

Acetaldehyde Stimulation of Net Gluconeogenic Carbon Movement from Applied Malic Acid in Tomato Fruit Pericarp Tissue^{1,2}

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ABSTRACT

Applied acetaldehyde is known to lead to sugar accumulation in fruit including tomatoes (*Lycopersicon esculentum*) (O Paz, HW Janes, BA Prevost, C Frenkel [1982] J Food Sci 47: 270–274) presumably due to stimulation of gluconeogenesis. This conjecture was examined using tomato fruit pericarp discs as a test system and applied *i*-[U-¹⁴C]malic acid as the source for gluconeogenic carbon mobilization. The label from malate was recovered in respiratory CO₂, in other organic acids, in ethanol insoluble material, and an appreciable amount in the ethanol soluble sugar fraction. In Rutgers tomatoes, the label recovery in the sugar fraction and an attendant label reduction in the organic acids fraction intensified with fruit ripening. In both Rutgers and in the nonripening tomato *rin*, these processes were markedly stimulated by 4000 ppm acetaldehyde. The onset of label apportioning from malic acids to sugars coincided with decreased levels of fructose-2,6-biphosphate, the gluconeogenesis inhibitor. In acetaldehyde-treated tissues, with enhanced label mobilization, this decline reached one-half to one third of the initial fructose-2,6-biphosphate levels. Application of 30 micromolar fructose-2,6-biphosphate or 2,5-anhydro- α -mannitol in turn led to a precipitous reduction in the label flow to sugars presumably due to inhibition of fructose-1,6-biphosphatase by the compounds. We conclude that malic and perhaps other organic acids are carbon sources for gluconeogenesis occurring normally in ripening tomatoes. The process is stimulated by acetaldehyde apparently by attenuating the fructose-2,6-biphosphate levels. The mode of the acetaldehyde regulation of fructose-2,6-biphosphate metabolism awaits clarification.

AA⁴ is a common volatile in plants that accumulate during physiological disorders (25) and in ripening fruit (7, 14). Though the metabolic role of AA is not clear, applied AA

appears to stimulate a respiratory upsurge in climacteric and nonclimacteric fruit including blueberry and strawberry (13) as well as in potato tubers (24) and an enhanced metabolite turnover in ripening fig (9). The action of AA may be independent of ethylene, because AA was shown on one hand to inhibit ethylene biosynthesis (E Pesis, personal communication) and on the other to promote softening and degreening in pear even when ethylene biosynthesis and action were arrested (14).

The finding that AA application is accompanied by an increase in the total sugars content in tomato (19, 21) raises the possibility of AA mediation of carbohydrate turnover and net carbon reallocation from reserve compounds to sugars. In banana, gluconeogenesis from starch degradation products was shown to be stimulated by ethylene (1). By comparison, AA stimulation of sugar accumulation is not restricted to fruit with pronounced ethylene synthesis and action (13, 19) or fruit enriched in reserve carbohydrates and may, therefore, entail the utilization of other reserve compounds for sugar synthesis. The AA-induced accumulation of sucrose in tomato fruit (21) may be particularly revealing because sucrose accumulation is generally construed as an index of gluconeogenic activity (30). This suggestion, together with the observation that in fruit AA applications are accompanied by a reduction in the organic acids content (19, 20), may indicate that AA stimulates gluconeogenic carbon flow from organic acids.

This hypothesis was tested by determining if the net label movement from applied ¹⁴C-labeled malic acid to the sugar fraction in tomato pericarp discs could be stimulated by AA and, conversely, if the process is arrested by inhibition of gluconeogenesis. In addition, we tested the possibility that AA stimulation of gluconeogenesis may be by mediating the level of Fru 2,6-P₂, the glycolysis-gluconeogenesis modulator in plants (12, 27).

MATERIALS AND METHODS

Chemicals

L-[U-¹⁴C]Malic acid (Amersham Corp.) with specific activity of 53 mCi/mol was used as a source metabolite for gluconeogenic sugar production. MS medium was purchased from Sigma and the gluconeogenesis inhibitor 2,5-AM-ol (22) from Calbiochem. Fru 2,6-P₂ was obtained from Sigma and organic acids standards were purchased from Merck, Sigma, and Aldrich.

¹ Supported by State funds and by grant I-900–85, The United States-Israel Binational Agricultural Research and Development Fund. New Jersey Agricultural Experiment Station, Publication No. D-12140–8–90.

² This work is in commemoration of Professor Jacob Biale, who inspired the present and other studies.

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⁴ Abbreviations: AA, acetaldehyde; 2,5-AM-ol, 2,5-anhydro- α -mannitol; Fru 2,6-P₂, fructose-2,6-biphosphate; TCAC, tricarboxylic acid cycle; MS medium, Murashige and Skoog basal medium.

Plant Material

Tomato (*Lycopersicon esculentum* Mill) fruit including Rutgers, a normally ripening cultivar, and the nonripening tomato mutant *rin* (23) were field or greenhouse grown. Rutgers tomatoes were harvested at mature-green, breaking (ripening), and the red ripe stage, and *rin* fruit were harvested 45 to 50 d following anthesis. Whole fruit were conditioned for a few days at room temperature and then placed in 4 L glass jars and ventilated for 24 h with air or air containing 4000 ppm AA as previously described (19). The concentration of AA was estimated by GC and verified against known concentration of AA standards (13, 14). These fruit were used for the studies outlined below.

Metabolism of 1-[U-¹⁴C]Malic Acid

Discs, 1 cm in diameter, were excised from the equatorial pericarp region of tomato fruit previously held in air or in AA. Six discs were placed in a sterile stoppered 50 mL Erlenmeyer flask together with a total of 1 mL MS medium containing 0.25 μ Ci of L-[U-¹⁴C]malic acid, representing 4.7 nmol malate. Alternatively, the labeled malate was applied together with 30 μ mol 2,5-AM-ol or 30 μ mol of Fru 2,6-P₂. Incubation was carried out in a water bath with gentle shaking at 25°C and at the end of the incubation periods four fractions were obtained for analysis: (a) released respiratory ¹⁴CO₂, (b) the soluble sugar fraction, (c) the organic acid fraction, and (d) a residual nonsoluble fraction. The respiratory CO₂ was collected on Whatman filter paper saturated with 200 μ L of 2 N KOH, kept in a well attached to the flask's stoppers to avoid contact with the incubation medium. To obtain the other fractions, the tomato discs were held in 5 mL 85% ethanol for 1 h at 60°C and the liquid fraction collected. The process was repeated twice more. The discs were then ground with a Virtis homogenizer in additional 5 mL 85% ethanol. The homogenate was filtered, and the ethanol-insoluble solid material (fraction d) was rinsed off the filter paper with 85% ethanol. The resulting ethanolic extracts were combined and evaporated *in vacuo* at 55°C to a 3 to 5 mL final volume water phase containing the soluble sugar and the organic acids fractions. The solution thus obtained was applied to a coupled 1 mL Dowex 50(H⁺) upper column and a lower Dowex 1 (formate) column. The columns were washed with 20 mL distilled water to elute the sugars (fraction b). The upper column (Dowex 50) was then drained dry and discarded. To obtain the bound organic acids (fraction c), the lower Dowex 1 column was eluted with 10 mL of 6 N formic acid followed by 10 mL of 12 N formic acid (29).

The water and formic acid eluents, containing the sugars and the acids, respectively, were collected separately in a 50 mL round bottom flask, and brought to dryness under vacuum. The residue was dissolved in 2 mL of distilled water and 20 μ L aliquots from the aqueous solution were used for the determination of the radioactivity in the sugars or the organic acids fractions using a Beckman LS-3801 liquid scintillation counter.

The composition of the sugar fraction (b) was verified by converting the sugars to their trimethyl silyl derivatives and analysis by gas chromatography as outlined previously (21)

and essentially as described by Beaudry *et al.* (1). This fraction was found to contain fructose and glucose as major components in addition to sucrose (Fig. 1).

Organic Acids Analysis

HPLC chromatography with a Dionex, Series 4000i, ion chromatograph equipped with a conductivity detector was used to separate and quantify the organic acids. Two types of columns were used: a high performance ion chromatography exclusion column, ICE-ASI (Dionex), and Polypore H (Rainin), a resin-based column, both with a dynamic suppressor to attenuate the ionization of the organic acid and improve the conductometric detection. The eluant for both columns was 1 mM HCl and the regenerant for the suppressor was 5 mM tetrabutylammonium hydroxide. The eluted compounds were recorded and quantified by a 4270 Dionex integrator. The eluants were identified based on the peak retention time and elution patterns from the HPLC column. When necessary, mass spectroscopy was used for verification.

For the determination of radioactivity in each of the individual peaks, the organic acid samples (fraction c) were run on the preparative Polypore H column, which was disconnected from the detector, and samples of specific acids were collected from the column end based on the predetermined retention time. The obtained ¹⁴C label was measured with a liquid scintillation counter as described above.

Assay of Fru 2,6-P₂

Fru 2,6-P₂ was assayed based on the activation of pyrophosphate-dependent fructose-6-phosphate kinase (31). Known amounts of Fru 2,6-P₂ were used to obtain a standard curve for the compound in a reaction mixture containing 0.33 unit aldolase, 0.33 unit glycerol phosphate dehydrogenase, 3.3 units triosephosphate isomerase, 0.008 unit P_i-phosphofructokinase, the coupling enzymes (Sigma), in addition to 2.5 mM fructose-6-phosphate, 2.5 mM pyrophosphate di-

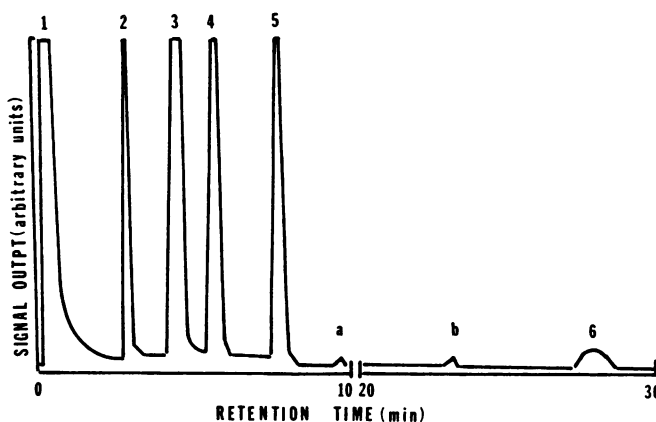


Figure 1. Gas chromatogram showing a profile of the ethanol-soluble sugar fraction. The resolved peaks represent: 1, the solvent front; 2, an internal xylose standard; 3, fructose; 4 and 5, the α - and β -glucose isomers, respectively; and 6, sucrose. Two additional peaks, a and b, appeared occasionally. Compound "a" was tentatively identified as *myo*-inositol. The other compound is an unknown.

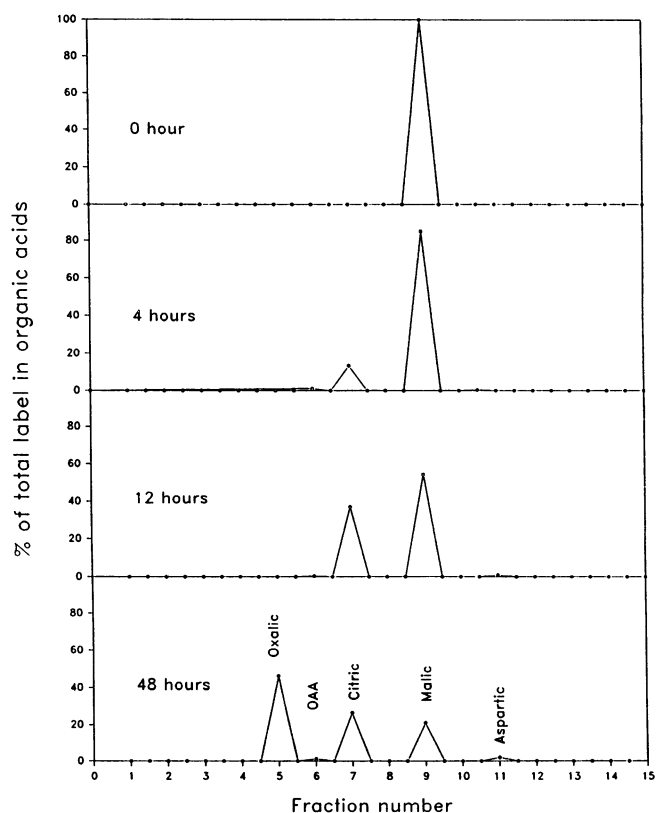


Figure 2. Time-dependent distribution of ^{14}C -label from applied L-[U- ^{14}C]malic acid in organic acids from tomato fruit pericarp discs. The indicated label distribution represent the percentage from the total in the organic acids fraction. The fruit discs were incubated with the malic acid and afterward used for obtaining and separating the organic acids as described in "Materials and Methods."

sodium in 50 mM Tris-HCl (pH 8.0), 0.14 mM NADH, and 20 μL solution of Fru 2,6-P₂ or plant extracts. Care was taken to remain within the linear response range of up to 10 pmol/mL. These conditions were used for estimating the Fru 2,6-P₂ obtained from the tissue extracts.

Fru 2,6-P₂ Extraction

Fruit pericarp discs weighing 1.5 to 2.0 g were quick-frozen in liquid N₂. The frozen tissue was homogenized with Polytron homogenizer in 3 mL of 0.05 N NaOH, and the homogenate was centrifuged at 14,000g for 30 min. The supernatant was used for the Fru 2,6-P₂ assay. Using this procedure and the outlined Fru 2,6-P₂ assay method, recovery of authentic Fru 2,6-P₂, added to the extraction medium before homogenization, was in the range of 85 to 90%.

RESULTS AND DISCUSSION

Excised tomato pericarp discs can be used to study fruit ripening because these tissue explants have been shown to undergo characteristic ripening processes including changes in pigmentation, softening, and ripening-associated respiratory and ethylene bursts (5). This test system was found to be

Table 1. Time-Course Pattern of ^{14}C -Incorporation from Applied L-[U- ^{14}C]Malic Acid into Various Metabolic Fractions in Tomato Fruit Discs

Values in each row show the percentage of the applied label recovered in each assayed fractions and in addition the combined total. The portion of the applied label not taken up by the fruit discs was removed by washing the discs at the end of the incubation period. Additional material was retained by the Dowex column used for separating the sugar and the acid fractions. No attempt was made to determine the makeup of the unrecovered labeled materials.

Time	Applied Label				
	Organic acids fraction	Evolved CO ₂	Residual (nonsoluble) fraction	Sugar fraction	Combined total
<i>h</i>			%		
4	35.7	7.2	0.72	0.33	43.95
8	32.6	11.9	1.54	4.03	50.07
12	31.2	20.6	1.68	2.77	56.25
24	24.3	22.7	2.60	2.58	52.18
40	21.3	27.6	3.20	2.10	54.20

appropriate for the present work particularly since it allowed the application of test solutions. The fruit pericarp was used preferentially because it is known to contain 20% more sugars than the locular tissue (4) and thereby a possible sink for newly formed sugars.

Malic acid was used in this study because of the acid high metabolic mobility: malic acid is known to move freely across cellular membranes and is readily utilized for various synthesis and as source of reducing power in cellular metabolism (32) and was reported to be effectively metabolized in ripening fruit (17). Importantly, malic acid is known to serve as gluconeogenic substrate in CAM plants (11) and hence an appropriate compound for testing the conjecture that, in tomato fruit tissue, the acid may be utilized in sugar production.

When applied to the tomato discs the label from the applied [^{14}C]malic acid appeared to partition into other organic acids. A detailed analysis of the organic acids content and composition (Fig. 2) indicates that ^{14}C in the malate fraction declined continuously and was recovered sequentially in citrate and then other acids. The label in citrate increased up to 12 h of incubation and then subsided. After 48 h some label was recovered also in oxaloacetic acid, aspartic acid, and a substantial level in oxalic acid. The accumulation of oxalic acid is surprising because in whole tomatoes the acid is found in trace amounts (4). The identity of the compound, based on the retention time and elution profile by HPLC, was further tested and authenticated by mass spectroscopy. In excised tissues the onset of an extensive TCAC activity and apparently an attendant accumulation of TCAC constituents (18) may be accompanied also by the production of oxalate as a TCAC by-product (10) and may account for the increase in oxalate in excised fruit tissue. The biosynthetic pathway or the metabolic role of the produced oxalate are not known, however (10), and await further clarification. Nonetheless, these results show that applied malate was apparently recycled and converted to other constituents and byproducts of the TCAC pathway.

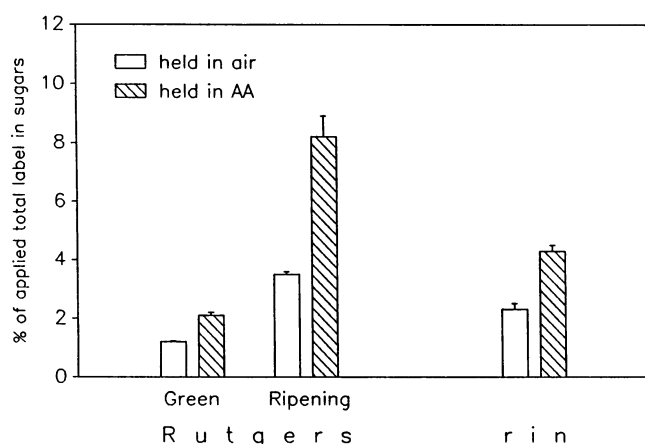


Figure 3. Incorporation of ^{14}C -label into the sugar fraction of fruit pericarp disks incubated with applied L-[U- ^{14}C]malic acid. Pericarp disks were obtained from whole tomatoes kept for 24 h in air and air containing 4000 ppm acetaldehyde. The label distribution is in percentage of the applied [^{14}C]malate. The methods for malate application, sugar extraction, and label estimation are described in "Materials and Methods."

Table I shows that the combined label recovered in the test pericarp tissue was around one-half of the applied [^{14}C]malate. The balance apparently represents the malate not taken up by the tissue and rinsed off following incubation as well as some unknown materials retained by the Dowex column during the separation of the organic acids and sugar fractions. In the recovered portion, the organic acids initially represented a major fraction, but with time of incubation the malic acid-derived ^{14}C in the combined organic acids fraction appeared to decline, suggesting that malate and other TCAC constituents derived from the applied acid (Fig. 2) were metabolized. The ^{14}C partitioned also into additional fractions; the continuous and progressive increase of ^{14}C in respiratory CO_2 suggested, not surprisingly, that the applied malate or other acids were expended as respiratory substrate (17). Interestingly, malate serves as precursor compounds for alcohol insoluble cellular material likely to be polysaccharides including cell wall components. Though the nature of this fraction was not analyzed further there was a continuous and progressive label accumulation in the insoluble fraction in agreement with other observations that malate is readily metabolized due to interconversion to other organic acids and utilization in respiration and in synthesis (17).

A small but significant fraction of the label was recovered in the soluble sugar fraction. There was only little label incorporation in this fraction for the first 4 h, an upsurge in the label recovery after 8 h, and a decline afterward. We made use of this phenomenon to test if this process is related to gluconeogenic carbon mobilization and whether it could be stimulated in tomato tissue by applied AA.

To examine if this process is ripening-dependent, the flow of the ^{14}C label from malate to sugars was compared in the normally ripening Rutgers variety and in *rin*, a nonripening tomato mutant (23). Figure 3 shows that there was a small but significant label movement to the sugar fraction in both

the unripe (green) Rutgers tomato and the nonripening *rin* fruit. In the ripe Rutgers tomato, however, the label recovery in the sugars fraction roughly doubled, suggesting that the normally occurring malate interconversion to sugars in the fruit is intensified with ripening.

Applied AA led to further increase in the label mobilization to the sugar fraction in either the unripe and the ripe Rutgers fruit as well as in *rin* tomatoes. The increase was particularly obvious in the ripe Rutgers tomato, presumably due to the fruit tissue predisposition to sugar synthesis as shown above. The response of the *rin*, behaving like nonclimacteric fruit (8), is also significant; although in this tissue, the magnitude of the ^{14}C recovery in the sugar fraction is equivalent to that of the green Rutgers fruit, the AA-treated *rin* mimicked a metabolic activity found in climacteric fruit. In ripening banana, starch is degraded to triose or hexose phosphates which diffuse from the amyloplast to the cytosol and are metabolically apportioned for glycolysis and in addition for gluconeogenesis (1). The present work is an additional example of ripening-related gluconeogenesis suggesting that the process may be common in ripening fruit. However, the carbon interconversion from organic acids to sugars, rather than from starch as found in banana, represents an alternative carbon source for sugar synthesis. This pathway may be particularly important in nonclimacteric fruit because these fruits are usually not enriched in starch (2), their ethylene production and activity are not pronounced, and the phytohormone is not known to mobilize ripening processes in these fruit (3). The AA stimulation of sugar production is therefore an alternative mechanism to the ethylene-associated sugars synthesis (1) and may be substantial, amounting to a 50% increase in total sugars and quadrupling in the sucrose content in tomatoes (21) and roughly a 20% increase in the soluble solids in grape (20).

An expected consequence of the organic acids utilization in sugar formation would be a decrease in the organic acid content as previously shown (19, 20). Figure 4 indicates that ripening-associated sugar accumulation in Rutgers tomato was accordingly attended by a decrease in the organic acids

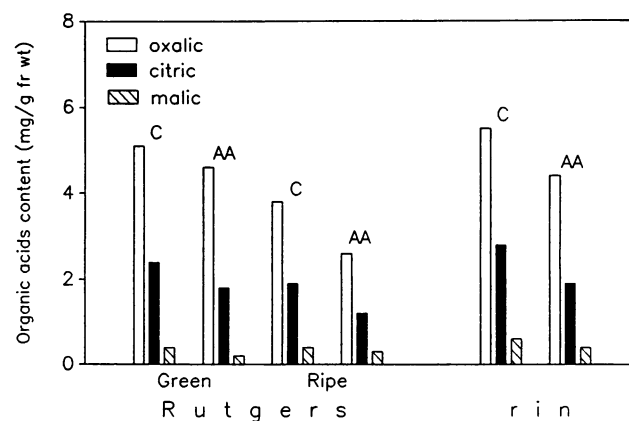


Figure 4. Organic acid content in tomato pericarp discs from fruit held in air (C) and in air containing 4000 ppm AA. The extraction and the estimation of the organic acids are outlined in "Materials and Methods."

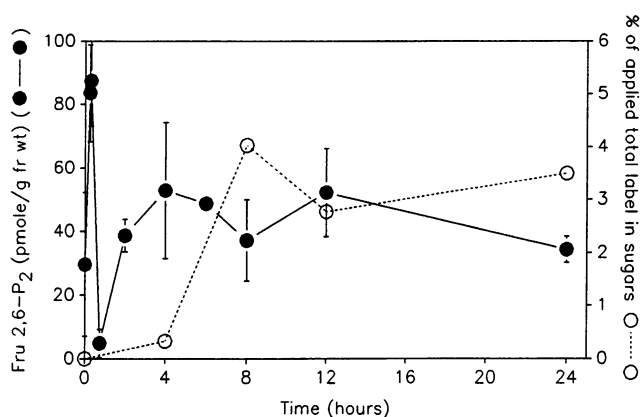


Figure 5. Time-dependent changes in Fru 2,6-P₂ levels and the accompanying changes in label recovery from applied L-[U-¹⁴C]malic acid, in the total sugar fraction in tomato pericarp discs. The employed procedures were as outlined in "Materials and Methods."

level, and that AA, furthermore, accentuated this trend apparently due to enhanced acids interconversion to sugars. This response was found also in the *rin* tomato where the AA-stimulated sugar production (Fig. 3) is appropriately associated with diminished levels of organic acids. AA applications are invariably associated with the reduction in the organic acid content (19, 20) in part due to malic acid decarboxylation (E Pesis, personal communication), the utilization of the acids in respiration (Table I) and in gluconeogenesis, as the present work suggests. A similar utilization of TCAC intermediates was found also in germinating castor bean seedlings where the massive conversion of fat to sugars in the endosperm involves the synthesis of oxaloacetate and malate and the utilization of these acids in gluconeogenesis (16). The volatile stimulation of sugar production from organic acids circumvented the dependence of the process on carbohydrates as reserve materials and is found, therefore, in both climacteric and in nonclimacteric fruit (19, 20, 21) where reserve carbohydrates are usually low.

We reasoned that AA stimulation of gluconeogenesis may entail modulation of the Fru 2,6-P₂ levels, a key metabolite controlling the glycolysis-gluconeogenesis balance (12, 27). Fru 2,6-P₂ stimulates the activity of PPI-dependent phosphofruktokinase and hence of glycolysis. When the Fru 2,6-P₂ declines, gluconeogenesis ensues, apparently due to release from inhibition of fructose 1,6-bisphosphatase activity (27). To analyze this conjecture, inferring AA-induced Fru 2,6-P₂ depletion, we estimated in the tomato tissue time-dependent changes in the Fru 2,6-P₂ levels and related these changes to the ¹⁴C mobilization from the applied malic acid to the sugar fraction. Figure 5 shows that the concentration of Fru 2,6-P₂ increased sharply during the first 30 min of incubation followed by a rapid decrease. Stitt (27) argued that these short-term changes in the Fru 2,6-P₂ concentration in excited or stressed tissues are associated with glycolytic carbon mobilization for respiration and synthesis. The Fru 2,6-P₂ level subsequently increased for the next 4 h followed by a decline during an additional 4 h period. While the Fru 2,6-P₂ level was increasing the ¹⁴C flow to the sugar fraction remain low.

It began increasing for the next 4 h period as the levels of Fru 2,6-P₂ were declining. These results are consistent with and may explain the pattern of the label mobilization from the applied malate to the sugar fraction in Table I; the peak in the label recovery in the sugar fraction after 8 h incubation reflects apparently diminished Fru 2,6-P₂ levels during this period.

For the remainder of the experiment (up to 24 h), the Fru 2,6-P₂ levels fluctuated but the oscillations were progressively attenuated with time of incubation. Similar fluctuations in Fru 2,6-P₂ levels were reviewed and reported by Stitt (27) in carrot and sugarbeet when transferred from 7°C to higher temperatures and also in leaves and pea roots upon flooding as well as in barley leaf segments during photosynthesis.

In the tomato the pattern of the label mobilization to sugars was consistently in inverse proportion to the Fru 2,6-P₂ levels, in keeping with the view that decline in the tissue levels of the compound led to release of gluconeogenesis from inhibition (27, 28). A similar relationship was found in germinating castor bean endosperm where massive gluconeogenesis is also attended with the decrease in the level of Fru 2,6-P₂ (16) as well as in CAM plants where the diurnal Fru 2,6-P₂ fluctuations correlate with day-night cycles, showing a decline when photosynthesis is engaged and sugars are synthesized and increasing again in the dark when carbon is mobilized by glycolysis for respiration and synthesis (6).

To examine if the Fru 2,6-P₂ activity is related to the AA stimulation of the label flow to the sugar fraction, we assayed for Fru 2,6-P₂ levels in pericarp tissues from fruit allowed to ripen normally in air and by comparison in tissues from AA-held tomatoes. The results in Figure 6 revealed that after 8 h incubation the concentration of the compound in the pericarp from air-held green fruit was around 40 pmol/g fresh weight of tissue. The progressive reduction in the Fru 2,6-P₂ levels as the fruit ripen is in agreement with the view that the intensification of ripening-associated gluconeogenesis (Fig. 3) is predicted on declining Fru 2,6-P₂ activity. In AA-held fruit there was in addition further and substantial diminution in the Fru 2,6-P₂ concentration amounting to one-half to one-third of the level found in air-held fruit. In mature banana fruit,

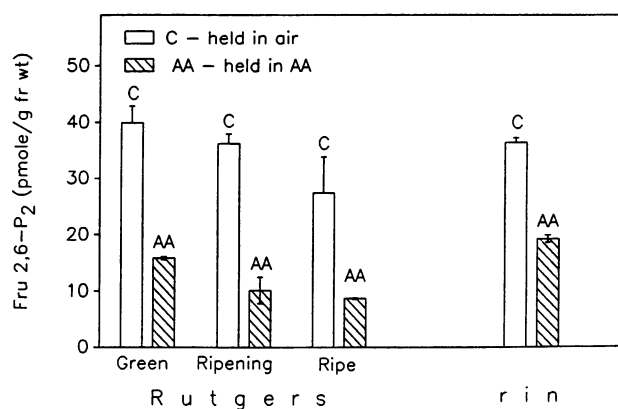


Figure 6. Fru 2,6-P₂ levels in fruit pericarp tissue after 8 h incubation. The discs were obtained from whole tomatoes kept for 24 h in air or air containing 4000 ppm AA.

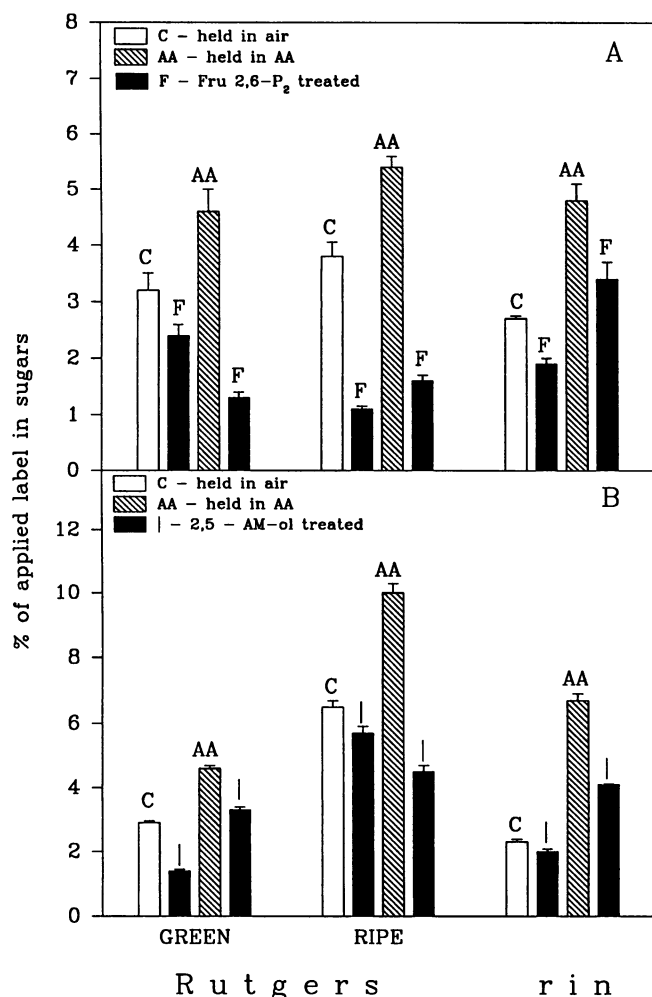


Figure 7. Recovery of ^{14}C -label in the sugar fraction from tomato pericarp disks incubated with $\text{L-}[U\text{-}^{14}\text{C}]\text{malic acid}$ as influenced by a coapplication of (A) Fru 2,6-P₂ and (B) 2,5-AM-ol. Whole green and ripe Rutgers tomatoes and *rin* fruit were held for 24 h in air and air containing 4000 ppm AA and were then used to obtain pericarp discs. The excised tissue was held in flasks containing 1 mL MS medium and 0.25 μCi $\text{L-}[U\text{-}^{14}\text{C}]\text{malic acid}$ and additional samples containing the $[\text{C}^{14}]\text{malate}$ supplemented with 30 μmol Fru 2,6-P₂ or 2,5-AM-ol. After 8 h incubation, the total sugar fraction was extracted, and the label was estimated as described in "Materials and Methods."

though the estimated levels of the compounds were in the range of 200 to 400 $\mu\text{g/g}$, and roughly an order of magnitude higher than in the tomato tissue, a reduction by one-half of the initial Fru 2,6-P₂ level was attended with a precipitous shift in the carbon flux from a glycolytic to the gluconeogenic mode (1). Figure 6 shows that in the tomato pericarp tissue the AA-induced reduction in Fru 2,6-P₂ by similar ratios is associated with a comparable change, namely, stimulation of gluconeogenesis-driven carbon movement (Fig. 3). It appears that ripening in tomatoes is associated with a propensity for gluconeogenesis, apparently due to the reduction in the Fru 2,6-P₂ levels. These metabolic conditions appear to be markedly intensified by the action of acetaldehyde.

To further test the view that the carbon mobilization from

malate to sugars is not merely coincidental to and furthermore is dependent on the tissue depletion in Fru 2,6-P₂, we examined if gluconeogenesis, as occurring normally or as stimulated by AA, may be arrested by the application of Fru 2,6-P₂. Figure 7A shows that applied Fru 2,6-P₂ inhibited the label flow to sugars in green and in ripening Rutgers tomato as well as in the *rin* tissue. In AA-treated tissues, with depressed Fru 2,6-P₂ levels and subsequently augmented gluconeogenic activity, the inhibition of this process by Fru 2,6-P₂ was particularly obvious.

Though Fru 2,6-P₂ can be taken up by plant tissues (26) we are aware that the employed levels (30 μM) are supraphysiological. To ascertain that the arrested label mobilization is caused by the Fru 2,6-P₂ inhibitory action, rather than metabolic abnormality, we applied also 2,5-AM-ol. The compound is known to arrest gluconeogenesis in isolated rat hepatocytes by inhibiting the activity of fructose-1,6-bisphosphatase and also of pyruvate kinase (22). In tomato tissue the applied 2,5-AM-ol (Fig. 7B) appeared to inhibit the label recovery in the sugar fraction in both the green and normally ripening Rutgers tomato as well as in *rin* tissue. Moreover, AA stimulation of this process, presumably due to diminished inhibitor level, was substantially attenuated by 2,5-AM-ol. Hence, the decline in Fru 2,6-P₂ in tomatoes may not be merely coincidental to the onset of gluconeogenesis, because an increase in the Fru 2,6-P₂ levels, as well as another compound with a similar inhibitory function, antagonized gluconeogenic carbon flow from malate as occurring normally and particularly when stimulated by AA.

The findings in the present study are consistent with the perceived role of Fru 2,6-P₂, namely arrest of gluconeogenesis when sufficiently high and release of the process from inhibition as the level of the compound declines (27). Accordingly, ^{14}C mobilization from malic acid to sugars remained low when the level of Fru 2,6-P₂ in the tomato tissue was high due to the application of the compound (Fig. 7A), or as occurred during the first 4 h in incubated tomato discs (Fig. 5). When the level of Fru 2,6-P₂ declined, during extended incubation period, the interconversion of malate to sugars increased by more than 10-fold (Fig. 5). It is interesting to note also that the ripening-associated intensification of gluconeogenesis (Fig. 3) is appropriately associated with a progressive decline in Fru 2,6-P₂ levels (Fig. 6).

The AA stimulation of the label flow from malate to sugars (Fig. 3), presumably by gluconeogenic mode, appears to be related to a pronounced decline in the Fru 2,6-P levels. This effect could be antagonized by increasing the tissue levels with gluconeogenesis-arresting compounds including Fru 2,6-P₂ and 2,5-AM-ol. The synthesis and degradation of Fru 2,6-P₂ are controlled by fructose-6-phosphate-2-kinase and fructose-2,6-bisphosphatase, respectively (27), and it is not clear how AA activity may relate to this regulatory scheme. Future studies on the mode of AA action may reveal if the volatile action consists of modulating the activity or alternatively, the synthesis of these enzymes.

It is also important to consider the relevance of AA action to normally occurring ripening processes. A considerable production of AA ensues with the onset of fruit ripening (7, 14), and the present work suggest that the tissue-produced AA may mobilize reserve compounds for sugar synthesis. Early

on, Professor Biale noted the importance of respiration-driven metabolism in ripening fruit (2) and later proposed (3) that while in climacteric fruit ethylene synthesis and action provide a frame of reference for a respiratory upsurge and the attendant acceleration of ripening, in nonclimacteric fruit a similar role may be found by "exploration into unique volatiles or into substances that impart aroma to ripe fruit." The proposed role of AA, namely, the mobilization of reserve compounds, particularly in nonclimacteric fruit where the synthesis and action ethylene is not pronounced (2, 3), may be a realization of the seminal view by Biale on volatile-regulated ripening processes.

Stress conditions in plants are associated with the synthesis of sugars presumably because sugars are protectants of biopolymers (15). From this perspective the AA inducement of sugar accumulation may add to the survivability of tissues, suggesting that the view of the volatile as stress agent (25) may be reevaluated and AA and perhaps related volatiles may be regarded as a stress antagonist. Indeed, additional studies (not shown) confirm that volatiles are potent antagonists of chilling stress in plants.

ACKNOWLEDGMENT

The authors wish to acknowledge the guidance by Dr. S. Kowalczyk, Department of Biochemistry, Nikolaj Kopernik University, Torun, Poland, on the role and metabolism of fructose-2,6-bisphosphate.

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