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Characterization of a biologically active pectin from *Plantago major* L.

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PMII isolated from the leaves of *Plantago major* L. is a pectin type polysaccharide with anti-complementary activity. It is highly esterified and partly *O*-acetylated with regions of 1,4 linked polygalacturonic acid and at least two different hairy regions. The galactose side chains are linked to position 4 of rhamnose in the main chain. The structure of the galactan side chains is complex, but 1,3,6 linkages are dominating in one of the isolated hairy regions. Arabinose is attached to position 3 and 6 of galactose. In the other hairy region arabinose is attached to position 3 of galacturonic acid.

De-esterification and de-acetylation do not alter the anti-complementary activity of PMII. Different parts of PMII were shown to have different activities. The smooth regions are only slightly active in contrast to the hairy regions which had significantly higher activity. The hairy regions of highest molecular weight (PVa) with 1,3,6 linked galactose side chains were found to be the most active fraction. The importance of arabinose for the activity seems to depend on the site of substitution. Removal of arabinose terminally linked to galactose increases the activity slightly while removal of arabinose linked to the galacturonic acid backbone decreases the activity.

INTRODUCTION

Pectins are composed of a partly methylesterified α-1,4 linked D-galacturonic acid backbone with a small proportion of α-1,2 linked L-rhamnose residues. Side chains are attached to C-4 of some rhamnose residues and have also been found at C-2 or C-3 of galacturonic acid (Talmadge *et al.*, 1973). The neutral side chains consisting of arabinose and galactose are located in hairy regions of the polysaccharide. Unbranched regions of polygalacturonic acid are referred to as smooth regions (De Vries *et al.*, 1982).

A number of different biological activities of pectins have been reported previously: antitussive effect (Nosalova *et al.*, 1993), prevention of oxidative damage in rat jejunal mucosa (Kohen *et al.*, 1993), antiulcer activity (Yamada *et al.*, 1991) and activation of the complement system (anti-complementary activity). Pectins with anti-complementary activity have been isolated from several plants such as the roots of *Glycyrrhiza uralensis* Fisch *et* D.C. (Zhao *et al.*, 1991), *Bupleurum falcatum* L. (Yamada *et al.*, 1989) and *Angelica acutiloba* Kitagawa (Kiyohara *et al.*, 1988) which are plants used in traditional Chinese and Japanese herbal medicine.

A biologically active pectin type polysaccharide, PMII, has been isolated from the leaves of *Plantago major* L. which is used in traditional medicine in wound healing. PMII consists of 71.7% galacturonic acid, 4.2% rhamnose, 8.8% arabinose and 8% galactose. It has anti-complementary activity and induces production of tumour necrosis factor alpha (TNF-α) from human monocytes (Samuelsen *et al.*, 1995).

Not all pectins have anti-complementary activity. An interesting aspect of biologically active polysaccharides is to determine which parts of the polymer that are important for the activity. This paper presents certain structural and anti-complementary activity studies of PMII.

METHODS

Materials

PMII was isolated from *Plantago major* L. as described by Samuelsen *et al.* (1995).

Methanolysis and GC

The method of methanolysis and GC analysis used is described by Samuelsen *et al.* (1995).

Reduction

The uronic acids of polymer fractions were reduced with sodium borodeuteride after activation with carbodiimide as described by Kim & Carpita (1992). The reduction was followed by methylation and GC-MS as described below.

Methylation analysis

The polysaccharides were methylated by the method described by Harris *et al.* (1984) modified by Kvernheim (1987) using the lithium salt of methylsulphinyl carbanion (Blakeney & Stone, 1985). The partially methylated alditol acetates were analysed by GC-MS. The gas chromatograph (GC-MS) was fitted with a split-splitless injector, used in the split mode and a Hewlett-Packard methyl silicone capillary column (12 m × 0.22 mm i.d.) with film thickness 0.33 μm. The column was inserted directly into the ion source of the mass spectrometer. The injector temperature was 250°C, the detector temperature was 300°C and the column temperature was 80°C when injected, then increased with 30°C/min to 170°C, followed by 0.5°C/min to 200°C and then 30°C/min to 300°C. Helium was the carrier gas and the flow rate was 0.9 ml/min. E.I. mass spectra were obtained using Hewlett-Packard Mass Selective Detector 5970 with a Hewlett-Packard 5890 GC.

Periodate oxidation

Polysaccharide samples (75 μmol) were subjected to oxidation with 0.01 M $NaIO_4$ in 0.1 M NaOAc buffer pH 4 at 4°C in the dark. The reaction was terminated after 24 h with ethylene glycol and the solution was neutralized with $NaHCO_3$ prior to reduction overnight with $NaBH_4$. After neutralizing with dilute acetic acid and desalting by dialysis (cut off 3500 Da), the procedure was repeated to achieve complete oxidation (Painter & Larsen, 1970).

Degree of acetylation

Substitution by acetyl groups was confirmed by IR spectroscopy. The polysaccharide sample (5 mg) was dried at 40°C in vacuum over P_2O_5 for 48 h before preparing a KBr tablet. The IR spectra were obtained using a Beckman AccuLab 2 IR spectrophotometer.

The degree of acetylation was determined as described by Tomoda *et al.* (1974). The polysaccharide sample was dried over P_2O_5 and hydrolysed with 1 M HCl at 100°C for 2 h. Propionic acid was added as an internal standard. The hydrolysate was applied directly to analysis on a Carlo Erba 6000 Vega series gas chromatograph with control module ICU 600. The chromatograph had a split-splitless injector, a flame ionisation detector and was coupled to a Hewlett Packard HP 3396A integrator. The column was a glass column (2 m×3 mm i.d.) packed with 10% FFAP (Supelco) on 80/100 Chromosorb WAW. Nitrogen was used as the carrier gas at a flow rate of 50 ml/min. The column temperature was 150°C and the injector and the detector temperature was 200°C.

De-acetylation

The pH of the PMII solution (1.7 mg/ml) was raised to 12.5 using 0.2 M NaOH. After 30 min at 25°C the pH was lowered to 6.5 with 0.2 M HCl. The solution was then dialysed (molecular weight cut off 3500) against distilled water and freeze dried. A successful de-acetylation was confirmed by IR spectroscopy.

Degree of esterification

The polysaccharide sample was hydrolysed with 70% perchloric acid at 50°C using propanol as an internal standard. Liberated methanol was determined by gas chromatography using a Carbowax 5% 20 M column. The analysis was performed at the National institute of Forensic Toxicology in Oslo, Norway.

Determination of reducing sugars

The cleavage of the glycoside linkages was followed by measuring the increase of reducing sugars in the reaction mixture using dinitrosalicylic acid (DNS) (Miller, 1959 modified by Knutsen, 1991). The DNS reagent was made by adding NaOH (8 g), potassium sodium tartrate (150 g) and dinitrosalicylic acid (5 g) to 400 ml of distilled water at 60°C. Finally the solution was diluted with water to 500 ml.

The test was performed by adding 500 μl sample to 500 μl DNS reagent. If the sample is acidic it must be neutralized before addition to the DNS reagent otherwise precipitation will occur. The sample and reagent were mixed and boiled in a water bath for 5 min. Finally 2.5 ml water was added and the absorbance measured at 540 nm.

Enzymic hydrolysis

PMII (2 mg/ml) in 0.1 M phosphate buffer pH 7.5 was treated with pectinesterase (EC 3.1.1.11) from orange peel (Sigma Chemical co.) at 30°C for 7.5 h. A few drops of toluene was added as an antimicrobial agent. The solution was then heated to 100°C to denature the enzyme, dialysed (cut off 3500) and freeze dried.

De-esterified PMII (0.8 mg/ml) in 50 mM acetate

buffer pH 4.0 was treated with pectinase (EC 3.2.1.15) from *Aspergillus niger* (Sigma Chemical Co.) at 30°C for 26 h. The reaction was terminated by heating at 100°C.

Selective acid hydrolysis

Arabinose was removed from the polymer fractions by hydrolysis with 50 mM oxalic acid at 100°C for 2 h followed by dialysis and freeze drying. (Cartier *et al.*, 1987)

Size exclusion chromatography

The enzyme degraded and partially hydrolysed material was fractionated by size exclusion chromatography on a Bio Gel P2 and/or a Bio Gel P10 (Bio Rad) column. The columns (2.6 × 90 cm) were coupled to a Peristaltic pump P-3 (Pharmacia) and a LKB-Super Frac fraction collector (Pharmacia) and eluted with 50 mM ammonium hydrogen carbonate at 20 ml/h. The carbohydrate profile obtained was determined using the phenol-sulphuric acid reaction (Dubois *et al.*, 1956) or using a Shimadzu RID-6A refractive index detector.

HPAEC-PAD

High performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was carried out on a LC-system (Dionex Corporation) equipped with a CarboPac PA1 column (9 × 250 cm) coupled to a Spectra System AS 3500 auto sampler. The detection was carried out using pulsed amperometric detection (PAD-II). The eluent was 100 mM NaOH with a 1 M NaOAc gradient (2–60%) starting after 5 min and run at 1 ml/min.

Molecular weight determination

The molecular weight was determined by size exclusion chromatography coupled to LALLS (Low Angle Laser Light Scattering) as described by Christensen *et al.* (1993). A B-44 column and pullulan standards were used. This was performed at Institute for Biotechnology, The Norwegian Institute of Technology, University of Trondheim, Norway.

^{1}H-NMR

PMII was dissolved in D_2O (10 mg/ml) at pD 7 and the NMR spectrum was acquired on Jeol DX270 at 90°C with a 0.5 sec pulse delay and 1.4 sec acquisition time, using 3K sweep width and 8K data points. Chemical shifts were set relative to residual water (4.2 ppm).

Anti-complementary activity

Samples were subjected to an assay for their anti-complementary activity as previously described by Yamada *et al.* (1985) using a mixture of pectic polysaccharides form *Angelica acutiloba* as positive control.

RESULTS AND DISCUSSION

PMII, the main fraction isolated from the leaves of *P. major* L. constitutes 37% (w/w) of the carbohydrates in the crude extract. The monosaccharide composition is typical for pectins: 71.7% (mol%) galacturonic acid, 4.2% rhamnose, 8.8% arabinose, 8.0% galactose and 7.3% glucose. Glucose is probably a contaminant. According to the phenol-sulphuric acid test (Dubois, 1956) and methanolysis PMII contains a total of 75.5% carbohydrate.

The molecular weight (MW) of PMII is 46–48,000 with a polydispersity of MW/Mn = 1.4–1.9.

Acetylation

The presence of ester groups were stated by IR spectroscopy. After de-acetylation a reduction of the absorbance band at 1740 cm^{-1} was observed (spectra not shown). This band is related to the carboxylgroups of acetylated pectins. A band at about 1370 cm^{-1} from the vibration of methyl groups disappeared after de-acetylation. An intensive band at 1240 cm^{-1} was reduced, and this band is associated with the vibrations involving bonds CC and CO and angles OCO and CCO of the acetyl groups (Filippov, 1992).

PMII was hydrolysed with 1 M HCl and the liberated acetic acid was measured by GC analysis. According to this method PMII is acetylated at every 5th galacturonic acid residue in average. Acetylation is also shown in the ^{1}H-NMR spectrum (Fig. 1), the signals for acetyl are found at 2.1–2.2 ppm.

The actual position of the acetyl groups is not known. Several attempts were made to determine the acetyl position using a modified version of the procedure described by Tomoda *et al.* (1980), without succeeding. In this method all free hydroxyl groups on the carbohydrate polymer should be blocked by acetals, then the acetyl groups are hydrolysed off, and the residual product methylated followed by GC-MS. To give reliable results all the hydroxyl groups must be acetalated but this was impossible to achieve. The presence of galacturonic acid in the polymer will probably interfere with the introduction of acetal groups. Earlier work using this method in determination of the positions of acetyl groups have only been done on neutral polysaccharides such as glucomannans (Paulsen *et al.*, 1978, Paulsen & Folkedal, 1992). Reduction of the galacturonic acid carboxyl groups will cause some hydrolysis of the acetyl groups. Accordingly physical methods such as NMR should be used for determination of the position of the acetyl groups.

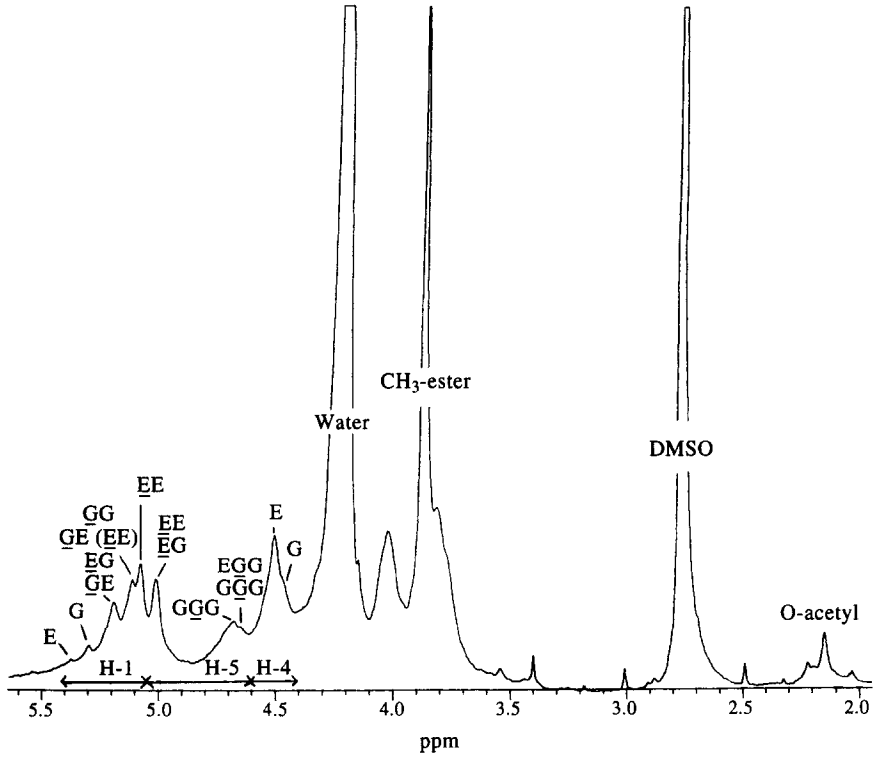

Fig. 1. ^{1}H-NMR spectrum of PMII. G = Galacturonic acid. E = Methylesterified galacturonic acid. The residue responsible for the resonance in the observed diads and triads are underlined.

Degree of esterification

PMII was hydrolysed with perchloric acid and the methanol liberated was measured by GC. According to this method the degree of esterification (d.e.) is 67%. This is in agreement with the ^{1}H-NMR spectrum (Fig. 1). Resonances from H-4 of all esterified galacturonic acid residues (E) and H-1 of E in diads (EE) are dominant in this spectrum. The H-5 resonances are split due to esterification. Signals from H-5 in E appear at a lower field than those from H-5 in non-esterified galacturonic acid units (G). Signals from H-5 of EE and EG cooccur at about 5.02 ppm whereas the GGG triad at 4.68 ppm is partly separated from EGG and GGE at about 4.64 ppm. A d.e. of 67% was determined by the signal intensities from H-1 and H-5 (Grasdalen *et al.*, 1988). At a higher field strength the spectrum of PMII would contain more information regarding esterification sequence.

Pectinase requires de-esterified 1,4 linked galacturonic acid for efficient hydrolysis (Rombouts & Thibault, 1986). Pectinase catalysed hydrolysis of native and de-esterified PMII, a simultaneous measurements of the liberation of reducing sugars may give an indication of d.e. As shown in Fig. 2 about 720 μg/ml galacturonic acid was liberated from de-esterified PMII after 26 h. Then the reaction was complete since the amount of liberated reducing sugars had reached a plateau at this time. In contrast 230 μg/ml galacturonic acid was liberated from native PMII. According to this the d.e. seem to be 68%, assuming that all the reducing sugars are galacturonic acid.

Enzyme hydrolysis

PMII was de-esterified with pectinesterase and then hydrolysed with pectinase. The hydrolysis proceeded until it was complete (26 h, Fig. 2). During this process

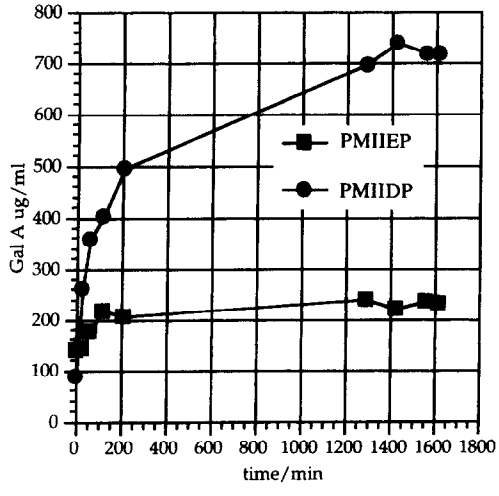

Fig. 2. Liberation of reducing sugars measured as galacturonic acid during pectinase catalysed hydrolysis of native PM II (PMIIEP) and de-esterified PM II (PMIIDP).

a part of PMII precipitated like a gel, and it was separated from the rest by centrifugation. The gel consists of 4.9% arabinose, 4.6% galactose, 3.4% rhamnose, 80.2% galacturonic acid and 6.9% glucose.

After periodate oxidation of the gel three times, 74% of the galacturonic acid was oxidized. The remaining material was reduced with $NaBD_4$ and methylated. The results show that this fraction consists mainly of polygalacturonic acid: 80% 1,4 linked galacturonic acid of which 6% is branched in position 3 and 5% in position 2.

Pectinase hydrolyses 1,4 linkages of de-esterified smooth regions of polygalacturonic acid for isolation of the hairy regions of the polymer. During this process blocks of 1,4 linked galacturonic acid will form and it seems that the polymer lacking the neutral side chains tends to precipitate at pH 4. However, the isolated precipitate dissolves by an increase of pH.

The d.e. of the pectin esterase treated material was not determined. Enzymic de-esterification is known to generate a block structure of de-esterified polygalacturonic acid (Rexová-Benková & Markovic, 1976). In the case of incomplete de-esterification blocks of esterified galacturonic acid will not be susceptible to hydrolysis by pectinase, and then the precipitate consists of esterified polygalacturonic acid. Possibly the formation of precipitate could be avoided by de-esterification by alkali instead of enzymic treatment.

Pectins low in methoxyl can form gels in the presence of calcium by the 'egg box' mechanism (Morris *et al.*, 1982). But calcium should not be present since none of the buffers employed in the isolation of PMII, which include extraction and ion-exchange chromatography, or in the enzyme treatment contained calcium ions. Therefore the precipitate formed is not likely to be a calcium gel.

Even though precipitation occurred at pH 4 this pH was used because it is the optimum pH value for pectinase. The formation of a precipitate probably made the enzyme unable to act on this part of PMII.

The soluble part of the pectinase hydrolysate was applied to a BioGel P2 column. One fraction consisting of monogalacturonic acid was separated from the pectinase resistant high molecular weight fraction which appeared in the void volume. The free galacturonic acid was confirmed by HPAEC-PAD analysis. The void fraction was applied to a Bio Gel P10 column and eluted with ammonium hydrogen carbonate. Two fractions were obtained, one of high molecular weight (PVa) and one of lower molecular weight (PVb) (Fig. 3).

Structural analysis was performed on these fractions by methanolysis and reduction, methylation and GC-MS. The position of arabinose in these fractions was determined by selective hydrolysis to remove arabinose, followed by reduction, methylation and GC-MS (Table 1).

The molar ratio of galacturonic acid to rhamnose of PVa is 1.7 and about 60% of the rhamnose residues are substituted with galactose at position 4. A large part of the galactose residues (40%) are 1,3,6 linked. There are also some (23%) 1,4 linked galactose present in addition to smaller amounts of branching in position 2 or 3. In average the side chains contain 3 galactose residues attached to each rhamnose residue in the backbone. Arabinose is linked to galactose at positions 3 and 6. The reduction in the amount of 1,3,6 linked galactose after hydrolysis equals the increase of 1,3 and 1,6 linked galactose after hydrolysis. 18% of the galactose residues have arabinose linked at position 3 while 10% of the galactose residues have arabinose attached at position 6. The molar ratio of galactose to arabinose is 1.5 in PVa.

In PVb the molar ratio of galacturonic acid to rham-

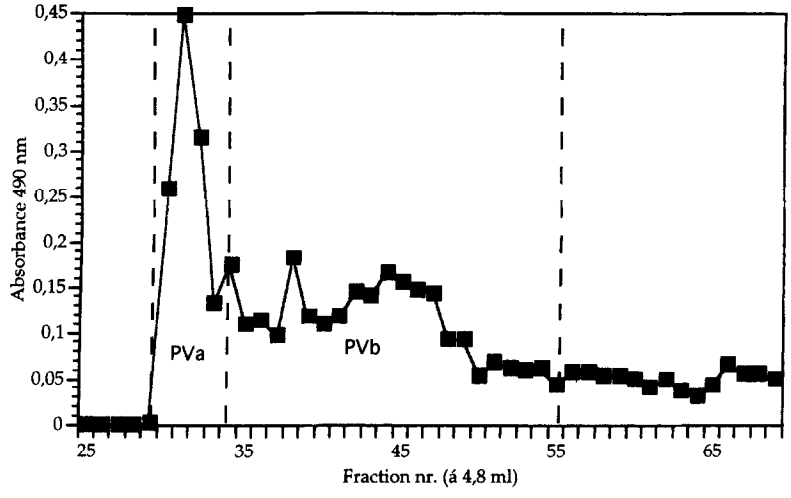

Fig. 3. Elution profile of the pectinase resistant part of PMII on a Bio Gel P10 column (90 × 2.6 cm), eluted with 50 mM ammonium hydrogen carbonate at 20 ml/h. The carbohydrate profile was determined by the phenol-sulphuric acid test. PVa is in the void volume (fractions 29–34) while fractions 35–55 were designated PVb.

nose is 3.7 and branches are found at 40% of the rhamnose residues. Smaller amounts of 1.3, 1.4 and 1.6 linked galactose were found with and without branching in position 2, 3, 4 or 6. The arabinose residues are attached to position 3 of the galacturonic acid backbone. Some arabinose residues may also be attached to position 3 of galactose, but this is not certain because of the small amount of each galactose linkage type found.

These results demonstrate the presence of at least two types of hairy regions in PMII. PVa is more branched than PVb and contains more arabinose and galactose. This can explain the fact that PVa has a higher hydrodynamic volume than PVb. In addition PVa contains less galacturonic acid than PVb which may indicate that the neutral side chains are closer together in PVa than in PVb.

Structure activity relationships

The anti-complementary activity of PMII was only slightly reduced after de-acetylation and de-esterification (A and B Fig. 4). The activity was retained in the pectinase resistant hairy regions of PMII. It was slightly higher when PMII was de-esterified prior to pectinase treatment (D) compared to the hairy regions isolated without de-esterification (C). DPV was separated into the two hairy regions PVa and PVb, and PVa has higher activity than PVb. A high degree of branching must be important for the activity. After removal of the arabinose residues, the activity of PVa was slightly increased (F). In PVa the arabinose residues are situated terminally on the galactan side chains. In contrast removal of arabinose from PVb reduced the activity from 61% to 47% (H). In this fraction arabinose is substituted directly on the galacturonic acid backbone and is therefore more important for the activity than arabinose as a part of neutral arabinogalactan side chains as in PVa.

The gel formed during pectinase digestion had low activity (I). This fraction is mainly composed of smooth regions which are inactive. The same conclusions have been drawn in earlier structure activity studies of pectic polysaccharides. The hairy region of an anti-complementary pectin from *Angelica acutiloba* and *Bupleurum*

Table 1. The linkages (mol%) of PMII fractions determined by reduction, methylation and GC-MS. PVa and PVb are the fractions obtained after separation of the pectinase treated PMII on a Bio Gel P10 column. These were hydrolysed to remove arabinose yielding PVa H and PVb H. P6V is he void fraction of PMII hydrolysed with 0.5 M TFA at 100°C for 1 h and fractionated on a Bio Gel P6 column

Carbohydrate	Linkage	PVa	PVa H	PVb	PVb H	P6V
Ara	T*f*	3,6	4,2	2,1	2,9	-
	T*p*	3,7	-	3,2	-	-
	1,2+1,3	3,1	-	1,5	-	-
	1,5	8,6	-	4,1	-	-
total		19,0	4,2	10,9	2,9	-
Gal	T	4,2	4,7	2,8	1,6	0,9
	1,3	-	2,8	1,0	1,9	0,9
	1,4	6,7	5,5	1,0	1,2	-
	1,6	-	5,2	1,9	3,7	1,8
	1,2,4	0,6	1,0	1,4	-	-
	1,3,6	11,8	3,6	2,9	1,8	0,6
	1,4,6	2,2	4,6	0,5	2,8	-
	1,3,4	3,4	2,0	0,9	-	-
total		28,9	29,4	12,4	13,0	4,2
GalA	T	1,0	0,8	5,7	3,3	4,7
	1,3	-	0,7	0,9	-	-
	1,4	25,4	30,5	35,4	49,9	85,2
	1,2,4	2,2	2,9	2,9	5,2	-
	1,3,4	-	3,9	10,3	-	-
total		28,6	38,8	55,2	58,4	89,9
Rha	T	1,1	1,8	1,1	1,2	-
	1,2	4,8	5,5	5,4	4,5	1,7
	1,3	1,2	-	2,6	0,8	
	1,2,4	10,0	11,4	5,9	4,7	1,0
	1,2,3	-	2,0	-	1,5	
total		17,1	20,7	15,0	12,7	2,7
Glc	T	3,4	4,1	2,3	6,0	2,5
	1,4	2,8	2,8	1,6	6,8	0,8
	1,3,4,6	-	-	2,2	-	-
total		6,2	6,9	6,1	12,8	3,3

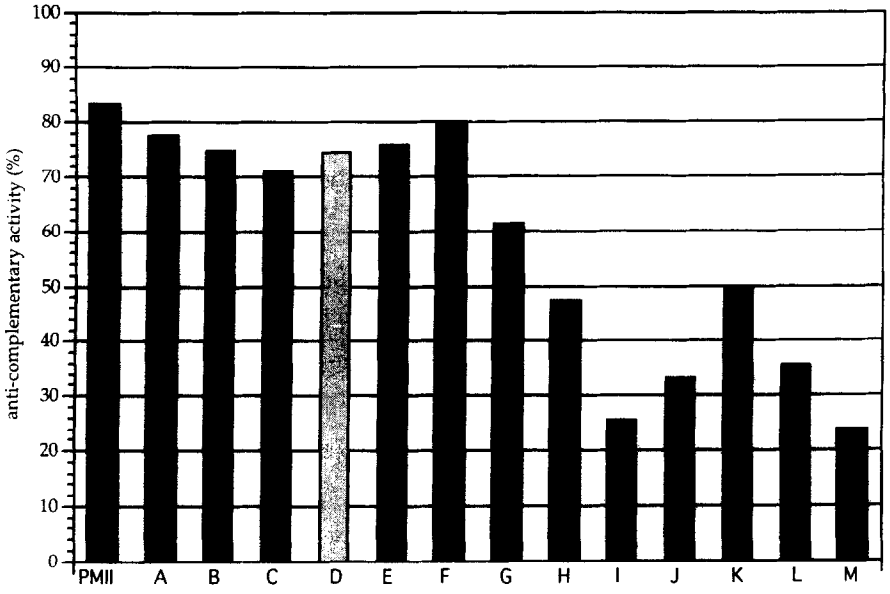

Fig. 4. Anti-complementary activity (%) of PMII and derivatives. **PMII** (83.7 ± 9.6). **A** (77.6 ± 0.6) = de-acetylated PMII. **B** (74.7 ± 3.6) = de-esterified PMII. **C** (71.2 ± 2.3) = EPV, the ramified regions not deesterified. **D** (74.5 ± 0.4) = DPV, the ramified de-esterified regions of PMII. **E** (75.8 ± 1.0) = PVa. **F** (80.0 ± 0.15) = PVa H. **G** (61.3 ± 4.3) = PVb. **H** (47.6 ± 1.2) = PVb H. **I** (25.7 ± 4.3) = gel fraction isolated during pectinase hydrolysis. **J** (33.3 ± 6.6) = pectolyase treated PMII. **K** (49.4 ± 6.3) = periodate oxidised PMII. **L** (35.6 ± 1.9) = PMII hydrolysed with 0.5 M TFA 100°C 1 h. **M** (24.0 ± 2.9) = PMII hydrolysed with 0.5 M TFA 100°C 5 h.

falcatum was shown to be essential for the activity while oligogalacturonides had none or only weak activities (Yamada, 1994).

Weak hydrolysis with 0.5 M TFA at 100°C for 1 h resulted also in a decrease of activity (L). This is in agreement with the above conclusions since the neutral side chains in the hairy regions are more easily hydrolyzed than the polygalacturonic acid backbone. The hydrolysate was separated on a BioGel P6 column and the void fraction subjected to reduction and methylation analysis. The unhydrolysed material consisted of 85% 1,4 linked galacturonic acid and only small amounts of terminal, 1,3, 1,6 and 1,3,6 linked galactose, not enough to express any anti-complementary activity (P6V in Table 1). Hydrolysis for 5 h leads to even lower activity (M).

Pectolyase is a mixture of pectin lyase (EC 4.3.3.10) and pectinase (EC 3.2.1.15) and will cause cleavage of the 1,4 galacturonic acid linkage by β-elimination and hydrolysis respectively. The activity of PMII is reduced after treatment with pectolyase (J).

Degradation of PMII by periodate oxidation causes some reduction in the activity. By this degradation 54% of the total arabinose and 20% of the galactose were oxidised. As shown in Table 1, the neutral side chains are heavily branched and will only be partially oxidised. This explains why some activity still is retained after degradation. 80% of the galacturonic acid residues and 23% of the rhamnose residues were oxidised.

CONCLUSION

A biologically active pectin, PMII isolated from the leaves of *P. major* L. consists of 75.5% carbohydrate and has a molecular weight of 46–48,000. The carbohydrate part is composed of 71.7% galacturonic acid, 4.2% rhamnose, 8.8% arabinose, 8.0% galactose and 7.3% glucose. PMII is partly *O*-acetylated and has a high d.e. (67%). The neutral side chains of the hairy regions have complex structures. In one of the hairy regions isolated 1,3,6 linked galactan side chains dominate with arabinose attached to position 3 and 6 of galactose. Arabinose is attached to position 3 of galacturonic acid in other parts of PMII.

The following structural parts are of major importance for the anti-complementary activity of PMII: The hairy regions of high molecular weight with mainly 1,3,6 linked galactan side chains. Arabinose attached terminally to the galactan side chains reduces the activity while arabinose side chains attached directly to position 3 of the 1,4 linked galacturonic acid backbone increases the activity. Esterification and acetylation do not alter the activity of PMII.

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