Sucrose Synthase in Wild Tomato, *Lycopersicon chmielewskii*, and Tomato Fruit Sink Strength

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ABSTRACT

Here it is reported that sucrose synthase can be readily measured in growing wild tomato fruits (Lycopersicon *chmielewskii*) when suitable methods are adopted during fruit extraction. The enzyme also was present in fruit pericarp tissues, in seeds, and in flowers. To check for novel characteristics, the wild tomato fruit sucrose synthase was purified, by $(NH_4)_2SO_4$ fraction and chromatography with DE-32, Sephadex G-200, and PSA-60, to one major band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The following characteristics were obtained: native protein relative molecular weight 380,000; subunit relative molecular weight 89,000; K_m values with: sucrose 53 millimolar, UDP 18.9 micromolar, UDP-glucose 88 micromolar, fructose 8.4 millimolar; pH optima between 6.2 to 7.3 for sucrose breakdown and 7 to 9 for synthesis; and temperature optima near 50°C. The enzyme exhibited a high affinity and a preference for uridylates. The enzyme showed more sensitivity to divalent cations in the synthesis of sucrose than in its breakdown. Sink strength in tomato fruits also was investigated in regard to sucrose breakdown enzyme activities versus fruit weight gain. Sucrose synthase activity was consistently related to increases in fruit weight (sink strength) in both wild and commercial tomatoes. Acid and neutral invertases were not, because the published invertase activity values were too variable for quantitative analyses regarding the roles of invertases in tomato fruit development. In rapidly growing fruits of both wild and commercially developed tomato plants, the activity of sucrose synthase per growing fruit, i.e. sucrose synthase peak activity X fruit size, was linearly related to final fruit size; and the activity exceeded fruit growth and carbon import rates by at least lo-fold. In mature, nongrowing fruits, sucrose synthase activities approached nil values. Therefore, sucrose synthase can serve as an indicator of sink strength in growing tomato fruits.

In working on sucrose breakdown in various types of plant tissues, we have sought to identify metabolic activities that act as control points and that may be useful as biochemical indicators of plant sink strength. The enzyme activities identilled include sucrose synthase or acid invertase in specific plant sink tissues along with the PPi-dependent phosphofructokinase and fructokinase in some sink tissues (20-22, 28).

For example, from work with various plant sinks, it was concluded that sucrose synthase activity was a biochemical indicator of sink strength in growing *Solanum tuberosum* tubers, in developing lima bean seeds, and seasonally in the vascular cambial tissue of tree roots (20, 2 1, 28). In contrast, recent work on carbohydrate assimilation with tomato fruits (29, 30) reported that sucrose synthase activity was not detectable at any time during fruit development with the wild tomato *(Lycopersicon chmielewskii)*, and acid invertase activity was low throughout fruit development. In the same studies, fruits of commercial tomato varieties *(Lycopersicon esculentum)*, expressed both an active sucrose synthase and an acid invertase. With commercial tomatoes, the specific activity of sucrose synthase also changed markedly during fruit development in correlation with ADP-glucose pyrophosphorylase; and both of these activities correlated with a transient starch accumulation pattern found in developing fruits (17). Other work also suggests that ADP-glucose pyrophosphorylase activities may correlate with sink development, particularly in starch-accumulating sinks (15).

The apparent absence of sucrose synthase in a low-yielding wild tomato plant fruits and its presence at good activity levels in high-yielding commercial tomato varieties appeared to be related to yield and sink strength in tomatoes (17, 29, 30). However, a recent report (IO) with another wild tomato *(Lycopersicon hirsutum)* demonstrated a low-activity sucrose synthase throughout fruit development. In earlier sink strength work with tomato fruits, other workers concluded that "sink activity" was a more primary determinant of sink strength than sink size (1, 26). Most enzyme work on tomato fruit sink activity suggested that invertase activity may be one determinant. A few reports have appeared on sucrose synthase in tomato fruits (8, 10, 17, 29, 30), but none concluded that its activity was an indicator of sink strength or that it is strongly related to fruit growth. Indeed, the possible absence of sucrose synthase in wild tomato fruit could strengthen the theory that invertase activity correlates with tomato fruit sink growth (8, 9, 27).

Because of our studies on identifying metabolic components of sink strength to use in genetic and molecular modifications of plant sinks, we investigated the three initial enzymes of sucrose breakdown in developing tomato fruits. In addition, the inability to detect sucrose synthase in wild tomato (30) concerned us because we had found an active enzyme in growing tubers of the wild potato *(Solanum acule)*,

which also is a low-yielding noncultivated plant in the Andes (25). Therefore, in 1988 sucrose synthase was assayed in wild tomato fruits and the activity was found immediately when we employed the protein extraction procedures used with potatoes. In this work, we investigated the problem of obtaining an active sucrose synthase from wild tomato fruits; then we asked if the wild fruit sucrose synthase possessed any novel biochemical characteristics, so the enzyme was purified and partially characterized; and finally we attempted to clarify whether or not sucrose synthase and acid or neutral invertase were fruit sink strength indicators in either wild or commercial tomatoes.

MATERIALS AND METHODS

Wild tomato *(Lycopersicon chmielewskii)* seed *was* obtained from The University of California, Davis, planted, and grown in the greenhouse under standard cultural conditions to produce vigorous plants. All chemicals and coupling enzymes were purchased from Sigma or Boehringer.

Sucrose Synthase Extraction

Freshly harvested fruits were homogenized with a Waring blender for 1 min at top speed or with a mortar and pestle followed by OMNI 1000 portable mini-homogenizer (Biotech) for 1 min. All steps were carried out at 4°C. The standard extraction solution (buffer A) contained 200 mM Hepes-KOH (pH 7.0), 3 mM Mg-acetate, 0.5 mM EDTA, 0.5 mM PMSF, 5 mM DTT, 20 mM 2-mercaptoethanol, 5% (v/v) glycerol, 1% (w/v) insoluble PVP-360, and 1% (w/v) Dowex-1 chloride form. The ratio of fruit fresh weight(g) to extraction solution volume (mL) was 1:5. The homogenate was passed through one layer of nylon cloth and was centrifuged at 34,000g for 20 min. The supematant was desalted through Sephadex G-25 and this extract was used to assay initial activities of sucrose synthase and soluble invertases.

Sucrose Synthase Purification

For further study on the biochemical characteristics and regulation of sucrose synthase, the enzyme was purified by solid $(NH_4)_2SO_4$ fraction, DEAE-cellulose ion exchange column chromatography, Sephadex G-200 gel filtration, and PBA affinity column chromatography in a procedure similar to that of Morell and Copeland (11). Fifty-six grams of freshly harvested young fruits (<0.4 g each) were homogenized with 280 mL of buffer A as described above. After passing through one layer of nylon cloth and centrifuging at 10,000g for 20 min, the supematant was fractionated with 40 to 70% $(NH_4)_2SO_4$. The 70% $(NH_4)_2SO_4$ pellet, after centrifuging at 10,000g for 10 min, was resuspended in a solution of 200 mM Hepes-KOH (pH 7.0), 0.5 mM EDTA, 3 mM Mg-acetate, 20 mM 2-mercaptoethanol, and 20% (v/v) glycerol. Before loading on a DE-32 column (1.5 x 7 cm), the preparation was dialyzed against 10 mM K-phosphate (pH 7.2) containing 0.5 mM EDTA and 5 mM 2-mercaptoethanol (buffer B). The column was washed with buffer B and then eluted with a gradient produced by introducing 250 mL of 0.3 M KCl in buffer B into 250 mL of buffer B. Fractions of 5.1 mL were

collected. Fractions with activity (Nos. 29-60) were pooled and $(NH_4)_2SO_4$ was added to 70%. After centrifugation at 10,000g for 10 min, the pellet was resuspended in 2 mL of 50 mM Hepes-KOH buffer (pH 7.0) containing 0.5 mM EDTA, 50 mM KCl, 1 mM Mg-acetate, and 5 mM 2-mercaptoethanol (buffer C). Then the supematant was loaded on a Sephadex G-200 column (2.2 x 85 cm) and eluted with buffer C. Fractions of 3.1 mL were collected. Active fractions with the least contamination of unwanted proteins, determined by SDS-PAGE, were pooled and dialyzed against 50 mM Hepes-KOH (pH 8.5) containing 5 mM sucrose, 10 mM Mg-acetate, and 5 mM 2-mercaptoethanol (buffer D). The preparation was applied to a PBA-60 affinity column equilibrated with 100 mL of buffer D containing 200 mM sucrose. Unbound proteins were washed out with 10 mL buffer D. Sucrose synthase was eluted with 100 mM Tris-HCl (pH 8.5) containing 1 mM Mg-acetate and 5 mM 2-mercaptoethanol. The active fractions were collected and dialyzed against buffer B. This preparation was used for the biochemical characterization of the wild tomato fruit sucrose synthase.

Sucrose Synthase "Inactivator" Preparation

Freshly harvested wild tomato fruits were homogenized with 100 mM Hepes-NaOH (pH 7.5), 0.5 mM EDTA, 0.5 mM PMSF. The homogenate was passed through one layer of nylon cloth and was centrifuged at 34,000g for 20 min. Sucrose synthase activity was not detectable in the preparation.

Enzyme Assays

Sucrose synthase activity in the breakdown direction was assayed in a 1 mL reaction mixture containing 100 mM Mes (pH 6.5), 3 mM Mg-acetate, 0.5 mM EDTA, 5 mM 2-mercapmethanol, 0.02 mM glucose 1,6-diP, 0.5 mM NAD, 1 mM UDP, 1 mM PPi, 50 mM sucrose (for crude enzyme), or 200 mM sucrose (for purified enzyme), 1 unit phosphoglucomutase, 2 units glucose&P dehydrogenase (from *Leuconostoc*), and 1 unit UDP-glucose pyrophosphorylase was added for purified enzyme assays. NADH production was monitored continuously with a Beckman DU-7 spectrophotometer at 340 nm. Sucrose synthase activity in the synthetic direction was assayed in a 1 mL reaction mixture containing 100 mM Bicine (pH 8.5), 25 mM Mg-acetate, 75 mM KCl, 0.2 mM UDP-glucose, 4 mM P-*enol*pyruvate, 15 mM fructose, 0.15 mM NADH, 2 units pyruvate kinase, 2 units lactic dehydrogenase. A control, without fructose, was run simultaneously. The oxidation of NADH was followed continuously at 340 nm. For studies on the regulation of purified sucrose synthase, the enzyme was incubated with substrates at 25°C for 30 min (sucrose breakdown) or 15 min (sucrose synthesis) and then boiled for 5 min and assayed by the coupling enzymes stated above. To determine the nucleotide diphosphate specificity of purified sucrose synthase breakdown activity, the enzyme was assayed in 1 mL reaction mixtures containing 100 mM Mes (pH 6.5), 200 mM sucrose, 1 mM ATP, 0.5 mM NAD, 1 unit hexokinase, 1 unit phosphoglucose isomerase, 2 units glucose-6-P dehydrogenase, and 1 mM nucleotide diphosphate was added to start the reaction. The soluble invertases were as-
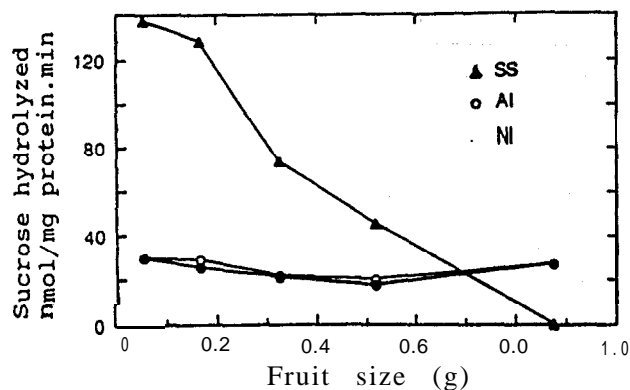
Figure 1. Wild tomato fruit size versus the specific activities of sucrose synthase (A), acid invertase (0), and neutral invertase(●).

sayed in K-phosphate/citrate buffer at pH 5.0 and 7.0 for acid and neutral invertase, respectively, as described in more detail by Xu et al. (28). All enzyme assays were done at 25°C unless otherwise stated. Protein content was determined using the Bradford procedure with BSA as the standard (2).

RESULTS

Sucrose Synthase Extraction and Measurement in Wild Tomato Fruits

Sucrose synthase activity was not detectable when the Yelle et al. (30) extraction method was used with freshly harvested wild fruits. Protein samples extracted without PVP and/or reducing agents (see "Materials and Methods") also contained no detectable sucrose synthase activity; in addition, the extracts inactivated sucrose synthase from other plants. The inactivation effect could be removed by dialysis and desalting the extracts. Sucrose synthase inactivation by the extract was independent of preincubation time and was irreversible (data not shown). The apparent "inactivator" in wild tomato seemed different from that in wheat, in which a proteinaceous inhibitor was found and purified (4, 14). The main reason for the lack of sucrose synthase was the high content of phenolic type substances in wild tomato fruits. In addition, the Yelle et al. (30) buffer concentration was only 50 mM, which did not maintain the extract pH when their extraction procedures were followed. Therefore, upon varying the extraction buffer

(pH and concentration); the ratio of fruit fresh weight to extraction buffer; plus adding PVP; and increasing the reducing agent concentration, sucrose synthase activity could be readily assayed in wild tomato fruits.

Suitable sucrose synthase extraction procedures and assay reaction mixtures are given in "Materials and Methods." Using these techniques, sucrose synthase was measured at various fruit growth stages. The specific activity of sucrose synthase decreased with increasing fruit fresh weight (Fig. I). In full-sized wild fruits (0.8-0.9 g), sucrose synthase activity was extremely low or not detectable. This pattern of decreasing sucrose synthase specific activity was quite similar to that reported with commercial tomato fruits at various periods after anthesis (17, 30).

Sucrose synthase was measured in freshly separated pericarp tissues and seeds of wild tomato fruits and found in both tissues (Table 1). On a per milligram protein and on a per fruit basis, sucrose synthase breakdown and synthetic activity was higher in seeds than in pericarp. In contrast, both acid and neutral invertase activities were higher in pericarp tissues than in seeds. Sucrose synthase was active in wild tomato flowers also (data not shown).

Storage Stability of Sucrose Synthase Activity in Detached Fruits and Extracts

In the work of Yelle et al. (30), the fruits were harvested over a 64-d period and stored at -70'C. Later, the fruits were extracted and enzyme activities were determined. We also examined the stability of sucrose synthase in detached fruits and in storage. Wild tomato fruits, stored in liquid N_2, lost as much as half of their sucrose synthase activity within 2 weeks, whereas fruits kept at 4°C for 5 d to 2 weeks, with humidity, had no significant loss of sucrose synthase activity. In another study, if detached fruits (about 0.4 g) were kept humidified (in an H_2O-saturated atmosphere), sucrose synthase activity decreased slowly, about 17% within several days at 25°C, whereas in nonhumidified fruits sucrose synthase activity decreased about 89% by 7 d after detachment (Fig. 2). Using identical conditions, sucrose synthase activity was assayed in detached fruits of commercial tomato varieties; no significant differences were observed between humidified and nonhumidified environments, with fruit in both conditions losing about 20% of their activity in 1 week (data not shown). Hence, the Yelle et al. (30) storage procedures should not have

Table I. Sucrose Synthase and Invertase Activities in Pericarp and Seeds of Wild Tomato Fruits
The fruit size was near 0.45 g.

Tissue	Sucrose Synthase				Invertases			
	per mg protein		per fruit		per mg protein		per fruit	
	Breakdown	Synthesis	Breakdown	Synthesis	AI'	NI[b]	AI	NI
				nmol/min				
Pericaro	61.4 (±0.1)	171.8 (±7.8)	27.7 (±0.1)	77.7 (±3.5)	66.8 (±9.5)	61.8 (±5.7)	30.2 (±4.3)	28.0 (±2.6)
Seeds	88.6 (k1.7)	205.4 (±12.1)	36.3 (±0.7)	84.1 (±4.9)	13.5 (±0.3)	11.5 (±0.6)	5.5 (±0.1)	4.7 (±0.2)

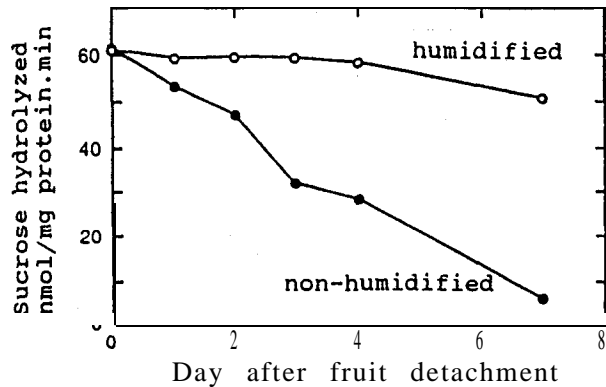[a] Acid invertase.   [b] Neutral invertase.

Figure 2. Storage of wild tomato fruits (about 0.4 g each) and stability of sucrose synthase. Detached fruits were stored at room temperature (near 22%) either in air (●) or in an atmosphere saturated with an open pan of water (0).

Figure 3. Responses of the wild tomato fruit purified sucrose synthase to pH for sucrose breakdown (open symbols) and synthesis (closed symbols). The pH values are for the reaction mixture at the start of the reaction. The buffers were Mes (circles), Hepes (triangles), and Bicine (squares).

reduced activity to zero. Sucrose synthase activity in detached wild and commercial tomato fruits was relatively unstable but was much more stable than, for example, sucrose synthase activity in detached potato tubers, which lost 95% activity in 3 d after detachment (22). Higher soluble solids content in wild tomato (5, 30), or a larger surface area per unit of fresh weight than market tomato fruits, or after-ripening process differences may be reasons for the unstable sucrose synthase in wild fruits, but these were not investigated.

Wild fruit protein extracts prepared as already described for sucrose synthase extraction (see "Materials and Methods"), when kept at -80°C for 1 week, showed no loss of sucrose synthase activity. After $(NH_4)_2SO_4$ fraction and dissolving in a small amount of 200 mM Hepes-KOH (pH 7.0) containing 3 mM Mg-acetate, 20 mM 2-mercaptoethanol, 20% glycerol, and 0.5 mM EDTA, the enzyme was stable and activity only decreased about 10% when stored for 1 month at 4°C. Each of these tests, with the extracted sucrose synthase of wild tomato fruits, demonstrated that the enzyme was easily stabilized.

Purification and Characterization of Wild Tomato Sucrose Synthase

Sucrose synthase was purified about 100-fold by the procedures stated in "Materials and Methods" and one major protein band was visible on SDS-PAGE. The specific activity was near 5.5 for breakdown and 7.5 for synthesis in μmol sucrose mg^{-1} protein min^{-1}. The molecular mass of the native enzyme was near 380 kD, determined by gel filtration. The molecular mass of the subunit was 89 kD, determined by SDS-PAGE. These specific activity and molecular mass values are similar to those found in other studies (3) such as with corn (19, 24), peach fruits (12), or soybean nodules (11). Hyperbolic activity responses were obtained with all substrates and the respective K_m values for sucrose, UDP, fructose, and UDP-glucose were 53 mM, 18.9 μM, 8.4 mM, and 88 μM, respectively. In comparison with the range of K_m values in the literature, 10 to 290 mM for sucrose, 0.1 to 6 mM for UDP, 2 to 8 mM for fructose, and 0.01 to 8 mM for UDP-
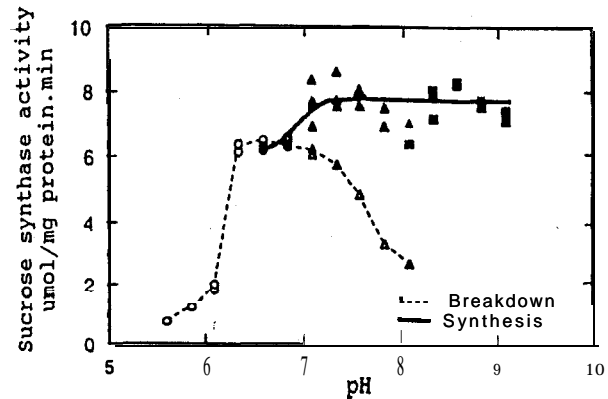
glucose (3, I 1– 13, 16, 18, 19, 24), wild tomato sucrose synthase had low K_m values for UDP and UDP-glucose. The enzyme was specific for uridylates, with less than 6% of the uridylate activity with other nucleotides.

Also with the purified protein, the following characteristics were obtained. The optimum pH was between 6.2 and 7.3 for sucrose breakdown and between 7 and 9 for sucrose synthesis (Fig. 3). Whether or not the small fluctuations of peak synthetic activity in Figure 3 resulted from the effects of buffers was not tested. Between 25 and 40°C, both synthetic and breakdown activities showed similar, almost linear, increasing responses to temperature (Fig. 4). From 40 to 50°C, the synthetic activity increased less than the breakdown activity. The enzyme was stable at 50°C for at least 15 min. Both activities showed a maximum near 50°C and deactivated completely at 60°C.

The synthetic activity of sucrose synthase was more sensitive to divalent cations than the breakdown activity. Synthetic activity was increased 100% by 10 mM Mg^{2+}, 37% by 10 mM
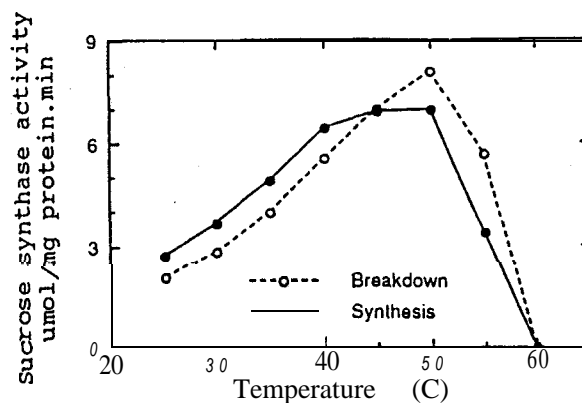

Figure 4. Temperature responses of the wild tomato fruit purified sucrose synthase for sucrose breakdown (0) and synthesis (0). The protein was incubated at each temperature for 15 min, the substrates were added, and the enzyme assayed.

Mn^{2+}, and 36% by 0.5 mM Ba^{2+}. Ca^{2+} inhibited synthetic activity about 15% at 0.5 mM and 40 to 60% at 5 mM. Only slight cation interactions were observed during synthesis in the presence of K', 1 to 7.5 mM, or NH_4^+, 1 to 5 mM. Sucrose synthase breakdown activity changed little, less than 5% of the control, in the presence of the same mono- and divalent cations. In addition, 100 mM Tris at pH 7.0 inhibited both the synthetic and breakdown activity by about 20%, and 100 mM Na-phosphate at pH 7.0 inhibited synthetic and breakdown activity by 14 and 35%, respectively. Both buffer inhibitions were reversible, These biochemical characteristics of the purified wild tomato fruit enzyme were similar to those summarized by Copeland (3) for other plants.

Changes in Sucrolytic Enzyme Activities during Fruit Growth and Fruit Sink Strength

Sucrose synthase activity was higher than both acid invertase and neutral invertase activities throughout most growing stages of wild tomato fruits. At maturity and ripening, near a 0.9 g fruit, the invertases retained activity but sucrose synthase was nearly absent. There was no detectable relationship between increasing fruit size and the small activity changes of either acid invertase or neutral invertase in the wild tomato (Fig. 1).

In a comparison study, we repeated the same experiments with a small market tomato available to us. This tomato had several dominant sucrose-cleaving activities during fruit development (Fig. 5). In the youngest fruits, sucrose synthase was the most prominent enzyme; in middle-size fruits, all three sucrolytic enzymes were active; in mature and ripening fruits, acid and neutral invertases were prominent enzymes. Sucrose synthase decreased its specific activity with fruit growth, whereas both acid and neutral invertase activities increased during earlier fruit development, then decreased somewhat before reaching full size. During ripening, acid invertase increased sharply, as many workers have reported (7–10, 27, 29). But sucrose breakdown activities, particularly for invertases, in the wild tomato (Fig. 1) were clearly different from a commercial tomato (Fig. 5), as others have reported (10, 17, 29, 30).

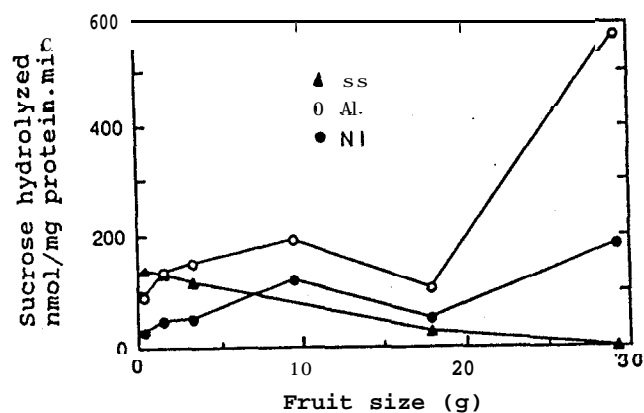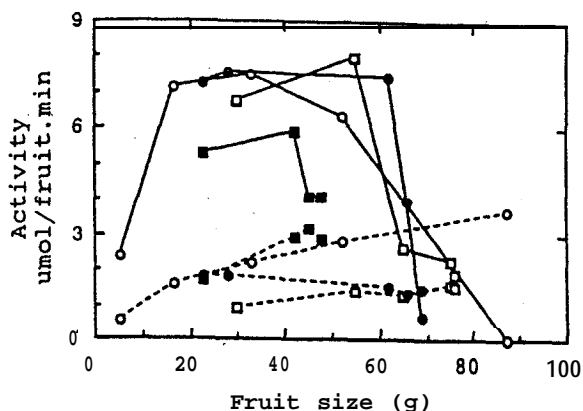The specific activity values in Figures 1 and 5 were con-

Figure 6. Total fruit activity for sucrose synthase (solid lines) and acid invertase (dashed lines) versus fruit size. Note the wild tomato values were multiplied by 100. Final fruit size was the fresh weight when growth weight curves plateaued. The tomato species or cultivar and the source of the data used to calculate the values in Figure 6 are: wild tomato (0, Fig. 1); UC82B (■, 17); LA1563 (●, 17); and VF145–17879 (□, 17).

vet-ted to total enzyme units per fruit (fresh weight basis) and plotted versus fruit size. We then compared the wild tomato data (Fig. 1) and the published data of other workers (10, 17, 29, 30). We found that all of the tomato sucrose synthase activity data were similar when plotted as in Figure 6. Note that values for wild tomato fruit size and enzyme activities were multiplied by 100 to plot on Figure 6. The only datum not used was a single 10^3 higher value (8). The curves of sucrose synthase, total activity per fruit versus fruit size, show the same pattern of a rapid early increase, remaining near a plateau during most of rapid fruit growth, and then declining to almost nil at mature full fruit size.

When we calculated total fruit invertase, acid or neutral, in a similar fashion from the same studies and plotted those data *versus* fruit size, we obtained the results plotted as dotted lines in Figure 6. These results show a gradual increase in invertase as fruit size increased. We knew that there were many more tomato fruit invertase data in the literature than sucrose synthase data. But, unfortunately, when we tried to compare the literature invertase activities, we found such wide fluctuations in these values that we were unable to make a critical quantitative evaluation of invertase activities and fruit sink strength in tomatoes. If these data were plotted, as in Figure 6, a scattergram-type plot was obtained.

DISCUSSION

In our studies, now covering three tomato growing seasons, sucrose synthase activity was readily detectable in actively growing wild tomato fruits when suitable extraction procedures were employed. The inability to detect the activity in this wild tomato earlier (30) likely was caused by unsuitable extraction procedures. Whole fruit homogenation using those procedures (30) led to a pH drop (data not shown) that acted like a reversible inhibition (23), but this could be remedied by increasing the buffer concentration. An unidentified "inactivator" was present in wild tomato fruit but this was

Figure 5. A commercial tomato fruit size versus the specific activities of sucrose synthase (▲), acid invertase (O), and neutral invertase (●).

overcome with PVP and reducing agents. A study of the biochemical properties of the purified wild fruit sucrose synthase did not reveal novel traits, except a high affinity and strong preference for uridylates. Hence, this research then focused on biochemical aspects of sink strength changes in developing tomato fruits.

During tomato fruit development, two stages can be distinguished: **first** the growth of the fruit to near full size, and second the fruit ripening process. This work does not attempt to explain sucrose metabolism during the fruit ripening process. Rather, the work attempts to clarify the role of sucrose synthase and invertases during fruit growth as "sink strength indicators." Sink strength has been considered as the product of sink size and sink activity (26). To determine sink activity, much work has been done on carbon import into developing tomato fruits (6, 26) and a few reports are available on enzymes that may be components of sink activity (7-10, 17, 29, 30). Previously, we reported that sucrose synthase was an indicator of sink strength in growing potato tubers (2 I) and in developing lima bean seeds (28). A hypothesis in our work is that the primary role of sucrose synthase is to cleave sucrose in the cytoplasm of growing sink cells to feed both hexoses into intermediary metabolism via the sucrose synthase pathway (2 1). Here, the work shows (a) that sucrose synthase, total peak activity per fruit during maximum fruit growth, was highly related to final fruit size, and (b) that sucrose synthase. can serve as one indicator of sink strength with growing tomato fruits. However, the available data on invertases were very inconsistent and a relationship to sink activity could not be discerned for growing fruits.

As we analyzed the sucrose synthase data such as that plotted in Figure 6, we realized that the plateau activity values were quite similar; particularly considering that the assay conditions in individual studies varied factors such as assay temperature (Fig. **4),** or direction of assay (Table I, Fig. **3),** or the **pH** (Fig. **3),** or the substrate concentration. Taking these factors into consideration, and knowing that final tomato fruit size varied from less than **l** g in wild tomatoes to values in the hundreds of grams in commercial varieties, we plotted the average plateau sucrose synthase activity values (Fig. 6) during maximum fruit growth *versus* the final fruit size for wild and commercial tomatoes and obtained the linear relationship shown in Figure 7, solid line. The correlation between sucrose synthase activity during most of fruit growth (Fig. 6) and the final fruit weight is strong (r = 0.9938). However, near mature fruit size, sucrose synthase activities approached nil values as shown in Figure 6 and plotted in Figure 7, dashed line. Earlier we demonstrated, with sinks such as tubers and seeds, a similar relationship with sucrose synthase having a high activity during maximum sink growth followed by a quick loss of activity at maturity (21, 22, 28). Hence, we propose that sucrose synthase is a biochemical indicator of sink strength during maximum tomato fruit growth.

The slope of the relationship in Figure 7 during maximum fruit growth can be calculated as a specific activity of 132 nmol g^{-1} fresh weight min^{-1} for sucrose synthase measured as breakdown activity at **25°C.** With this sucrose synthase activity (132 nmol g^{-1} fresh weight min^{-1}), a commercial tomato fruit of 50% maximum size could hydrolyze about 3 g of sucrose/day. Johnson *et* al. (8) reported a maximum dry
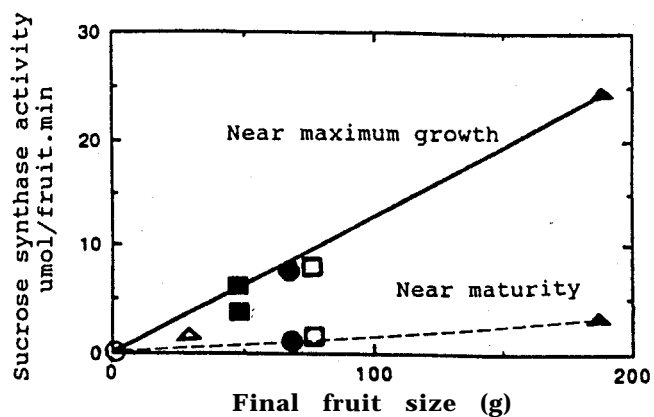

Figure 7. Relationships between tomato fruit weight at maturity and the total sucrose synthase activity near maximum fruit growth (——) or near fruit maturity (- - -). The total activity values were obtained by averaging the values near maximum growth or near maturity (as in Fig. 6) and correcting for differences in assay conditions such as temperature, substrate concentration, and reaction direction. The tomatoes were: wild (0); **UC82B (■);** LA1 563 (0); **VF145-17879 (□);** variety **Bigboy** (A); and a local variety **(A).**

matter accumulation rate of 0.37 g d^{-1} and **Walker and Ho** (26) obtained a carbon import rate of 0.08 g d^{-1} for a fruit of 50% maximum size. We recognize that sucrose synthase was assayed under optimum conditions *in vitro,* but the *in vivo* substrate concentrations, **pH,** temperature, etc. are unknown also. Even with these reservations, these enzyme activities are at least lo-fold greater than maximum growth rates. Thus, we conclude that the sucrose synthase activity reported here is more than sufficient to hydrolyze the sucrose imported for growth, including respiration.

In previous plant improvement work with tomatoes to increase fruit size, we speculated that selection followed the linear relationship between fruit size and activity during rapid fruit growth (Fig. 7, solid line). We propose that further work could continue this or one could endeavor to increase the slope by increasing the sucrose synthase activity in rapidly growing tomato fruits. Sucrose synthase is a readily reversible enzyme; hence, one can imagine it being involved in both the breakdown and the synthesis of sucrose. However, in growth sinks, such as a rapidly growing tomato fruit or a lima bean seed **(28),** which are not accumulating sucrose, we propose that the principal role of sucrose synthase is to break down sucrose and to feed it into the cellular metabolism supporting sink growth. In other words, sucrose synthase is a biochemical determinant of sink strength in growing tomato fruits.

We analyzed all of the available data on invertase activity as sink strength indicators; but combining these studies was not possible due to the wide variability in the reported enzyme activity values. For example, in work from the same laboratory on the same tomato variety, **UC82B,** acid invertase values were reported of: 75 to 100 **(17),** 400 to 1,200 **(30),** and 500 to 22,000 (29) nmol g^{-1} fresh weight min^{-1}. Other literature values contain similar orders of magnitude variations. **Invertase** activities are low in some wild tomato genotypes and sucrose-accumulating tomato genotypes, but high in common commercial tomato and nonsucrose-accumulating tomato

(10, 29, 30). Invertase activities generally peak later than sucrose synthase activity during fruit growth, which, perhaps is related to enlargement of sink cells, and usually have a sharp rise during the ripening process (8, 30), which seems related more to ripening or after-ripening of tomato fruits. Manning and Maw (9) and Iki *et* (7) reported a constant rise of acid invertase activity with fruit development; Johnson *et al. (8)* reported a constant stable acid invertase activity during most of fruit development, regardless of fruit growth rate, and then a sixfold increase of activity at ripening; whereas Yelle *et al. (30)* found a single peak curve with highest acid invertase activity at mid fruit development. It seems that the sharp rise in invertase activity at maturity may be related to the ripening process in tomato fruits. However, we conclude that the roles of invertases in tomato fruit development cannot be analyzed now using currently available work because (a) the specific activity values for acid invertase are too variable when one compares different studies; (b) the patterns of activity changes during fruit growth also vary greatly and in several instances show no relationship to fruit growth rates; and (c) neutral invertase has not been assessed yet in a variety of tomato plants.

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