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The Role of β-Galactosidases in the Modification of Cell Wall Components during Muskmelon Fruit Ripening

Anil P. Ranwala[1], Chiyuki Suematsu, and Hiroshi Masuda*

Department of Agricultural Chemistry, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080, Japan

ABSTRACT

The changes in activities of soluble β-galactosidase and two forms of wall-bound β-galactosidases extracted with NaCl and EDTA were investigated throughout the development of muskmelon (*Cucumis melo* L. cv Prince) fruits. DEAE-cellulose ion-exchange chromatography of soluble β-galactosidase revealed the presence of two isoforms. Soluble isoform I was detected in all stages throughout the fruit development, whereas soluble isoform II appeared around 34 d after anthesis when fruit ripening initiated. Both NaCl- and EDTA-released β-galactosidase activities also increased as ripening proceeded. The soluble and wall-bound forms behaved differently upon ion-exchange chromatography. Enzymological properties such as optimum pH, optimum temperature, K_m values for *p*-nitrophenyl β-D-galactopyranoside, and inhibition by metal ions were nearly similar in all forms. Molecular sizes of pectic polymers and hemicelluloses extracted from fruit mesocarp cell walls were shifted from larger to smaller polymers during ripening, as determined by gel filtration profiles. NaCl-released β-galactosidase from cell walls of ripe fruits had the ability to degrade in vitro the pectin extracted from preripe fruit cell walls to smaller sizes of pectin similar to those that were observed in ripe cell walls in situ. Both soluble isoform I and II were able to degrade in vitro the 5% KOH-extractable hemicellulose from preripe fruit cell walls to sizes of molecules similar to those that were observed in ripe cell walls in situ. Soluble isoform I and the NaCl-released form from ripe fruits were able to modify in vitro 24% KOH-extractable hemicellulose from preripe cell walls to sizes of molecules similar to those that were observed in ripe fruits in situ.

Ripening of most of the fleshy fruits is often characterized by softening or loss of firmness. It is assumed that modification and degradation of cell wall components underlie the process of softening in ripening fruits. The polysaccharide components that form the network of the cell wall possess bonds that are susceptible to the action of various degradative enzymes. Degradation and solubilization of pectic polymers accompanied by the dramatic increase in PG[2] activity have often been observed at softening stages of many fruits (5, 10, 12, 24, 27). However, a recent study in tomato (9) suggests that only polyuronide degradation by PG is not sufficient for the induction of softening in ripening fruits. Apart from the changes in pectic polymers, the modifications of other cell wall components, such as hemicelluloses, occur during ripening in hot pepper (11), apple (15), and muskmelon (20). Moreover, a net loss of cell wall noncellulosic neutral sugar has been observed during ripening in apple (15) and muskmelon (20).

Muskmelon (*Cucumis melo* L.) is a fleshy fruit that softens extensively during ripening. The softening of the edible mesocarp of melon fruit begins in the middle of the development period (around 30 DAA) together with other characteristic changes associated with ripening. McCollum et al. (20) reported that softening of muskmelon was related to modifications of pectic and hemicellulosic polysaccharides and loss of noncellulosic neutral sugars. However, the enzyme activities that lead to the modifications of pectins and hemicelluloses have not been identified yet. Investigations on softening-associated mechanisms in muskmelon mesocarp have revealed no detectable PG activity at any stage of development and ripening (13, 17, 20). Additionally, cellulase activity has shown a decline as ripening proceeded (17). These results necessitate the investigation of the presence and action of other possible wall-degrading enzymes in muskmelon mesocarp to better understand the enzymic control of degradation and modification of cell wall components.

β-Galactosidases (EC 3.2.1.23) are widely distributed in various plant tissues, including developing fruits (2–4, 6, 11, 16). Studies on apple (2) and hot pepper (11) have indicated remarkable increases in the activity of β-galactosidase during ripening. One β-galactosidase isozyme in tomato that increases its activity during ripening has exhibited the ability to hydrolyze galactose-rich polysaccharide in vitro from its cell walls (23). These findings suggest possible involvement of β-galactosidases in modification of cell wall components during the ripening of fruits.

In this study, the changes in the activities of soluble and wall-bound β-galactosidases were determined throughout the development of muskmelon fruits. Isoforms of β-galactosidases were identified and partially purified by ion-exchange chromatographic procedures, and their enzymological properties were determined. In vitro modifications of pectic and hemicellulosic components from cell walls by these β-galactosidase isozymes were investigated in detail.

MATERIALS AND METHODS

Plant Material

Muskmelons (*Cucumis melo* L. cv Prince) were grown in the field at the Experimental Farm of Obihiro University

[1] On study-leave from the Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.

[2] Abbreviations: PG, polygalacturonase; DAA, days after anthesis.

during the summer seasons of 1990 and 1991. Female flowers were hand-pollinated and tagged at anthesis. Fruits were harvested at 14, 24, 34, 44, and 50 DAA. The exocarp and endocarp were discarded, and the mesocarp was chopped into small pieces and used immediately for the extraction of enzymes.

Extraction and Partial Purification of Soluble β-Galactosidases

Mesocarp tissues (200 g fresh weight) at each stage of development were homogenized in a Polytron homogenizer (Kinematica) for 2 min in 2 volumes of 50 mM phosphate buffer (pH 7.4) that contained 1 mM 2-mercaptoethanol. The homogenate was squeezed through two layers of cotton cloth to separate the crude soluble extracts from cell walls. Solid ammonium sulfate was added to the crude extracts to 80% saturation. Precipitated proteins were collected by centrifugation at 12,000*g* for 20 min and dissolved in a small volume of 5 mM phosphate buffer (pH 7.4) that contained 1 mM 2-mercaptoethanol. The resultant solution was dialyzed against the same buffer. Insoluble material was removed by centrifugation, and the supernatant was applied to a column of DEAE-cellulose (2.5 × 25 cm) that had been pre-equilibrated with the same buffer. Proteins were eluted with a linear gradient of 0 to 0.3 M NaCl in 1 L of buffer.

Extraction and Partial Purification of Wall-Bound β-Galactosidases

Cell walls obtained after extraction of the soluble fraction were washed thoroughly with 10 L of distilled water and suspended in 200 mL of homogenizing buffer that contained 2 M NaCl, and the suspension was kept for 24 h at 4°C. The extracts were collected by vacuum filtration through two layers of cotton cloth and designated the NaCl-released fraction. Proteins in the extract were precipitated by addition of solid ammonium sulfate to 80% saturation. The protein pellet obtained after centrifugation was dissolved in a small volume of 5 mM acetate buffer (pH 5.6) that contained 1 mM 2-mercaptoethanol, and the resultant solution was dialyzed against the same buffer with two changes. The dialyzate was centrifuged and the supernatant was applied to a column of CM-cellulose (2.5 × 25 cm) that had been pre-equilibrated with the same buffer as that used for dialysis. Adsorbed proteins were eluted with a linear gradient of 0 to 0.5 M NaCl in 1 L of buffer.

After the extraction with NaCl, the cell walls were washed thoroughly with 10 L of distilled water. The cell walls were then suspended in 0.5% (w/v) EDTA in 50 mM K-phosphate buffer (pH 6.8), and the suspension was kept for 24 h at 30°C. The extracts were collected under vacuum filtration and designated the EDTA-released fraction. Proteins in the extract were precipitated by 80% saturation with ammonium sulfate, and further purification was carried out in the same way as the purification of soluble β-galactosidase on a column of DEAE-cellulose, with the exception that proteins were eluted with a linear gradient of 0 to 0.5 M NaCl.

Assay of β-Galactosidase and Other Glycosidases

β-Galactosidase activity was assayed by following the release of *p*-nitrophenol from *p*-nitrophenyl β-D-galactopyranoside substrate. Other glycosidase activities were assessed by the use of appropriate *p*-nitrophenyl glycoside substrates. The reaction mixture consisted of 0.5 mL of 5 mM substrate, 0.5 mL of 0.1 M McIlvaine buffer (pH 5.0), and 0.5 mL of enzyme solution. The reaction was allowed to proceed for 10 to 30 min at 37°C and was terminated by adding 1.5 mL of 0.2 M sodium carbonate. The concentration of liberated *p*-nitrophenol was determined by measuring the absorbance at 410 nm. One unit of enzyme activity was defined as that amount of enzyme that catalyzes the liberation of 1 nmol of *p*-nitrophenol/min at 37°C.

Assay of PG

The assay of PG was performed with incubations of 2 h at 37°C in a total volume of 1 mL of reaction mixture, which consisted of 300 μL of enzyme extract, 40 mM McIlvaine buffer (pH 5.0), and the substrate, 0.2% (w/v) polygalacturonic acid that had been washed with ethanol. Enzyme activity was determined by the reductometric method of Milner and Avigad (21) using galactouronic acid as a standard.

Estimation of Protein

Protein concentration was determined by the method of Bradford (1), with BSA as standard. The amount of protein in chromatographic fractions was measured by absorbance at 280 nm.

Fractionation of Cell Wall Components

Mesocarp tissues (500 g fresh weight) from preripe fruits (20 DAA) and ripe fruits (50 DAA) were homogenized in 2 volumes of 50 mM phosphate buffer (pH 7.4) in a Polytron homogenizer for 3 min. The cell walls were collected by filtration and washed thoroughly with distilled water. The cell walls were treated two times with 500 mL of 0.5% (w/v) EDTA in 50 mM K-phosphate buffer (pH 6.8) for 4 h at 100°C. The extracts were designated EDTA-soluble fraction, which corresponded to pectic substances. The residual cell walls were washed thoroughly with distilled water and incubated with 5% KOH for 24 h at 30°C. After extraction, the residual cell walls were further incubated with 24% KOH for 24 h at 30°C.

The extracts were designated 5% KOH-soluble and 24% KOH-soluble hemicelluloses, respectively. Each extract was dialyzed against distilled water for 2 d (KOH extracts were neutralized with acetic acid before dialysis). The polysaccharides in each fraction were precipitated by the addition of 2.5 volumes of ethanol and collected by centrifugation. The pellets were dissolved in distilled water and dialized against distilled water for 2 d. After centrifugation to remove insoluble material, each sample was lyophilized.

Gel Chromatography of Cell Wall Components

Each cell wall component (15 mg each) was dissolved in 2 mL of elution buffer and applied to a column of Sephacryl S-300 (60 cm × 1.5 cm), pre-equilibrated with 25 mM Na-acetate buffer (pH 5.0) that contained 100 mM NaCl and 3 mM EDTA, and was eluted with the same buffer. Total carbohydrates in column fractions were measured using the phenol-sulfuric acid method (8). Uronic acid content was measured using the Carbazole method (7).

β-Galactosidase Treatment of Cell Wall Components

Each cell wall component (15 mg) from preripe fruits was suspended in 2 mL of reaction mixture consisting of 50 mM McIlvaine buffer (pH 5.0) containing 200 units of partially purified β-galactosidases and was incubated for 2 d at 30°C. The reaction mixture was overlaid with toluene to prevent microbial contamination. The reaction was terminated by heating in boiling water for 3 min. Then, the sample was subjected to Sephacryl S-300 gel filtration as described above.

RESULTS

Changes in the Activities of Four Glycosidases during Fruit Development

Figure 1 shows the changes in the activities of β-galactosidase, α-galactosidase, α-mannosidase, and β-glucosidase in soluble, NaCl-released, and EDTA-released wall-bound fractions extracted from muskmelon mesocarp throughout development. In the soluble fraction, α-galactosidase activity was the highest at all stages of development and exhibited fluctuation during fruit development. β-Galactosidase activity was fairly constant up to 44 DAA and increased markedly at 50 DAA. β-Glucosidase activity was high in immature fruits and declined dramatically after 24 DAA. α-Mannosidase activity was relatively low and constant throughout development.

In the NaCl-released wall-bound fraction, β-galactosidase activity exhibited a steady increase throughout fruit development. α-Galactosidase and β-glucosidase activities declined during fruit development, and α-mannosidase activity was very low and constant throughout fruit development. In the EDTA-released wall-bound fraction, β-galactosidase activity was predominant at all stages of development and it increased steadily, exhibiting a drastic rise from 44 to 50 DAA. Other glycosidase activities were more or less constant or had little fluctuations.

The activity of PG was not detected at any stage of development either in soluble or wall-bound fractions.

Partial Purification of Different β-Galactosidase Isozymes

The activities of soluble α-galactosidase, α-mannosidase, and β-glucosidase exhibited similar behavior during DEAE-cellulose chromatography throughout fruit development, whereas β-galactosidase exhibited changes in the chromatography profile at the ripening stage. Figure 2 shows the activity profiles of soluble β-galactosidase at different stages of fruit development. Soluble β-galactosidase at 14 and 24 DAA was

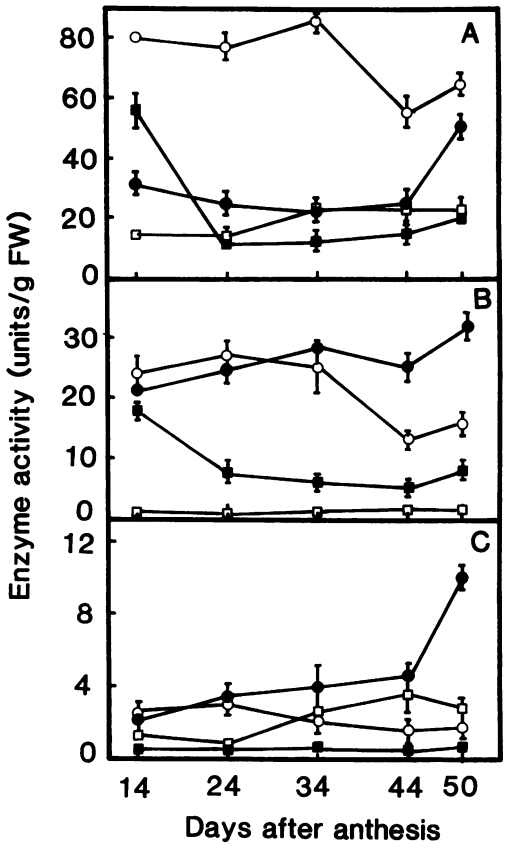

Figure 1. Changes in activities of soluble and wall-bound glycosidases in developing fruit mesocarp. ○, α-Galactosidase; ●, β-galactosidase; □, α-mannosidase; ■, β-glucosidase. A, Soluble; B, NaCl-released; C, EDTA-released. Soluble and EDTA-released enzyme activities were determined after dialysis of crude extracts in 5 mM phosphate buffer (pH 7.4) that contained 1 mM 2-mercaptoethanol. NaCl-released enzyme activity was measured without dialysis. Each value is the mean of results from two replicates, each of which consisted of at least two fruits. Vertical bars represent ± SE.

eluted in a single peak that was unabsorbed to DEAE-cellulose (soluble isoform I). At 34 DAA, a new peak appeared (soluble isoform II) that was adsorbed to DEAE-cellulose and eluted at 0.05 M NaCl, and the level of the new peak thereafter increased steadily.

The active fractions of soluble isoform I in DEAE-cellulose chromatography were pooled, dialyzed against 5 mM acetate buffer (pH 5.6) that contained 1 mM 2-mercaptoethanol, and applied to a column of CM-cellulose (2.5 × 25 cm) equilibrated with the same buffer. The absorbed proteins were eluted with a NaCl gradient of 0 to 0.5 M. Soluble isoform I was eluted in a single peak at 0.1 M NaCl (data not shown). The elution pattern was the same for isoform I in the case of enzymes from 14 and 50 DAA. The pooled fractions of soluble isoform II in DEAE-cellulose chromatography were applied to a column of Phenyl-Toyopearl (1.5 × 10 cm) equilibrated with 10 mM phosphate buffer (pH 7.4) that contained 1 mM 2-mercaptoethanol and 30% ammonium sulfate. Proteins were eluted with a decreasing gradient of 30 to 0% ammonium sulfate. Soluble isoform II was eluted in a

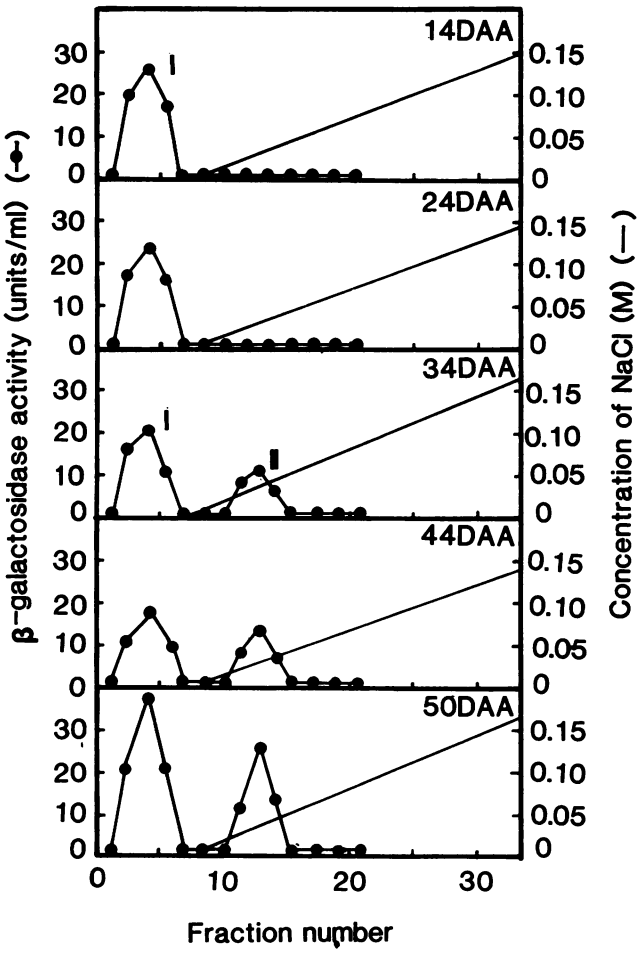

Figure 2. Chromatography on DEAE-cellulose of soluble β-galactosidase from the mesocarp of fruits at different stages of development. Chromatography was performed as described in "Materials and Methods" and fractions of 20 mL were collected.

single peak at the concentration of 10% ammonium sulfate (data not shown).

NaCl-released wall-bound β-galactosidase was adsorbed to a CM-cellulose column and eluted in a single peak at 0.1 M NaCl at each stage of development (data not shown). EDTA-released β-galactosidase was not adsorbed to DEAE-cellulose column (data not shown).

All four partially purified β-galactosidase isoforms were used to investigate their properties. Soluble isoforms I and II and the NaCl-released form were concentrated using a column of Phenyl-Toyopearl for incubation with cell wall components. These partially purified β-galactosidase enzyme preparations contained no detectable activity of PG or other tested glycosidases. However, SDS-PAGE analysis revealed the presence of about 5 to 10 different proteins in these preparations.

Enzymological Properties of β-Galactosidases

All soluble and wall-bound β-galactosidases had a pH optimum of 4.0 to 4.4 when tested with McIlvaine buffer in the range of 2.4 to 8.0. In temperature-dependence studies, soluble isoform II exhibited the highest activity at 60°C, whereas other forms exhibited their highest activities at 50°C. K_m values of different forms for *p*-nitrophenyl β-D-galactopyranoside were determined and the values were 0.5, 1.66, 1.66, and 0.56 mM for soluble I, soluble II, NaCl-released, and EDTA-released β-galactosidases, respectively. The effects of metal ions and EDTA on the enzymic activities are shown in Table I. $HgCl_2$ and $AgNO_3$ completely inhibited all β-galactosidases, and $CuSO_4$ partially inhibited all β-galactosidases.

Action of Three β-Galactosidase Isoforms on Cell Wall Components

Gel filtration of pectin (EDTA-soluble component) extracted from preripe fruits (20 DAA) and ripe fruits (50 DAA) revealed that modification of pectic polymers occurred during ripening (Fig. 3, A and E). From the preripe fruits to ripe fruits, there was a decrease in large polymers and a concom-

Table I. *Effects of Metal Ions and EDTA on Activities of Various β-Galactosidases*

Results are expressed as relative activity (percent).

Compound[a]	Soluble Isoform I	Soluble Isoform II	NaCl-Released Wall-Bound	EDTA-Released Wall-Bound
None	100	100	100	100
$CoCl_2$	91	104	102	98
$MnCl_2$	93	116	88	100
$CaCl_2$	87	98	98	97
$ZnCl_2$	93	103	98	105
$CuSO_4$	63	70	68	66
$MgSO_4$	95	103	94	106
$HgCl_2$	0	0	0	0
$AgNO_3$	0	0	0	0
EDTA	95	98	94	90

[a] Each compound (1.25 mM) was preincubated with enzyme for 5 min before substrate was added to the reaction mixture.

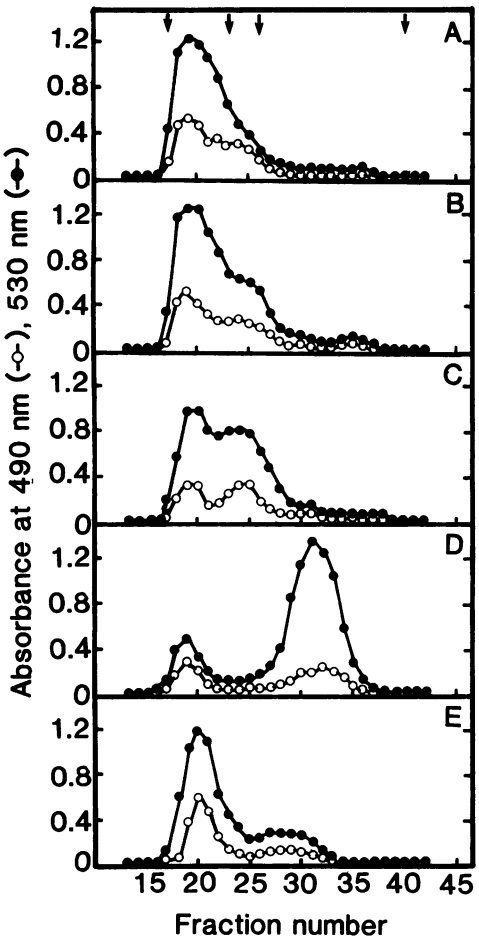

Figure 3. Sephacryl S-300 gel filtration profiles of pectin (EDTA-soluble fraction) extracted from muskmelon mesocarp cell walls. A, Extracted from preripe fruits without treatment; B–D, extracted from preripe fruits treated with soluble I and II and NaCl-released β-galactosidases, respectively; E, extracted from ripe fruits without treatment. Gel filtration was preformed as described in "Materials and Methods," and fractions of 4 mL were collected. Column fractions were assayed for total carbohydrate using the phenol-sulfuric acid method (8) (A_{490}) and for uronic acid content using the carbazole method (7) (A_{530}). Arrows at the top of the figure represent the elution positions of (from left to right) blue dextran 2000, dextrans of average molecular mass of 500 and 70 kD, and glucose.

itant increase in low mol wt polymers. Gel filtration of the pectin from preripe fruits that had been treated with three different β-galactosidase forms (soluble I and II and NaCl-released) from ripe fruits indicated that these enzymes had the ability to modify pectic polymers (Fig. 3, B–D). Especially, NaCl-released wall-bound β-galactosidase from ripe fruits had very high ability to degrade the high mol wt pectic polymers to low mol wt ones, whereas NaCl-released β-galactosidase from preripe fruits only slightly degraded pectic polymers (Fig. 4). This result suggests that NaCl-released wall-bound β-galactosidase from preripe fruits and ripe fruits may be different with regard to substrate specificity.

Hemicelluloses extracted with 5% KOH also exhibited dramatic changes in the polymer size during ripening. The level of large hemicellulose polymers declined and that of small polymers increased concomitantly as ripening progressed (Fig. 5, A and E). Soluble β-galactosidase I and II had the ability to modify the hemicellulose fraction in vitro, and the resultant gel filtration profile resembled the profile from ripe fruits in situ (Fig. 5, B, C, and E). NaCl-released β-galactosidase showed no or very little effect on the modification of this hemicellulose fraction.

Hemicelluloses extracted with 24% KOH showed a slight change in the molecular size during ripening (Fig. 6, A and E). Treatment of hemicellulose with β-galactosidases indicated that the soluble isoform II and the NaCl-released form had the ability to degrade this fraction of hemicellulose in vitro (Fig. 6, C and D) in a pattern similar to that observed in situ.

DISCUSSION

Previous work done on the ripening of muskmelon fruits has demonstrated that modifications of cell wall components such as polyuronides and hemicelluloses occurred during the softening of muskmelon (20). However, the enzymic control of these modifications has not yet been identified. The data presented in this report provide strong evidence for the involvement of β-galactosidase isoforms in the modification of cell wall components during the ripening of muskmelon fruits.

To investigate whether or not some glycosidases, namely, α- and β-galactosidases, β-glucosidase, and α-mannosidase, are involved in the softening of melon fruits, the changes in their total activities throughout fruit development were determined. Moreover, the changes in the levels of β-galactosidase isoforms that were separable with ion-exchange chromatography were examined throughout fruit development.

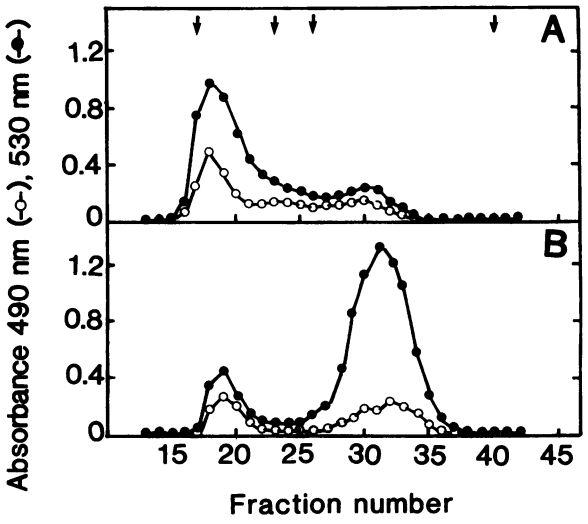

Figure 4. Sephacryl S-300 gel filtration profiles of EDTA-soluble pectins extracted from preripe muskmelon mesocarp cell walls and treated with NaCl-released β-galactosidase extracted from fruits at different stages. A, β-Galactosidase from preripe (10 DAA) fruits; B, β-galactosidase from ripe (50 DAA) fruits. Methods, symbols, and molecular mass calibration standards are as in Figure 3.

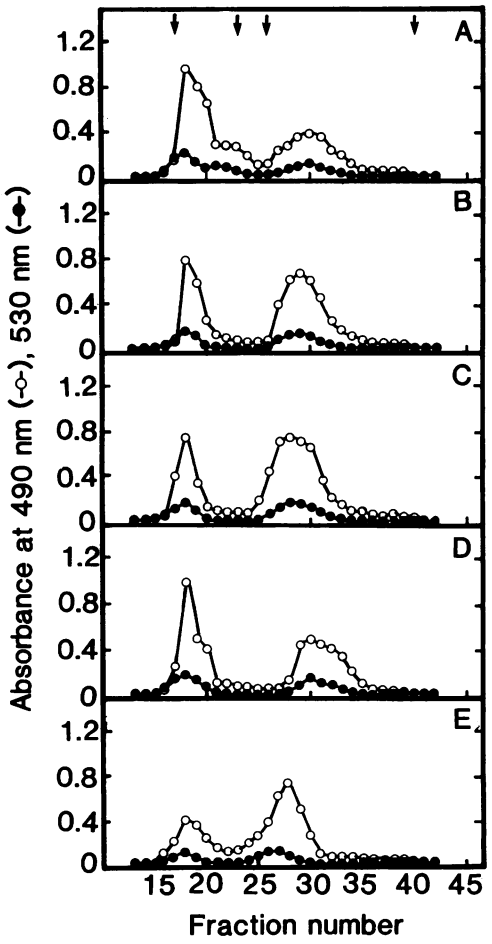

Figure 5. Sephacryl S-300 gel filtration profiles of 5% KOH-extractable hemicelluloses from muskmelon mesocarp cell walls. A, Extracted from preripe fruit without treatment; B–D, extracted from preripe treated with soluble I and II and NaCl-released β-galactosidases, respectively; E, extracted from ripe fruits without treatment. Methods, symbols, and molecular mass calibration standards are as in Figure 3.

Among the glycosidase activities tested in this study, β-galactosidase activity showed a notable increase in its level at late ripening stages. The individual levels of two soluble β-galactosidase isozymes (soluble isoform I and II) that had been separated from one another by DEAE-cellulose chromatography changed remarkably (Fig. 2). The soluble isoform II was not detectable at 14 and 24 DAA, appeared at 34 DAA when softening of melon mesocarp began, and increased until abscission. Comparable results have been reported by Pressey (23) in tomatoes, where total β-galactosidase activity remained relatively constant throughout ripening, whereas individual levels of activity of different isozymes changed differentially during ripening. Other glycosidases examined in this study, namely α-galactosidase, β-glucosidase, and α-mannosidase, did not show a notable increase during ripening.

The wall-bound β-galactosidases have been readily solubilized by high molar salt solutions in most cases (4, 6, 18, 19). β-Galactosidases that are tightly bound to cell walls and that could not be released by salt solutions have been successfully extracted by 0.5% EDTA solutions in the case of suspension cultured sugar beet cells (18). In this study, after the extraction of soluble enzyme, wall-bound enzymes were extracted successively with 2 M NaCl and 0.5% EDTA. Considerable activity of wall-bound β-galactosidase could be solubilized with the above two treatments, confirming the usefulness of both extraction procedures. Moreover, a drastic increase in EDTA-released β-galactosidase was observed at 50 DAA.

The soluble and two wall-bound β-galactosidases behaved differently upon ion-exchange chromatography, indicating that they have different properties. However, other enzymological properties such as optimum pH, optimum temperature, and K_m values for *p*-nitrophenyl β-D-galactopyranoside were in a similar range, and the effects of metal ions on activity were nearly similar in all forms.

Pectic polysaccharides are the major constituents of the

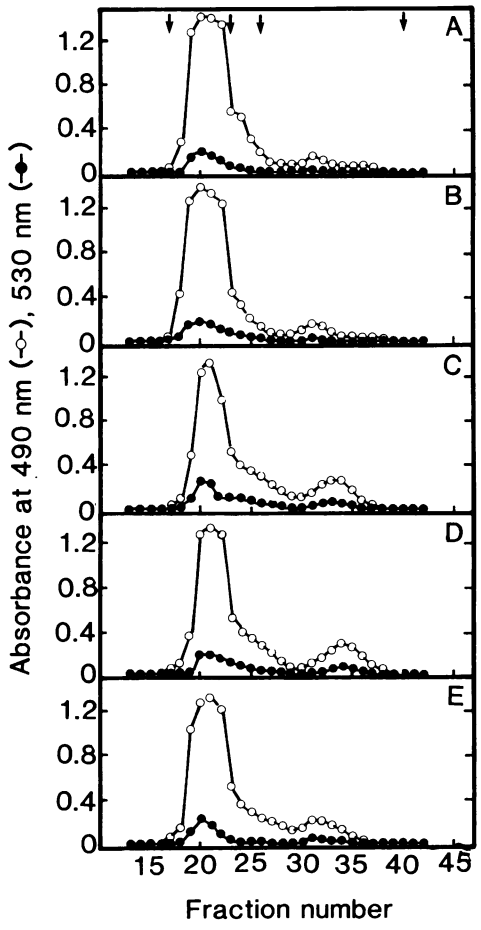

Figure 6. Sephacryl S-300 gel filtration profiles of 24% KOH-extractable hemicelluloses from muskmelon mesocarp cell walls. A, Extracted from preripe fruit without treatment; B–D, extracted from preripe treated with soluble I and II and NaCl-released β-galactosidases, respectively; E, extracted from ripe fruits without treatment. Methods, symbols, and molecular mass calibration standards are as in Figure 3.

primary cell walls and are interconnected to the other polysaccharide polymers such as hemicellulose and cellulose, forming a tight cross-linked matrix network. Galactan, xyloglucan, and arabinan appear to be integral parts of an intermeshed pectic network in primary cell walls (14, 22). The breakdown of the bonds holding this structure will lead to loosening of the stability of this network, which results in the degradation of the cell wall components, causing loss of tissue firmness. Both the degradation of the polygalacturonide backbone and the hydrolysis of other intermeshed cross-linked carbohydrates are involved in this process. The role of PG in the degradation of this network has long been recognized due to its ability to attack polygalacturonide chains, thus solubilizing the pectic polymer. However, enzymes that can possibly reduce the stability of the cell wall through their action on the other linkages have received relatively less attention. McCollum et al. (20) reported that an increase in solubility and a decrease in molecular size of polyuronides in the cell walls of muskmelon fruit occurred during ripening with the apparent absence of PG activity.

In this study, NaCl-released β-galactosidase extracted from cell walls of ripe fruits was able to degrade larger pectin polymers from preripe fruit cell wall to smaller size polymers in vitro as determined by gel filtration profiles. The NaCl-released β-galactosidase may possibly attack 1,4-β-galactosidic cross-linkages in pectic polymers, resulting in the breakdown of large polymers to smaller ones. Investigations carried out on apple fruits (2), *Lilium* pollen (25), and tomato fruits (26) have led to the proposal that there is a hydrolytic effect of β-galactosidase on the neutral glycosidic linkages of pectic polysaccharides. Konno et al. (16) reported that β-galactosidase from carrot cell suspension culture had the ability to degrade pectic polymer extracted from carrot cell wall.

On the other hand, β-galactosidase isoforms showed their ability to degrade the hemicellulose fraction of the cell wall. Especially, soluble isoform I and II from ripe fruits were able to attack hemicellulose extracted by 5% KOH and to reduce the size of the polymer. The gel filtration profiles of the hemicellulose that had undergone the degradation by β-galactosidase slightly resembled the profile observed in situ in the ripened fruit. In hot pepper fruits, where PG activity was not detectable, a shift of the mol wt of hemicellulose from large polymers to small polymers has been observed concomitant with a dramatic rise of β-galactosidase activity as ripening proceeded (11). In apple fruits, an increase in β-galactosidase activity was accompanied by a decrease in galactose content of the cell wall (2). Pressy (23) demonstrated that one β-galactosidase isoform in tomato, which increased in its level during ripening, was able to hydrolyze the galactose-rich polysaccharide from its cell walls in vitro.

In muskmelons, McCollum et al. (20) reported a shift of the molecular size of hemicelluloses from larger to smaller polymers in situ during ripening, and also a decline in galactose content in the melon cell wall as ripening progressed. The results in the present study confirmed the appearance of a new soluble β-galactosidase isoform (isoform II) at the ripening stage that had the ability to modify hemicellulose polymers in vitro in a pattern similar to that observed in situ. Thus, the changes observed in hemicelluloses during the ripening of muskmelons can be, even partly, attributed to the action of β-galactosidases.

Observation of the gel filtration of hemicelluloses extracted by 24% KOH also showed slight changes in their molecular size from unripe fruits to ripe fruits. Soluble isoform II and NaCl-released β-galactosidases were able to act in vitro on 24% KOH-extractable hemicellulose from preripe fruits and modify it to molecules similar to those observed in situ in ripe fruits.

The results reported here demonstrate that β-galactosidases are involved in the modification of pectic polymers and hemicellulose components from preripe muskmelon mesocarp according to the results obtained in vitro. The gel filtration profiles of pectins and hemicelluloses that have been modified by β-galactosidases are comparable to the profiles observed in situ in ripe fruits. However, partially purified β-galactosidase preparations used in this study still contained many proteins, as determined by SDS-PAGE analysis. Therefore, we cannot rule out the possibility of the involvement of other degradative enzymes in combination with β-galactosidase in the observed in vitro modifications of cell wall components. Further purification of these different β-galactosidase isoforms and detailed analysis of the cell wall structure of muskmelon will provide a better picture of the exact functions of different β-galactosidase isoforms in cell wall degradation of muskmelon.

The idea that PG may be a major determinant of fruit softening has been substantially modified by a recent study. Giovannoni et al. (9) reported that polyuronide degradation by PG was not sufficient for the induction of softening in tomato fruits. Additionally, there are some fruits, including muskmelon, that undergo extensive softening in the absence of PG activity. The results presented here indicate the involvement of β-galactosidases in the modification of the cell wall structural polysaccharides of muskmelon fruits, suggesting its important role in softening.

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