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Note

Characterization of a methyl-esterified tetragalacturonide fragment isolated from a commercial pectin with a medium degree of methyl-esterification

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ABSTRACT

A galacturonan fragment was obtained from a 38% DM commercial apple pectin by endopolygalacturonase digestion followed by separation using ion exchange chromatography. By NMR, MALDI-TOF MS and chemical analysis the structure of this oligomer was found to be a tetramer of galacturonic acid with a single methyl ester on the GalA next to the reducing end residue. Assignment of all the ^{13}C and ^{1}H chemical shifts for this oligomer will be helpful in determining the structure of more complex pectin fragments.

Methyl-esterification is a common feature of both the homogalacturonan and xylogalacturonan regions in pectins.[1–3] Methyl-esterification masks the negative charge that would be present on each GalA residue in homogalacturonans at physiological pH. The degree of esterification and pattern of esterification have a great influence on the interactions of homogalacturonans with each other and between homogalacturonans and ions. These properties are hypothesized to be tightly regulated by the plant in a developmental and tissue-specific manner.[4] Over expression, and mutation or suppression, of pectin methyl esterases have dramatic effects on plant growth.[5–7] Underscoring the potential importance of methyl-esterification there are 58 annotated pectin methyl-esterases in the *Arabidopsis* genome.[8] We are attempting to develop a comprehensive model of the structure of pectins, especially how the different distinct structural regions are linked together. There are several models of how the different regions are linked together.[9] Since pectin is upward of 50% of primary cell walls of dicots[10] it must play a major role in cell wall structure and growth. Knowing the arrangement of the various regions of pectins will help in the development of hypothesis on how pectin functions in cell walls.

NMR spectroscopy is one of the major approaches we use to determine the structures. Here we present the complete ^{1}H and ^{13}C chemical shift assignments for a methyl-esterified galacturonan oligomer isolated from a commercial apple pectin.

Ló et al.[11] have made assignments for both ^{1}H and ^{13}C chemical shifts for non-esterified GalA oligomers. Tjan et al.[12] reported ^{1}H assignments for non-esterified GalA oligomers, totally and partially methyl-esterified oligomers, and oligomers with 4,5 unsaturations introduced during digestion with pectate lyase. Grasdalen et al.[13] have used ^{1}H NMR spectra to investigate the pattern of methyl esterification in pectin. Unfortunately some of their chemical shift assignments are at odds with those of Tjan et al.[12]

Pectin Classic AY 802 was chosen for the production of partially esterified oligomers because it has an intermediate degree of methyl esterification. Assuming that the esters are fairly randomly distributed, and expecting that the EPG needs four adjacent non-esterified GalA residues to be able to act,[14] we could predict that many small esterified oligomers should be generated from it by EPG digestion. The elution profile of the oligomers is shown in Figure 1. Comparison of the migration rate of standard GalA, $GalA_2$, and $GalA_3$ in capillary zone electrophoresis (CZE) and analysis of their sugar composition indicated that peaks 2, 3, and 5 were GalA, $GalA_2$, and $GalA_3$ respectively. Peak 4 eluted from the ion exchange column between the dimer and trimer of GalA suggesting that it had a smaller charge to mass ratio than the $GalA_3$. On CZE peak 4 migrated a little slower than $GalA_4$. The mass of the sodiated adduct of P4 was determined by MALDI-TOF MS to be m/z 759.075 corresponding to the mass of a methyl-esterified GalA tetramer+ Na^+.

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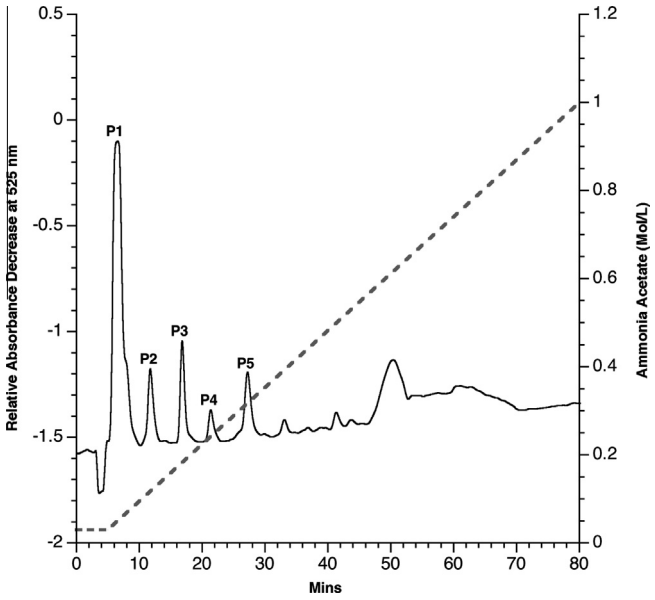

Figure 1. Anion-exchanged chromatography profile of Classic AY apple pectin 802 after degradation with EPG. The peak labeled by P4 is oligomer methyl esterified tetra-galacturonan.

Elucidation of the structure of the oligomer was accomplished by assigning all of the signals in the TOCSY, HMQC, and HMBC spectra of the oligomer. The 2D data obtained were analyzed with the help of NMR-View (Bruce A. Johnson, Merck and Co., Whitehouse Station, NJ, USA), a computer program providing linked crosshairs on all of the spectra displayed on the screen allowing precise correspondence of signals at the same chemical shifts. The assignments are shown in Table 1 and allowed us to deduce the structure shown in Figure 4. The TOCSY spectrum (Fig. 2) clearly shows the spin systems for the α and β reducing GalA IV stretching out from H-1 at 5.32 and 4.60 ppm, respectively. The vertically matched correlations starting from 5.15 and 5.17 ppm were assigned to H-1 and H-5 of GalA III. The downfield shift of H-5 compared to those in the other residues is consistent with the results of Tjan et al.[12] and reflects the lack of an ionizable carboxyl adjacent to it. The H-1 of GalA II is upfield of the others. Tjan et al.[12] suggested that the methyl group on GalA III is close enough to shield this proton. It appears that in their work Grasdalen et al.[13] interchanged the signals for H-5 of methyl esterified GalA and the H-1 of the GalA adjacent to a methyl esterified GalA. GalA I has almost identical chemical shifts to those reported by Ló et al.[11] for non-reducing terminal GalA residues in non-esterified GalA oligomers. From the HMQC spectrum (not shown), we could identify all of the ^{13}C chemical shifts except the C-6s. In the HMBC

Table 1
Assignments of chemical shifts of P4[a]

	H1	H2	H3	H4	H5	C1	C2	C3	C4	C5	C6
α-GalA I	5.06	3.72	3.89	4.25	4.76	99.41	69.85	69.89	71.50	72.59	176.39
α-GalA II	4.91	3.77	4.05	4.41	4.74	100.27	68.40	68.36	79.57	71.75	176.15
α-GalA III	5.15	3.74	4.04	4.49	5.17	99.93	68.45	69.00	78.68	71.23	171.33
α-GalA IV	5.32	3.83	4.01	4.42	4.43	92.90	68.27	68.95	78.95	70.71	175.31
β-GalA IV	4.60	3.49	3.75	4.35	4.06	96.88	71.88	72.83	78.83	74.85	175.09

[a] Identified chemical shifts (in ppm) of the methylated tetramer of GalA. NR end= GalA I, GalA II, GalA III, GalA IV =R end.

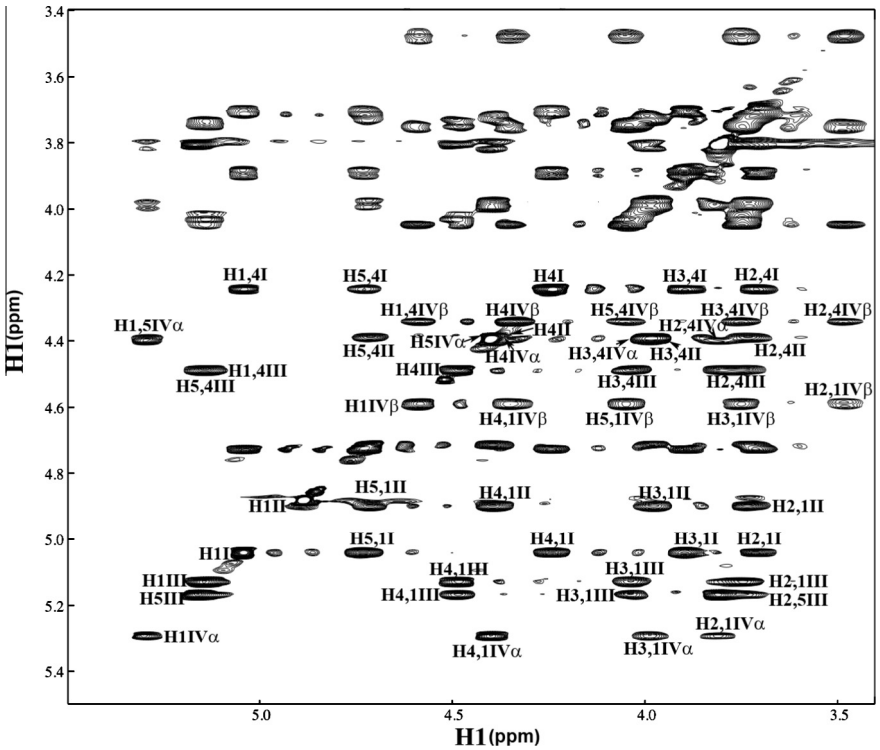

Figure 2. TOCSY spectrum of methyl esterified tetra-galacturonan (P4). NR end= GalA I, GalA II, GalA III, GalA IV =R end.