoglucomutase, ADP-Glc pyrophosphorylase, and soluble and insoluble starch synthases. The outer pericarp exhibits comparatively lower activities of ADP-Glc pyrophosphorylase and insoluble starch synthase only. These data are discussed in terms of the developmental and tissue-specific coordinated control of Suc-to-starch metabolism.

Young tomato fruits undergo a transient period of starch accumulation (see Ho and Hewitt, 1986). Starch concentrations may increase to 20% dry weight in the young fruit but are negligible in the mature fruit. The hypothesis has been presented that this transient starch may function as a carbohydrate reservoir in the developing fruit and may contribute to the soluble hexose level in the mature fruit (Dinar and Stevens, 1981).

The tomato plant translocates Suc (Walker and Ho, 1977), which can enter metabolism via either invertase or Suc synthase. Suc synthase is often associated with Suc cleavage in starch metabolism (Quick and Schaffer, 1996) (Fig. 1), and its activity pattern (but not that of invertase) follows the transient starch accumulation pattern of tomato fruit (Robinson et al., 1988). Suc synthase activity has accordingly been linked to sink strength in the developing tomato fruit (Ho et al., 1991; Sun et al., 1992; Wang et al., 1993; Ho, 1996). The enzyme ADP-Glc PPase catalyzes the synthesis of ADP-Glc in starch-synthesizing tissue. Robinson et al. (1988) reported that its activity, too, follows the transient starch accumulation pattern, although this loss of activity was not observed by Guan and Janes (1991). The other enzymes in the Suc-to-starch metabolic pathway during the transient starch accumulation period have not yet to our knowledge been studied, nor have their relative activities been compared.

Starch level is a function of starch synthesis and breakdown. Regarding starch breakdown metabolism, Robinson et al. (1988) studied developmental changes in amylase and starch phosphorylase activity in developing tomato fruit. They reported that phosphorylase activity was overwhelmingly dominant and presumably responsible for starch breakdown throughout the development of the fruit, and that neither enzyme underwent developmental modulation of activity. Accordingly, our present study of the metabolism of transient starch accumulation is based on the assumption that the decline in starch content is primarily the result of changing starch synthesis.

Spatially, starch accumulation is pronounced in the inner pericarp and columella tissue of the developing tomato fruit, where Suc synthase is preferentially localized (Wang et al., 1994). However, the spatial localization of the subsequent steps in the starch biosynthetic pathway have not yet to our knowledge been studied.

The goal of the present study was to characterize the Suc-to-starch biosynthetic pathway in developing tomato fruit to determine developmental and spatial patterns of the enzymes in the pathway and to identify metabolic steps with activities that are potentially limiting to starch accumulation. In addition, we attempted to determine the extent of a coordinated control pattern of Suc-to-starch metabolism.

MATERIALS AND METHODS

Plant Material and Chemicals

Plants of tomato (*Lycopersicon esculentum* cv F144; a large-fruited cultivar) were grown under standard condi-

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Abbreviations: DH, dehydrogenase; DIECA, diethyldithiocarbamic acid; NDP, nucleoside diphosphate; PGI, phosphoglucoisomerase; PGM, phosphoglucomutase; PPase, pyrophosphorylase.

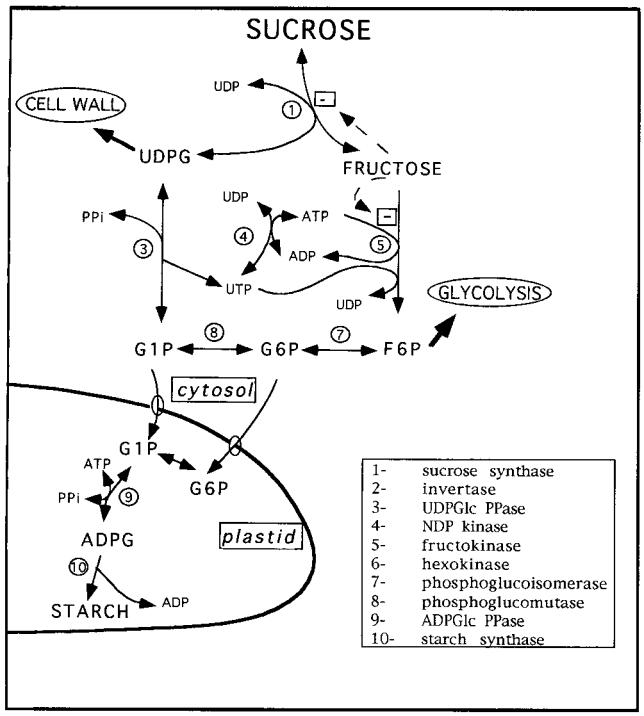

Figure 1. Schematic representation of the metabolic pathway of Suc-to-starch synthesis. Dashed lines leading from Fru indicate enzyme inhibition of the Suc synthase and fructokinase reactions by Fru. The invertase and hexokinase (phosphorylation of Glc) reactions are not included in the schematic.

tions in a greenhouse in Bet Dagan, Israel. Flowers were allowed to self-pollinate and were tagged at anthesis. Pericarp tissue was used in the developmental study of enzyme activities. For the study of the spatial distribution of enzyme activities, immature fruit was separated as described in "Results." Chemicals and enzymes were purchased from Sigma, Bio-Rad, and Boehringer Mannheim.

Sugar and Starch Assays

Soluble carbohydrates and starch were assayed from approximately 1 g fresh weight of tissue, as described by Miron and Schaffer (1991). Freehand sections of tomato fruit were stained with a 0.3% I_2/1.5% KI solution.

Enzyme Assays

Soluble acid invertase (EC 3.2.1.26) was assayed according to Miron and Schaffer (1991). Suc synthase (EC 2.4.1.13) was extracted from approximately 1 g fresh weight of pericarp tissue and its activity was measured in the Suc cleavage direction, as described by Schaffer et al. (1987). Fructokinase (EC 2.7.1.4) and hexokinase (EC 2.7.1.1) activities were assayed as follows: approximately 2.5 g fresh weight of fresh pericarp tissue was homogenized (Kinematica homogenizer, Littau, Switzerland) in 2 volumes of chilled extraction buffer containing 50 mM Hepes-NaOH (pH 7.5), 1 mM $MgCl_2$, 1 mM EDTA, 10 mM KCl, 2.5 mM DTT, 1% insoluble PVP, and 3 mM DIECA. After centrifugation at 18,000*g* for 30 min, the supernatant was precipitated with 80% ammonium sulfate and centrifuged for 10 min at 18,000*g*. The precipitate was resuspended in 1 mL of the extraction buffer and desalted on a Sephadex G-25 column with washing buffer containing 50 mM Hepes-NaOH (pH 7.5), 1 mM EDTA, and 1 mM DTT. The desalted extract was used as a crude enzyme.

Hexokinase activity was measured by an enzyme-linked assay, according to a modification of the method of Huber and Akazawa (1985). Assays contained, in a total volume of 1 mL, 30 mM Hepes-NaOH (pH 7.5), 1 mM $MgCl_2$, 0.6 mM EDTA, 9 mM KCl, 1 mM NAD, 1 mM ATP, and 2 units of NAD-dependent Glc-6-P DH (from *Leuconostoc*, Sigma). For the assay of Glc phosphorylation, the reaction was initiated with 2 mM Glc. For the assay of Fru phosphorylation, 2 units of PGI (Sigma, type III) was added and the reaction was initiated with 2 mM Fru. Reactions were carried out at 37°C and A_{340} was monitored continuously.

NDP kinase (EC 2.7.4.6) was assayed as the UTP-dependent formation of ATP from ADP. ATP was measured in a linked assay with hexokinase and Glc-6-P DH, according to a modification of the method of Dickinson and Davies (1971). One gram fresh weight was extracted in 2 volumes of the extraction buffer containing 50 mM Hepes-NaOH (pH 7.0), 5 mM $MgCl_2$, 2 mM DTT, and 3 mM DIECA. After centrifugation for 30 min at 18,000*g* the supernatant was heated for 4 min at 60°C, recentrifuged, and the supernatant used as an enzyme extract. Activity was assayed spectrophotometrically in a reaction mixture (1 mL) containing 100 mM Tris (pH 7.5), 0.8 mM EDTA, 0.8 mM $MgCl_2$, 0.4 mM ADP, 1 mM NAD, 0.5 unit of hexokinase (from yeast, Boehringer Mannheim), and 1 unit of Glc-6-P DH, and the reaction was initiated with 0.8 mM UTP. The addition of AMP (Dancer et al., 1990) reduced measurable ATP levels and was therefore not added to the assay mixture.

For the assays of UDP-Glc PPase (EC 2.7.7.9), PGM (EC 5.4.2.2), PGI (EC 5.3.1.9), and ADP-Glc PPase (EC 2.7.7.27), approximately 1 g fresh weight of fruit tissue was extracted in 2 volumes of chilled extraction buffer containing 50 mM Hepes-NaOH (pH 7.5), 1 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, and 3 mM DIECA. The supernatant after centrifugation (18,000*g*, 30 min) was used for assaying UDP-Glc PPase, PGM, and PGI. For the assay of ADP-Glc PPase, the crude enzyme extract was heated for 4 min at 60°C, centrifuged (18,000*g*, 5 min), and the supernatant used as the crude enzyme extract, according to the method of Chen and Janes (1995) (H. Janes, personal communication). The heat treatment increased extractable enzyme activity by approximately 10-fold.

UDP-Glc PPase was assayed by monitoring spectrophotometrically the pyrophosphate-dependent production of Glc-1-P via a continuous enzyme-linked assay coupling Glc-1-P to Glc-6-P (with PGM) and monitoring the Glc-6-P DH catalyzed production of NADH at 340 nm, as described by Doehlert et al. (1988) with minor modifications. The assay buffer contained 50 mM Hepes-NaOH (pH 7.8), 5 mM $MgCl_2$, 1 mM UDP-Glc, 1 mM NAD, 1 unit of Glc-6-P DH, 2 units of PGM (Sigma), 20 μM of Glc-1,6-bisP, and 3 to 5

μL of the enzyme extract. Following a 5-min incubation at 37°C, the reaction was initiated by the addition of 1 mM PPi for a total volume of 1 mL. ADP-Glc PPase was assayed as for UDP-Glc PPase, with the replacement of UDP-Glc by 1 mM ADP-Glc and the addition of 10 mM 3-phosphoglycerate and 10 mM NaF. PGI and PGM were assayed spectrophotometrically, as described by Doehlert et al. (1988).

Soluble and insoluble starch synthases (EC 2.4.1.21) were extracted according to a modification of the method of Ching et al. (1983). Two grams fresh weight of tomato fruit tissue was ground in 2 volumes of chilled extraction buffer consisting of 100 mM Hepes-NaOH (pH 7.5), 1 mM EDTA, 5 mM DTT, 10 mM K_2HPO_4, and 1 mM PMSF. After homogenization and centrifugation (18,000*g*, 40 min), the pellet was washed in the same buffer and centrifuged for 10 min at 18,000*g*. The supernatant from the wash was added to the initial supernatant and served as the crude soluble enzyme. The remaining pellet was suspended in 2 mL of the extraction buffer and served as the crude insoluble enzyme.

Starch synthase activity was assayed spectrophotometrically by measuring the ADP-Glc-dependent production of ADP. For the soluble enzyme the assay buffer contained, in a total volume of 0.75 mL, 100 mM *N*-tris(hydroxymethyl)methylglycine (pH 8.6), 25 mM potassium acetate, 2 mM DTT, 2 mM ADP-Glc, 3.75 mg of insoluble amylopectin, and 0.2 mL of enzyme extract. Reactions were carried out for 15 min at 30°C and terminated by boiling for 1 min. Blank reactions were performed and the enzyme was added immediately prior to boiling. Insoluble starch synthase was assayed as above, but without priming with amylopectin. The ADP product was measured via an enzyme-linked assay with pyruvate kinase/lactate dehydrogenase, according to a modification of the method of Jaworek and Welsch (1985), after centrifugation in a reaction mixture containing 50 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$, 20 mM KCl, 0.2 mM NADH, 0.8 unit of pyruvate kinase and 1.2 units of lactate dehydrogenase in a mixture (Sigma) at 37°C for 30 min. Reactions were begun with 1 mM PEP, and NAD^+ production was measured as A_{340}.

Electrophoresis and Immunoblotting

Protein was extracted in 50 mM Hepes (pH 7.5), 1 mM EDTA, 1 mM $MgCl_2$, 2.5 mM DTT, 3 mM DIECA, and 1 mM PMSF. Twenty to 30 μg of protein was subjected to SDS-PAGE (10–15% gradient, Mini-Electrophoresis System, Bio-Rad) according to the manufacturer's instructions, and proteins were transferred to nitrocellulose membranes (Electro Transblot apparatus, Bio-Rad). Western blots were performed with the following antibodies, kindly supplied by the respective researchers: fructokinase from pea seed (D.D. Randall), ADP-Glc PPase from tomato fruit (H. Janes), Suc synthase from corn endosperm (K. Koch), and acid invertase from tomato fruit (A.B. Bennett). Bands were visualized using 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (Bio-Rad) according to the manufacturer's specifications.

RESULTS

Transient starch accumulation in developing tomato fruit pericarp of cv F144 is presented in Figure 2, and cross-sections of the three developmental stages are shown following iodine staining in Figure 3. Starch levels reached approximately 25 mg/g fresh weight in the pericarp of immature fruit, but decreased to insignificant levels by the mature stage. The starch was predominantly localized in the columella and placental tissue and in the inner layers of the pericarp tissue, but the gelatinous tissue, seeds, and outer layers of the pericarp tissue were characterized by low levels of starch (Fig. 3B).

Enzyme Activities during Fruit Development

A developmental survey of the activities of the enzymes involved in the Suc-to-starch metabolic pathway (Fig. 1) is presented in Figure 4. Two distinct developmental patterns of activities could be discerned, which paralleled the decrease in starch levels. The enzymes Suc synthase, fructokinase, ADP-Glc PPase, and starch synthase all showed developmental losses of activity, whether presented on a fresh weight or protein basis, and paralleled the decrease in starch concentration. On the other hand, acid invertase, UDP-Glc PPase, hexokinase, NDP kinase, PGM, and PGI showed more moderate developmental decreases in activity. This is especially evident when activity is expressed on a protein basis, since protein levels of developing tomato fruits decrease significantly during development (Fig. 2b) and enzymes maintaining constant specific activity would

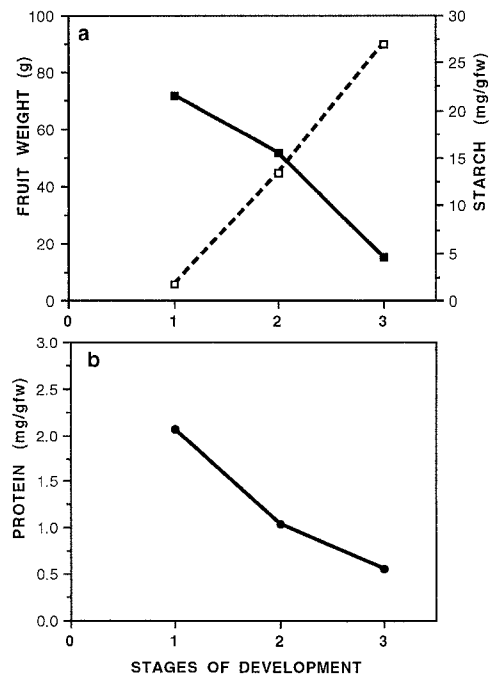

Figure 2. Fruit fresh weight and starch and protein concentrations in tomato cv F144 pericarp throughout development. a, ■, Pericarp starch concentration; □, fruit fresh weight; b, pericarp protein concentration. The developmental stages are shown in Figure 3. The data represent the average of six individual fruits. gfw, Gram fresh weight.

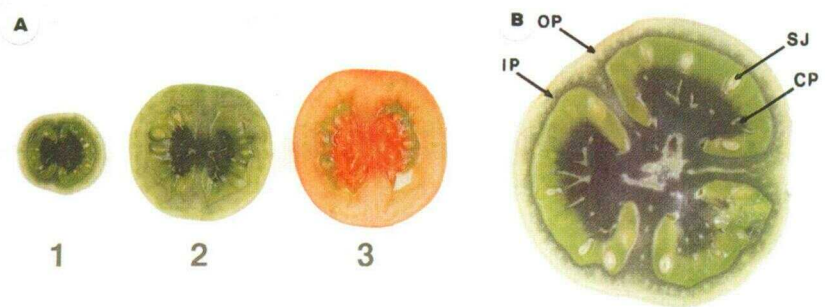

Figure 3. A, Three stages of tomato fruit development (cv F144) used in the present study. Freehand cross-sections were stained for starch with I_2/KI. B, Cross-section of immature tomato fruit stained with I_2/KI. The outer pericarp (OP) is delineated from the inner pericarp (IP) by a border of vasculature. The columella and placenta (CP) tissues are heavily stained and show nonstaining vasculature within. The gelatinous tissue and embedded seeds (SJ) are not stained.

appear to decrease on a fresh weight basis. Specific activities of UDP-Glc PPase, PGI, and invertase actually increased with development, whereas PGM and NDP kinase maintained constant specific activity between the first two stages studied, when starch content decreases.

The decline in specific enzyme activities was accompanied by decreases in the respective enzyme protein levels, as observed by immunoblotting performed with available polyclonal antibodies. Protein levels of Suc synthase, fructokinase, and ADP-Glc PPase decreased in the mature fruit, which contrasted with invertase, the activity and protein level of which dramatically increased during tomato fruit development (not shown).

A comparison of the maximum activity values that we observed during this study of the enzymes in the Suc-to-starch metabolic pathway is presented in Table I. These values are the enzyme activities at developmental stage 1 (Fig. 4). To relate these values to the approximate maximum rate of starch synthesis in tomato fruit, we have calculated the latter from data reported by Ho et al. (1983) and Ho and Hewitt (1986) as approximately 20 nmol Glc g^{-1} fresh weight min^{-1}.

Table I indicates that UDP-Glc PPase, NDP kinase, PGI, and PGM appeared to be in great excess compared with the estimated starch accumulation rate. Activity of Suc synthase was significantly higher than that of invertase, but both initial Suc cleavage enzymes appeared to be in excess in relation to the rate of starch accumulation, although less so than the previously mentioned enzymes. In vitro fructokinase activity, in the absence of inhibitory Fru levels (see "Discussion"), did not appear to be rate-limiting for starch synthesis. The enzymes ADP-Glc PPase and starch synthase seem the most obvious candidates for limiting steps based on comparative in vitro extractable activities, although their observed in vitro activities were sufficient to account for the rate of starch accumulation.

Tissue Localization of Starch and of Suc-to-Starch Metabolism

Iodine staining of developing fruit (Fig. 3B) and measurement of starch levels (Table II) showed that starch content was relatively low in the seed cavity/jelly, the vascular bundles, and the outer portion of the pericarp of immature tomato fruit. High starch levels were observed in the inner portion of the pericarp, the radial pericarp, and the columella and placenta.

A comparison of enzyme activities in the various tissues showed that the low starch levels in the seed cavity/jelly were associated with lower activities of Suc synthase, UDP-Glc PPase, PGM, ADP-Glc PPase, and starch synthase compared with the activities in either of the high-starch-accumulating tissues, the columella and inner pericarp. The seed cavity was not characterized by low protein levels or low extractable enzyme activities in general, as shown by the comparative activities of acid invertase, which is highest in the seed cavity. Protein concentrations of the four tissues studied were not significantly different, and data are presented only on a fresh weight basis.

In the low-starch-accumulating outer pericarp, only the activities of ADP-Glc PPase and the insoluble starch synthase were significantly lower than those in the high-starch-accumulating tissues. Activities of the remaining enzymes assayed were similar in the outer pericarp and in the columella and inner pericarp tissues. With regard to the starch synthases, activity of insoluble starch synthase was highest in the columella and placenta and approximately equal to the activity of the soluble starch synthase in this tissue. In the other tissues, activity of the soluble enzyme was 10 to 30 times higher than that of the insoluble enzyme.

Results of immunoblots of protein from the four tissues, using antibodies to invertase, Suc synthase, fructokinase, and ADP-Glc PPase, are presented in Figure 5. Most striking is the decreased abundance of ADP-Glc PPase protein in the outer pericarp and the seed cavity/jelly, corresponding to its reduced activity in these tissues. Suc synthase and fructokinase showed somewhat less protein in the seed cavity/jelly tissue compared with the other tissues. The 52-kD band of invertase (Yelle et al., 1991) is more abundant in the seed cavity/jelly tissue. We did not further investigate the significance of the approximately 48-kD band and the tissue-specific differences in the relative staining of the two bands.

DISCUSSION

The Suc-to-starch synthetic pathway (Fig. 1) in tomato fruit appears to be under developmentally coordinated control. Four of the enzymes in the pathway—Suc synthase, fructokinase, ADP-Glc PPase, and starch synthase—show dramatic decreases in activity that occur concomitant with the decrease in starch content. The coordination spans organellar compartmentation, since the initial two enzymes in the pathway are purportedly cytosolic (Kruger, 1990)

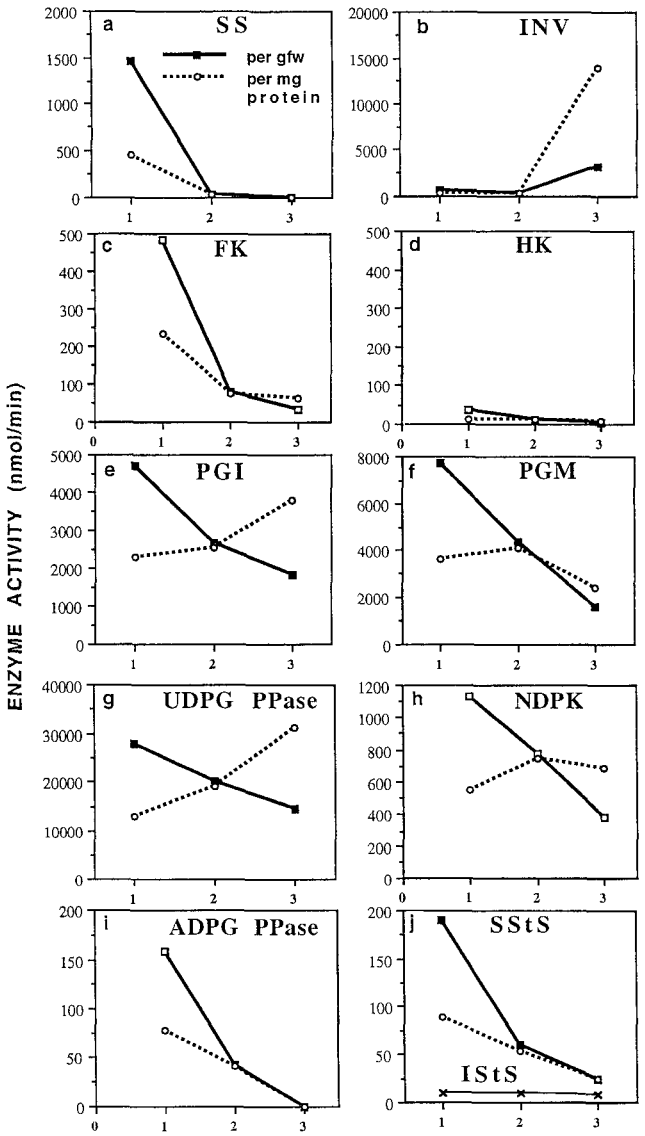

Figure 4. Enzyme activities in developing pericarp (outer plus inner) of fruit of cv F144. The three stages studied correspond to those pictured in Figure 3A. Solid lines represent activities expressed on a fresh weight basis; broken lines represent activities expressed on a protein basis. a, Suc synthase (SS); b, acid invertase (INV); c, fructokinase (FK); d, hexokinase (HK); e, PGI; f, PGM; g, UDP-Glc PPase; h, NDP kinase; i, ADP-Glc PPase; j, soluble starch synthase (SStS) and insoluble starch synthase (IStS). Insoluble starch synthase is presented only on a fresh weight basis and is represented by the symbol ×. Each data point is the average of a minimum of three assays from individual fruits. gfw, Gram fresh weight.

and the latter two are presumably plastidic (Boyer, 1996), although recent evidence indicates a cytosolic ADP-Glc PPase in maize endosperm (Denyer et al., 1996). Furthermore, these same four enzymes are also potentially limiting to Suc-to-starch flux, as indicated by comparison of their in vitro activities (Table II). With all of the limitations inherent in basing conclusions on in vitro activities (see ap Rees and Hill, 1994), such a comparison can nevertheless be useful in pointing to potentially limiting steps. Although we cannot be certain that our observed "maximal" extractable activities are in fact such, ADP-Glc PPase and starch synthase are closest to the rate of starch accumulation, as calculated from previous reports (Ho et al., 1983; Ho and Hewitt, 1986). Starch synthase activities often appear limiting to the starch accumulation rate in tissues such as maize endosperm (Ozbun et al., 1973) and pea embryo (Edwards et al., 1988; see Kruger, 1990). With regard to ADP-Glc PPase, transgenic potato tubers (Stark et al., 1992) and tomato fruit (Stark et al., 1996) with a nonallosterically controlled ADP-Glc PPase enzyme had increased starch levels, indicating that the in vivo flux through this step in biosynthesis is limiting to starch synthesis.

The activities of Suc synthase and fructokinase appear to be in excess of the starch accumulation rate, although significantly less so than those of the other enzymes in the pathway (UDP-Glc PPase, PGI, and PGM). However, the in vivo activities of Suc synthase and fructokinase may be well below their in vitro extractable activities, since both may be significantly inhibited by physiological levels of Fru in tomato fruit. Both fructokinase and Suc synthase may be inhibited by Fru (i.e. Renz and Stitt, 1993; Sebkova et al., 1995), and we have observed that the high physiological concentrations of Fru in tomato fruit (in excess of 30 mM) inhibit both fructokinase and Suc synthase activities by at least 70% (Schaffer and Petreikov, 1993; A.A. Schaffer and M. Petreikov, unpublished data). Accordingly, all four of the enzymes in which the developmental loss of activity is correlated with the cessation of starch synthesis may potentially be limiting to the flux of starch synthesis in tomato fruit.

The results of the present study support the notion that Suc-to-starch metabolism in young tomato fruit occurs via the Suc synthase pathway. This is indicated by the temporal developmental pattern of Suc synthase activity and its coordination with the activities of other enzymes of

Table I. *Maximum catalytic activities of enzymes of Suc-to-starch metabolism and the rate of starch accumulation in immature tomato fruits*

Data for enzyme activities are taken from the developmental study shown in Figure 4 (stage 1) and are based on a minimum of three individual extractions from individual fruits. The starch accumulation rate is an approximate calculation from Ho et al. (1983) and Ho and Hewitt (1986).

Enzyme	Activity
	nmol g^{-1} fresh wt min^{-1}
Suc synthase	1,800
Invertase	920
Fructokinase	783
Hexokinase	43
UDP-Glc PPase	35,400
NDP kinase	1,300
PGI	5,300
PGM	10,000
ADP-Glc PPase	160
Starch synthase (soluble)	232
Starch synthase (insoluble)	17
Starch accumulation rate	20

Table II. *Distribution of carbohydrates, protein, and enzyme activities in tissue portions of immature (approximately 14 d after flowering) tomato fruit (cv F144)*

Each value is the average of measurements from three individual fruits. Values followed by the same letter within a row are not statistically different at the 5% level using Duncan's multiple comparison test.

Component	Outer Pericarp	Inner Pericarp	Columella + Placenta	Jelly + Seed Cavity
		mg g^{-1} fresh wt		
Starch	8.6 b	40.6 a	52.6 a	12.5 b
Glc	6.1 b	11.9 a	6.9 b	7.1 b
Fru	5.4 c	11.0 a	8.0 b	5.4 c
Protein	2.1 a	1.9 a	2.0 a	2.2 a
		nmol g^{-1} fresh wt h^{-1}		
Enzyme				
Suc synthase	1,000 a	870 a	1,150 a	330 b
Invertase	490 a	950 a	760 a	1380 a
Fructokinase	780 a	767 a	689 a	425 b
Hexokinase	45 a	57 a	67 a	40 a
UDP-Glc PPase	21,400 a	26,440 a	15,750 a	3340 b
NDP kinase	4,470 a	4,670 a	4,400 a	4200 a
PGI	6,870 a	5,320 a	5,330 a	5360 a
PGM	5,410 a	6,750 a	5,500 a	1210 b
ADP-Glc PPase	33 b	257 a	261 a	44 b
Starch synthase (soluble)	131 ab	210 a	119 ab	16 b
Starch synthase (insoluble)	6 b	21 ab	93 a	3 b

starch synthesis, particularly fructokinase. Relatively high fructokinase-to-hexokinase ratios are characteristic of other starch-synthesizing tissues such as lima bean seeds and potato tubers (Xu et al., 1989; Renz et al., 1993; Ross et al., 1994). However, a contributory role for invertase in Suc metabolism in the young fruit, particularly that not related to the synthesis of starch, cannot be ruled out, and the relative contribution of invertase to Suc metabolism in the young fruit still remains to be determined. Ho and Hewitt (1986) deduced that at its peak, starch accumulation could account for 30% of the dry weight accumulation in the tomato fruit. If Suc synthase were part of a dedicated metabolic pathway of starch accumulation and cell wall synthesis (Amor et al., 1995), there would still be room for a role for invertase hydrolysis as part of Suc metabolism in the young tomato fruit.

The localization of starch in young fruit is tissue-specific. Wang et al. (1994) also reported that starch is accumulated in the columella, radial pericarp, and inner pericarp, with lower levels in the outer pericarp and seed jelly, although there have been reports that the seed cavity does contain starch (Janes, 1941; Davies and Cocking, 1965). Our results indicate that the spatial distribution of starch can be accounted for by Suc-to-starch metabolic enzyme activities. The low starch level in the jelly is related to a decreased activity of most of the starch synthesis pathway, both cytosolic and plastidic. On the other hand, the outer pericarp is characterized by low activities of only the presumably plastidic ADP-Glc PPase and insoluble starch synthase, whereas the cytosolic enzyme activities are similar to those in the starch-accumulating tissues.

The results of the present study suggest the potential for fruit anatomical variability in the contribution to total fruit starch content. It might be expected that tomato types with a high ratio of columella + placenta to jelly would have a higher starch content in the whole fruit. It is interesting that the temporal metabolic control of starch synthesis is not identical to the spatial control. Whereas

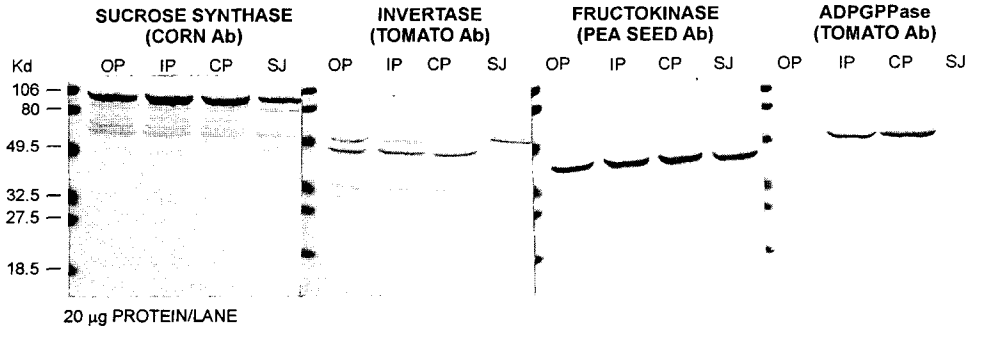

Figure 5. Immunoblots of proteins extracted from the four tissues of immature tomato fruit pictured in Figure 3B. OP, Outer pericarp; IP, inner pericarp; CP, columella + placenta; and SJ, gelatinous tissue + seeds. Immunoblots are with polyclonal antibodies to acid invertase, fructokinase, Suc synthase, and ADP-Glc PPase. Ab, Antibody.

temporal changes are manifest in the four enzymes with activities that are potentially limiting to starch synthesis, the spatial differences are not limited only to these enzymes.

The contribution of starch accumulation in the immature fruit to soluble sugar levels in the mature fruit will depend on the size of the transient reservoir of polysaccharide. This in turn will be a function of the concentration of starch and the size of the reservoir, which itself will be determined by the length of the starch accumulation period, the growth rate of the fruit, and the anatomical distribution of starch within the fruit. A simple calculation (ignoring the role played by daily starch turnover) shows that a mid-size tomato cultivar (100 g) that accumulates a transient maximum of 2% starch on the basis of the fresh weight of the bulk fruit tissue when the fruit is small (i.e. 10 g) will contain a potential reservoir of only 200 mg of starch, an insignificant contribution to the carbon economy of the mature fruit. However, a genotype that continues active starch accumulation for an extended period and reaches 3% starch when the fruit weight is 50 g will have a reservoir that could affect final fruit sugar content. Thus, multiple strategies are available for increasing the starch reservoir of developing fruit: increasing the rate of starch synthesis, expanding the relative contribution of starch-synthesizing tissues to the fruit bulk, and extending the period of starch accumulation.

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