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Occurrence of pectic hairy regions in various plant cell wall materials and their degradability by rhamnogalacturonase *

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ABSTRACT

Pectic polysaccharide fractions of high molecular weight, resistant to further degradation by pectolytic, hemicellulolytic, and cellulolytic enzymes, were isolated from potato fibre and from pear, carrot, leek, and onion tissue by the liquefaction process. The fractions, referred to as modified hairy regions (MHR), were characterized by the determination of their sugar composition, linkage type composition, degree of esterification (methyl ester and *O*-acetyl groups), and molecular weight distribution. Galacturonic acid, galactose, and rhamnose were found to be the major sugar residues in most of the MHR preparations, while arabinose was the main sugar in pear MHR. The rhamnose–galacturonic acid ratio ranged between 0.44 for pear MHR to 0.63 for MHR from leek. High degrees of acetylation (da) were calculated assuming that acetyl groups were only attached to galacturonic acid residues. All MHR fractions had a similar molecular weight distribution which was rather heterogeneous. It was observed that all MHR preparations were degraded by RGase in a similar fashion. In all digests, a characteristic population of reaction products having a molecular weight of ca. 1000–2000, representing rhamnogalacturonan oligomers, was present. It was concluded that pectic hairy regions with comparable structural features are common to a variety of fruit and vegetable tissues.

INTRODUCTION

For many years, pectins and pectin-related substances have been investigated minutely for their structural features[1] and their functions within the plant cell wall[2]. O'Neill et al.[1] stated that so far only three pectic polysaccharides have been isolated from the primary cell walls of plants. These are homogalacturonan, rhamnogalacturonan I (RG-I), and a substituted galacturonan referred to as rhamnogalacturonan II (RG-II). RG-I was pictured as a long repeating sequence of alternating L-rhamnose and D-galacturonic acid residues with a variety of L-arabinosyl-, D-galactosyl-, and L-fucosyl-containing side chains[1]. RG-II has an extremely complex glycosyl-residue and glycosyl-linkage composition, including

* Hairy (Ramified) Regions of Pectins, Part IV. For Part III, see ref. 21.

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such rare sugars as aceric acid, 2-keto-3-deoxyoctulosonic acid, 2-*O*-methyl-L-fucose, 2-*O*-methyl-D-xylose, and D-apiose. For apple pectin, a general model was proposed by De Vries[3], containing a main "smooth region" [a linear α-(1 → 4)-galacturonan] and "hairy regions" containing most of the neutral sugars. These hairy regions or polysaccharide fractions having similar structures have been reported to be present in pectins isolated from grapes[4], carrot[5–8], pear[9,10], kiwi[11,12], onion[13–15], potato[16–18], and many other plant tissues.

Recently, we determined the structure of the hairy regions of apple pectin isolated from apple juice manufactured by the liquefaction process[19]. This pectic fraction, referred to as modified hairy regions (MHR), was characterized as a branched, highly acetylated rhamnogalacturonan, carrying side chains of arabinose, galactose, or xylose. The length of these side chains varied. Also, we described the isolation and characterization of an enzyme rhamnogalacturonase (RGase)[20], which was able to hydrolyse up to 4% of all glycosidic linkages present in apple MHR. After degradation of MHR with RGase, various fractions could be isolated, including residual, high molecular weight galacturonan polymers rich in xylose and arabinose, and oligomeric fragments consisting of rhamnose, galactose, and galacturonic acid[20]. NMR analysis of a low molecular weight fraction[21] revealed the presence of a basic alternating tetramer α-Rha*p*-(1 → 4)-α-GalA-(1 → 2)-α-Rha*p*-(1 → 4)-GalA. A β-Gal*p* unit was linked to C-4 of approximately half of the terminal Rha*p* and to α-(1 → 2)-linked Rha*p* residues.

Based on these results, a hypothetical model was proposed[19] for MHR, which was updated recently[22] by the introduction of subunits of xylogalacturonan and of stubs of the rhamnogalacturonan backbone rich in arabinose.

It is suggested that rhamnogalacturonan-rich hairy regions play a role in the manufacture of fruit juices produced with the use of enzymes. Schols et al.[23] claimed the hairy regions to be the origin of haze formation in juices manufactured with the assistance of enzymes. Will and Dietrich[24] reported that rhamnogalacturonans are responsible for membrane fouling in the clarification of fruit juices by ultrafiltration.

In this paper, we report the isolation and characterization of MHR fractions after enzymic liquefaction of the dicotyledonic tissue of potato (*Solanum tuberosum*), carrot (*Daucus carota*), and pear (*Pyrus communis*), and monocotyledonic tissue of leek (*Allium porrum*) and onion (*Allium cepa*). All MHR preparations were characterized chemically and enzymically using RGase.

EXPERIMENTAL

Isolation of MHR.—Leek, carrots, onions, and pears (Conference) were bought at the local market. Potato fibres (remaining after starch removal) were a gift of Avebe, The Netherlands. The carrots were steam-peeled (30–40 s, 10 bar; PKC, Pöttesfeld/Neuwied). All fresh products were sliced (< 3 cm), steam-blanched (100°C, 3 min), and then milled to a particle size of 4 mm (Fryma perforated-disc

mill). Pears were directly milled without blanching. Approximately 8 kg of the pulp obtained was mixed with 2 kg of water in a thermostated vessel (Terlet, Zutphen, The Netherlands). Potato fibres (0.2 kg) were suspended in 10 kg of water. The pH of the products was adjusted to 5.0 ($\pm$0.3), the enzyme (0.1% Rapidase C600; Gist Brocades, Delft, The Netherlands) was added, and the mixtures were held at 45°C for 4 h under continuous stirring. After inactivation of the enzyme (30 min, 90°C) and centrifugation (15 000*g*, 15 min), the supernatant solutions were subjected to ultrafiltration using a tubular system (Kidney dialysis system; Nephross™ Andante HF; molecular weight cut-off 5000; Organon Teknika, The Netherlands). The ultrafiltration retentates were diluted three times with distilled water, ultrafiltered again, and lyophilized.

Saponification of the MHR.—The methyl ester and *O*-acetyl groups in MHR were removed by treatment with 0.05 M NaOH (1.7% MHR, 0°C, 24 h), followed by dialysis and lyophilization, to yield MHR-S.

Analytical methods.—Samples were hydrolysed by 2 M CF_3CO_2H at 121°C for 1 h and neutral sugars were converted into their alditol acetates[19] to determine the sugar composition. The uronic acid content was determined colorimetrically using *m*-hydroxybiphenyl[19]. Methylation analysis and the reduction of uronides were performed as described previously[19]. Protein content was determined as described[25].

Chromatography.—High-performance size-exclusion chromatography (HPSEC) on three Bio-Gel TSK columns in series (40XL, 30XL, and 20XL; calibration was performed using pectins) and size-exclusion chromatography (SEC) over Sephacryl S200 and S500 were carried out as described[19].

Enzymic hydrolysis.—Solutions of the various MHR and MHR-S samples (0.25%) in 0.05 M NaOAc (pH 5.0) were incubated with RGase for 20 h at 30°C as described[20]. After incubation, the enzymes were inactivated (5 min, 100°C) and the digests were analyzed by HPSEC.

RESULTS

Characterization of MHR from various sources.—Tissues of leek, onion, carrot, and pear, and potato fibres were treated with a technical enzyme preparation under conditions used in the liquefaction process[19]. From the juices obtained, pectic polysaccharides were isolated by ultrafiltration. Since these pectic substances were isolated similarly to the MHR from apple pulp[19], we used the same name for the isolated pectic polymers. The isolated MHR from potato fibre accounted for 4.1% of the starting material and ca. 0.04% of the initial fresh tissue. For the other plant materials under investigation, the MHR fractions represented 0.13–0.25% of the fresh weight.

The sugar composition of MHR from the various sources are shown in Table I, together with that of apple MHR for comparison. Although there are differences between the composition of the materials, they all have rhamnose, arabinose,

galactose, and galacturonic acid as main sugar residues. Arabinose is the major sugar in the MHR from pear tissue (40 mol%), while onion MHR contained only 7 mol% of arabinose. The lower arabinose levels in some MHR explain the relative higher amounts of the other sugar residues. Xylose was almost absent in carrot, leek, and potato MHR. The rhamnose content varied between 12 mol% for pear MHR to 22% for MHR from carrot and leek. The galacturonic acid content fluctuated between 27 (pear MHR) and 39 mol% (carrot MHR). All MHR preparations had a remarkably high rhamnose–galacturonic acid ratio, which varied from 0.44 to 0.63. The sugar composition of the MHR from apples varied slightly from the data published before[19] where sulfuric acid hydrolysis was used. CF_3CO_2H hydrolysis used in this study gave a slightly higher rhamnose content. Obviously, also the rhamnose–galacturonic acid ratio increased: 0.44 instead of the value 0.29 as found before. It can also be observed from Table I that the percentage of xylose showed great variation depending on the origin of the tissue. As shown in Table I, all newly isolated MHR preparations had a high degree of acetylation (mol of acetic acid per 100 mol of galacturonic acid) similar to data previously published for apple MHR[19]. The degree of acetylation (da) varied from 33% for pear MHR to 90% for the MHR fraction isolated from potato fibre. These da values are high, although for the corresponding rhamnogalacturonan I isolated from suspension-cultured cotton cells it was reported[26] that 30% of the galacturonosyl residues carried an acetyl group. In most studies in which similar pectin fragments have been studied, acetyl groups were lost during alkaline extraction or the presence of acetyl groups was neglected completely. The degree of methylation (dm) is significantly lower (6–42%) than the degree of acetylation. The dm not only depends on the type of starting material, but is also influenced by the action of pectin methyl esterase during the liquefaction process. From Table I, it can be seen that all MHR fractions tested contained low amounts of protein.

TABLE I

Sugar composition (mol%) of Modified Hairy Regions isolated from various sources

Sugar	Source					
	Pear	Carrot	Leek	Onion	Potato	Apple
Rha	12	22	22	21	16	9
Ara	40	12	18	7	16	51
Xyl	7	1	1	3	2	8
Man	0	1	0	1	1	0
Gal	14	24	24	30	24	10
Glc	0	1	0	0	6	0
GalA	27	39	35	38	35	22
OMe	21	6	11	6	13	42
OAc	33	44	77	54	90	60
Rha/GalA	0.44	0.56	0.63	0.55	0.46	0.41
Total carbohydr. (%w/w)	75	72	69	74	67	86
Protein (%w/w)	1	3	7	6	4	1

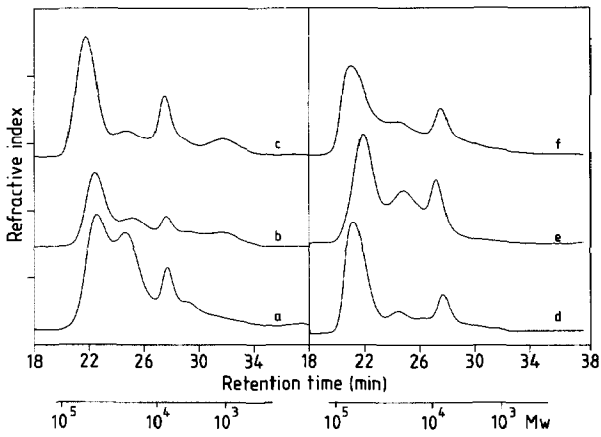

Fig. 1. High-performance size-exclusion chromatography elution patterns of MHR isolated from various sources: a, carrot; b, onion; c, leek; d, potato fibre; e, pear; f, apple.

The molecular weight and the molecular weight distribution of MHR were determined by HPSEC as shown in Fig. 1. The various MHR preparations all had a fairly similar elution pattern in which three populations (A, B, and C) can be recognized. Only the relative amounts of the three populations varied. Next to these three populations, in most elution patterns, a rather broad fourth peak was present in minor quantities. The different populations were isolated using chromatography over Sephacryl S500 and S200 and characterized by determining their sugar composition and degrees of esterification (Table II). Since population D of most MHR could not be isolated in sufficient quantities, their characteristics were not included in Table II. Sometimes, regularities could be recognized in the sugar composition of the corresponding populations. For both apple and pear MHR, the xylose content decreased with decreasing molecular weight; for onion MHR, this trend was not observed. The number of rhamnose residues in MHR from pear, carrot, and apple was higher in the high molecular weight populations than in the lower molecular weight populations. The relative proportion of arabinose varied significantly. MHR from pear, carrot, leek, and potato contained relatively more arabinose in the higher molecular weight populations, while MHR from onion and apple showed a higher proportion of arabinose in the lower molecular weight fractions. In general, it could be concluded that the high molecular weight populations were relatively rich in galactose and acetyl groups, and relatively low in galacturonic acid and methanol. Notable is the high degree of acetylation of the populations of potato MHR. Despite the observed differences in molecular weight and sugar composition of the populations within MHR from one source, MHR can be considered to be a mixture of similar polysaccharides.

Table III shows the results of the methylation analysis of the MHR from various sources. Despite differences in origin and sugar composition, no significant varia-

TABLE II

Sugar composition (mol%) of the fractions of MHR from various sources, isolated over Sephacryl S200 and S500

MHR		Rha	Ara	Xyl	Man	Gal	Glc	GalA	OMe	OAc	Rha/GalA
Pear											
Population	A	11	41	10	0	9	0	29	22	39	0.38
	B	14	38	3	0	22	0	23	24	32	0.61
	C	17	25	3	0	7	1	47	29	20	0.36
Carrot											
Population	A	23	12	2	0	30	0	33	2	60	0.70
	B	23	11	2	0	28	1	35	2	34	0.66
	C	27	14	2	0	12	0	45	7	12	0.60
	D	26	19	1	1	9	2	42	n.d. [a]	n.d.	0.62
Leek											
Population	A	20	21	1	0	23	1	34	7	99	0.59
	B	20	18	1	2	27	2	30	11	69	0.67
	C	21	17	1	1	12	0	48	15	27	0.44
	D	18	15	2	1	11	3	50	n.d.	n.d.	0.36
Onion											
Population	A	23	3	4	0	29	1	40	4	69	0.58
	B	20	3	5	1	27	2	42	10	52	0.48
	C	21	8	4	1	19	1	46	15	36	0.46
Potato											
Population	A	21	22	1	0	28	1	27	3	155	0.78
	B	23	20	1	1	26	1	28	8	136	0.82
	C	21	14	1	1	9	2	52	32	29	0.40
Apple											
Population	A	8	49	10	0	9	0	24	28 [b]	55 [b]	0.33
	B	8	55	5	0	11	0	21	84 [b]	57 [b]	0.38
	C	10	58	2	0	6	1	23	100 [b]	21 [b]	0.43

[a] Not determined. [b] Degree of methylation and acetylation as determined before[19].

tions were observed for the sugar linkage composition. Remarkably high values were found for terminally linked rhamnose and galacturonic acid residues. This might be caused by some degradation of the backbone during the methylation analysis. However, not all galacturonic acid residues were recovered after reduction and methylation. This also influenced the molar ratio of the other sugar residues, which are somewhat higher as expected. Rhamnose residues were mainly (1 → 2)-linked, some of them branched at C-4. Xylose was mainly present as terminally linked residues. Arabinose was found to be present as (1 → 5)-linked chains, occasionally branched at C-3. A rather high proportion of the galactose residues were terminally or (1 → 4)-linked, next to other types of linkages and branching points. Most of the galacturonosyl residues recovered were (1 → 4)-linked while 10–25% of these residues were branched at C-3.

Enzymic degradation of the various MHR.—Since MHR was isolated during a liquefaction process using a crude enzyme preparation containing pectolytic, hemicellulolytic, and cellulolytic enzymes, it was expected to be resistant to further

TABLE III

Sugar glycosidic linkage composition of MHR isolated from various sources

Sugar linkage	Glycosidic linkage composition [a]				
	Pear	Carrot	Leek	Onion	Potato
Rhamnose					
T-Rha*p* [b]	1.7	1.7	1.0	0.9	1.0
1,2-Rha*p* [b]	7.7	13.1	11.4	9.8	5.0
1,3-Rha*p*	0.3	0.2	0.2		
1,2,4-Rha*p*	4.6	14.7	12.6	19.5	18.1
	(14.3)	(25.2)	(25.2)	(30.2)	(24.1)
Arabinose					
T-Ara*f*	9.9	4.0	3.3	2.1	3.7
1,5-Ara*f*	33.4	11.0	19.2	1.8	22.4
1,3,5-Ara*f*	2.7			0.3	
1,2,3,5-Ara*f*	1.0	0.4	0.5		
	(47.0)	(15.4)	(23.0)	(4.2)	(26.1)
Xylose					
T-Xyl*p*	5.5	1.7	2.2	3.9	1.8
1,4-Xyl*p*	1.5	tr [d]	1.6	tr	
1,2-Xyl*p*	0.3				
	(7.3)	(1.7)	(3.8)	(3.9)	(1.8)
Galactose					
T-Gal*p*	5.0	12.7	11.3	15.4	16.5
1,2-Gal*p*	0.6	0.9	2.0	1.4	
1,4-Gal*p*	3.0	5.6	2.7	5.0	5.0
1,6-Gal*p*	2.1	3.2	1.4	1.6	0.6
1,2,4-Gal*p*			1.4	1.6	1.4
1,3,4-Gal*p*		0.2	0.5	0.5	0.3
1,3,6-Gal*p*	3.2	1.9	1.7	0.8	
	(13.9)	(24.5)	(21.0)	(26.3)	(23.8)
Glucose					
T-Glc*p*	0.7	3.0			0.7
1,4-Glc*p*	1.2	1.3	0.7		0.8
	(1.9)	(4.3)	(0.7)	(0)	(1.5)
Galacturonic acid [c]					
T-Gal*p*A	2.7	4.5	6.1	9.2	3.9
1,4-Gal*p*A	7.4	14.7	16.8	18.4	15.0
1,3,4-Gal*p*A	3.7	3.3	2.2	4.1	2.3
	(13.8)	(22.5)	(25.1)	(31.7)	(21.2)
Ratio terminal/branching	1.77	1.17	1.23	1.18	1.22

[a] Linkage types in mol%; the totals after methylation analysis are given in brackets. [b] T, terminal; 1,2-linked Rha, etc. [c] Determined as galactose residues after carboxyl reduction. [d] Traces.

enzymic degradation. In incubation experiments, it was indeed confirmed that both the technical enzyme preparation Rapidase C600 and a variety of purified pectolytic, hemicellulolytic, and cellulolytic enzymes were not able to degrade the

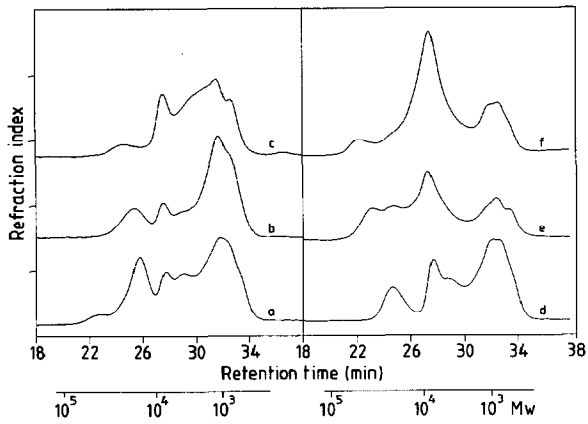

Fig. 2. High-performance size-exclusion chromatography elution patterns of MHR isolated from various sources after saponification and degradation with RGase at 30°C and pH 5 for 20 h: a, carrot; b, onion; c, leek; d, potato fibre, e, pear; f, apple.

isolated MHR polysaccharides, even after prolonged incubation times. Since apple MHR could be degraded by a specific rhamnogalacturonase[19], the various MHR were also incubated with RGase after removal of the ester groups by chemical saponification. Characteristic HPSEC elution patterns are shown in Fig. 2, which indicated that RGase was active towards all MHR tested. However, small differences could be observed in the extent of degradation. The elution patterns showed small proportions of residual high molecular weight material, followed by a distinct population of fragments at a retention time of 27 min and a characteristic population of fragments at retention times of 32–33 min. The latter population represented oligomers, composed of rhamnose and galacturonic acid, with struc-

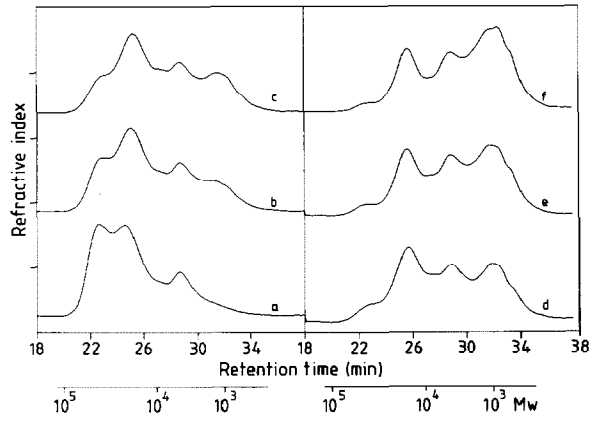

Fig. 3. High-performance size-exclusion chromatography elution patterns of saponified carrot MHR after treatment with RGase at 30°C and pH 5 for various times: a, blank; b, 5 min; c, 15 min; d, 60 min; e, 300 min; f, 20 h.

tures similar to oligomers identified in RGase digests of apple MHR[20,21]. The total amount of RG-oligomers varied for the different MHR fractions and seemed to be dependent on the amount of rhamnogalacturonan in the MHR.

It was calculated with GPC software that RGase was able to hydrolyse all MHR preparations to similar levels as previously found for apple MHR[19]. The degrees of degradation (percentage of glycosidic bonds hydrolysed) for MHR from pear, leek, onion, carrot, and potato were 7, 9, 9, 10, and 12%, respectively.

RGase degradation of MHR monitored with time.—RGase was added to all saponified MHR samples, and the reaction products formed were monitored after fixed time intervals as shown for MHR of carrot in Fig. 3. The fraction with the highest molecular weight in MHR was degraded directly after addition of RGase with formation of fragments having lower molecular weight. The other two populations seemed to be less accessible for RGase and were not totally degraded, even after prolonged incubation times. The peaks representing the characteristic RG-oligomers became more prominent with time. The degradation by RGase of all MHR samples tested was similar.

DISCUSSION

From the results presented, it can be concluded that, by liquefaction of tissues of a wide variety of plants using the technical enzyme preparation Rapidase C600, a high M_w pectin fraction could be isolated, representing 0.04–0.25% of the starting material. The pectic fractions obtained from the various plant tissues show a remarkable analogy with the Modified Hairy Regions described from apple tissue[19]. We therefore designated all fractions as Modified Hairy Regions. They all have a high content of rhamnose and galacturonic acid. Similar to apple MHR and rhamnogalacturonan I[1] also, the ratio between these sugars is high and varies between 0.44 and 0.63. Pectic polysaccharides with similar structures have been isolated before from the materials studied here[6,7,10,14,17]. However, the methods of isolation in those studies were rather diverse, which prohibits comparison of the results.

Using size-exclusion chromatography, it was shown that all MHR preparations, in spite of differences in sugar composition and degrees of esterification, had the same elution behaviour and consisted of at least three populations. The various populations were isolated and further characterized. The populations within the MHR of one source showed almost the same sugar composition as the starting MHR although differences occurred. For some MHR preparations, it can be noticed that, with decreasing molecular weight, the proportion of ester groups or certain sugars decreases or increases (e.g., rhamnose in pear MHR increasing from 11 mol% in population A to 17 mol% in populations C). In most cases, the sum of the concentrations of a specific sugar in the subpopulations was consistent with the concentration in the total MHR.

Major variations were found in the dm and da values for the different populations. The excessively high da values for potato population A and B (155 and 137% respectively) gave rise to doubts on the reliability of these values. For this reason, the average da and dm were calculated from the weight percentage of each individual population (as established by HPSEC) and the dm and da values of the individual populations. This average value was compared with the measured dm and da value for the total MHR fraction. In most cases, there was good agreement. For the da of leek MHR, a value of 78% was calculated, while the measured value was 77%. The extraordinary high values of 155 and 136% for population A and B of potato MHR resulted in a calculated value of 113% while the measured value was 90%. Since the values for the populations were reproducible and both calculated and measured da values for potato MHR had the same order of magnitude, the high da values were thought to be representative for these specific populations. Rhamnogalacturonan I[1] was reported to be acetylated as well, although the position of the ester groups was not exactly established[1]. However, Komalavilas and Mort[26] reported that up to 30% of the galacturonic acid residues in an RG-I like polymer isolated from suspension-cultured cotton cells had acetyl groups linked through O-3. In the near future, more information might become available on the position and distribution of the acetyl groups, since the presence of a rhamnogalacturonan acetylesterase in some industrial enzyme preparations has been reported[27]. This enzyme was found to be specific for rhamnogalacturonan regions and not for homogalacturonan (smooth) regions.

Methylation analysis resulted in qualitative rather than quantitative information. The efficiency of carboxyl-reduction was low and variable as reflected in the low yield of galactose residues after reduction. However, colorimetric determination of the carboxyl-reduced material showed that only small amounts of uronides were left when carboxyl-reduction was carried out three times. This could reflect a tendency of pectic polymers to undergo β-elimination during the methylation procedure. In general, the same types of sugar linkages were found in all MHR fractions.

Comparison of our results for pear MHR with data in the literature dealing with pectin fractions rich in neutral sugars[10,28] showed good agreement, although MHR contained more rhamnose and galactose residues, while the degree of methylation was rather low. This might be caused by enzymic demethylation during the liquefaction process, while Yoshioka et al.[28] suggested that "native" pectin molecules might be de-esterified during ripening from ca. 90% to less than 40%.

Many recent publications deal with pectic substances extracted from carrot tissue both before[5,8,29–32] and after enzymic treatment[6,7]. A wide range of different pectic molecules have been described including fractions having a rhamnose–galacturonic acid ratio of 1[8]. This latter fraction was isolated without the use of enzymes, which is quite remarkable. Also, treatment of carrot cell wall polysaccharides with endo-pectin lyase[7] and endo-pectate lyase[6] resulted in the same type of hairy regions. It should be mentioned that some studies of carrot pectic material

were carried out using suspension-cultured cells[6,33], which might give different characteristics for the corresponding polysaccharides[34]. Although the presence of acetyl groups in hairy regions is not often mentioned in the literature, Massiot et al.[30] reported a da value of 29%.

Pectins isolated from onion tissue have been reported[13–15] to be relatively rich in galacturonic acid. Treatment of onion cell wall polysaccharides with pectin lyase[14] resulted in fractions with similar characteristics to our MHR; a fraction was described[14] having a rhamnose–galacturonic acid ratio of 0.4.

The characteristics of potato MHR were compared with data in the literature regarding chemically[16,18] as well as enzymically[17,35] extracted pectins. The former pectins were rich in galacturonic acid and were believed[16,18] to represent homogalacturonan chains with some neutral sugar side-chains attached. Treatment of potato tissue resulted in rhamnogalacturonan-rich polysaccharides[17,35] which resembled our MHR. Differences were found for the relative amount of galactose residues in the various fractions.

Enzymic degradation by RGase.—Since our isolation procedure of MHR included the use of a broad range of enzymes, our MHR preparations were resistant to a variety of pure carbohydrases present in our laboratory except towards rhamnogalacturonase. This enzyme was isolated specifically for its ability to degrade the backbone of apple MHR[19]. As discussed previously[20], no degradation by RGase was observed when the MHR fractions were not saponified before incubation. This is explained by the hindrance of RGase by *O*-acetyl groups.

Monitoring the degradation at various time intervals by HPSEC revealed that the elution patterns were about the same at all stages and showed great similarities in the various MHR. However, MHR from carrot tissue was found to be somewhat more resistant to degradation then the other MHR fractions. Population B especially was degraded slowly and not so completely as the others. Although the degree of degradation (9–12%) seemed to be related to the amount of rhamnose and/or galacturonic acid in this type of polymer, no suitable correlation was found. Linear regression of the degree of degradation against the relative amount of rhamnose, galacturonic acid, or the sum of both resulted in correlation factors of ca. 0.7. Since we do think that the degradation of MHR is coupled to the amount of rhamnose and/or galacturonic acid, the poor correlation might be explained by the assumption that only part of the rhamnose and galacturonic acid residues were present in an alternating form, while segments of the backbone have another sequence of both sugars. Previous results[22] based on studies of the degradation products of apple MHR by RGase action pointed to the presence of three different subunits: a xylogalacturonan, an arabinan-rich stub of the rhamnogalacturonan, and sections in which the rhamnogalacturonan oligomers are dominantly present. In the accompanying paper[36], the presence of these rhamnogalacturonan oligomers will be discussed in more detail. Although MHR from carrot, leek, and potato were found to contain hardly any xylose, analogous subunits having various ratios of rhamnose to galacturonic acid might be present in all

MHR. This idea is strengthened by the limited action of both polygalacturonase and rhamnogalacturonase towards the backbone of the various MHR.

In conclusion, it can be stated that the enzymic liquefaction process resulted in populations of specific pectin fragments. Although slight alterations may occur during this isolation procedure, the pectic hairy regions obtained showed great similarity to fractions isolated in a different way, as described in the literature. Due to the identical isolation procedure, the (modified) hairy regions obtained in this study can be more readily compared. Rhamnogalacturonase proved to be a powerful tool for the elucidation of pectin structures, since it is able to hydrolyse selectively in the backbone of the MHR.

Such characteristics as the sugar composition, da, dm, molecular weight distribution, and degradability by RGase showed great resemblances between MHR from pear, carrot, leek, onion, and potato tissue. Since the occurrence of the MHR seemed to be widespread, as it is found to be present in tissues of both monocotyledons and dicotyledons, a specific role for such molecules in plant growth or defence should not be excluded.

There is still a difference of opinion about whether the hairy regions and homogalacturonans are connected to each other in the cell wall. The model of De Vries et al.[3] began from the principle that both molecules are interlinked; also, rhamnogalacturonan I is usually isolated by the action of polygalacturonase[1]. On the other hand, Dick and Labavitch[10] and Dongowski and Anger[8] isolated their hairy regions without the use of enzymes.

More research is needed to reveal the exact structure of the (modified) hairy regions and their position within the cell wall framework.

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