Control of Mung Bean Pectinmethylesterase Isoform Activities

INFLUENCE OF pH AND CARBOXYL GROUP DISTRIBUTION ALONG THE PECTIC CHAINS*

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Well-characterized pectin samples with a wide range of degrees of esterification (39–74%) were incubated with the solubilized pure α and γ isoforms of pectinmethylesterase, from mung bean hypocotyl (Vigna radiata). Enzyme activity was determined at regular intervals along the deesterification pathway at pH 5.6 and pH 7.6. It has been demonstrated that the distribution of the carboxyl units along the pectin backbone controls the activity of the cell wall pectinmethylesterases to a much greater extent than the methylation degree, with a random distribution leading to the strongest activity. Polygalacturonic acid was shown to be a competitive inhibitor of the α isoform activity at pH 5.6 and to inhibit the γ isoform activity at both pH 5.6 and pH 7.6. Under these conditions, the drop in enzyme activity was shown to be correlated to the formation of deesterified blocks of 19 ± 1 galacturonic acid residues through simulations of the enzymatic digestion according to the mechanisms established previously (Catoire, L., Pier- ron, M., Morvan, C., Herve du Penhoat, C., and Goldberg, R. (1998) J. Biol. Chem. 273, 33150–33156). However, even in the absence of inhibition by the reaction product, activity dropped to negligible levels long before the substrate had been totally deesterified. Comparison of α and γ isoform cDNAs suggests that the N-terminal region of catalytic domains might explain their subtle differences in activity revealed in this study. The role of pectinmethylesterase in the cell wall stiffening process along the growth gradient is discussed.

Pectinmethylesterases (PMEs)† are cell wall-bound proteins present in almost all plants and phytopathogenic microorganisms. They modify pectic homogalacturan chains, generating free carboxyl groups along the polygalacturonic backbone and releasing protons into the apoplasm. Their action can therefore lead to antinomical effects, especially in primary cell walls. On one hand, the pH decrease should enhance expansin activity and in turn increase the cell wall extensibility (1), but, on the other hand, the generation of carboxyl blocks would be expected to allow the formation of multichain structures via calcium bridges (2). Such structures would greatly affect the physical properties of pectin, due to the assembly of pectic chains into expanded, highly hydrated gel networks decreasing cell wall porosity and also cell wall extensibility (3). Such Ca2+ bridging requires the occurrence of nearly 10 successive carboxyl groups (4) and implies that the PMEs work processively along the galacturonan chain. Unfortunately, although PME biochemical and molecular characteristics have been widely investigated (5–8), a limited number of studies of their action pattern have been reported (9–13). Recently, using partially depolymerized pectin samples and different PME isoforms extracted from mung bean hypocotyl cell walls, we (14) determined both enzyme activity and average product structure at regular intervals along the deesterification pathway. Simulations of different mechanisms by fitting to the experimental data provided information on the possible action patterns of three isoforms. In the case of two of them, the so-called PEα (pI around 7.5) and PEγ (pI above 9) isoforms, a single-chain mechanism (SCM) associated with a free carboxyl group at the second nearest neighbor position was postulated at pH 5.6, whereas some multiple attack mechanism (MAM) was required to reproduce the experimental data at pH 7.6. Although these action patterns reproduced the free carboxyl and methylester distribution in pectin incubated with PEα and PEγ at both pHs for the entire degree of esterification (DE) range observed experimentally, they did not explain the marked decrease in activity for DE values below 50–60%. Indeed, in the simulated digestions, deesterification occurred smoothly down to DE values between 2–4%.

In the present study, we set out to further investigate the effects of neutral and acidic pHs on the development of the deesterification process catalyzed by the neutral and alkaline PME isoforms (PEα and PEγ, respectively). We wanted to determine what limits the deesterification process in situ because, in young cell walls, active PMEs coexist with highly methylated pectins. Three well-characterized commercial pectin samples differing in their DE were chosen for these investigations. We endeavored (a) to determine the kinetic constants of deesterification, (b) to establish the activity profiles as a function of the deesterification pathway for all three samples,
was stopped by lowering the pH to pH 3 with 0.05 N HCl. The protons were titrated with 10 mM NaOH under nitrogen, the pH was maintained at pH 5.6 or pH 7.6 with an automatic titrator (TTT 80; Radiometer), and the reaction rate was expressed as μmol of H⁺ released/min. The reaction was stopped by lowering the pH to pH 3 with 0.05 N HCl.

**Experimental Procedures**

**Pectin Samples**—Three pectin samples PS1, PS2, and PS3, which differed in their DE, were graciously supplied by Herbert Streith & Fox KG (Neuenbürg, Germany).

**Enzyme Isolation and Assay**—Cell walls were isolated from the upper 2.5 cm of hypocotyl tissues of 3-day-old seedlings of mung bean (Vigna radiata L. Wilzeck) according to a procedure described previously (15). PMEs bound to the cell wall fragments were solubilized with 1 N NaCl, and the different isoforms were recovered as described by Bordenave and Goldberg (16, 17). PME activity was measured titrimetrically by following the release of protons from the pectin sample in the presence of 150 mM NaCl (total volume of the assay, 6 ml). The protons were titrated with 10 mM NaOH under nitrogen, the pH was maintained at pH 5.6 or pH 7.6, interaction experiments (18) were run as follows. Pectins with a DE of 74% were treated with PEa and PEγ. For each cloned PCR product, three independent clones were sequenced on both strands using the T7 Sequenase Quick denature vector (Amersham Pharmacia Biotech) and used to screen the cDNA library. Upon sequencing, this fragment was shown to include the coding regions of several internal peptides. The 3'-5'-rapid amplification of cDNA ends system (Life Technologies, Inc.) was then used to obtain the full-length cDNA of PMEα. A cDNA library was also constructed in AZAP II (Stratagene) from polyadenylated RNA of mung bean hypocotyls using XL1-Blue cells as indicated by the manufacturer. The PCR fragment was labeled with digoxigenin (Roche Molecular Biochemicals) and used to screen the cDNA library.

**Results**

**Characteristics of the Pectin Samples Used in the Assays**—The pectin samples used in the assays have been described previously (22). The three samples (PS1, PS2, and PS3) had average molecular masses of 188, 148, and 154 kDa and DEs of 74%, 54%, and 39%, respectively. These pectins presented some side chains (9%, 5%, and 5%, respectively), and the methoxyl distribution of PS1 and PS2 was random, whereas that of PS3 was slightly blockwise. This latter information could be reliably obtained from 13C NMR spectra because it was possible to establish triad populations (i.e., the relative fractions of EEE, EEU + UEE, and UEU trimers within the polymer could be evaluated by integration of the C6 signals). The percentages of methylester groups suitable for PME binding in PS1, PS2, and PS3 (i.e., satisfying the UXE constraint) were estimated to be 27%, 46%, and 60%, respectively, from the UXE populations of the theoretical pectins.

**Activity of PME Isoforms toward Pectins with Different DEs**—The kinetic parameters of the two PME isoforms, PEa and PEγ, acting on various pectin samples differing in their.

| Pectin sample | pH | PEa | | | PEγ |
|--------------|----|-----|-----|----|
| PS1         | 5.6 | 1.85 | 0.50 | 0.35 | 0.63 |
|             | 7.6 | 1.97 | 0.53 | 0.43 | 1.31 |
| PS2         | 5.6 | 0.77 | 0.33 | 0.47 | 0.16 |
|             | 7.6 | 0.48 | 0.22 | 0.42 | 0.24 |
| PS3         | 5.6 | 0.21 | 0.19 | 0.41 | 0.08 |
|             | 7.6 | 0.31 | 0.19 | 0.42 | 0.19 |
methylesterification degrees have been collected in Table I. With both enzyme fractions and at pH 5.6 as well as at pH 7.6, the lower the DE, the higher the affinity. When the $K_m$ was calculated for the esterified residues suitable for enzyme binding, i.e., the esterified residues satisfying the requirement that a free carboxyl group be located at the second nearest neighbor position (UXE constraint), the affinity also increased when the DE decreased. However, these increases were smaller than those calculated for the total methylester populations. With regard to the effects of pH, with the neutral isoform, PE$_\alpha$, whose activity is known to be modified relatively little by the ionic conditions (pH or saline concentration (20)), the kinetic parameters ($V_{max}$ and $K_m$) were rather similar at pH 5.6 and pH 7.6. In contrast, for PE$_\gamma$, both the affinity and the maximal activity were higher at pH 5.6, whatever the DE of the substrate.

Interaction Experiments—Preliminary data indicate that upon incubation with PE$_\alpha$, pectins formed a gel that can be pelleted only at CaCl$_2$ concentrations higher than 0.5 mM. Moreover, in the presence of 2.5 mM or more calcium ions, 80 ± 3% of the pectins sedimented at pH 5.6 and only 72 ± 4% of the pectins sedimented at pH 7.6. These results are in agreement with those of Penel et al. (18), who conducted similar experiments with polygalacturonic acid (PGA). The differences, although small, were observed repeatedly, whatever the concentration of CaCl$_2$ (from 0.5 to 5 mM), indicating a difference in the distribution of the negative charges produced by the enzyme.

Time Course of Enzymatic Deesterification of Pectins with Different DEs—To visualize the effects of DE on the time course of the reaction rate of the deesterification process, plots of activity versus DE have been traced for pectin samples differing in their DE (Fig. 1). Incubations were performed either at pH 5.6 or pH 7.6. The plots obtained with the two isoforms differed, but whatever the incubation conditions, none of the enzymatic fractions were able to generate totally deesterified pectins, and the final DE depended on the nature of the substrate. At pH 7.6, with the neutral isoform PE$_\alpha$, two successive phases were observed (Fig. IA): during the first phase, the reaction rate was nearly constant, whereas during the second one, it decreased rapidly. The DE value that corresponds to the greatest change in slope of the activity profiles is referred to as the slope change point (i.e., SCPE$_\alpha$ or SCPE$_\gamma$ in Tables II and III) and is indicated by filled (pH 5.6) and open (pH 7.6) arrows.

The pH of the incubation medium was maintained at either pH 5.6 (filled symbols) or pH 7.6 (open symbols). The activity (in $\mu$eq of H released/min/mg protein) was estimated from the time necessary to release 2.5 $\mu$eq of H$^+$ from the pectin sample. For each enzyme, the initial reaction rate for DE 70–75% at pH 5.6 has been normalized to parameters (i.e., ionic conditions (pH or saline concentration (20)), the kinetic distribution of the carboxyl and methoxyl groups along the polymer backbone is an important factor for the activity, with a random distribution of the carboxyl units inducing in all cases a higher activity than a blockwise one.

Deesterification Limits—Assays were performed to identify the factors responsible for the drop in activity at both pHs that were investigated. They consisted of attempts to restore the activity at the end of the deesterification process either by adding new substrate or enzyme molecules in the incubation medium or by changing the pH.
Addition of Enzymes—Whatever the pH, the addition of new enzyme molecules at the end of the PEα-catalyzed deesterification did not allow resumption of the enzymatic reaction (Fig. 2), indicating that the modified substrate generated upon PEα action cannot be further processed.

Addition of Substrate—PEα was incubated either at pH 5.6 or at pH 7.6 with PS1 (DE, 74%), and the reaction was allowed to progress until the rate became negligible (Fig. 3). PS1 in NaCl was then added to the assay so that the initial conditions were restored. At pH 7.6, the deesterification started again and developed as seen initially. In contrast, at pH 5.6, the reaction started again but progressed much more slowly than it did initially (initial velocity was only a third of that developing at the beginning of the experiment). In this case, the presence in the assay of the enzymatically deesterified substrate, i.e. the presence of acidic blocks, inhibited the deesterification of the native pectin sample. Similar observations were made for PEγ at acidic pH (data not shown) but at pH 7.6, contrary to PEα, the addition of pectin induced no or only a very attenuated resumption of the deesterification process (Fig. 3).

Modification of the pH—PEα was incubated at pH 5.6 with PS1 until the DE was lowered to nearly 60%, and the pH was then adjusted to pH 7.6. The deesterification started again and proceeded rather rapidly until a DE of ~38% (Fig. 4) was reached. In contrast, if the pH change was made when the reaction rate had become very insiginificant, which was achieved for a DE around 50%, the deesterification started again but was very short-lived (Fig. 4). However, addition at that time of new pectin molecules induced a strong resumption of the deesterification process (data not shown), similar to the one observed in Fig. 3.

Effect of Galacturonic Units—At pH 5.6, with both isoforms, addition of PGA in the assay induced a decrease in the velocity. Preliminary experiments carried out with PEα showed that the inhibition was competitive with a $K_i$ estimated to be 4.2 mM galacturonic acid residues. In contrast, at pH 7.6, the two isoforms exhibited a different sensitivity to PGA. With PEα, even high concentrations of PGA (i.e. 7-fold the substrate concentration) did not modify the reaction rate. At this pH, PEγ activity was inhibited by PGA (i.e. 10 mM galacturonic units induced a 50% decrease of activity). With both isoforms, whatever the pH value, addition of galacturonic acid (the monomer) was without effect. These data confirm the observations made with respect to Fig. 3. At pH 7.6, the presence of new carboxyl groups on the pectin substrate that resulted from enzyme action inhibits the deesterification of new pectin molecules by

### Table III

**Distribution of U blocks during digestion by MAM**

Distribution of U blocks of various lengths (12–22 residues) for 1,000 chains of PS1, PS2, and PS3 (degree of polymerization = 100) during digestion by the UXE MAM at the beginning (DE of the native polysaccharide) and at DE values corresponding to the slope change points for the incubations with PEα (SCPEα) and PEγ (SCPEγ).

| Pectin | DE % | Number of U-Blocks |
|--------|------|---------------------|
|        | 12   | 13                  |
|        | 14   | 15                  |
|        | 16   | 17                  |
|        | 18   | 19                  |
|        | 20   | 21                  |
|        | 22   |                      |
| PS1    | 73   | 0                   |
| Native | 63   | 13                  |
| SCPEγ  | 44   | 10                  |
| SCPEα  | 53   | 2                   |
| PS2    | 48   | 12                  |
| Native | 28   | 5                   |
| SCPEα  | 38   | 4                   |
| PS3    | 18   | 1                   |

*The points of greatest change of slope, SCPEα and SCPEγ, were extracted from the activity versus DE profiles at pH 7.6 in Fig. 1. These curves were separated into two regions that were equated to two straight lines: a plateau region (activity/DE = 0) followed by the region with maximum slope (activity/DE max), and the slope change points correspond to the intersection of these lines.*
PE\(\gamma\) but not by PE\(\alpha\), whereas at acidic pH, the activity of both isoforms is reduced.

Simulations—The numerical simulations of the U block populations during deesterification by either SCM or MAM requiring a free carboxyl group at the second nearest neighbor position (UXE constraint) were based on five runs with 1,000 chains of polymer (PS1, PS2, or PS3 with chain lengths of 100 residues). Turnover of pectin during the deesterification process has been evaluated to be 300–400 molecules (or successful pectin/PME encounters/enzyme molecule/s) from the specific activity (20) and the molar mass and this turnover was in agreement with turnovers reported for orange PMEs (10). Considering that the substrateenzyme ratio in the activity assays was roughly the same as that in the simulations (1,000:1), such a turnover implies that as soon as a single pectin with a U block sufficiently long to cause inhibition of the enzyme is formed, a change of slope should be observed in the activity profiles in Fig. 1.

The sampling in the numerical simulations is too small to give statistical populations and the standard deviations for significant populations are in the 5–10% range (i.e. 12-residue U blocks of PS2 at DE 28% in the MAM simulations; Table III, 221 ± 9 chains). In the case of the smallest populations, these standard deviations are close to 100% (i.e. the 19-residue U blocks in the SCM simulation; Table II, at the slope change points of PME activity at pH 5.6 in Fig. 1 (PS1, 4 ± 1.5; PS2, 1.5 ± 0.5; PS3, 1 ± 1.3)). Thus, the block lengths with the smallest populations at the change of slope points for the incubations with PE\(\alpha\) (SCPE\(\alpha\)) and PE\(\gamma\) (SCPE\(\gamma\)) that would be expected to cause inhibition of enzymatic activity cannot be given with greater accuracy than ± 1 residue. Moreover, considering the deviations of the experimental points in Fig. 1 with respect to a smooth regular curve with two well-defined regions (a plateau and a region with a steep slope), these slope change points cannot be determined more precisely than within 1% in the more favorable cases. These deviations of 1% in DE correspond to a variation of 1 residue in the length of the largest U block. Reasonable U block ranges that take into account these deviations of the experimental points in Fig. 1 with respect to a smooth regular curve with two well-defined regions (a plateau and a region with a steep slope), these slope change points cannot be determined more precisely than within 1% in the more favorable cases. These deviations of 1% in DE correspond to a variation of 1 residue in the length of the largest U block. Reasonable U block ranges that take into account these deviations of the experimental points in Fig. 1 with respect to a smooth regular curve with two well-defined regions (a plateau and a region with a steep slope), these slope change points cannot be determined more precisely than within 1% in the more favorable cases. These deviations of 1% in DE correspond to a variation of 1 residue in the length of the largest U block.

Comparison of PE\(\alpha\) and PE\(\gamma\) Peptide Sequences—The cDNA-deduced amino acid sequence of isoform PE\(\gamma\) has been determined previously (20). The mature PE\(\gamma\) polypeptide is composed of 318 amino acids with a calculated molecular mass of 34.7 kDa and an estimated pi of 9.84. These values are consistent with those observed by SDS-polyacrylamide gel electrophoresis and isoelectric focussing (16). In the present study, we describe the isolation of a partial cDNA clone encoding the PE\(\alpha\) isoform. This clone was obtained by reverse transcription-PCR using two degenerate primers that were designed from the results of protein sequencing (20). Attempts to get the full-length cDNA were done using the 3′-5′-rapid amplification of cDNA ends technique, but only the 3′-rapid amplification of cDNA ends was successful. Although 5′-rapid amplification of cDNA ends was performed repeatedly on different RNA preparations and using commercial kits from different suppliers, we were unable to get the missing 5′-end. Even the plaque blotting method using the digoxigenin-labeled DNA fragment of PE\(\gamma\) to screen a cDNA library failed to give the complete cDNA. Nevertheless, the partial amino acid sequence of PE\(\gamma\) deduced from the cDNA clone (Fig. 5) perfectly matches the internal peptide sequences determined previously from the purified authentic enzyme (20). The sequence comprises 277 amino acids, with a calculated molecular mass of 30.9 kDa. Comparison of all the known PME protein sequences reveals the presence of a highly conserved C-terminal catalytic domain with an average size of 320 amino acids. Therefore, the partial PE\(\alpha\) protein sequence covers a very large part of the catalytic domain of the protein (estimated to more than 85%). This partial PE\(\alpha\) sequence con-
tains four putative N-glycosylation sites located predominantly in the second half of the catalytic domain, whereas only one is present in the mature PEγ protein. Because the purified PEα isoform has an apparent molecular mass of 45 kDa (16), this may signify that PEα, but not PEγ, is highly glycosylated. The partial PEα sequence has a calculated pI of 8.34, whereas the pI of the native enzyme was previously estimated to be 7.5 (16). Therefore, the missing N terminus of the catalytic domain contributes greatly to the neutral pI value observed for PEα. By comparison, the removal of the first 40 residues of the mature PEγ (Fig. 5) protein resulted in a polypeptide with a lower calculated pI value (8.9 instead of 9.84). These observations indicate that the N-terminal portion of the mature polypeptides confers mainly the basic or neutral character of the two PME isoforms.

Comparison of the partial PEα protein sequence with that of PEγ revealed 57% identity and 69% similarity. When considering all the known PME sequences from plants, fungi, and bacteria, including PEα and PEγ, 12 residues located in the four most conserved regions (Fig. 5) were found to be invariant. These residues, which include 1 arginine residue, 2 aromatic residues, and 2 aspartate residues, are likely to be involved in enzyme function and in maintaining a functional conformation. In contrast, the residues that could explain the differences in the action patterns of the PME isoforms probably reside in more variable regions. The inhibition experiments with PGA, coupled with the results shown in Figs. 2–4, suggest the implication of one or more likely several basic residues in both proteins. For PEα, residues exhibiting a pK_a in the 6–7 range (only histidine has such a pK_a) are good candidates to explain the lack of inhibition of the enzyme by PGA observed only at pH 7.6 as well as the kinetic behavior of the enzyme at this pH. At the same position(s), arginine or lysine residues are expected to occur in PEγ. The examination of both peptide sequences did not reveal the location of such residues, suggesting that they may reside in the missing N terminus of PEα, the portion of the catalytic domain that contributes greatly to the final pI of the enzyme.

DISCUSSION

It was demonstrated previously (14) that PEα and PEγ from V. radiata deesterify PS1 (DE, 74%) according to the SCM and MAM at pH 5.6 and pH 7.6, respectively, with the requirement that a free carboxyl group be located at the second nearest neighbor position (UXE constraint). The activity versus DE profiles in Fig. 1 confirm this dichotomy in the action pattern with a change in pH for substrates with a wide range of DE values (39–74%). Moreover, results of interaction experiments suggest that the distribution of the newly formed charges resulting from incubation at either pH 5.6 or pH 7.6 is effectively different. Higher affinity for Ca^{2+} in the pectins incubated with PEα at pH 5.6 as opposed to those incubated with PEγ at pH 7.6 is consistent with the formation of longer U blocks, corroborating the more pronounced blockwise nature of the deesterification process at lower pH.

However, a comparison of the curves in Fig. 1 for PS1, PS2, and PS3 reveals new paradoxes. Indeed, both enzymes show enhanced activity for a native substrate with a random distribution of carboxyl groups as opposed to a pectin of identical DE that has undergone enzymatic deesterification, leading to a blockwise distribution of pendant groups. The greater activity with the former substrates undoubtedly reflects the higher probability of satisfying the required UXXE condition for pectin binding when these polar groups are randomly distributed along the pectic chain rather than localized in acidic blocks.

The present study has also shown through the inhibition assays that chains with long U blocks such as polygalacturonic acid are competitive inhibitors of PEα at pH 5.6. Simulations of the deesterification process for all three pectic samples (PS1, PS2, and PS3) with the SCM have revealed that the slope change in both PEα and PEγ activity is concomitant with the formation of U blocks of roughly 19 ± 1 residues. It is tempting to relate this block length to either the number of contiguous unesterified residues necessary for the formation of aggregates or the number of residues in the binding site. The former hypothesis is plausible because the formation of calcium linkages between adjacent regions of 15–20 galacturonic acid residues on pectic chains has been reported for calcium pectate gels (23). It has been demonstrated recently by small angle x-ray scattering (24) that these gels can correspond to bundles of 2–20 chains. It is more difficult to examine the latter hypothesis because no crystal structures of plant PME have yet been published. However, it should be noted that Catoire et al. (22) have recently shown that PEα can only work on galacturonan whose degree of polymerization was around or higher than 20, in agreement with the second hypothesis.

At pH 7.6, inhibition of PEγ by PGA is observed, and this also corresponds to the formation of U blocks of roughly 19 ± 1 residues during deesterification simulated with the MAM. In contrast, PEα is not inhibited by PGA under neutral conditions (pH 7.6), and very long U blocks (30–50 residues) are formed before a significant loss of activity is observed. Nonetheless, the deesterification process stops before its theoretical completion. The termination of the reaction may be due to the lack of hydrophobic residues that might be necessary either for binding or to diminish the substrate/solvent interactions, thus indirectly promoting binding. Indeed, in the case of PEα, addition of substrate at pH 7.6 led to a complete resumption of enzyme activity.

At the molecular level, the explanation for the pH effect is still difficult to assess. Differences in the kinetic parameters for PEα and PEγ as a function of pH, as well as the observed competitive inhibition by PGA, suggest the involvement of basic residues (histidine residue(s) for PEα and arginine or lysine residue(s) for PEγ) that are presumably located in the N terminus of the catalytic domain. Although the PME sequences show no obvious homology to other proteins, the recent crystal structure of a PME from Erwinia chrysanthemi (25) reveals that this protein adopts the right-handed parallel β-helix architecture that is common to other pectinolytic enzymes such as pectin and pectate lyases (26–30), polygalacturonases (31, 32), and rhamnogalacturonase (33). In addition, endopolygalacturonase is also competitively inhibited by cross-linked pectic acid (34), suggesting analogous inhibition patterns for PEα and PEγ and polygalacturonase. The availability in the coming months of the coordinates of the first crystal structure of PME (25) will be of great value to further our understanding of the deesterification process by PEα and PEγ and will serve as a template for molecular modeling studies.

The results described in the present study underline the importance of the distribution of the carboxyl units along the pectin backbone in the enzymatic deesterification of pectins. This parameter controls the activity of the cell wall PMEs to a much greater extent than the methylesterification degree, with a random distribution being the most favorable situation. Moreover, it has been clearly demonstrated that whatever the pH or the enzyme used in the assay, the deesterification process progressively drops to a negligible level, even though the pectins are far from being totally deesterified. This observation can explain the coexistence in most cell walls of methylesterified pectins and active PMEs. Considering that the cell wall pH

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2 Peter Swarén, personal communication.
is around 5.6, irregardless of the PME isoforms present in planta, it can be predicted that the enzymatic deesterification will stop abruptly after giving rise to U blocks of roughly 20 residues. The occurrence of relatively short acidic blocks interspacing the pectic backbone has already been suggested (35). Calcium ions, which are abundant in the cell walls, will then induce the formation of junction zones, giving rise to a gel network. Recent data (36) showed that in mung bean hypocotyl, calcium ions are present mostly in elongated cell walls and colocalized with acidic polygalacturonan blocks in tricellular junctions. The formation in precise cell wall domains of multichain structures will modify the cell wall cohesion and restrict extensibility. The feedback control of the demethyl esterification might represent a kind of protection because totally deesterified homogalacturonan could disturb the apoplasmic traffic. Occurrence inside the cell walls of microdomains is now documented (37), and the localization in precise areas of acidic pectin blocks results in part from the specific mechanisms of the enzymatic demethyl esterification process. Further investigation of cell wall rheological properties of transgenic plants with reduced PME activity might provide useful information for a better and more precise understanding of PME functions in planta.

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