Changes in Sugar Uptake by Excised Discs and its Stimulation by Absciscic and Indoleacetic Acids during Melon Fruit Development

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Summary

To clarify how ABA and IAA may mediate sugar uptake during fruit development, the kinetics of sugar accumulation by excised discs of melon (*Cucumis melo* L. cv. Prince) was studied.

Uptake kinetics for glucose and fructose in discs of young and premature fruit, 14 and 28 days after pollination, respectively, and for sucrose in young tissues have biphasic (curvilinear) concentration curves. This indicates the presence of both carrier-mediated and simple diffusional uptake systems. The carrier-mediated system occurs at lower sugar concentrations for the three sugars in young fruit, but for glucose and fructose in premature fruits; it was not detectable in mature fruit 42 days after pollination. The K_m value for carrier-mediated sugar uptake ranged from 6 to 14 mM and did not change with fruit development and among sugars.

ABA at 10^{-5} M enhanced the simple diffusional uptake of the three sugars, whereas IAA at the same concentration stimulated the carrier-mediated system of the three sugars but not the simple diffusional uptake system for sucrose.

The roles of ABA and IAA on the carrier-mediated and simple diffusional uptakes systems for sugars seem to be very important during the growth and maturation of the melon fruit.

Kew Words: abscisic acid, indoleacetic acid, melon fruit, membrane transport, sugar uptake.

Introduction

Sugar accumulation during fruit growth is important because it is used as the principal criterion of melon fruit quality (Pratt, 1971). Developmental studies have shown that total sugars increase rapidly 15 days prior to the full-slip stage (Bianco and Pratt, 1977; Lester and Dunlap, 1985). The sugars accumulate mainly in the vacuole and to a lesser degree in the apoplast; their concentration increased with fruit maturation (Ofosu-Anim and Yamaki, 1994b). Properties of sugar uptake across the membranes may vary with maturation. In general, sugar transport systems across the membrane consist of carrier-mediated and diffusional systems. The carrier-mediated transport is inhibited by *p*-chloromercuribenzenesulfonic acid (PCMBS), a sulfhydryl modifying reagent that is relatively impermeable to a membrane. Application of PCMBS allows one to discriminate between the diffusional transport from the carrier-mediated transport systems (Schmitt et al., 1984; Yamaki and Asakura, 1988; Felker et al., 1991).

Absciscic acid (ABA) stimulated phloem unloading (Tanner, 1980) and the accumulation of sucrose and other assimilates in sink tissue of sugar beet (Saftner

and Wyse, 1984), apple (Berüter, 1983) and strawberry (Archbold, 1988). We reported that ABA restricted efflux of sugars from strawberry and melon fruit discs (Ofosu-Anim and Yamaki, 1994a and b) and stimulated the uptake of sugar by strawberry fruit disc (Ofosu-Anim et al., 1996). However, ABA inhibited sucrose uptake in *Ricinus communis*, *Beta vulgaris*, *Petunia hybrida* and *Phaseolus vulgaris* (Vreugdenhill, 1983; Estruch et al., 1989). Indoleacetic acid (IAA) stimulated the accumulation of photoassimilates in cellular tissues (Patrick, 1979). IAA increased the H^+ -pump activity by stimulating synthesis of plasma membrane H^+ -ATPase (Hager et al., 1991). On the contrary, Saftner and Wyse (1984) reported an inhibition of sucrose uptake in sugar beet root by IAA.

We have already shown that ABA and IAA stimulated the uptake and accumulation of sugars into the disc tissue of strawberry fruit, thus influencing fruit enlargement (Ofosu-Anim et al., 1996). Melon fruit can grow larger than strawberry fruit, and seems to have the stronger sink activity than one. Not much is known about the mechanism of sugar uptake across the membrane of melon tissue and fruit growth and how IAA or ABA influences this process. In this paper, we report the change in properties of sugar uptake across the membrane in the developing of melon and the effects of ABA and IAA on the process.

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Materials and Methods

Plant materials

Melon (*Cucumis melo* L. cv. Prince) seedlings were raised in plastic pots in the glasshouse and transplanted at the four-leaf stage to the field during the summer of 1994. At anthesis, pistillate flowers on secondary lateral branches were hand pollinated and tagged. Five fruits were harvested in the morning for each sample of young (14 days after pollination), premature (28 days) or mature (42 days) fruits. Young, premature, and mature fruits weighed about 324, 589 and 753g, respectively. Cylinders (10 mm in diam.) were removed from the fruit cortex with a cork borer and disc tissues of 3 mm thick were prepared from the mid-sections of the cylinders with a razor.

Sugar uptake into disc tissues

Thirty disc tissues, weighing of about 10g, pooled from five fruits were preincubated for 30 min in 5 ml of 20 mM Tris-Mes buffer, pH6.0, containing 10 mM sucrose for sucrose uptake, 2 mM CaCl_2 and 200 mM mannitol at 25 °C on a rotary shaker. After incubation, the medium was replaced with the incubation medium containing ^{14}C -sucrose (13 MBq/ μmol , Amersham, UK) for sucrose uptake study and incubated for 1 hr. For glucose or fructose uptake study, the preincubation medium contained 10 mM glucose and ^{14}C -glucose (8.5 MBq/ μmol , Amersham, UK), respectively, or 10 mM fructose and ^{14}C -fructose (11.2 MBq/ μmol , Amersham, UK), respectively. At the end of the uptake period, the discs were rinsed free of ^{14}C -sugar medium and placed in an iced medium without radiolabel for 5 min to remove the labelled compounds on the surface and in the apoplast. The washing was repeated three times.

Assay of absorbed sugar

Discs were extracted in 5 ml of 80% ethanol at 80 °C for 30 min (almost all of labelled compounds were recovered) and radioactivity in 1-ml aliquots, after adjusting to the original volume, was determined with a scintillation counter.

Plant growth regulators effect on sugar uptake

To study the effect of (+)-2-cis, 4-trans-abscisic acid (ABA) (Toray Co., Tokyo, Japan) or indoleacetic acid on uptake, the plant growth regulators in concentrations of 10^{-4} , 10^{-5} or 10^{-6} M were added to the incubation medium described above for 1 hr.

Calculation of carrier-mediated and simple diffusional uptake

Sugar uptake in the presence of PCMBs was considered as simple diffusional uptake; carrier-mediated uptake was obtained by subtracting simple diffusional uptake from total uptake in the absence of PCMBs.

Results

Time course of sugar uptake

The uptake rate of 10 mM fructose, glucose and sucrose was relatively constant for 60 min in young fruit tissue (Fig. 1). Fructose accumulated fastest in the young fruit tissue but glucose was in the premature fruit tissue (data not shown).

Kinetics of sugar uptake

In discs of young fruit, the kinetics of glucose, fructose and sucrose uptake is biphasic or curvilinear (Fig. 2). The carrier-mediated system is more efficient at lower sugar concentrations. Furthermore, the ratio of carrier-mediated uptake to total uptake is smaller for fructose and sucrose at the same sugar concentration. The Lineweaver-Burk plot of carrier-mediated uptake revealed that at this stage of development, K_m values for sucrose, glucose and fructose, respectively, were 6.9 mM, 12.9 mM and 13.7 mM, whereas their V_{max} were 0.06, 0.14 and 0.13 $\mu\text{mol/gFW/hr}$ for sucrose, glucose and fructose, respectively (Table 1).

In discs of premature fruit, a biphasic curve was observed for glucose and fructose (Fig. 3), however, uptake of sucrose at this stage was entirely linear and a carrier-mediated uptake was not detectable. K_m values of 12.6 and 11.1 mM for glucose and fructose, respectively, do not differ much from those of young fruit tissues. V_{max} values at this stage were 0.18 and 0.15 $\mu\text{mol/gFW/hr}$ for glucose and fructose, respectively, which are slightly higher than those for young fruit tissues. In discs of mature fruit, PCMBs had almost no effect on sugar uptake, indicating that sugar accumula-

Fig. 1. Time-dependency curves of sugar uptake by discs of young melon fruit. Sucrose (10 mM) (○), glucose (10 mM) (△) and fructose (10 mM) (□). Bars = $\pm$ SE.

Fig. 2. Concentration-dependent curves of sugar uptake by discs of young melon fruit.

Total uptake for 1 hour in the absence of PCMBS ($\circ$, $\bullet$); simple diffusional uptake in the presence of 1 mM PCMBS ($\square$, $\blacksquare$) and carrier-mediated uptake by their difference ($\triangle$, $\blacktriangle$). Open and closed symbols represent duplicate experiments (1 and 2). ^{14}C -sucrose (upper), ^{14}C -glucose (middle) and ^{14}C -fructose (lower) were the substrates.

Fig. 3. Concentration-dependent curves of sugar uptake by discs of premature melon fruit.

The symbols are the same as those in Fig. 2.

Table 1. Changes in K_m and V_{max} of carrier-mediated uptake into melon fruit.

	K_m (mM)			V_{max} ($\mu\text{mol. gFW}^{-1}\text{. hr}^{-1}$)		
	Young	Premature	Mature	Young	Premature	Mature
Sucrose	6.9 ± 0.2^x	ND	ND	0.06 ± 0.01	ND	ND
Glucose	12.9 ± 0.4	12.6 ± 0.3	ND	0.14 ± 0.03	0.18 ± 0.01	ND
Fructose	13.7 ± 0.6	11.1 ± 0.1	ND	0.13 ± 0.01	0.15 ± 0.01	ND

ND : not detected

^x Mean $\pm$ SD

tion at this stage was by simple diffusion (Table 1).

Stimulation of sugar uptake by ABA

At 10^{-4} , 10^{-5} and 10^{-6} M ABA the uptake of 10 mM sucrose, glucose and fructose by discs of young fruit (Table 2) was stimulated, the stimulation being most effective at a concentration of 10^{-5} M. At 10^{-5} M ABA the simple diffusional uptake of sucrose, glucose and fructose was increased by 85, 56 and 132%, respectively. ABA did not stimulate carrier-mediated uptake of the three sugars.

Stimulation of sugar uptake by IAA

IAA at 10^{-6} M was the most effective concentration to stimulate the uptake of three sugars (Table 3). IAA enhanced both the carrier-mediated and simple diffusional uptakes for 10 mM fructose and glucose, by only

Table 2. Effect of ABA on the uptake of sucrose, glucose and fructose by excised discs of young melon fruit.

	ABA (M)	Carrier- mediated	Simple diffusional	Total
		(μmol. gFW ⁻¹ . hr ⁻¹)		
Sucrose uptake	0	0.09 ± 0.01 ^x	0.26 ± 0.01	0.35 ± 0.02
	10^{-4}	0.09 ± 0.02	0.33 ± 0.01	0.42 ± 0.01
	10^{-5}	0.10 ± 0.02	0.48 ± 0.01	0.58 ± 0.01
	10^{-6}	0.12 ± 0.01	0.41 ± 0.02	0.53 ± 0.01
Glucose uptake	0	0.16 ± 0.01	0.39 ± 0.01	0.55 ± 0.01
	10^{-4}	0.12 ± 0.01	0.50 ± 0.02	0.62 ± 0.01
	10^{-5}	0.16 ± 0.03	0.61 ± 0.01	0.77 ± 0.02
	10^{-6}	0.12 ± 0.01	0.57 ± 0.05	0.69 ± 0.03
Fructose uptake	0	0.09 ± 0.02	0.34 ± 0.02	0.43 ± 0.02
	10^{-4}	0.10 ± 0.02	0.54 ± 0.06	0.64 ± 0.04
	10^{-5}	0.08 ± 0.02	0.79 ± 0.01	0.87 ± 0.02
	10^{-6}	0.08 ± 0.01	0.71 ± 0.03	0.79 ± 0.02

^x Mean ± SD

Table 3. Effect of IAA on the uptake of sucrose, glucose and fructose by excised discs of young melon fruit.

	IAA (M)	Carrier- mediated	Simple diffusional	Total
		(μmol. gFW ⁻¹ . hr ⁻¹)		
Sucrose uptake	0	0.09 ± 0.01 ^x	0.17 ± 0.01	0.26 ± 0.01
	10^{-4}	0.25 ± 0.02	0.14 ± 0.01	0.39 ± 0.01
	10^{-5}	0.28 ± 0.13	0.13 ± 0.02	0.41 ± 0.01
	10^{-6}	0.38 ± 0.16	0.16 ± 0.01	0.54 ± 0.01
Glucose uptake	0	0.09 ± 0.02	0.33 ± 0.01	0.42 ± 0.01
	10^{-4}	0.23 ± 0.01	0.37 ± 0.01	0.60 ± 0.03
	10^{-5}	0.26 ± 0.01	0.38 ± 0.05	0.64 ± 0.03
	10^{-6}	0.27 ± 0.05	0.44 ± 0.02	0.71 ± 0.04
Fructose uptake	0	0.12 ± 0.03	0.26 ± 0.01	0.38 ± 0.02
	10^{-4}	0.22 ± 0.01	0.37 ± 0.01	0.59 ± 0.01
	10^{-5}	0.19 ± 0.04	0.41 ± 0.01	0.60 ± 0.02
	10^{-6}	0.34 ± 0.01	0.59 ± 0.02	0.93 ± 0.02

^x Mean ± SD

the carrier-mediated uptake for 10 mM sucrose. IAA enhanced the total uptake of fructose, glucose and sucrose by 200, 183 and 322%, respectively.

Discussion

In the absence of PCMBs, a membrane impermeable inhibitor of sugar carrier protein, uptake of sugars by discs of the melon fruit revealed a biphasic pattern for sugar concentration, whereas with PCMBs, the uptake curve was linear. The biphasic uptake of sucrose by isolated protoplasts of soybean cotyledons was interpreted by Schmitt et al. (1984) to represent simultaneous carrier-mediated transport, composed of both active-, facilitated diffusional-, and simple diffusional-transport across the plasma membrane. A similar biphasic system was reported for sugar uptake by protoplasts of apple fruit flesh (Yamaki and Asakura, 1988) and from maize endosperm (Felker et al., 1991). Therefore, this biphasic pattern suggests that both carrier-mediated and simple diffusional transports participate in sucrose, glucose and fructose accumulation in melon fruit tissue. The contribution of carrier-mediated and simple diffusional uptake to total uptake varied with the apoplast sugar concentration. If sugar concentration is low, sugar uptake occurs through the carrier-mediated system.

The Km values of sugar carriers in young fruit tissue were almost equal, being 12.9 mM for glucose, 13.7 mM for fructose and 6.9 mM for sucrose. These values did not differ from those in premature fruit tissue except for sucrose. These values are higher than the Km values reported for sucrose, glucose and fructose for sugar beet root and apple fruit (Saftner et al., 1983; Yamaki and Asakura, 1988). Similar Km values for hexose uptake by storage tissue of carrot (Daie, 1984) and strawberry fruit (Forney and Breen, 1986; Ofosu-Anim et al., 1996) have been reported. The ratio of the contribution of carrier-mediated uptake to total sugar uptake was higher in young tissues than in mature ones, which means that sugar carriers on the plasma membrane of young fruit play a greater role in the transport of sugars in developing fruit than mature fruit. The reduction in carrier-mediated uptake for glucose and fructose and its virtual absence in premature fruit for sucrose as well as its total absence in mature fruit may be predictable because the fruit had attained almost maximum size accompanied by a reduction in sugar accumulation.

Abscisic acid, especially at 10^{-5} M, stimulated the simple diffusional uptake of all sugars by disc of melon fruit but not carrier-mediated system as in the strawberry fruit (Ofosu-Anim et al., 1996). It is not clear why ABA enhanced only simple diffusional uptake, because it enhanced the membrane permeability by making the lipid of the plasma membrane rigid (Leshem et al., 1990). If permeability of the plasma membrane were to increase in the presence of ABA, the efflux of sugar may be expected to increase. However, in the present

study, net uptake of sugar increased which means a reduction of sugar efflux occurred. Furthermore, we reported that ABA reduced the loss of sugars from discs of strawberry and melon fruit by restricting the rate of efflux across the plasma membrane (Ofosu-Anim and Yamaki, 1994a and b). These results imply that other systems within the cytosol or on the tonoplast may be affected by ABA and may restrict the efflux of sugars. ABA has also been reported to stimulate the H^+ -pumps of the tonoplast of barley roots (Kasai et al., 1993) and sorbitol uptake through the H^+ -pump of tonoplast of apple (Yamaki and Asakura, 1991). Thus, the stimulatory effect of ABA on the uptake of sugar into melon fruit tissue may be attributed to a reduced efflux of sugar which is countered by sugar accumulation in the vacuole through the activation of H^+ -pump of tonoplast.

IAA at $10^{-6}M$ greatly stimulated the uptake of glucose and fructose by enhancing the carrier-mediated and simple diffusional transport systems but auxin stimulated sucrose only by the carrier-mediated uptake system. IAA was found to stimulate 1) the carrier-mediated uptake of glucose and the simple diffusional uptake of all three sugars in the strawberry fruit (Ofosu-Anim et al., 1996); 2) the active rather than the passive uptake of sorbitol by the isolated protoplast of apple (Yamaki and Asakura, 1991); and 3) proton excretion in many plants by promoting the synthesis of plasma membrane H^+ -ATPase (Hager et al., 1991). Thus, the stimulative effect of IAA to carrier-mediated uptake of glucose, fructose and sucrose may be the consequence of the accelerated proton excretion which leads to increased sugar and proton cotransport into the fruit tissue.

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メロン果実組織への糖吸収の生長に伴う変動と ABA と IAA による促進

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摘 要

メロン果実 (*Cucumis melo* L. 'プリンス') への糖の吸収機構を明らかにするために、果肉組織への糖の透過特性の生長に伴う変動および ABA や IAA の透過機構に対する影響について検討した。

糖透過のキネティックス解析では、幼果 (受粉後 14 日) と未熟果 (28 日目) からの組織へのグルコースとフルクトース、および幼果からの組織へのスクロースの吸収は biphasic 曲線となり担体輸送と単純拡散輸送の存在を示した。それ故、幼果においてはグルコース、フルクトース、スクロースに対して、未熟果においてはグルコース、フルクトースに対して担体輸送が存在した、しかしながら成熟果 (42 日目) では担体輸送は検知できなかった。そして、幼果および未熟果において、担体輸送による吸収は低糖濃度でより効率的であった。この担体輸送の K_m 値は糖の種類および果実の生

長段階にかかわらず 6-14 mM の範囲で大きな変化はなかった。

ABA (10^{-5} M) はグルコース、フルクトース、スクロースに対する単純拡散輸送を促進することによって、果実組織への糖の吸収を促進した。一方、IAA (10^{-5} M) は上記 3 糖に対する担体輸送と、グルコース、フルクトースに対する単純拡散輸送を促進することによって、果肉組織への糖の吸収を促進した。

糖の担体輸送および単純拡散輸送の生長に伴う変化、そしてそれらに対する ABA や IAA の促進効果は、メロン果実の生長や肥大と密接な関係をもつものと思われる。

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