Fructose Content and Fructose–Related Enzyme Activity during the Fruit Development of Apple and Japanese Pear

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Summary

Fructose content and fructose–related enzyme activity based on fresh weight were measured during fruit development to characterize the metabolic properties responsible for fructose accumulation in apple (Malus domestica Borkh.) and Japanese pear (Pyrus pyrifolia Nakai). When fructose content gradually increased from 66 to 92 days after flowering (DAF) in Japanese pear fruit, NAD–dependent sorbitol dehydrogenase (SDH) and fructokinase (FK) activities decreased simultaneously. Subsequently, while fructose content rapidly increased from 92 to 126 DAF, the decrease in FK activity continued, whereas SDH activity increased. In apple fruit, FK activity throughout fruit development was lower than 10% of the maximum activity in Japanese pear fruit. Therefore, it seems that the low FK activity contributes to fructose accumulation while SDH activity increased in apple and Japanese pear fruits. Fructose–6–phosphatase (F6Pase) activity was much lower than FK activity during the fruit development of apple and Japanese pear, which indicates that FK plays a more important role than does F6Pase as a determinant of fructose level in conversion between fructose and fructose–6–phosphate. Phosphoglucose isomerase (PGI) activity was similar or higher compared with FK activity during the fruit development of apple and Japanese pear, suggesting that the isomerization by PGI is not a rate–limiting step from fructose to glucose–6–phosphate. Thus, it is not likely that PGI is responsible for the regulation of fructose levels.

Key Words: apple, fructokinase, fructose, fruit, Japanese pear.

Introduction

Sweetness is a major determinant of fruit quality. Although sweetness is generally determined by the total amount of soluble sugars, it is well appreciated that sugar composition also influences this sensory property because of the high sweetness value of fructose relative to other sugars.

In apple and Japanese pear fruits, Yamaki and Ishikawa (1986), Yamaki and Moriguchi (1989), and Moriguchi et al. (1992) have investigated seasonal changes in the activity of sorbitol–related enzymes that are sorbitol–6–phosphate dehydrogenase, sorbitol dehydrogenase, and sorbitol oxidase, and sucrose–related enzymes that are sucrose synthase, sucrose phosphate synthase and invertase. Moriguchi et al. (1992) disclosed a correlation between sucrose accumulation and sucrose synthase activity during fruit maturation. In this study, we focused on the relationship between fluctuations of fructose content and fructose–related enzyme activity to characterize the metabolic properties responsible for fructose accumulation in apple and Japanese pear fruits, because the two species show drastic increases in the fructose level during fruit development.

To our knowledge, few studies have focused on fructose accumulation in fruits. Although some groups have suggested a positive correlation between fructose accumulation and NAD–dependent sorbitol dehydrogenase (SDH) activity in apple and Japanese pear fruits (Yamaki and Ishikawa, 1986; Yamaki and Moriguchi, 1989; Beruter and Studer Feusi, 1997), the changes in the activity do not always correspond to those in fructose throughout the developmental stages of fruits. Besides, relationships between other enzymes and fructose accumulation have not been discussed. Recently, Schaffer et al. (1999) proposed that the potential accumulation of fructose may involve fructokinase, which had been cloned from tomato plants (Kanayama et al., 1997). In fact, Davies and Oparka (1985) showed that the phosphorylation of fructose could regulate the level of fructose in potato tubers. Therefore, FK and other enzymes involved in the metabolism of sorbitol, which is known to be a primary translocated sugar in apple and Japanese pear, were investigated.

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Materials and Methods

Plant Materials

Four-year-old ‘Fuji’ apple trees (*Malus domestica* Borkh.) on *M. 9/Malus prunifolia* Borkh. var. *ringo* Asami and 4-year-old ‘Hosui’ Japanese pear trees (*Pyrus pyrifolia* Nakai) on *Pyrus betulaefolia* Bunge grown in the orchard of Tohoku University were used in 1999. The flowering dates and harvesting times were April 25 and mid-November for ‘Fuji’, and April 20 and mid-September for ‘Hosui’, respectively. Fruits were sampled approximately a month after flowering to 185 days after flowering (DAF) for ‘Fuji’ or 126 DAF for ‘Hosui’ because fructose content increased during these stages of fruit development. The collected pulp was frozen in liquid nitrogen and stored at -80 °C until analyzed.

Determination of sugar content

Sugars were extracted three times successively with 80 °C ethanol. The combined extract was dried in vacuo and redissolved in water. The solution was passed through a Sep-Pak C18 Cartridge (Waters, USA) to remove non-sugars and the filtrate partitioned with chloroform. The chloroform phase was washed twice with water and the combined water phase was dried in vacuo. Sugars were dissolved in water and analyzed by high-performance liquid chromatography equipped with a RI detector and Asahipak NH2 P-50 4E column (Showa Denko, Tokyo).

Extraction and assays of SDH

SDH was extracted, following the protocol of Lo Bianco et al. (1999). The prepared enzyme solution was desalted by a NAP 10 column (Amersham Pharmacia Biotech, Sweden). SDH activity was assayed by the method of Yamaki and Ishikawa (1986).

Extraction and assays of fructokinase (FK), fructose-6-phosphatase (F6Pase) and phosphoglucone isomerase (PGI)

Extraction was carried out at 4 °C or on ice, according to the modified method of Schaffer and Petreikov (1997). Fruit flesh was homogenized to powder with a mortar and pestle using liquid nitrogen, and the powder was extracted by a vortex mixer and high speed homogenizer with 50 mM Hepes–NaOH (pH 7.5) containing 1 mM MgCl2, 1mM EDTA, 10 mM KCl, 2.5 mM dithiothreitol, 3 mM sodium N,N-diethylthiodiglycerol (PVPP) and 1% (w/v) bovine serum albumin (BSA). The homogenate was centrifuged for 15 min at 10,000 × g and the supernatant was filtrated. The filtrate was brought to 80% saturation with ammonium sulfate, then centrifuged at 10,000 × g for 15 min. The precipitate was dissolved in the extraction buffer without PVPP and BSA, and the solution desalted by a NAP 10 column.

FK activity was assayed by the method described in Martínez–Barajas and Randall (1996) except for the fructose concentration. Kanayama et al. (1998) reported two Fks, Frk1 and Frk2, from tomato plants. Frk 1 has a high Km value (1.3 mM) for fructose and does not show substrate inhibition, whereas Frk2 has a low Km value (0.054 mM) for fructose and is inhibited to approximately 25% of the maximum activity by 10 mM fructose. Therefore, 50 mM or 0.5 mM fructose was added to the reaction mixture.

F6Pase activity was assayed according to the method described by Morton et al. (1985) except that reaction buffer was 60 mM Hepes–NaOH (pH 7.2) or 60 mM acetate–NaOH (pH 5.0). PGI activity was assayed according to the method of Doehlert et al. (1988). The activities of the enzymes based on fresh weight were compared with fructose content in this study.

Results

Fructose accumulation

Fructose increased until 152 DAF and comprised 65% of total sugars in apple fruit whereas, in Japanese pear fruit, fructose gradually increased until 92 DAF and then rapidly increased to 58% of total sugars (Fig. 1). The increase patterns are similar to those in ‘Jonagold’ and ‘Kosui’ fruits, respectively (Yamaki and Ishikawa, 1986; Yamaki and Moriguchi, 1989). The fluctuations in glucose, sorbitol, and sucrose contents were also similar to those in the previous reports. Glucose, sorbitol, and sucrose did not significantly accumulate during the experimental periods, differently from fructose.

Enzyme activity

SDH activity was initially undetectable, and then

![Graph](image-url)
Fig. 2. Seasonal changes in SDH activity during the fruit development of apple and Japanese pear.

Fig. 3. Seasonal changes in FK activity during the fruit development of apple and Japanese pear. FK activity was measured in the reaction mixtures containing 50 mM or 0.5 mM fructose. FK activity (nmol min⁻¹ gFW⁻¹) by 50 mM and 0.5 mM fructose in ‘Fuji’ are 0.35 and 0.30 at 33 DAF, 0.23 and 0.34 at 66 DAF, 0.53 and 0.50 at 185 DAF, respectively.

Fig. 4. Seasonal changes in F6Pase activity during the fruit development of apple and Japanese pear. The activity was measured at pH 5.0 and 7.2.

Fig. 5. Seasonal changes in PGI activity during the fruit development of apple and Japanese pear.

increased to a maximum 128 DAF in apple fruit (Fig. 2). An increase in its activity at the very late stage of fruit development which has been previously reported (Yamaki and Ishikawa, 1986; Yamada et al., 1999) was not found in this study because sampling was halted 185 DAF. The fluctuation in SDH activity in ‘Hosui’ fruit was similar to that in ‘Kosui’ fruit (Yamaki and Moriguchi, 1989).

FK activity was high from 38 to 66 DAF, when the activity was evoked by 0.5 mM fructose; it then decreased rapidly in Japanese pear fruit (Fig. 3). FK activity was low throughout the fruit development of apple amounting to less than 10% of the maximum activity in Japanese pear fruit.

F6Pase activities measured at two different pH changed similarly in Japanese pear fruit (Fig. 4). The activity at pH 5.0 was higher than that at pH 7.2 during pear development. In apple fruit, F6Pase activity at pH 5.0 was also higher than that at pH 7.2, whereas the activity at pH 7.2/pH 5.0 ratios changed from 0.16 to 0.84. Although the fluctuations in F6Pase activity differed between apple and Japanese pear fruits, its activity was much lower than that of FK throughout the developmental stages of both fruits, i.e., F6Pase activity at pH 5.0 was less than 3% of FK activity (0.5 mM) at any stage.

PGI activity increased from 66 to 152 DAF, then decreased in apple fruit, whereas in Japanese pear fruit, it attained maximum activity at 66 DAF (Fig. 5).

Discussion

Possible sources of fructose in fruit are through sorbitol dehydrogenase, sucrose hydrolysis, and starch degradation. Sucrose content in peduncles is much lower than sorbitol content during fruit development of apple (Yamaki and Ishikawa, 1986). In addition, changes in the activity, based on fresh weight, of sucrose synthase, acid invertase, and neutral invertase that convert sucrose to fructose do not correspond to fructose accumulation in apple and Japanese pear fruits (Yamaki and Ishikawa,
1986; Moriguchi et al., 1992; Beruter and Studer Feusi, 1997). As for starch, it increases while fructose is accumulating; fructose does not increase during starch hydrolysis in apple fruit (Beruter and Studer Feusi, 1997). These reports support that sorbitol is a much more important source than sucrose and starch as a substrate for fructose. Thus, we focused on the sorbitol-related pathway.

Yamaki and Ishikawa (1986) and Yamaki and Moriguchi (1989) suggested that SDH was a determinant of fructose accumulation as well as the primary enzyme for sorbitol metabolism. However, they also found that SDH activity decreases while fructose continued to increase along with growth of apple and Japanese pear. In this study, SDH activity also decreased rapidly from 66 to 92 DAF in Japanese pear fruit, whereas fructose content gradually increased. The increase in fructose content could be related to a simultaneous decrease in FK activity because the phosphorylation capacity for fructose-related with glucose/fructose ratios as shown in potato tubers (Davies and Oparka, 1985). Furthermore, it is likely that an increase in SDH activity and a decrease in FK activity are responsible for the increase in fructose from 92 to 126 DAF in Japanese pear fruit. In apple fruit, the early flux (40–128 DAF) of fructose content corresponds with that of SDH activity but not the late one (152–185 DAF). In addition to SDH, the lower FK activity throughout the fruit development of apple, compared with FK activity in Japanese pear at 38 and 66 DAF, could be related to the increase in fructose content.

If F6Pase activity is comparatively high, its fluctuation should affect the level of fructose. However, F6Pase activity was much lower than FK activity during the fruit development of apple and Japanese pear which suggests that FK plays a greater role than does F6Pase as a determinant of fructose level in conversion between fructose and fructose-6-phosphate (F6P). Little is known regarding hexose-phosphate specific phosphatase in plants, so that the activities detected in apple and Japanese pear fruits do not always establish the existence of specific cytosolic phosphatase for F6P.

The reversible isomerization of glucose-6-phosphate (G6P) and F6P is catalyzed by PGI. Kruckenberg et al. (1989) demonstrated that in Clarkia, mutants with decreased PGI activity changed G6P/F6P ratios in leaves. Based on this observation, it is possible that levels of PGI activity could alter fructose/glucose ratios. When the relationship between PGI activity and fructose content was analyzed, PGI activity was found to be higher than FK activity during the fruit development of apple, whereas in Japanese pear fruit, it was equal to or higher than the FK activity. These results indicate that the isomerization by PGI is not a rate-limiting step from fructose to glucose-6-phosphate which leads us to believe that PGI is unlikely responsible for the regulation of fructose levels.

The ratios of FK activity by 0.5 mM fructose/the activity by 50 mM fructose changed from approximately 4 to 1 with the fruit growth of Japanese pear. The activity by 0.5 mM fructose is attributed to hypothetical Frk2 activity that is induced at the early stage of fruit development in tomato in contrast with Frk1 expressed constitutively (Kanayama et al., 1997). Therefore, an inducible type of FK may be functioning at the early stage of Japanese pear development.

It is possible that hexose transporters in vacuoles of fruit cells are involved in the regulation of fructose accumulation. However, the uptake is higher for glucose than for fructose in tonoplast vesicles from pear fruit in which fructose predominantly accumulates (Shiratake et al., 1997). Thus, fructose-related enzymes are probably more important in fructose accumulation than are the hexose transporters in vacuoles. Further biochemical and genetic studies regarding fructose-related enzymes, especially SDH and FK, are required to establish the mechanism of fructose accumulation in fruits of Rosaceae plants.

**Literature Cited**


リンゴおよびニホンナシ果実の生長過程におけるフルクトース含量とフルクトース関連酵素活性の変動

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摘要

リンゴおよびニホンナシ果実でのフルクトース積累に関与する酵素を検討するため、フルクトース含量とフルクトース関連酵素活性の変動を新鮮果当たりで比較した。ニホンナシ果実において、開花後66から92日以降でフルクトース含量は増加したが、NAD依存性ソルビトール脱水素酵素 (SDH)活性は低下した。その間フルクトキナーゼ (FK)活性の顕著な低下がみられた。さらに開花後92から126日までフルクトース含量が急激に上昇した際、SDH活性の上昇とともにFK活性の低下もみられた。リンゴ果実においては、生育期間を通じてFK活性は低く推移した。以上のことより、SDHに加えてFK活性の低さがフルクトース積累に関与する可能性が示された。リンゴおよびニホンナシ果実において、フルクトース6リン酸ホスファターゼ (F6Pase)活性はFK活性に比べて顕著に低かったため、フルクトースレベルの決定要因としてはFKの方が重要であると考えられた。一方、ホスホグルコースイソメラーゼ (PGI)活性はFK活性に比べて同等か高かったため、PGIが律速段階とはならないと考えられた。従って、PGIはフルクトースレベルの決定要因ではないことが示唆された。