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Minireview

Changes in Cell Wall Polysaccharides During Fruit Ripening

Kazuyuki Wakabayashi

Department of Biology, Faculty of Science, Osaka City University, Sugimoto 3, Sumiyoshi-ku, Osaka 558-8585, Japan

Softening is typically involved in the ripening process of many fruits and usually represented by a decrease in the firmness of the tissues. Softening of most fruits is accompanied by the dissolution of cell adhesion, which is brought about by the degradation of the middle lamella. In cell wall polymers, pectic polysaccharides, particularly polyuronides, have been known to be major constituents of the middle lamella. The relationship between the degradation of pectic polyuronides and the decrease in the firmness of fruit tissues has been extensively documented. Recently, in addition to the pectin degradation, xyloglucan breakdown has been observed in the early stage of softening in some fruits. These findings suggest that the degradation of both xyloglucans and polyuronides is cooperatively involved in fruit softening processes: the xyloglucan breakdown may contribute to the initiation process, while the polyuronide degradation to the excessive softening process.

Key words: Cell wall polysaccharides — Degradation — Fruit — Polyuronides — Softening — Xyloglucans

Fruit ripening is often looked upon as the start of fruit senescence and, previously, the deteriorative aspects had been emphasized. In the recent view, however, ripening is regarded as well-coordinated processes of organ differentiation, which are genetically programmed (Fischer and Bennett 1991). In various biochemical transitions, such as the pigment synthesis and the production of volatiles, softening of the tissues typically accompanies the ripening of many fruits. Fruit softening is a developmentally programmed process and brought about by the modifications in the structure of cell walls, particularly by the degradation of cell wall polymers. The mechanical strength and the texture of cell walls change dramatically during fruit softening process. In addition, ripe fruit contains a large amount of hydrolases that are involved in the degradation of cell wall polymers. Thus, it has been considered that fruit softening is a good model system to study the relationship between the mechanical strength and the structural feature of cell walls and also the function and regulation of cell wall hydrolases in the degradation of cell walls.

Abbreviations: CDTA, *trans*-1, 2-cyclohexanediamine-*N, N, N', N'*-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; PG, polygalacturonase; PME, pectinmethylesterase; XET, xyloglucan endotransglycosylase.

Generally, fruit cell walls contain a large amount of pectins. Also pectic polymers are major constituents of the middle lamella and thus contribute to the cell adhesion mechanism. Degradation of pectins, particularly that of polyuronides, may cause the collapse of the cell adhesion and thereby decreasing the tissue strength. The relationship between the polyuronide degradation and the softening has been extensively studied in many fruits. On the other hand, recent molecular genetic approaches, such as transgenic plants, have been shown that the degradation of polyuronides is not sufficient for the induction of softening in tomato fruit (Giovannoni *et al.* 1989, Smith *et al.* 1990). These findings have led to the proposal that the degradation of other components in cell walls may be required for the induction process of softening and that the cooperative degradation of such components and polyuronides brings about sufficient relaxation for fruit to soften.

Fruit cell walls contain a significant amount of xyloglucans. Recently, the degradation, particularly depolymerization, of xyloglucans has been observed in the early stage of softening in some fruits, such as tomato, avocado, melon and kiwifruit. The present review focuses on the degradation of xyloglucans and polyuronides in fruit tissues, the possible role of these polymers in the regulation of fruit softening, and the regulation mechanism of the degradation of these polymers.

Xyloglucans

Xyloglucans are a predominant hemicellulosic polysaccharide and they account for as much as 20-25% of the constituents of primary cell walls of dicotyledonous plants (Darvill *et al.* 1980, Hayashi 1989).

Generally, xyloglucans are attached to cellulose microfibrils by hydrogen bonds and cross-link between cellulose microfibrils (Hayashi and Maclachlan 1984, Fujino *et al.* 2000). The reported values of the weight-average molecular masses of xyloglucans in cell walls of stem tissues, such as azuki bean and pea epicotyls and squash hypocotyls are in the range of 500-1500 kDa (Nishitani and Masuda 1981, Hoson *et al.* 1991, Wakabayashi *et al.* 1991, Miyamoto *et al.* 1997). The estimated lengths of xyloglucan molecules are about 400-600 nm (Sakurai 1991, Vincken *et al.* 1997). The lengths are much longer than the distance between adjacent cellulose microfibrils (10-15 nm; Fujino *et al.* 2000) and comparable to those of the cellulose molecules in primary cell walls, 250-1000 nm (Blaschek *et al.* 1982). Therefore, the formation of the cellulose-xyloglucan network probably gives

strength (rigidity) to cell walls. Indeed, the treatment of stem tissues with auxin promotes the depolymerization of xyloglucans as well as the decrease in the mechanical rigidity of cell walls, and thereby induces cell elongation of stem tissues (azuki bean epicotyls, Nishitani and Masuda 1981, Hoson *et al.* 1991; pine hypocotyls, Lorences and Zarra 1987; squash hypocotyls, Wakabayashi *et al.* 1991). On the other hand, close correlations between the increase in the molecular mass of xyloglucans and the increase in the mechanical rigidity of cell walls have been observed in pea epicotyls (Miyamoto *et al.* 1997) and in azuki bean epicotyls (Soga *et al.* 1999). These studies show, from elution profiles of xyloglucan molecules after gel-permeation chromatography, that xyloglucans are certainly shifted to higher or lower molecular mass regions after the treatment, and the molecular masses of xyloglucans increase or decrease about 20-40% from the control level. These findings suggest that changes in the molecular mass of xyloglucans are involved in the regulation of the mechanical strength of cell walls.

Xyloglucan degradation in ripening fruit

In fruit tissues, xyloglucans account for 20-30% of the constituents of cell walls in pericarp of tomato (Sakurai and Nevins 1993), 5-10% in mesocarp of avocado (Sakurai and Nevins 1997), about 10% in mesocarp of grape berry (Nunan *et al.* 1998), 20-25% in pericarp of melon (Rose *et al.* 1998) and about 7% in pericarp of persimmon (Cutillas-Iturralde *et al.* 1994). Xyloglucans from pre-ripe fruits are high molecular mass polymers (the weight-average molecular masses are in the range of hundreds - 1000 kDa) and the values are comparable to those of stem tissues. These findings indicate that fruit cell walls contain a significant amount of xyloglucans with high molecular mass.

Table 1 summarizes the xyloglucan breakdown in fruit tissues. Using the stress-relaxation method, Sakurai and Nevins (1993) showed that tissues of red (ripe) tomato fruit were loosened as compared with those of mature green (unripe) fruit and suggested that fruit softening is caused by the changes in the viscosity and elasticity in the tissues by the degradation of cell wall polymers. In cell wall components, levels of xyloglucans decreased about 30% from the mature green to the red stage fruit. In addition, the molecular mass of hemicellulosic polysaccharides, particularly that of xyloglucans, of red fruit walls was reduced to 50% of that of mature green fruit, while the molecular mass of chelator (EDTA)-soluble pectins was almost similar in the mature green and red fruit (Sakurai and Nevins 1993). Maclachlan and Brady (1994) also showed that the molecular mass downshift of xyloglucans was observed in the early stage of tomato fruit softening and continued in parallel with the decrease in the tissue firmness, although the amounts of xyloglucans remained constant during the softening.

In tomato fruit ripening mutants, *rin* (ripening inhibitor) mutation impairs many aspects of fruit ripening processes, such as softening, polyuronide degradation and ethylene biosynthesis, and *rin* fruit contains less than 1% of the wild-type level of polygalacturonase (PG) (Giovannoni *et al.* 1989). The reduced PG level may cause the limited degradation of polyuronides and thereby inhibits fruit softening. However, when low PG levels in *rin* mutant were transformed to nearly normal levels by the PG gene transfer, with the result that polyuronides were extensively degraded and solubilized (Giovannoni *et al.* 1989, DellaPenna *et al.* 1990), fruit softening was still inhibited (Giovannoni *et al.* 1989). This result indicates that in *rin* fruit, the high levels of PG activity and the resultant degradation of polyuronides were not sufficient to stimulate softening. In *rin* fruit, the levels and the molecular mass of xyloglucans were almost equivalent to those in wild-type fruit, and the molecular mass of xyloglucans in *rin* fruit did not change during ripening, while that in wild-type fruit extensively decreased (Maclachlan and Brady 1994). The firmness of *rin* fruit only slightly decreased during ripening (Maclachlan and Brady 1994). Therefore, it is possible that the limited xyloglucan breakdown in *rin* fruit is involved in the inhibition of tissue softening.

Melon fruit (Charentais variety) undergoes remarkably rapid ripening, with the transition from the pre-ripe to the over-ripe stage occurring within 24 to 48 hr, while tomato fruit generally develops from the pre-ripe to the over-ripe stage over a period of 10 to 18 days (Rose *et al.* 1998). In cell walls of melon fruit, depolymerization of hemicellulosic polysaccharides took place throughout ripening, and the degradation of tightly bound (24% KOH-extractable) xyloglucans was detected at the early onset of softening, while polyuronide depolymerization occurred in the later ripening stages (Rose *et al.* 1998).

Avocado fruit also softens extensively during ripening. When cold-storage pre-ripe fruit was ripened in 25 C, fruit firmness rapidly decreased (O'Donoghue and Huber 1992, Sakurai and Nevins 1997, Wakabayashi *et al.* 2000). The molecular mass of strong alkali (4 M NaOH)-extractable xyloglucans decreased extensively in the early stage of fruit

Table 1. Xyloglucan breakdown in ripening fruit.

Fruit	Xyloglucan breakdown	Onset of breakdown	Reference
Tomato	Depolymerization and decrease in amount	During softening	Sakurai and Nevins (1993)
	Depolymerization	Early stage of softening	Maclachlan and Brady (1994)
Avocado	Depolymerization	Early stage of softening	O'Donoghue and Huber (1992)
	Depolymerization and decrease in amount	Early stage of softening	Sakurai and Nevins (1997)
Melon	Depolymerization	Early stage of softening	Rose *et al.* (1998)
Persimmon	Depolymerization and decrease in amount	Extensive softening period	Cutillas-Iturralde *et al.* (1994)
Kiwifruit	Depolymerization	Early stage of softening	MacRae and Redgwell (1992)

softening (O'Donoghue and Huber 1992, Sakurai and Nevins 1997). In addition, the levels of xyloglucans in avocado fruit decreased during the period (Sakurai and Nevins 1997).

In persimmon fruit, the levels and the molecular mass of xyloglucans decreased to about 70% and 30% of those of the pre-ripe fruit during the excessive softening period, respectively (Cutillas-Iturralde *et al.* 1993, 1994). Also, in kiwifruit, depolymerization of xyloglucans was observed during softening (MacRae and Redgwell 1992). These observations clearly show that the degradation of xyloglucans in cell walls is associated with significant changes in fruit texture. The breakdown of xyloglucan molecules in the early (initial) stage of softening may partially disrupt the cellulose-xyloglucan network which, in turn, probably causes a decrease in the rigidity of cell walls in fruit tissues. In addition, partial breakdown of the cellulose-xyloglucan network decreases the integrity of cell wall architecture, which may increase the wall pore size and thereby enhances the diffusion (mobility) of hydrolases in cell walls. Indeed, the stimulation of the degradation of xyloglucans in cell walls of stem tissues by auxin treatment increases the size of wall pore (Hoson 1993). Since the breakdown of xyloglucans is often accompanied by the extensive degradation of polyuronides in ripening fruit (O'Donoghue and Huber 1992, Huber and O'Donoghue 1993, Sakurai and Nevins 1993, 1997, Rose *et al.* 1998), the xyloglucan breakdown may contribute to the stimulation of the polyuronide degradation via increasing mobility of hydrolases, such as PG.

Xyloglucan depolymerizing activity in ripening fruit

Depolymerization of xyloglucan molecules has been explained by the action of cell wall hydrolases. Levels of β-1, 4-glucanase (Cx-cellulase) activity have been shown to increase during tomato fruit ripening (Campbell *et al.* 1990). Tomato fruit contains at least three types of β-1, 4-glucanase that can hydrolyze xyloglucans (Maclachlan and Brady 1992). On the other hand, Cx-cellulase purified from ripe avocado fruit did not promote the xyloglucan depolymerization and the protein fraction depleted of Cx-cellulase was capable of the degradation of xyloglucans (O'Donoghue and Huber 1992). This result suggests that Cx-cellulase is not involved in the ripening-related depolymerization of xyloglucans in avocado fruit.

In recent aspect of hemicellulose metabolism, the endotransglycosylation of xyloglucans has attracted the attention (Nishitani and Tominaga 1991, 1992, Fry *et al.* 1992). The enzymic activity responsible for the transglycosylation has been characterized and described as xyloglucan endotransglycosylase (XET). The proteins with XET activity were purified from azuki bean stems (Nishitani and Tominaga 1992). Xyloglucan endo-hydrolases that had been purified from azuki bean stems (xyloglucan hydrolase/endotransferase, Tabuchi *et al.* 1997) and from nasturtium seeds (Farkas *et al.* 1992, Fanutti *et al.* 1993) were found to possess both hydrolase and transglycosylase activity. XETs that have both hydrolase and transglycosylase activity can use xyloglucan oligosaccharides and/or H_2O as acceptors for the reaction, and xyloglucan oligosaccharides are capable of stimulating the activity of XETs (Tabuchi *et al.* 1997, Nishitani 1997). Therefore, XETs have been proposed to depolymerize xyloglucans. Tomato fruit contained a xyloglucanase (xyloglucan-depolymerizing) activity that was substantially stimulated by xyloglucan oligosaccharides, and its activity was highest at the early stage of fruit softening and declined during aging (Maclachlan and Brady 1994). Also, the onset of xyloglucan breakdown synchronized with the period of the highest xyloglucanase activity. Thus, it is possible that XETs are responsible for the xyloglucan-depolymerizing activity in fruit tissues. The higher activity of XET was reported during the softening of persimmon fruit (Cutillas-Iturralde *et al.* 1994). In kiwifruit, XET activity increased throughout tissue softening period (Redgwell and Fry 1993) and the activity was also correlated with the depolymerization of xyloglucans (MacRae and Redgwell 1992). XET has been purified from ripe kiwifruit, and the purified enzyme is capable of hydrolyzing (depolymerizing) high molecular mass kiwifruit xyloglucans in the presence or absence of xyloglucan oligosaccharides (Schröder *et al.* 1998). The enzyme had maximum activity between pH 5.5 and 5.8. The molecular weight of XET of kiwifruit (34 kDa, Schröder *et al.* 1998) is comparable to that of azuki bean stems (33 kDa, Nishitani and Tominaga 1992) and small green tomato fruit (34 kDa, de Silva *et al.* 1994). In addition, kiwifruit XET shows high amino acid identity to XETs of small green tomato fruit and azuki bean stems, although tomato and azuki bean XETs cannot act as a hydrolase (Schröder *et al.* 1998). It is possible that XETs possessing both hydrolase and transglycosylase activity play a principal role in the degradation of xyloglucans in ripening fruit.

Polyuronides

Polyuronides consist of linear polymers of α-(1, 4)-linked galacturonic acid (homogalacturonans) and rhamnosyl residue-containing galacturonans (rhamnogalacturonans). Rhamnogalacturonans usually contain arabinose- and galactose-rich side chains (arabinogalactans) (Darvill *et al.* 1980, Fischer and Bennett 1991). Galacturonic acid residues in polyuronides are partly methylesterified. Polyuronides are major components in fruit cell walls.

Polyuronide degradation in ripening fruit

The extensive solubilization and depolymerization of polyuronides have been observed during the ripening of many fruit types (Huber 1983). In ripe avocado fruit, more than 90% of polyuronides in cell walls were extracted with water or chelator (CDTA) solution, and their molecular masses were extraordinarily small compared to polyuronides from firm (pre-ripe) fruit (Huber and O'Donoghue 1993, Wakabayashi *et al.* 2000). The increase in the levels of water-soluble polyuronides during ripening was accompanied by the decrease in the levels of water-insoluble polyuronides (Campbell *et al.* 1990, Wakabayashi *et al.* 2000), indicating that polyuronides solubilized during ripening originated from polymers more tightly integrated in the walls or perhaps linked to hemicelluloses (Redgwell *et al.* 1997a). Similarly, tomato, kiwifruit, blackberry, persimmon, plum and

strawberry fruit show the dramatic increases in water-soluble polyuronides during ripening (Redgwell *et al.* 1997b). Depolymerization of chelator-soluble polyuronides during softening has been shown in many fruit types, such as tomato, avocado, kiwifruit, persimmon, apple, watermelon, plum and nashi pear (Redgwell *et al.* 1992, 1997b). Increased solubilization and depolymerization of polyuronides are usually correlated with the decrease in fruit tissue firmness (Huber 1983, Redgwell *et al.* 1997b). Thus, the polyuronide degradation has been considered to be involved in the processes of fruit softening.

PG catalyzes the hydrolytic cleavage of α-(1, 4)-galacturonan linkages. PG activity has been shown to increase during ripening of many fruit types and there is a correlation between the PG activity and the degradation of polyuronides (Huber 1983). The observations that *in vitro* degradation of isolated cell walls by PG mimics the polyuronide degradation that occurs *in vivo* (Wallner and Bloom 1977, Huber 1981, Themmen *et al.* 1982, Chun and Huber 1997, 1998, Wakabayashi *et al.* 2000) indicate the central role of PG in polyuronide degradation in ripening fruit.

On the other hand, the molecular genetic approaches, antisense technique, showed that suppression of tomato fruit PG mRNA accumulation by constitutive expression of an antisense transgene reduced the levels of PG mRNA and enzyme activity (Smith *et al.* 1990). PG activity in transgenic fruit was reduced to less than 1% of wild-type fruit. In transgenic fruit, suppression of PG mRNA accumulation inhibited the ripening-related depolymerization of chelator-soluble polyuronides, but it did not prevent the increases in the levels of polyuronide solubilization and fruit softening (Smith *et al.* 1990). This result suggests a possibility that depolymerization of polyuronides solely is not sufficient for the induction of tissue softening. However, ripe transgenic tomato fruit had beneficial postharvest-handling characteristics; measurable improvements in storage life, in solids content and in consistency and viscosity of processed juice (Fischer and Bennett 1991). In addition, transgenic fruit remained slightly firmer than wild-type fruit during the period from the full-ripe to the over-ripe stage (Brummell and Labavitch 1997). These findings suggest that in late ripening stages, PG-mediated polyuronide depolymerization plays a role in reducing fruit integrity and firmness.

In tomato *rin* mutant, low PG levels in the mutant were recovered to nearly control levels by the PG gene transfer, and increased PG activity caused the extensive depolymerization and solubilization of polyuronides but not the promotion of fruit softening (Giovannoni *et al.* 1989). As described above, in *rin* fruit, xyloglucan breakdown was substantially inhibited during ripening (Maclachlan and Brady 1994). Thus, the limited xyloglucan breakdown may intercept the tissue softening in transgenic *rin* fruit.

Regulation of polyuronide degradation in ripening fruit

The increasing activity of PG is brought about by the increases in levels of PG proteins and PG mRNAs (Fischer and Bennett 1991). In tomato, PG mRNA accumulated to extremely high levels in ripening fruit, accounting for ca. 2% of the total mRNA (DellaPenna *et al.* 1987). The extractable PG activity increased about 10-fold during ripening of tomato and avocado fruit (Campbell *et al.* 1990, Huber and O'Donoghue 1993). Generally, there is a correlation between PG levels and degradation of polyuronides (Fischer and Bennett 1991), indicating that the induction of *de novo* synthesis of PG proteins is involved in the stimulation of polyuronide degradation.

In addition to PG levels, various factors, such as cell wall (apoplastic) environment and regulators, appear to be involved in the regulation of activity of PG. In cell wall environment, pH and ionic concentrations deeply contribute to the catalytic properties of tomato PG (Chun and Huber 1998). Tomato PG was nearly inactive at pH 6.0 and above and low ionic strength *in vitro*, but decreasing pH value (to pH 4.5) and increasing ionic strength dramatically increased the activity of PG (Chun and Huber 1998). In tomato fruit, the pH of the apoplastic fluid decreased from 6.7 in mature-green fruit to 4.4 in fully-ripe fruit and also the ionic strength, especially potassium concentration, increased about three-fold during the period (Almeida and Huber 1999). Since the extensive depolymerization of polyuronides was observed during the period from the fully-ripe to the over-ripe stage *in vivo* (Huber and O'Donoghue 1993), the lowered apoplastic pH and the increased potassium concentration in fully-ripe fruit may stimulate the activity of PG already present in cell walls and thereby induce such an extensive polyuronide depolymerization.

In the case of avocado fruit, purified PG substantially promoted the release of polyuronides from cell walls of pre-ripe fruit, concomitant of marked downshift in molecular mass *in vitro* (Wakabayashi *et al.* 2000). Polyuronides released by PG, however, were relatively high molecular mass polymers as compared with those from fully-ripe fruit. Repeated treatment of the released polyuronides with PG did not promote further depolymerization. On the other hand, when the released polyuronides were treated with pectin-methylesterase (PME), extensive molecular mass downshift occurred in response to incubation with PG. These observations suggest that partial de-esterification by PME is required for PG to carry out extensive depolymerization of polyuronides. Thus, the degree of methylesterification of polyuronides may be a factor contributing to the regulation of polyuronide degradation, and both PG and PME may be cooperatively involved in the degradation process of polyuronides in avocado fruit. Similarly, the increase in polyuronide release by PG after the treatment of cell walls with PME has been reported in mature-green tomato fruit (Koch and Nevins 1989).

Fruit cell walls contain significant amounts of galactose and arabinose. These neutral sugars are constituents of arabinogalactans, which are usually attached to rhamnogalacturonans (Darvill *et al.* 1980). The decreases in the amounts of galactose and arabinose during ripening has been shown in many fruit types (Fischer and Bennett 1991, Redgwell *et al.* 1997a). A temporal association between polyuronide solubilization and galactose loss has been observed during the early stage of melon fruit ripening (Rose

et al. 1998). In addition, β-galactosidases and β-galactanases had a capacity to alter the molecular mass of polyuronides (de Veau *et al.* 1993, Ali *et al.* 1998). Therefore, the removal of galactose-containing side chains, probably arabinogalactans, may facilitate the polyuronide degradation in ripening fruit.

Concluding remarks

Fig. 1 shows the schematic representation of the xyloglucan breakdown and the polyuronide degradation during fruit ripening. Xyloglucans and polyuronides in fruit tissues undergo an active turnover during ripening, and the degradation of both polymers seems to be involved in reducing the cell wall integrity, thereby decreasing firmness of fruit tissues. The regulation of breakdown of cell wall polysaccharides in fruit tissues is performed by many factors, such as the levels and activity of hydrolases and the interaction of hydrolases with polysaccharide complex. Induction of *de novo* synthesis is involved in the increase in hydrolase activity. In addition, cell wall environments, especially pH conditions, deeply contribute to the regulation of hydrolase activity. The integrity of cell wall architecture, wall pore size, and modification of polymers (addition and removal of side chains and methylesters) are involved in the regulation of the mobility of hydrolases and the susceptibility of polysaccharides to hydrolases. These factors also influence the catalytic potential of hydrolases.

Regulation mechanisms of the degradation of cell wall polysaccharides in fruit tissues are almost comparable to those in stem tissues (Sakurai 1991, Hoson 1993, Soga *et al.* 2000). In general, the turnover of cell wall polysaccharides in fruit tissues occurs rapidly on a large scale. Therefore, information on the regulation of cell wall breakdown in fruit tissues may provide us with clues to a further understanding of regulation of cell wall degradation in stem tissues. In addition, ripe fruit contains a large amount of various kinds of cell wall hydrolases. These hydrolases in fruit tissues are available for the study of the breakdown of cell wall polysaccharides in stem tissues. Integration and coordination of many pieces of knowledge obtained from studies of fruit ripening as well as stem growth should enhance our understanding of how cell wall breakdown is regulated by various factors in plant.

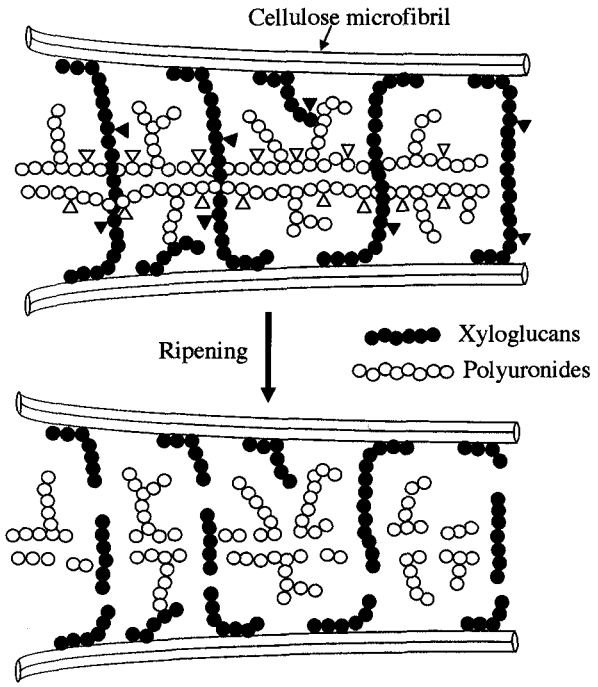

Fig. 1. Schematic representation of the xyloglucan breakdown and the polyuronide degradation during fruit ripening. Xyloglucan-hydrolyzing enzymes (▲) and PG (△) are involved in the breakdown of xyloglucans and polyuronides, respectively. Activities of these enzymes are enhanced by the induction of *de novo* synthesis and by the changes in cell wall environments, such as the acidification. Also, the increase in wall pore size and the modification of polymers (removal of side chains and methylesters) are involved in the stimulation of the catalytic potential of enzymes via increasing the mobility of enzymes and the susceptibility of polymers to enzymes. Breakdown of both xyloglucans and polyuronides reduces the integrity of cell walls and thereby causes a decrease in the firmness of fruit tissues.

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References

Ali, Z.M., Ng, S.-Y., Othman, R., Goh, L.-Y. and Lazan, H. 1998. Isolation , characterization and significance of papaya β-galactanases to cell wall modification and fruit softening during ripening. Physiol. Plant. **104**: 105-115.

Almeida, D.P.F. and Huber, D.J. 1999. Apoplastic pH and inorganic ion levels in tomato fruit: A potential means for regulation of cell wall metabolism during ripening. Physiol. Plant. **105**: 506-512.

Blaschek, W., Koehler, H., Semler, U. and Franz, G. 1982. Molecular weight distribution of cellulose in primary cell walls. Planta **154**: 550-555.

Brummell, D.A. and Labavitch, J.M. 1997. Effect of antisense suppression of endopolygalacturonase activity on polyuronide molecular weight in ripening tomato fruit and in fruit homogenates. Plant Physiol. **115**: 717-725.

Campbell, A.D., Huysamer, M., Stotz, H.U., Greve, L.C. and Labavitch, J.M. 1990. Comparison of ripening processes in intact tomato fruit and excised pericarp discs. Plant Physiol. **94**: 1582-1589.

Chun, J.-P. and Huber, D.J. 1997. Polygalacturonase isozyme 2 binding and catalysis in cell walls from tomato fruit: pH and β-subunit effects. Physiol. Plant. **101**: 283-290.

Chun, J.-P. and Huber, D.J. 1998. Polygalacturonase-mediated solubilization and depolymerization of pectic polymers in tomato fruit cell walls. Regulation by pH and ionic conditions. Plant Physiol. **117**: 1293-1299.

Cutillas-Iturralde, A., Zarra, I. and Lorences, E.P. 1993. Metabolism of cell wall polysaccharides from persimmon fruit. Pectin solubilization during fruit ripening occurs in apparent absence of polygalacturonase activity. Physiol. Plant. **89**: 369-375.

Cutillas-Iturralde, A., Zarra, I., Fry, S.C. and Lorences, E.P. 1994. Implication of persimmon fruit hemicellulose metabolism in the softening process. Importance of xyloglucan endotransglycosylase. Physiol. Plant. **91**: 169-176.

Darvill, A., McNeil, N., Albersheim, P. and Delmer, P.D. 1980. The primary cell walls of flowering plant. *In* E.N. Tolbert, ed., The Biochemistry of Plants Vol. 1, Academic Press, New York, pp. 91-192.

DellaPenna, D., Kates, D.S. and Bennett, A.B. 1987. Polygalacturonase gene expression in Rutgers, *rin*, *nor*, and *Nr* tomato fruits. Plant Physiol. **85**: 502-507.

DellaPenna, D., Lashbrook, C.C., Toenjes, K., Giovannoni, J. J., Fischer, R.L. and Bennett, A.B. 1990. Polygalacturonase isozymes and pectin depolymerization in transgenic *rin* tomato fruit. Plant Physiol. **94**: 1882-1886.

de Silva, J., Arrowsmith, D., Hellyer, A., Whiteman, S. and Robinson, S. 1994. Xyloglucan endotransglycosylase and plant growth. J. Exp. Bot. **45**: 1693-1701.

de Veau, E.J., Gross, K.C., Huber, D.J. and Watada, A.E. 1993. Degradation and solubilization of pectin by β-galactosidases purified from avocado mesocarp. Physiol. Plant. **87**: 279-285.

Fanutti, C., Gidley, M.J. and Reid, J.S.G. 1993. Action of a pure xyloglucan endo-transglycosylase (formerly called xyloglucan-specific endo-(1-4)-β-D-glucanase) from the cotyledons of germinated nasturtium seeds. Plant J. **3**: 691-700.

Farkas, V., Sulova, Z., Stratilova, E., Hanna, R. and Maclachlan, G. 1992. Cleavage of xyloglucan by nasturtium seed xyloglucanase and transglycosylation to xyloglucan subunit oligosaccharides. Arch. Biochem. Biophys. **298**: 365-370.

Fischer, R.L. and Bennett, A.B. 1991. Role of cell wall hydrolases in fruit ripening. Annual Rev. Plant Physiol. Plant Molec. Biol. **42**: 675-703.

Fry, S.C., Smith, R.C., Renwick, K.F., Martin D.J., Hodge, S.K. and Matthews, K.J. 1992. Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. Biochem. J. **282**: 821-828.

Fujino, T., Sone, Y., Mitsuishi, Y. and Itoh, T. 2000. Characterization of cross-links between cellulose microfibrils, and their occurrence during elongation growth in pea epicotyl. Plant Cell Physiol. **41**: 486-494.

Giovannoni, J.J., DellaPenna, D., Bennett, A.B. and Fischer, R.L. 1989. Expression of a chimeric polygalacturonase gene in transgenic *rin* (ripening inhibitor) tomato fruit results in polyuronide degradation but not fruit softening. Plant Cell **1**: 53-63.

Hayashi, T. 1989. Xyloglucans in the primary cell wall. Annual Rev. Plant Physiol. Plant Molec. Biol. **40**: 139-168.

Hayashi, T. and Maclachlan, G. 1984. Pea xyloglucan and cellulose. I. Macromolecular organization. Plant Physiol. **75**: 596-604.

Hoson, T. 1993. Regulation of polysaccharide breakdown during auxin-induced cell wall loosening. J. Plant Res. **106**: 369-381.

Hoson, T., Masuda, Y., Sone, Y. and Misaki, A. 1991. Xyloglucan antibodies inhibit auxin-induced elongation and cell wall loosening of azuki bean epicotyls but not of oat coleoptiles. Plant Physiol. **96**: 551-557.

Huber, D.J. 1981. Polyuronide degradation and hemicellulose modifications in ripening tomato fruit. J. Amer. Soc. Hortic. Sci. **108**: 405-409.

Huber, D.J. 1983. The role of cell wall hydrolases in fruit softening. Hortic. Rev. **5**: 169-219.

Huber, D.J. and O'Donoghue, E.M. 1993. Polyuronides in avocado (*Persea americana*) and tomato (*Lycopersicon esculentum*) fruits exhibit markedly different patterns of molecular weight downshifts during ripening. Plant Physiol. **102**: 473-480.

Koch, J.L. and Nevins, D.J. 1989. Tomato fruit cell wall. I. Use of purified tomato polygalacturonase and pectinmethylesterase to identify developmental changes in pectins. Plant Physiol. **91**: 816-822.

Lorences, E.P. and Zarra, I. 1987. Auxin-induced growth in hypocotyl segments of *Pinus pinaster* Aiton. Changes in molecular weight distribution of hemicellulosic polysaccharides. J. Exp. Bot. **38**: 960-967.

Maclachlan, G. and Brady, C. 1992. Multiple forms of 1,4-β-glucanase in ripening tomato fruits include a xyloglucanase activatable by xyloglucan oligosaccharides. Austral. J. Plant Physiol. **19**: 137-146.

Maclachlan, G. and Brady, C. 1994. Endo-1, 4-β-glucanase, xyloglucanase, and xyloglucan endo-transglycosylase activities versus potential substrates in ripening tomatoes. Plant Physiol. **105**: 965-974.

MacRae, E. and Redgwell, R.J. 1992. Softening in kiwifruit. Postharvest News and Information. **3**: 49N-52N.

Miyamoto, K., Mitani, Y., Soga, K., Ueda, J., Wakabayashi, K., Hoson, T., Kamisaka, S. and Masuda, Y. 1997. Modification of chemical properties of cell wall polysaccharides in the inner tissues by white light in relation to the decrease in tissue tension in *Pisum sativum* epicotyls. Physiol. Plant. **101**: 38-44.

Nishitani, K. 1997. The role of endoxyloglucan transferase in the organization of plant cell walls. Int. Rev. Cytol. **173**: 157-206.

Nishitani, K. and Masuda, Y. 1981. Auxin-induced changes in the cell wall structure: Changes in the sugar compositions, intrinsic viscosity and molecular weight distributions of matrix polysaccharides of the epicotyl cell wall of *Vigna angularis*. Physiol. Plant. **52**: 482-494.

Nishitani, K. and Tominaga, R. 1991. In vitro molecular weight increase in xyloglucans by an apoplastic enzyme preparation from epicotyls of *Vigna angularis*. Physiol. Plant. **82**: 490-497.

Nishitani, K. and Tominaga, R. 1992. Endo-xyloglucan transferase, a novel class of glycosyltransferase that catalyzes transfer of a segment of xyloglucan molecule to another xyloglucan molecule. J. Biol. Chem. **267**: 21058-21064.

Nunan, K.J., Sims, I.M., Bacic, A., Robinson, S.P. and Fincher, G.B. 1998. Changes in cell wall composition during ripening of grape berries. Plant Physiol. **118**: 783-792.

O'Donoghue, E.M. and Huber, D.J. 1992. Modification of

matrix polysaccharides during avocado (*Persea americana*) fruit ripening: an assessment of the role of Cx-cellulase. Physiol. Plant. **86**: 33–42.

Redgwell, R.J. and Fry, S.C. 1993. Xyloglucan endotransglycosylase activity increases during kiwifruit (*Actinidia deliciosa*) ripening. Implications for fruit softening. Plant Physiol. **103**: 1399–1406.

Redgwell, R.J., Melton, L.D., and Brasch, D.J. 1992. Cell wall dissolution in ripening kiwifruit (*Actinidia deliciosa*). Solubilization of the pectic polymers. Plant Physiol. **98**: 71–81.

Redgwell, R.J., Fischer, M., Kendal, E. and MacRae, E.A. 1997a. Galactose loss and fruit ripening: high-molecular-weight arabinogalactans in the pectic polysaccharides of fruit cell walls. Planta **203**: 174–181.

Redgwell, R.J., MacRae, E.A., Hallett, I., Fischer, M., Perry, J. and Harker, R. 1997b. In vivo and in vitro swelling of cell walls during fruit ripening. Planta **203**: 162–173.

Rose, J.K.C., Hadfield, K.A., Labavitch, J.M. and Bennett, A. B. 1998. Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. Plant Physiol. **117**: 345–361.

Sakurai, N. 1991. Cell wall functions in growth and development. A physical and chemical point of view. Bot. Mag. (Tokyo) **104**: 235–251.

Sakurai, N. and Nevins, D.J. 1993. Changes in physical properties and cell wall polysaccharides of tomato (*Lycopersicon esculentum*) pericarp tissues. Physiol. Plant. **89**: 681–686.

Sakurai, N. and Nevins, D.J. 1997. Relationship between fruit softening and wall polysaccharides in avocado (*Persea americana* Mill) mesocarp tissues. Plant Cell Physiol. **38**: 603–610.

Schröder, R., Atkinson, R.G., Langenkämper G. and Redgwell, R.J. 1998. Biochemical and molecular characterisation of xyloglucan endotransglycosylase from ripe kiwifruit. Planta **204**: 242–251.

Smith, C.J.S., Watson, C.F., Morris, P.C., Bird, C.R., Seymour, G.B., Gray, J.E., Arnold, C., Tucker, G.A., Schuch, W., Harding, S. and Grierson, D. 1990. Inheritance and effect on ripening of antisense polygalacturonase genes in transgenic tomatoes. Plant Molec. Biol. **14**: 369–379.

Soga, K., Wakabayashi, K., Hoson, T. and Kamisaka, S. 1999. Hypergravity increases the molecular mass of xyloglucans by decreasing xyloglucan-degrading activity in azuki bean epicotyls. Plant Cell Physiol. **40**: 581–585.

Soga, K., Wakabayashi, K., Hoson, T. and Kamisaka, S. 2000. Changes in the apoplastic pH are involved in regulation of xyloglucan breakdown of azuki bean epicotyls under hypergravity conditions. Plant Cell Physiol. **41**: 509–514.

Tabuchi, A., Kamisaka, S. and Hoson, T. 1997. Purification of xyloglucan hydrolase/endotransferase from cell walls of azuki bean epicotyls. Plant Cell Physiol. **38**: 653–658.

Themmen, A.P.N., Tucker, G.A. and Grierson, D. 1982. Degradation of isolated tomato cell walls by purified polygalacturonase *in vitro*. Plant Physiol. **69**: 122–124.

Vincken, J.-P., York, W.S., Beldman, G. and Voragen, A.G.J. 1997. Two general branching patterns of xyloglucan, XXXG and XXGG. Plant Physiol. **114**: 9–13.

Wakabayashi, K., Chun, J.-P. and Huber, D.J. 2000. Extensive solubilization and depolymerization of cell wall polysaccharides during avocado (*Persea americana*) ripening involves concerted action of polygalacturonase and pectinmethylesterase. Physiol. Plant. **108**: 345–352.

Wakabayashi, K., Sakurai, N. and Kuraishi, S. 1991. Differential effect of auxin on molecular weight distributions of xyloglucans in cell walls of outer and inner tissues from segments of dark grown squash (*Cucurbita maxima* Duch.) hypocotyls. Plant Physiol. **95**: 1070–1076.

Wallner, S. J. and Bloom, H.L. 1977. Characteristics of tomato cell wall degradation in vitro. Implication for the study of fruit-softening enzymes. Plant Physiol. **60**: 207–210.

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