Sugar Content, Compartmentation, and Efflux in Strawberry Tissue

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Abstract. Using the compartmental analysis method, the distribution of sucrose, glucose, and fructose and their efflux from the free space, cytoplasm, and vacuole were determined in Nyoho strawberries (*Fragaria × ananassa* Duch.) picked 25 or 35 days after pollination (DAP). At both stages, >70% of total sugar accumulated in the vacuole. Concentration of sugar in the free space increased from 167 mM in fruit at 25 DAP to 217 mM at 35 DAP, whereas that within the cell (cytoplasm + vacuole) increased from 233 to 352 mM. Permeability of the plasma membrane to sucrose, glucose, and fructose was higher than that of the tonoplast and, except for that of fructose, the permeability of the plasma membrane to sugars increased with fruit maturation. ABA at 10^{-5} M compared to 10^{-4} M restricted the release of all sugars from fruit discs and was due mainly to reduced efflux across the plasma membrane rather than the tonoplast. Thus ABA may stimulate the accumulation of sugars in fruit flesh by restricting their efflux. Chemical name used: abscisic acid (ABA).

In producing soft fruit such as strawberries, quality in terms of sweetness and juiciness is very important, since these attributes affect consumer demand. Quality is influenced by the amount and composition of sugar accumulated in the fruit. While it is agreed that cell number is an important factor in fruit enlargement and contributes to variation in fruit size, there is little doubt that the increase in cell volume contributes the most to total fruit enlargement (Cheng and Breen, 1992; Havis, 1943). Coombe (1976) reviewed the development of fleshy fruit and compiled data on their sugar composition. The sugar composition of strawberries varies with the degree of fruit ripeness (Reyes et al., 1982), and the concentration of glucose and fructose is higher early in strawberry growth than in ripe fruit (Forney and Breen, 1986).

The expansion of fruit cells is reportedly influenced by turgor pressure, which to a large extent is determined by the concentration of solutes, including sugars. Most of the imported sugars accumulate in the vacuole of sink-tissue storage cells (Leigh et al., 1979; Yamaki and Ino, 1992). This implies that the vacuole is the primary source of turgor pressure formed against the cell wall leading to cell and fruit enlargement. Thus, fruit sweetness and enlargement are related to the active accumulation of sugars in the vacuole. On the other hand, strawberry development reportedly is influenced by increasing accumulation of sugars and ABA per fruit from middevelopment to ripening (Archbold and Dennis, 1984; Kano and Asahira, 1981). Externally applied ABA has also stimulated the uptake of sugar by some sink tissues (Archbold, 1988; Beruter, 1983; During and Alleweldt, 1984; Saftner and Wyse, 1984). However, this stimulatory effect of ABA has not been fully explained. It is therefore important to determine the composition and content of sugar distributed among the compartments of the cell as relating to fruit development and ABA application. The compartmental analysis method, although originally used for testing compartmentation models in algae (McRobbie and Dainty, 1958) and higher plants (Pallaghy et al., 1970), has been used

recently to characterize internal sugar pools in the vacuole, cytoplasm, and free space and membrane permeability (Saftner et al., 1983; Yamaki and Ino, 1992). This method therefore seems appropriate for investigating the accumulation and distribution of sugars in strawberry cells. In this paper we discuss the compartmentation of sugars in strawberry cells at two growth stages and how ABA affects the efflux of sugars from strawberry tissue discs.

Materials and Methods

Material. Strawberry plants, raised for ≈40 days under 20C days and 15C night, were grown in pots containing 1 peat : 1 vermiculite : 2 soil (by volume) in a glasshouse without supplemental lighting from August 1992 to February 1993. At anthesis, flowers were hand-pollinated and tagged. Fruit from the first-cycle flowers (late August to mid-September) were used for the compartmental analysis experiment and those from the second-cycle flowers (early October to late October) were used for the inhibitors experiment. Since primary fruit were irregular in size, secondary and tertiary fruit of similar size were used in both experiments.

Compartmental analysis method. Fruit were harvested 25 or 35 days after pollination (DAP) in the morning for each experiment. Fruit at 25 DAP were light green with an average weight of 10 g, while those at 35 DAP were slightly red at the peduncle end and averaged 15 g. Cylinders 5 mm in diameter were removed from the fruit cortex with a sharp cork borer and discs 3 mm thick were prepared by cutting them with a razor. In a preliminary experiment, we observed that incubating the discs at room temperature led to an abrupt release of sugars after 1 h, while at 0C a gradual release was observed after 2 h. It was also observed that a medium containing 2 mM CaCl_2 and 0.2 M mannitol was almost isotonic with the free-space sugar concentration, since preliminary experiments revealed that free-space sugar concentration was ≈0.2 M. In a solution of only 2 mM CaCl_2 , the release of sugar showed the same tendency as that at room temperature. Thus, the above medium and conditions were used for the experiment.

Five grams of discs was placed in a mesh container and quickly dipped in 25 ml of a medium containing 2 mM CaCl_2 and 0.2 M mannitol at 0C to remove surface sugar released from cut and damaged cells. The discs were then incubated in 100 ml of the same

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solution and aerated at 0C for various intervals for 2 h. Aliquots of 0.5 ml were withdrawn at the end of each interval for assay. The amount of sugar in each aliquot was determined by high-performance liquid chromatography (HPLC). At the end of the 2-h incubation period, the sugar remaining in the discs was extracted by boiling for 10 min after homogenizing in 5x the volume of 80% ethanol and the amount was determined by HPLC. Full details of the procedures for compartmental efflux analysis and the considerations involved are given by Macklon (1975).

Estimation of free-space volume. To determine the free-space volume, 5 g of discs was incubated in a medium containing 2 mM CaCl_2 , 0.1 M sorbitol, and 0.1 M mannitol for 1 h at 0C. The discs were then transferred to a medium containing 2 mM CaCl_2 and 0.2 M mannitol for 40 min, and aliquots were withdrawn as above at the same interval and data were subjected to compartmental analysis. The volume of free space was calculated by assuming that, after equilibrium, the sorbitol concentration in the free space is equal to the sorbitol concentration in the incubation medium (Yamaki and Ino, 1992).

Estimation of air-space, free-space, and within-cell volumes. The air-space volume was determined by the method described by Yamaki and Ino (1992) using a medium containing 2 mM CaCl_2 and 0.3 M mannitol. Within-cell volume was obtained by subtracting free space and air space from total tissue volume.

Effects of inhibitors on the efflux of sugars. In the experiment to

determine the effect of inhibitors on efflux of sugars from the discs, ABA (10^{-4} and 10^{-5} M), 100 μM carbonylcyanide m-chlorophenyl-hydrazone (CCCP), and 1 mM p-chloromercuri-benzenesulfonic acid (PCMBS) were added to the incubation medium and compartmental analysis was carried out as above. For this experiment, fruit from the second-cycle flowers were used since first-cycle fruit were unavailable. ABA; S-(+)-ABA [(+)-2-cis,4-trans-abcisic acid], a natural abscisic acid, was offered by Toray Co.

Sugar analysis. Aliquots containing the released sugar were passed through a membrane filter and 50 μl was injected into the HPLC system (model 655A-11; Hitachi, Tokyo) with a refractive index detector (RI-3H). Sugars were separated on a column (Shodex Sugar SC 1011; Showadenko Co., Tokyo) at 80C with distilled water ($0.8 \text{ ml} \cdot \text{min}^{-1}$).

Results

Compartmentation of sugar. Efflux curves for each sugar at both stages (Fig. 1) were constructed from the log values 3 of the amount of each sugar remaining in the discs at the end of each incubation period. The final linear part of the curve was equated with vacuolar efflux, which was assumed to be the compartment with the slowest efflux. Extrapolating to time zero gave the amount of sugar in the vacuole at the start of efflux. Subtracting the vacuolar component from the amount of sugar in the discs at each

Fig. 1. Time course reduction in the total amount of the various sugars in strawberry tissue discs during incubation. Estimation of amount of sugar in vacuole (A and D), cytoplasm (B and E), and free space (C and F) 25 and 35 days after pollination (DAP). Each point represents the mean of two replicates.

time interval gave an efflux curve representing loss from compartments other than the vacuole, the final phase representing efflux from the cytoplasm. Extrapolating this efflux curve to time zero gave the amount of sugar in the cytoplasm. Analyzing the curve in the same way revealed another phase considered as the amount relating to the free space (Pitman, 1963). Table 1 shows the amount of the various sugars in the different compartments. At both stages, >70% of the sugars accumulated in the vacuole, while the percent distribution in the free space and cytoplasm varied with fruit age. At 25 DAP, the amount of total sugar in the free space was higher than the cytoplasm; a reverse order was, however, observed in fruit at 35 DAP. The main sugar accumulated varied with the stage of development: it was fructose at 25 DAP and sucrose at 35 DAP. The amount of sugar accumulated in each compartment increased with maturation, especially in the cytoplasm and free space relative to the increase in the vacuole.

Volume of air space and free space. Free-space volume increased with fruit maturation (Table 2). Fruit at 35 DAP showed a 16% increase in free space over those at 25 DAP. Air space was determined in fruit at 25 DAP but could not be detected at 35 DAP. The within-cell volumes at the two stages were similar.

Concentration of sugar in free space and within the cell. Table 3 shows the concentration of the sugars in the free space and within the cell compartments calculated from data in Tables 1 and 2. Generally, there was an increase in the concentration of sugars in the various compartments with fruit maturation. Total sugar concentration in the free space increased from 167 mM in fruit at 25 DAP to 217 mM in fruit at 35 DAP, while that within the cell increased from 233 to 352 mM. That is, there was a greater percent increase in concentration within the cell than in the free space.

Effect of ABA, CCCP, and PCMBS on the rate of sugar efflux. The velocity constants of sugar efflux across the plasma membrane and tonoplast were calculated from the slopes of the efflux curves for cytoplasmic and vacuolar content (Table 4). The velocity constant for the release of the individual sugars across the tonoplast

was less than that across the plasma membrane. The velocity constants for the release of sucrose and glucose across the plasma membrane were higher in fruit at 35 DAP than at 25 DAP; that for fructose, however, decreased at 35 DAP. Although the rate of release of sucrose across the tonoplast increased at 35 DAP, that for glucose and fructose decreased. The effect of ABA, CCCP, and PCMBS on efflux of sugars from the discs is shown in Fig. 2. The *k* (velocity constant) values for the release of glucose and fructose across the tonoplast for the control in Tables 4 and 5 were different, although that for sucrose was similar. ABA at 10^{-5} M restricted the release of sugars from the discs, primarily the rate of release across the plasma membrane (Table 5). However, 10^{-4} M ABA was not as effective as 10^{-5} M ABA in restricting the release. CCCP also restricted the release of sugar and mainly affected the rate of release across both membranes. On the other hand, PCMBS increased the release of sugars from the discs, especially the rate of sugar efflux across the plasma membrane.

Discussion

The total amount of sugars in each compartment of the strawberry increased with maturation and likely increased fruit sweetness. The accumulation of >70% of total sugar in the vacuole at both stages of fruit growth shows that a lesser percentage of sugar is localized in cell compartments other than the vacuole. The increased accumulation of sugars in the vacuole is likely the source of turgor pressure for strawberry enlargement. The difference in sugar concentration between the free space and vacuole corresponds to 0.066 and 0.135 M at 25 and 35 DAP, respectively. These values are less than the 0.198 and 0.531 M obtained for immature and mature apples, respectively (Yamaki and Ino, 1992). In this study, the rate constant for the release of sugars across the tonoplast was less than that across the plasma membrane. This is consistent with the idea that the tonoplast is the most resistant barrier to sucrose release (Saftner et al., 1983; Yamaki and Ino, 1992). The

Table 1. Sugar content of the free space, cytoplasm, and vacuoles of strawberry flesh.

DAP ^z	Sugar	Free space	Cytoplasm	Vacuole	Total
<i>mg·g⁻¹ fresh wt</i>					
25	Sucrose	1.3 ± 0.2 ^y	1.1 ± 0.1	10.6 ± 0.7	13.0 ± 1.1
	Glucose	2.0 ± 0.2	1.7 ± 0.2	10.5 ± 0.7	14.2 ± 0.1
	Fructose	2.3 ± 0.1	1.2 ± 0.0	14.1 ± 0.6	17.6 ± 0.6
	Total	5.7 ± 0.3	4.0 ± 0.4	35.2 ± 0.9	44.8 ± 1.6
35	Sucrose	4.5 ± 0.3	5.9 ± 1.6	23.8 ± 1.1	34.2 ± 2.3
	Glucose	2.7 ± 0.5	3.3 ± 0.1	12.8 ± 2.8	18.8 ± 2.9
	Fructose	2.8 ± 0.1	3.0 ± 0.0	16.2 ± 0.7	21.9 ± 0.6
	Total	10.0 ± 0.1	12.1 ± 1.6	52.9 ± 4.0	75.0 ± 5.7

^zDAP = days after pollination.

^yMean ± SD.

Table 2. Free space, air space, and within-cell (cytoplasm + vacuole) volume in strawberries.

DAP ^z	Vol (ml·g ⁻¹ fresh wt)		
	Free space	Air space	Within cell ^y
25	0.169 ± 0.030 ^x	0.030 ± 0.002	0.802 ± 0.030
35	0.200 ± 0.028	Negligible	0.812 ± 0.029

^zDAP = days after pollination.

^yObtained by subtracting volume of free space and air space from the total volume of tissue.

^xMean ± SD.

Table 3. Concentration of sugar in the free space and within the cells of strawberry flesh.

DAP ^z	Sugar	Concn (mM)	
		Free space	Within cell
25	Sucrose	23	43
	Glucose	67	84
	Fructose	77	106
	Total	167	233
35	Sucrose	65	108
	Glucose	75	111
	Fructose	77	133
	Total	217	352

^zDAP = days after pollination.

Table 4. Velocity constant (*k*) of sugar across the plasma membrane (PM) and tonoplast (TP) of strawberry cells.

DAP ^z	Membrane	<i>k</i> (mg·min ⁻¹)		
		Sucrose	Glucose	Fructose
25	PM	0.098 ± 0.005 ^y	0.153 ± 0.002	0.236 ± 0.001
	TP	0.070 ± 0.033	0.126 ± 0.001	0.097 ± 0.011
35	PM	0.217 ± 0.001	0.200 ± 0.001	0.153 ± 0.002
	TP	0.026 ± 0.001	0.027 ± 0.002	0.031 ± 0.002

^zDAP = days after pollination.

^yMean ± SD

Fig. 2. Effect of ABA (A) and CCCP and PCMBs (B) on the time-course reduction in the amount of sucrose in strawberry tissue discs during incubation. Each point represents the mean of two replicates.

values obtained for the rate of release across both membranes differed from those reported for apples (Yamaki and Ino, 1992).

The absence of air space at 35 DAP may have resulted from its conversion to free space. This is supported by the fact that, although air space disappeared, the volume within the cell remained almost the same at 35 as at 25 DAP, whereas the free-space volume increased.

ABA stimulates the accumulation of sucrose and other assimilates in sugar beet root tissue (Saftner and Wyse, 1984) and strawberry cortex discs (Archbold, 1988). During and Allewelldt

(1984) clearly showed that there is a correlation between the ABA content in grape berries and the total sugar content. The accumulation of ABA in a maturing strawberries may therefore be important to the sugar accumulation and hence fruit enlargement. It is worth noting that in sucrose uptake experiments, the observed increase in uptake may be the net result of a concurrent influx and efflux. That ABA, especially at 10^{-5} M, restricted the release of sugars from the strawberry tissue discs suggests that one reason for the increase in uptake associated with ABA in sugar uptake experiments is a reduction in sucrose efflux. In particular, it may

Table 5. Effect of ABA, CCCP, and PCMBS on the velocity constant (k) of sugars across the plasma membrane (PM) and tonoplast (TP) of strawberry cells (at 25 DAP).

Treatment	Sugar	k (mg·min ⁻¹)	
		PM	TP
Control	Sucrose	0.123 ± 0.037 ^z	0.003 ± 0.001
	Glucose	0.118 ± 0.006	0.008 ± 0.001
	Fructose	0.112 ± 0.008	0.010 ± 0.000
ABA (10 ⁻⁴ M)	Sucrose	0.071 ± 0.006	0.004 ± 0.001
	Glucose	0.058 ± 0.005	0.008 ± 0.000
	Fructose	0.061 ± 0.017	0.008 ± 0.001
ABA (10 ⁻⁵ M)	Sucrose	0.011 ± 0.001	0.003 ± 0.001
	Glucose	0.043 ± 0.001	0.008 ± 0.001
	Fructose	0.039 ± 0.016	0.007 ± 0.002
Control	Sucrose	0.062 ± 0.037	0.005 ± 0.003
	Glucose	0.082 ± 0.004	0.017 ± 0.000
	Fructose	0.133 ± 0.016	0.015 ± 0.004
CCCP	Sucrose	0.045 ± 0.013	0.003 ± 0.001
	Glucose	0.047 ± 0.001	0.008 ± 0.001
	Fructose	0.063 ± 0.030	0.011 ± 0.002
PCMBS	Sucrose	0.090 ± 0.009	0.009 ± 0.001
	Glucose	0.127 ± 0.035	0.010 ± 0.001
	Fructose	0.120 ± 0.016	0.014 ± 0.002

^zMean ± SD.

depend on the reduction in sugar efflux across the plasma membrane rather than the tonoplast. Reduced efflux of sugar across the plasma membrane could occur by one of several mechanisms. ABA may alter the properties of the sugar carrier protein, H⁺-ATPase, or the structure of the plasma membrane. Archbold (1988) observed an increase in sucrose uptake with an ABA concentration of 10⁻⁴ M; however, in our study, ABA was more effective in restricting the efflux of sugar at 10⁻⁵ than 10⁻⁴ M. This event makes sense physiologically. By restricting the efflux of sugars from fruit tissues, ABA may contribute to sugar accumulation in fruit by stimulating the unloading of sugar from the phloem (Tanner, 1980).

CCCP, a metabolic uncoupler that reduces the electrochemical potential of H⁺ across the membrane, reduced the release of sugar from the discs, primarily by reducing sugar efflux across the plasma membrane rather than the tonoplast. This result suggests the involvement of an active efflux mechanism on the plasma membrane. This suggestion is supported by the reports that sugar efflux from wheat and tobacco protoplasts involve an energy-dependent process (Huber and Moreland, 1981) and that CCCP increased sucrose uptake by strawberry discs (Forney and Breen, 1986) possibly through a reduction of sucrose efflux. On the other hand, efflux of sugar from the discs was increased by PCMBS, an inhibitor of ATPase and other SH proteins like the suggested sugar carrier protein, and was most effective at increasing the efflux rate constant across the plasma membrane. Secor (1987) also reported that PCMBS increased the release of sucrose from soybean leaf disc while CCCP decreased it. However, it is unclear why PCMBS stimulated sugar efflux. First-cycle fruit were used for the experiment in Table 4 and second-cycle fruit for that in Table 5 and this may account for the differences in the rate of release of sugars observed for the control in the various experiments. Although fruit used were of the same age (25 DAP), first-cycle fruit were bigger

than the second-cycle fruit and may have been physiologically and/or developmentally dissimilar.

We have observed in a preliminary experiment (data not presented) that treating strawberries with ABA increased size and weight of the fruit at maturity. To improve the quality and production of strawberries, experiments that use chemicals such as ABA are worth pursuing because they may lead to producing sweeter and bigger strawberries to meet consumer demands.

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