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Pectic substances from red beet (*Beta vulgaris conditiva*). Part I. Structural analysis of rhamnogalacturonan I using enzymic degradation and methylation analysis

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Abstract

Cell wall material from ripe red beet (*Beta vulgaris* L. var. *conditiva*) was isolated as alcohol insoluble residue (AIR). The chelator-soluble pectin obtained by cyclohexane-*trans*-1,2-diaminotetraacetate (CDTA) extraction of the AIR was fractionated by anion exchange chromatography (AEC). The main fraction was further fractionated by gel filtration chromatography (GFC). Fractions from both chromatographic systems were stepwise degraded by endo-polygalacturonase, endo-β-(1→4)-D-galactanase, endo-α-(1→5)-L-arabinanase and α-L-arabinofuranosidase. Degradation products were fractionated by GFC or by AEC. Polymeric fractions were investigated by methylation analysis after carbodiimide-activated reduction with $NaBD_4$. Selected fractions were additionally methylated with trideuteromethyliodide to enable the detection of O-methyl substituted sugars. The results indicate that the CDTA-soluble pectins of red beet cell walls are composed of three different sub-units: a homogalacturonan, which accounts for about 75%, a highly ramified rhamnogalacturonan I (RG-I) and a typical rhamnogalacturonan II (RG-II). RG-I consists of a highly ramified backbone composed of nearly equal amounts of rhamnose and galacturonic acid. Side chains, mainly arabinans, galactans and type-II arabinogalactans are attached to the RG-I backbone. Some arabinans are connected via short galactan chains directly or indirectly to this backbone. Type-II arabinogalactans are formed by "inner" chains consisting of (1→3)-linked galactans and short "outer" chains composed of an average number of one to three (1→6)-linked galactose residues. Terminal arabinofuranoses are linked via the O-3-position to galactose residues. Nearly all non-reducing ends consist of glucuronic acid. Approximately 65% of the glucuronic acid residues are substituted by a methyl ether group and approximately 10%, most probably, by a terminally linked rhamnose.

Keywords: Red beet pectic substances; Rhamnogalacturonan I; Characterisation

1. Introduction

Pectins are a group of polysaccharides from the primary cell wall and the middle lamella of higher plants (Carpita & Gibeaut, 1993; John & Dey, 1986; McCann et al., 1995). Changes in the texture of fruits and vegetables and in the properties of their products are related to changes in pectic components. Pectins comprise a family of acidic polymers, like homogalacturonans and rhamnogalacturonans with several neutral polymers such as arabinans, galactans and arabinogalactans attached to them. They have been extensively investigated for their structure and functions within the plant cell wall using chemical analysis and enzymic degradation. Rhamnogalacturonan I (RG-I) is a polysaccharide solubilised from plant cell walls after treatment with polygalacturonase (PG). The RG-I polymer is composed of alternating L-rhamnose and D-galacturonic acid residues. L-Arabinosyl- and D-galactosyl-rich side chains are attached to this backbone. Occasionally the side chains are terminated by L-fucosyl, D-glucuronosyl or 4-O-methyl-D-glucuronosyl residues (Albersheim, Darvill, O'Neill, Schols & Voragen, 1996).

The aim of our work was the characterisation of the chelator-soluble pectic substances from ripe red beet (*Beta vulgaris* L. var. *conditiva*). For this purpose the alcohol-insoluble residue (AIR) was extracted with cyclohexane-*trans*-1,2-diaminotetraacetate (CDTA), the extracts were fractionated and characterised using enzymic degradation and methylation analysis. The isolation procedure and the characterisation of an RG-I are described below. The characterisation of an RG-II from ripe red beet will

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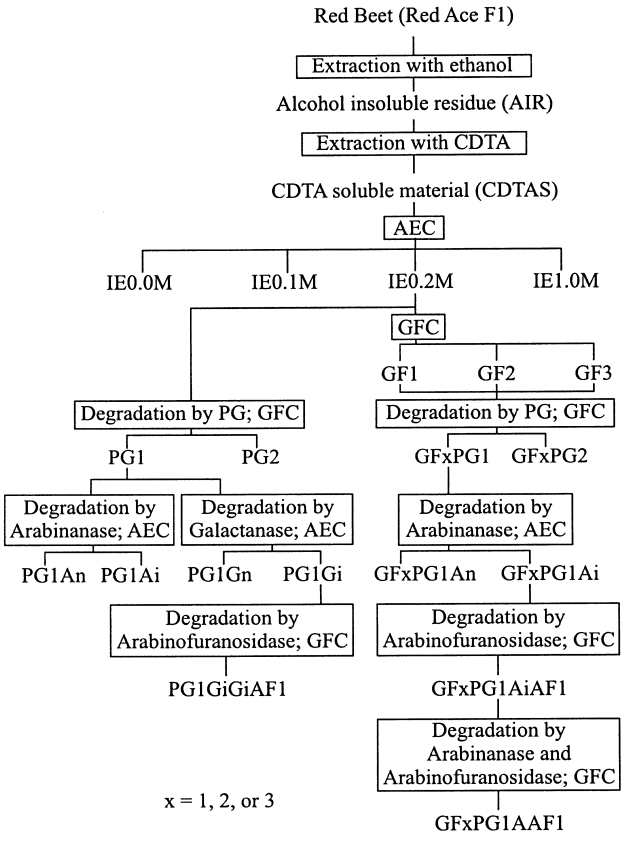

Fig. 1. Scheme for the isolation and fractionation of pectic substances from red beet cell wall material by CDTA-extraction, AEC, GFC and treatment with different pectin-degrading enzymes.

be described in part II of this paper (Strasser & Amadò, 2000).

2. Experimental

2.1. Isolation of pectic material

Cell wall material from ripe red beet of the variety Red Ace F1 was isolated as AIR. 15 kg of red beets were peeled, cut into small pieces and blended in 90% boiling ethanol for 10 min. The residue was homogenised with a commercial Waring blendor, filtered (Polyestergaze Polynom, Schweiz. Seidenfabrik AG, Zurich, CH), and washed 11 times with 70% ethanol.

AIR (30 g dry weight) was stirred in 3.0 l of 50 mM 1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA) solution (pH 6.5) at 20°C for 6 h. The residue was removed by filtration through a D3 glass filter funnel and washed with water. The residue was re-extracted under the same conditions for 2 h. The washings from both extractions were combined, filtered (0.45 μm, Millipore) and dialysed (Servapor 44146, Serva & Co, Heidelberg, G) first against tap water (3 days), and then against de-ionised water (4 days). After exhaustive dialysis the extract was concentrated under vacuum and freeze-dried (fraction: CDTAS, Fig. 1).

2.2. Fractionation of CDTAS

Anion exchange chromatography (AEC) and gel filtration chromatography (GFC) were performed as described earlier (Strasser, Wechsler & Amadò, 1996). Fractions IE0.0M, IE0.1M, IE0.2M, IE1.0M, and GF1, GF2, GF3, respectively, were pooled (Fig. 1).

2.3. Enzymic degradation with polygalacturonase

The methyl ester and O-acetyl groups were saponified prior to enzymic degradation with 150 ml 0.05 M NaOH at 0°C for 14 h. The pH was corrected to 4.5 with 0.1 M acetic acid containing 0.01% NaN_3. Fraction IE0.2M and the GF*x*-fractions (1.0 g) were incubated separately with a PG (90 IU, pH 4.5, 35°C, 6 h), heated to inactivate the enzyme (100°C, 10 min), filtered (0.45 μm, Millipore) and fractionated by GFC (Sephacryl S-300 HR, Pharmacia, 95 × 2.6 cm). A pH 5.0 sodium acetate buffer (0.05 M, containing 0.01% NaN_3) was used as eluent at a flow-rate of 1.8 ml/min. The separations were monitored with a HP 1037A RI-Detector (30°C). The corresponding fractions were pooled. High molecular weight fractions were dialysed and freeze-dried (fractions: PG1, PG2 and GF*x*PG1, GF*x*PG2, respectively, Fig. 1). The PG used for these degradation experiments had been purified and characterised by Elgorriaga (1994).

2.4. Enzymic degradation by an endo-α-(1 → 5)-L-arabinanase or an endo-β-(1 → 4)-D-galactanase

PG1- and PG1GF*x*-samples (100 mg in 30 ml 0.02 M sodium acetate buffer, containing 0.01% NaN_3) were either incubated with endo-arabinanase (gift from Novo Nordisk A/S, Bagsvaerd, DK; 50 IU, pH 5.5, 35°C, 12 h) or endo-galactanase (Megazyme Ltd, Boronia, AUS; 60 IU, pH 4.5, 45°C, 5 h). The solutions were filtered (0.45 μm, Millipore) after inactivation of the enzyme (100°C, 10 min) and fractionated by AEC (DEAE-Sepharose CL-6B, Pharmacia, 35 × 2.6 cm) using 170 ml 0.02 M and 200 ml 0.8 M sodium acetate buffer (pH 5.0 M, 0.01% NaN_3) for elution. Two fractions were collected, dialysed and freeze-dried (neutral fractions: An or Gn, ionic fractions: Ai or Gi, Fig. 1).

2.5. Enzymic degradation by α-L-arabinofuranosidase or by a combination of endo-arabinanase and α-L-arabinofuranosidase

PG1Gi- and GF*x*PG1Ai-samples (90 mg, 30 ml in 0.05 M sodium acetate buffer, containing 0.01% NaN_3) were incubated with arabinofuranosidase (gift from Novo Nordisk A/S, Bagsvaerd, DK; 60 IU, pH 4.5, 40°C, 5 h, additional 40 IU, 40°C, 48 h) or by arabinanase and arabinofuranosidase (50 mg GF*x*PG1AiAF1-sample in 30 ml 0.05 M sodium

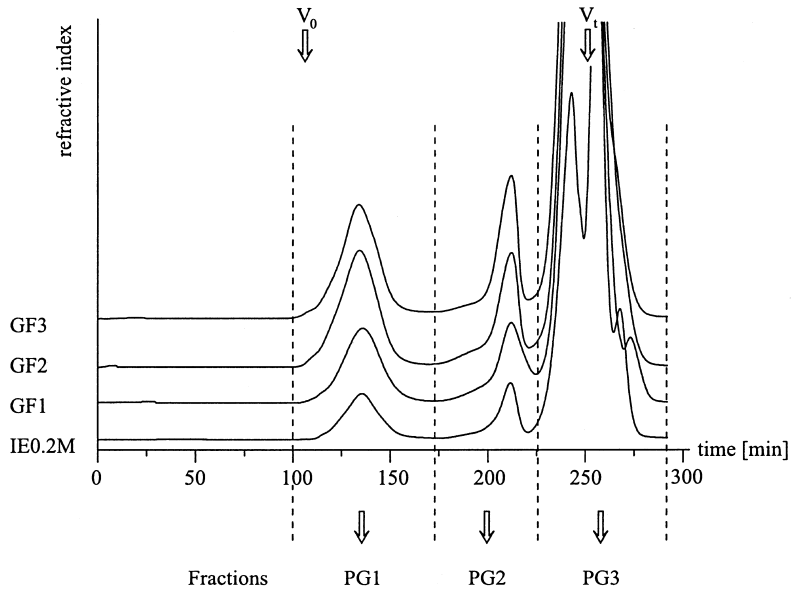

Fig. 2. GFC (Sephacryl S-300 HR) of CDTA-soluble red beet pectin fractions after degradation with a purified PG.

acetate buffer containing 0.01% NaN_3, 33 IU and 40 IU, pH 5.0, 40°C, 48 h). The solutions were filtered (0.45 μm, Millipore) after inactivation of the enzymes (100°C, 10 min) and fractionated by GFC (Sephacryl S-200, Pharmacia, 95 × 2.6 cm) using 0.05 M sodium acetate buffer (pH 5.0 containing 0.01% NaN_3) at a flow-rate of 1.8 ml/min. The separations were monitored using a HP 1037A RI-Detector (30°C). The corresponding fractions were pooled. High molecular weight fractions were dialysed (SpectraPor 3 Membranes, Socochim SA, Lausanne, CH) and freeze-dried (fractions: AF1, Fig. 1).

2.6. Analytical methods

Neutral sugars and uronic acids of enzymatically non-treated samples were determined by GC as alditol–acetates (Blakeney, Harris, Henry & Stone, 1983) and photometrically by the *m*-hydroxy-diphenyl method (Blumenkrantz & Asboe-Hansen, 1973), respectively. Analysis of the glycosidic linkages in polysaccharides was performed by methylation analysis after carbodiimide-activated reduction with $NaBD_4$. Carbodiimide-activated reduction was carried out by a method developed by Kim and Carpita (1992) and modified by Wechsler (1997). Methylation analysis was performed based on Harris, Henry, Blakeney and Stone (1984) and Kvernheim (1987), as modified by Wechsler (1997). The fraction PG1GiAF1 was additionally methylated using CD_3I instead of CH_3I.

2.7. Partial acid hydrolysis of RG-I

A solution of fraction PG1Ai (40 mg) in 2 M trifluoroacetic acid (TFA, 3 ml) was treated at 100°C for 1 h (An, O'Neill, Albersheim & Darvill, 1994). The solvent was removed by co-distillation with 2-propanol. A solution of the residue in water (1 ml) was applied to a DEAE-Sepharose CL-6B column (Pharmacia, 35 × 2.6 cm). The column was eluted with water (100 ml), and then with 1.5% formic acid (200 ml). The solvent of the acidic fraction was removed by co-distillation with 2-propanol.

2.8. Purification of the acidic oligosaccharides obtained by partial acid hydrolysis

The acidic oligosaccharides were purified using a semi-preparative CarboPac PA-100 column (9 × 250 mm, Dionex). The column was eluted at 4 ml/min with a gradient of NaOAc in 100 mM NaOH as follows: 0–50 mM NaOAc (0–5 min), 50–150 mM NaOAc (5–20 min), 150–200 mM NaOAc (20–30 min), 350–400 mM NaOAc (30–35 min), 0.9 mM NaOAc (35–40 min). The column was re-equilibrated in 100 mM NaOH for 15 min prior to the next injection. The column eluent was split, 25% going through a pulsed electrochemical detector (gold working electrode) and 75% collected by an automatic fraction collector (LKB Superrac 2211, Pharmacia). Fractions were desalted by an anion self-regenerating supressor (ASRS-I, Dionex) and dried by co-distillation with 2-propanol.

2.9. Glycosyl-residue composition analysis

The sample was hydrolysed in 2 M TFA (1 ml) at 120°C for 1 h. The solvent was removed by co-distillation with 2-propanol. A solution of the residue in water (1 ml) was analysed using a CarboPac PA-100 column (4 × 250 mm, Dionex) equipped with a pulsed electrochemical detector (gold working electrode). The column was eluted at 1 ml/min with a gradient as follows: 100–0 mM NaOH (0–0.1 min), 0–100 mM NaOH (0.1–20 min), 0–250 mM NaOAc in 100 mM NaOH (20–50 min), 0.9 mM NaOAc in 0.1 M NaOH (50–55 min). The column was re-equilibrated in 100 mM NaOH for 15 min prior to the next injection.

3. Results and discussion

3.1. CDTA-soluble pectins

Red beet pectins isolated by CDTA from the AIR were fractionated by AEC and GFC. Besides galacturonic acid, arabinose and galactose, typical residues known to be present in side chains of pectins (arabinan, galactan, and type-II arabinogalactan) were detected in the CDTA extract and in the fractions obtained by AEC and GFC. In addition, sugars indicating the presence of RG-II such as 1,3′-Api, T-Fuc, 1,3,4-Fuc and 1,3-Rha*p* were detected as well. The results of these investigations are summarised in the paper by Strasser et al. (1996). Incubation of the different chromatographic fractions with various enzymes followed by chromatographic fractionation of the degradation products (Fig. 1) led to a more detailed insight into the structure of CDTA-soluble pectic substances.

3.2. Degradation by PG and fractionation by GFC

Samples degraded by PG yielded three fractions each (PG1, PG2 and PG3, Fig. 2). PG3-fractions were analysed by HPAEC-PAD, and were shown to be composed of mono-, di- and trigalacturonic acid. This fraction was quantitatively predominant and indicated the presence of large homogalacturonan domains in the CDTA-soluble pectic substances of red beet. PG2-fractions contained high amounts of 1,3′-Api*p*, T-2-O-Me-Fuc*p*, T-2-O-Me-Xyl*p*, 1,3,4-Fuc*p* and 1,2-GlcA*p*, indicating the presence of an RG-II in the intermediate molecular weight fraction obtained by GFC (results are presented in part II of this study, Strasser & Amadò, 2000). Results of the methylation analyses of the PG1-fractions are presented in Table 1. All fractions contain high amounts of arabinose. The high molecular weight fraction GF1PG1 contains more and considerably more branched arabinans than the two fractions with lower molecular weights, GF2PG1 and GF3PG1. 1,3-Gal*p*, 1,6-Gal*p* and 1,3,6-Gal*p* residues indicate the presence of type-II arabinogalactan. Smaller molecules seem to contain more type-II arabinogalactan than larger molecules. Furthermore, surprisingly high values of terminally linked glucuronic acid were detected. The results obtained with the PG1-fractions strongly suggest the presence of RG-I-like structures in the not or only slightly PG-sensitive part of CDTA-soluble pectins from red beet. The absence of 1,3′-Api*p*, 1,3,4-Fuc*p* and 1,2-GlcA*p* in the PG1-fractions clearly indicate that they are not contaminated with RG-II.

3.3. Degradation of PG1-fractions by arabinanase or galactanase

Treatment of the different PG1-fractions by arabinanase or by galactanase led to a release of neutral polysaccharides. Methylation analysis of fractions obtained by AEC showed similar results for the ionic fractions (Ai and Gi) compared to their educts. Only slightly smaller amounts of arabinose could be detected (results not presented). The neutral fractions removed by either arabinanase (An) or galactanase (Gn) showed the presence of an arabinan in all the PG1-fractions (Table 1). This result suggests that some arabinans are linked directly or indirectly through a galactan to the pectic backbone. The arabinan removed by arabinanase was much more branched (resistant against further degradation) than the arabinan removed by galactanase. These results are consistent with the results published by Sakamoto and Sakai (1995), who found a fraction similar to our Gn-fraction in sugar beet pectin containing 95% of arabinose. Furthermore de Vries, den Uijl, Voragen, Rombouts and Pilnik (1983) have isolated fragments of arabinogalactans with side-chains consisting of arabinans with a degree of polymerisation of about 25 from apple pectins by treatment with a galactanase. Yamada, Kiyohara, Cyong and Otsuka (1987) found some arabinans directly attached to a 1,4-linked galactan chain. For CDTA-soluble pectins from red beet the amount of galactose remained nearly unchanged. Therefore, it can be concluded that the linking galactans must be relatively short. Arabinans liberated by the arabinanase treatment contained about 97 mol% arabinose, thus indicating that essentially no other sugars are attached to the outer regions (non reducing ends) of the arabinans.

3.4. Treatment of PG1Gi- and GFxPG1Ai-fractions by an arabinofuranosidase and by a combination of arabinanase and arabinofuranosidase

Degradation by arabinofuranosidase removed most terminally linked arabinose (Table 2), whereas the linear 1,5-linked arabinofuranose remained nearly unchanged. This might be because of sterical inaccessibility. Similar results are described in the literature (Cheetham, Cheung & Evans, 1993; McCleary, 1989; Renard, Voragen, Thibault & Pilnik, 1991). A dramatic change was observed for the patterns of the galactose residues. Since the arabinofuranosidase removed only single arabinose residues (as shown by HPAEC-PAD) this result indicates that arabinose residues were originally linked to galactans. The sum of galactose residues remained constant and is calculated to 100 mol% (Table 3). From Table 3 it can easily be concluded that the removal of arabinose leads to a decrease of 1,3,6-Gal*p*, 1,4-Gal*p* and 1,3-Gal*p* residues, which became 1,6-Gal*p* and T-Gal*p*, respectively. These results suggest the arabinose residues in type-II arabinogalactans to be linked to the position O-3 in galactans. Moreover the proportion of 1,6-Gal*p* (outer side-chains of type-II arabinogalactan) to 1,3,6-Gal*p* (branching points of type-II arabinogalactan) allows the estimation of an average number of residues within an outer side-chain of type-II arabinogalactan. In CDTA-soluble pectins of red beet approx. 1.5–2.1 1,6-linked galactose residues are linked to approx. 50% of the O-6-position of a 1,3-linked galactan chain (inner chain). The decrease of the residue 1,4-Gal*p* is an additional clue to the existence of arabinans linked through galactans.

Table 1
Glycosyl-linkage composition (mol%) of red beet RG-I-fractions obtained by enzymic treatment with PG, arabinanase and galactanase (indication of the different fractions, see text and Fig. 1)

	PG1	GF1PG1	GF2PG1	GF3PG1	PG1Gn	PG1An	GF1PG1An	GF2PG1n	GF3PG1An
T-Ara*f*	24.3	26.7	24.7	24.2	25.6	38.4	38.8	38.9	39.3
T-Ara*p*	0.3	0.3	0.3	0.4	0.2	0.2	0.3	0.5	0.3
1,2-Ara*f*	0.5	0.3	0.6	0.7	0.4	0.3	1.0	0.4	0.5
1,3-Ara*f*	2.2	2.5	2.1	2.0	2.1	3.2	1.6	3.4	3.5
1,5-Ara*f*	18.6	20.8	19.3	18.9	44.2	22.0	22.8	22.6	22.1
1,2,5-Ara*f*	2.2	1.7	2.6	2.7	2.5	1.1	1.1	1.2	1.3
1,3,5-Ara*f*	12.2	15.4	11.8	10.3	18.8	30.5	29.9	28.6	28.7
1,2,3,5-Ara*f*	2.1	2.0	1.5	1.8	1.8	3.5	3.3	2.9	3.1
T-Gal*p*	1.8	2.7	2.6	2.1	0.4	0.1	0.1	0.1	0.0
1,3-Gal*p*	3.0	1.7	2.6	3.4	0.8	0.0	0.0	0.5	0.1
1,4-Gal*p*	3.9	5.4	4.1	3.0	0.4	0.5	0.2	0.6	0.5
1,6-Gal*p*	1.8	1.7	2.2	2.2	0.3	0.0	0.0	0.0	0.0
1,3,4-Gal*p*	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0
1,3,6-Gal*p*	9.4	3.5	10.4	13.5	1.3	0.1	0.3	0.1	0.0
1,4-Glc*p*	0.6	0.3	0.3	0.3	0.7	0.2	0.3	0.3	0.2
1,4-Man*p*	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.3
T-Rha*p*	0.5	0.8	1.0	1.0	0.0	0.0	0.0	0.0	0.0
1,2-Rha*p*	1.5	1.5	1.3	1.3	0.0	0.0	0.0	0.0	0.0
1,2,4-Rha*p*	2.7	2.3	1.9	2.1	0.2	0.0	0.0	0.0	0.0
1,4-Xyl*p*	0.2	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0
1,4-GalA*p*	5.7	4.4	2.9	2.1	0.2	0.0	0.0	0.0	0.0
1,2,4-GalA*p*	0.2	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0
1,3,4-GalA*p*	1.2	1.5	1.4	0.9	0.0	0.0	0.0	0.0	0.0
T-GlcA*p*	4.2	3.3	5.6	6.2	0.0	0.0	0.0	0.0	0.0
1,4-GlcA*p*	0.6	0.4	0.6	0.7	0.0	0.0	0.0	0.0	0.0
Total	100	100	100	100	100	100	100	100	100
Yield (%)	13	11	15	15	4	24	20	16	10

Most of the remaining arabinose residues were removed by simultaneous incubation with arabinanase and arabinofuranosidase (Table 2). The removal of arabinose led to an enrichment of the other sugars. However, fucose, xylose, glucose and mannose were not affected, and are thus believed not to be part of the RG-I present in red beet. Rhamnose was mainly present as terminal-, 1,2- or 1,2,4-linked residue. The ratio of 1,2,4-Rha*p* to arabinose was much higher than one suggesting that most O-4-positions of rhamnose are substituted by galactose. During the different enzymatic degradation steps the ratio of the backbone sugars 1,2-Rha*p* and 1,2,4-Rha*p* remained nearly unchanged in all samples, indicating that sugars attached to the O-4-position of rhamnose were not removed. In all samples T-Rha*p* was found in almost equal amounts to 1,4-GlcA*p* suggesting a terminal disaccharide Rha*p*-(1 → 4)-GlcA*p*-(1 → as was described to be present in type-II arabinogalactan (Mollard & Joseleau, 1994; Pellerin, Vidal, Williams & Brillouet, 1995). Remarkably high amounts of terminally linked glucuronic acid residues were present in the enriched samples, probably attached to galactans (see below). Linkage analysis of the fraction PG1GiAF1 using CD_3I showed that two-thirds of the glucuronic acid residues were originally methylated at position O-4. Galacturonic acid was found in approx. equal amounts to the sum of 1,2-Rha*p* and 1,2,4-Rha*p*, thus indicating the presence of an alternating rhamnogalacturonan backbone. Considerable amounts of 1,3,4-GalA*p* were shown to be present in all samples. The almost total absence of xylose suggests that the 1,3,4-GalA*p*-residues are not part of a xylogalacturonan. Glucuronic acid does not seem to be a substituent either (see below). Because of the remaining residues, galactose as well as arabinose might possibly be attached to the O-3-position of galacturonic acid. Both linkages are already described to be present within the plant kingdom (Guillon & Thibault, 1989; Samuelson et al., 1996).

3.5. Semipreparative HPAEC-PAD fractionation and structural characterisation of the acidic material released by partial acid hydrolysis of fraction PG1GiAF1

The acidic material released by partial acid hydrolysis of PG1GiAF1 was isolated by AEC and then fractionated by HPAEC using a semipreparative CarboPac PA-100 column (Fig. 3). Some fractions were pooled, desalted using a supressor and analysed for their glycosyl residues by analytical HPAEC-PAD (Table 4). The values for each sugar and for each pool were calculated to the corresponding concentration of the respective sugar at a specific retention time. These results were graphically compared with the original HPAEC-PAD-chromatogram (Fig. 3).

Peak A is composed of rhamnose and galacturonic acid.

Table 2
Glycosyl-linkage composition (mol%) of red beet RG-I-fractions obtained by enzymic treatment with PG + arabinofuranosidase and PG + arabinofuranosidase + arabinanase, respectively (indication of the different fractions, see text and Fig. 1)

	GF1PG1AiAF1	GF2PG1AiAF1	GF3PG1AiAF1	GF1PG1AAF1	GF2PG1AAF1	GF3PG1AAF1
T-Ara*f*	4.8	3.9	3.9	2.3	2.5	2.9
T-Ara*p*	0.3	0.3	0.3	0.2	0.2	0.3
1,2-Ara*f*	0.7	1.1	1.7	0.7	1.0	1.1
1,3-Ara*f*	4.4	3.1	2.1	1.6	1.2	1.9
1,5-Ara*f*	17.4	10.5	4.4	1.4	1.4	3.9
1,2,5-Ara*f*	1.6	1.7	0.0	0.0	0.3	0.3
1,3,5-Ara*f*	2.2	1.6	0.9	0.4	0.0	0.8
1,2,3,5-Ara*f*	0.0	0.1	0.1	0.0	0.0	0.1
T-Fuc*p*	0.0	0.1	0.0	0.2	0.1	0.0
T-Gal*p*	8.0	6.6	7.2	10.8	8.2	8.3
1,3-Gal*p*	2.4	4.6	7.1	3.9	6.1	6.9
1,4-Gal*p*	11.1	7.0	5.2	14.5	8.0	5.4
1,6-Gal*p*	8.5	17.3	22.9	12.1	20.3	23.2
1,2,4-Gal*p*	0.0	0.0	0.0	0.0	0.0	0.7
1,3,6-Gal*p*	3.9	10.7	15.5	5.5	12.5	15.9
1,4-Glc*p*	0.6	0.4	0.3	0.3	0.7	0.5
1,6-Man*p*	0.0	0.0	0.0	0.0	0.0	0.2
T-Rha*p*	1.1	1.5	1.7	1.2	1.9	1.9
1,2-Rha*p*	2.8	2.9	1.7	4.6	2.9	1.4
1,3-Rha*p*	0.3	0.0	0.0	0.0	0.0	0.0
1,2,3-Rha*p*	0.2	0.0	0.0	0.0	0.0	0.0
1,2,4-Rha*p*	8.1	6.1	4.7	10.4	8.1	4.4
1,2,3,4-Rha*p*	0.2	0.1	0.1	0.6	0.2	0.1
T-Xyl*p*	0.2	0.1	0.0	0.0	0.1	0.0
1,4-Xyl*p*	0.2	0.1	0.1	0.2	0.0	0.0
T-GalA*p*	0.0	0.0	0.0	0.5	0.0	0.0
1,4-GalA*p*	8.6	5.0	3.4	11.1	5.4	3.2
1,2,4-GalA*p*	0.5	0.4	0.2	0.6	0.5	0.2
1,3,4-GalA*p*	3.7	2.6	1.9	5.1	3.2	2.0
1,4,6-GalA*p*	0.0	0.0	0.0	0.5	0.0	0.0
T-GlcA*p*	7.3	10.9	12.8	10.0	13.5	12.9
1,4-GlcA*p*	0.8	1.4	1.7	1.1	1.7	1.7
Total	100.0	100.0	100.0	100.0	100.0	100.0
Yield (%)	55	54	60	87	80	79

This peak is assumed to consist of the dimer α-D-GalA*p*–(1 → 2)-α-Rha*p*, which was confirmed by the formation of peak A during hydrolysis of peak F (see below). Peaks B and C contain more or less exclusively galactose and 4-O-methylglucuronic acid. Considering the results obtained by the linkage analysis, the measured retention time and the comparison with results by An et al. (1994), led to the conclusion that these peaks contain dimers composed of 4-O-Me–GlcA–Gal. The type of linkage of these dimers remains unknown. Peaks D and E contain galactose and glucuronic acid and suggest the presence of dimers of GlcA–Gal. In addition, peak D contains some monomeric galacturonic acid, which elutes at exactly the same retention time. The surprisingly high amounts of glucuronic acid (peak E) and 4-O-methylglucuronic acid (peak C) can also be explained by the presence of free monomeric uronic acids that co-elute with the dimers. Peak F contains predominantly rhamnose and galacturonic acid. Comparing with the results obtained by An et al. (1994), peak F could correspond to the tetramer α-D-GalA*p*–(1 → 2)-α-Rha*p*–(1 → 4)-α-D-GalA*p*–(1 → 2)-α-Rha*p*. In summary, these results demonstrate that 4-O-methylglucuronic and glucuronic acid are exclusively attached to galactose.

Table 3
Galactose residues of PG-treated red beet RG-I-fractions before and after arabinofuranosidase degradation (indication of the different fractions, see text and Fig. 1)

	GF1PG1			GF2PG1			GF3PG1		
	Ai	AF1	Differ.	Ai	AF1	Differ.	Ai	AF1	Differ.
T-Gal*p*	17	23	7	10	14	4	9	12	4
1,3-Gal*p*	10	7	−3	12	10	−2	18	12	−5
1,4-Gal*p*	35	33	−2	18	15	−3	12	9	−3
1,6-Gal*p*	11	25	14	9	38	28	9	40	31
1,2,4-Gal*p*	0	0	0	0	0	0	0	0	0
1,3,4-Gal*p*	1	0	−1	0	0	0	0	0	0
1,3,6-Gal*p*	26	12	−14	51	23	−28	53	27	−26
Total	100	100		100	100		100	100	

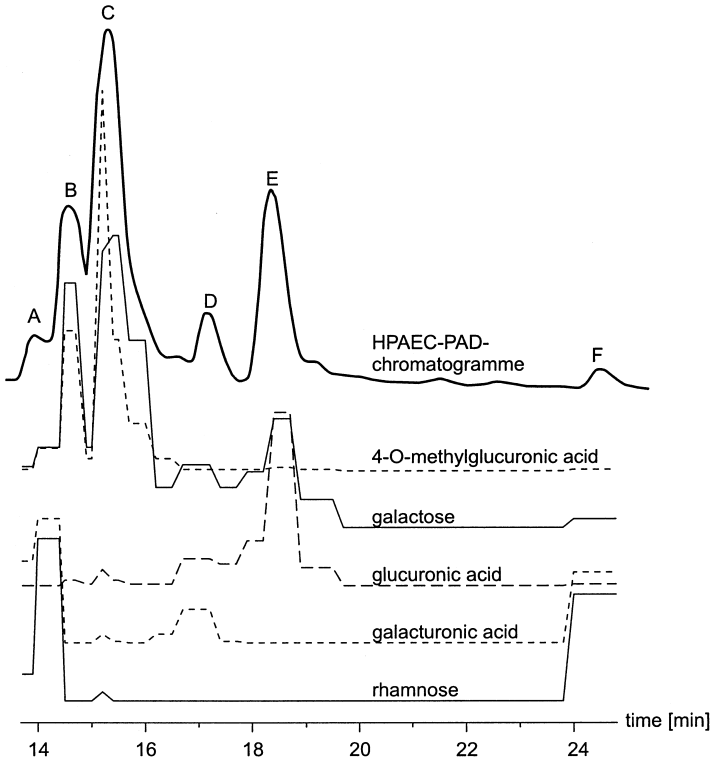

Fig. 3. HPAEC-PAD chromatograms of acidic material released from a (PG + galactanase + arabinofuranosidase)-treated red beet RG-I-fraction after partial acid hydrolysis (bold line), compared to calculated sugar concentrations at specific retention times (thin lines).

Oligosaccharides containing 4-O-methylglucuronic acid or glucuronic acid do not contain galacturonic acid or rhamnose.

4. Conclusion

Incubation of CDTA-soluble pectic substances of red beet with a PG yielded an enzyme-resistant fraction. Linkage analyses indicated the presence of an RG-I. The ratio of rhamnose to galacturonic acid and the results obtained using partial hydrolysis and semipreparative HPAEC-PAD suggest the presence of an alternating rhamnogalacturonan backbone. This backbone is highly ramified, since two-thirds of the rhamnose residues are branched. Side chains consisting of arabinans, galactans and type-II arabinogalactans are attached to the RG-backbone.

Arabinans have been shown to have an α-(1 → 5)-linked backbone. Single arabinose residues or small oligoarabinans are attached to the backbone at the O-3-position, and to a lower extent at the O-2-position. Some of the arabinans are indirectly attached through small galactans to the pectic backbone.

Short β-(1 → 4) galactan chains have been shown to occur in red beet RG-I as well. Most of them are attached directly to the pectic backbone.

Type-II arabinogalactans are the most complex side chains within red beet RG-I pectins. They consist of an

Table 4
Monosaccharide composition of acidic material released from a PG + galactanase + arabinofuranosidase-treated red beet RG-I-fraction after partial acid hydrolysis and HPAEC-PAD fractionation (indication of the different fractions, see text)

Pool number	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14
Rha	6	55	0	0	1	0	0	0	0	0	0	0	0	72
Gal	14	28	28	18	31	66	64	14	10	14	19	37	16	6
Me-GluA	0	8	16	3	43	30	16	4	0	1	1	1	1	1
GalA	18	42	0	0	1	0	0	3	5	1	0	0	0	48
GlcA	0	0	1	1	2	1	1	1	4	7	15	59	10	1
Total	39	132	44	22	78	97	81	21	20	22	35	97	27	129
Total/fract.1)	20	44	44	11	78	49	27	7	5	7	12	32	5	22
Num./fract.2)	2	3	2	2	1	2	3	3	4	3	3	3	5	6

inner chain composed of β-(1 → 3)-linked galactose residues. Approximately 50% of the galactose residues are substituted at the O-6-position by short outer side chains consisting of β-(1 → 6)-linked galactose residues (average: 1–3 residues). Some of these outer side chains are substituted by terminally linked glucuronic acid. Further work is needed to figure out the type and position of the glycosidic linkage between glucuronic acid and galactose (α- or β-, (1 → 3)-, (1 → 4)- or (1 → 6)-linked, respectively). The results obtained in this study suggest the presence of at least two differently linked glucuronic acid residues in red beet RG-I. Approximately 65% of the glucuronic acid residues are substituted by a methyl ether group and approx. 10% are most probably substituted by terminally linked rhamnose residues. Further work is needed to elucidate the fine structure of red beet RG-I.

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