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DAFTAR ISI

THE COMPLEXITY OF PECTIN: APPROACHES TO REVEAL ITS DISTRIBUTION AND STRUCTURE

Indira Prabasari

ABSTRACT

Pectin plays important roles both in plant science and industrial applications. The understanding about pectin structure nevertheless is unclear. In the plant science, pectin is believed to be synthesized in the Golgi apparatus in methyl-esterified form. To date, three major pectic polysaccharides are recognized: a fairly simple one called homogalacturonan (HG) which is composed entirely of GalA; a rather more complex pectic polysaccharide called rhamnogalacturonan I (RG I) which contains GalA, rhamnose (Rha), as well as a variety of other sugars as branches on the RG backbone, principally arabinan and galactan; and finally the most complex pectic polysaccharide of all, rhamnogalacturonan II (RG II) that also contains GalA to which a complex array of side chains containing several unusual monosaccharides are attached. Based on data accumulated over many years of dedicated research, a model of pectin structure was proposed in 1995 which became popular among plant and food scientists. A few years later, the model has been revisited. In order to have a better understanding of pectin, combinations of different approaches have been used. While enzymes and physicochemical analyses are most commonly used to elucidate the structure of pectin extracted from various plant materials, a range of antibodies have been developed to map the distribution of pectic polysaccharides in plant cell walls

1. INTRODUCTION

Pectin plays important roles, both in cell wall architecture and industrial applications; however, our understanding on the structure and function of pectin is still far from complete. One of the reasons is that pectin is a group of very complex biopolymers. In addition, the origin of pectin gives unique structural elements to the molecules, resulting in a diversity of physicochemical characters and functional properties.

2. PECTIN

2.1. Definition

The term "pectin" was first named by Broconnot in 1825 who carried out some of the first systematic studies on the subject. Pectin continued to be of academic as well as practical interest, and a brief review of early work is given in a major book by Kertesz in 1951 (May 1999). In fact, it is not easy to define pectin since it is a group of complex polysaccharides with more fine structural

details continue to be revealed; however in general term we can say that pectin is a mixture of heterogeneous, branched and highly hydrated polysaccharides rich in D-galacturonic acid (GalA) (Carpita 2000).

Voragen et.al. (2001) described pectin as a group of closely associated polysaccharides from the primary cell walls and intercellular regions of higher plants. In addition, the unspecific term protopectin is often used to designate the native pectin fractions in cell walls that can not be extracted by non degradative methods.

2.2. Structural elements of pectins

2.2.1. Homogalacturonan

Homogalacturonan (HG) is called as a 'smooth region' and believed to be synthesized originally in mostly methyl-esterified form. The GalA residues can be methyl-esterified at C-6 and may carry acetyl groups on O-2 and/or O-3. The methyl-esterification, in particular, has gained huge attention in pectin chemistry since it determines the physical properties of pectin to a large extent. Not only the amount of methyl-esterification is important, but also its distribution (Vincken 2003). Furthermore, the distribution found in specific pectins will depend on the action of endogenous enzymes such as pectin methylesterases (PME- which may cause a blockwise distribution of unesterified GalA residues) or by the condition under which the raw material is processed or the pectin is extracted (Voragen 1995).

There are a few reports describing HG with β-D-Xylp-(1→3) monoglycosyl units (Schols 1995; Albersheim 1996). This polysaccharide is referred to as xylogalacturonan (XGA). It seems that its presence to be confined to reproductive organs such as fruits and seeds (Albersheim 1996).

2.2.2. Rhamnogalacturonan

Rhamnogalacturonan I (RG-I) is with a backbone of alternating α-(1→2)-linked L-rhamnosyl and α-(1→4)-linked D-GalA residues ramified with different types of neutral oligo- and polysaccharides (e.g. arabinans, galactans and arabinogalactans), predominantly attached to O-4 of rhamnosyl residues.

The structure of rhamnogalacturonan II (RG-II) is highly conserved, as apparently identical structures have been obtained from the cell wall of a large variety of sources. The backbone of RG-II contains nine (1,4)-linked α-D-GalA residues to which four structurally distinct oligoglycosyl side chains are attached to O-2 or O-3 of four of the backbone residues such as apiose, 2-O-methyl-L-fucose, 2-O-methyl-D-xylose, aceric acid (3-C-carboxy-5-deoxy-L-xylose), KDO (3-deoxy-D-manno-octulosonic acid) and DHA (3-deoxy-D-lyxo-heptulosaric acid). RG-II is a low molecular weight (~4.8 kDa) complex polysaccharide segment present in primary walls predominantly as a dimmer that is covalently cross-linked by borate diesters (Pellerin 1996).

2.3. Chemical structure

A general representation of native pectins is shown in Figure 1. This has developed from the work of De Vries and colleagues (De Vries 1982; De Vries 1988), who used pure and well-characterised enzymes to study the structure of apple pectin extracted under mild condition. Occurrence, amount and chemical fine structure of the individual segments may vary significantly

depending on the source of the pectin and its developmental stage (Schols 1996; Voragen 2001).

3. CURRENT STUDIES ABOUT PECTIN STRUCTURE

As pectin is composed of as many as 17 different monosaccharides (Mohnen 1999; Ridley 2001), revealing its structure and how these polysachharides are linked is not easy.

Seven years after the model proposed in 1996 (Figure 1), the pectin structure has been revisited. The new findings seem to indicate that instead of an extended backbone consisting of homogalacturonan and rhamnogalacturonan regions, pectin is more likely a rhamnogalacturonan with neutral sugar and homogalacturonan side chains, as shown in Figures 2 and 3 may consider combining the two figures (Vincken 2003).

Although the new model is still speculative, several lines of evidence support the idea (Mc.Neil 1980; Renard 1998; Zhan 1998; Vincken 2003) and it seems to accommodate a number of observations which are more difficult to explain with the old model.

In addition, other evidence showed that pectin is much more hairy than thought before. Arabinan and arabinogalactan-I epitopes appear to be developmentally regulated in a tissue-specific manner (Bush 2001; Willats 2001). Figure 2A shows that in this pectin, different kinds of hairs are distributed in an at random fashion in the RG I backbone, whereas Figure 2B shows pectin that only contains two kinds of hairs which are attached to the RG I backbone in a cluster-like fashion.

4. PECTIN AND THE INDUSTRY

The ability of pectin to form gels is a property widely utilized in the food industry. Pectin is manufactured industrially by acid extraction, at an elevated temperature, of citrus peels (lime, lemon, orange) or apple pomace. Important characteristics of pectin preparations used as an ingredient in food industry include total GalA content, neutral sugar content and composition, degree of methyl esterification (DM), degree of acetyl esterification and protein and ash contents.

According to the new model (Figure 3), pectin is a comb-like molecule locking into each other upon gelation (Vincken 2003). This interaction is different from the other situation associated with the previous model, where pectin gels are simply a stack of homogalacturonans. The comb-like structures can acquire an increasingly intimate interaction when the DM decreases, especially when this is done in block-wise fashion. The newly proposed structure may help shed light on the behavior of industrial pectin molecules engaged in gel formation.

5. METHODS TO ANALYZ PECTIN DISTRIBUTION IN PLANT TISSUES

The use of monoclonal antibodies in mapping the distribution of pectin in plant tissue is very valuable since it will complement the in vitro data obtained from chemical extraction and structural elucidation studies. Several anti-pectin antibodies are now available for this purpose. Table 1 shows the major anti-pectin monoclonal antibodies that have been developed and characterized to date.

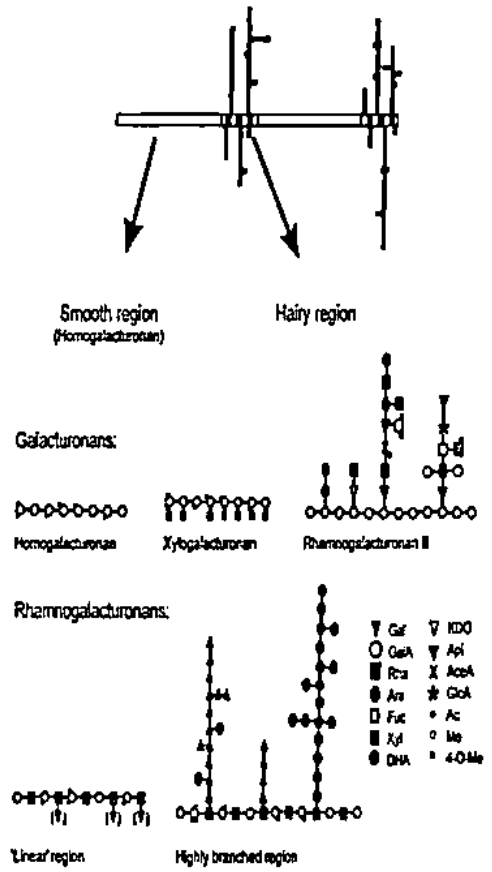

Figure. 1. Schematic structure of pectin, including the smooth region (homogalacturonan) and the hairy region. Various structural elements of pectin are also shown. Gal, galactose; GalA, galacturonic acid; Rha, rhamnose; Ara, arabinose; Fuc, fucose; Xyl, xylose; DHA, 3-deoxy-D-lyxo-2-heptulosaric acid; KDO, 2-keto-3-deoxy-D-mannooctulosonic acid; Api, apiose; AceA, aceric acid; GlcA, glucuronic acid, Ac, acetyl group; Me, methyl ester; 4-O-methyl ether (Voragen 2001).

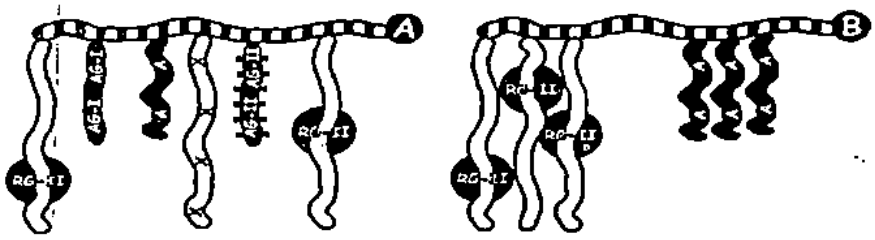

Figure 2. Two new tentative structures for pectin (Vincken 2003). For symbols, see Figure 3 below.

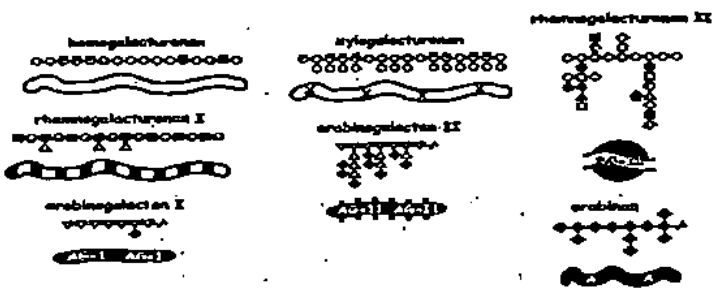

Figure 3. Schematic structures of the constituent polysaccharides of pectin (Vincken 2003)

Table 1. Anti-pectin antibodies

Antibodies	Epitope	References
JIM5	HG/low/not methyl-esterified	(Knox 1990; Willats 2000)
JIM7	HG/high methyl-esterified	(Knox 1990; Willats 2000)
2F4	HG/dimerised oligogalacturonides	(Liners 1989; Liners 1992)
PAM1	HG/blockwise pattern of de-esterification	(Willats 1999; Willats 2000)
LM7	HG/nonblockwise pattern of de-esterification	(Willats 2001)
CCRC-R1	RG-II monomer	(Williams 1996)
CCRC-M1	RG-I/t-α-fucose-(1→2)-linked to galactosyl[a]	(Puhlmann 1994)
CCRC-M2	RG-I/unknown[b]	(Puhlmann 1994)
CCRC-M7	RG-I/arabinosylated (1→6)-β-D-galactan	(Puhlmann 1994)
LM5	RG-I/(1→4)-β-D-galactan	(Jones 1997; Willats 1999)
LM6	RG-I/(1→5)-α-L-arabinan[b]	(Willats 1998; Willats 1999)

[a] Epitope also occurs in xyloglucan
[b] Epitope may also occur in arabinogalactan-protein proteoglycans (Knox 2002)

6. METHODS TO ANALYZE PECTIN EXTRACTED FROM PLANT TISSUES

Despite its limitation, chemical extraction and structural characterization of pectin continue to be important tools in understanding pectin. Various fractional extraction procedures have been developed for this purpose. The conventional procedures usually start with a stabilization and clean-up step of the plant material to inactivate endogenous degrading enzymes and to remove interfering compounds such as sugars, amino acids, organic acids, starch, proteins, nucleic acids, polyphenols, etc. Figure 4 shows the scheme commonly used for laboratory extraction of pectin, with each condition correspond to the removal of specific types of association and/or linkages. The extract obtained is then fractioned to homogeneity using size-exclusion and/or anion-exchange chromatography. For structure elucidation of such homogeneous fractions, sugar and glycosidic linkage composition and anomeric configuration of sugar residues are established. Strategies for establishing the fine structure further include fragmentation with pure, well-defined and highly specific enzymes, or with less-specific chemical reactions and fractionation to homogeneity of the fragments in the digests (Voragen 2000). These fragments often fit within the analytical range of advanced NMR and mass spectroscopi

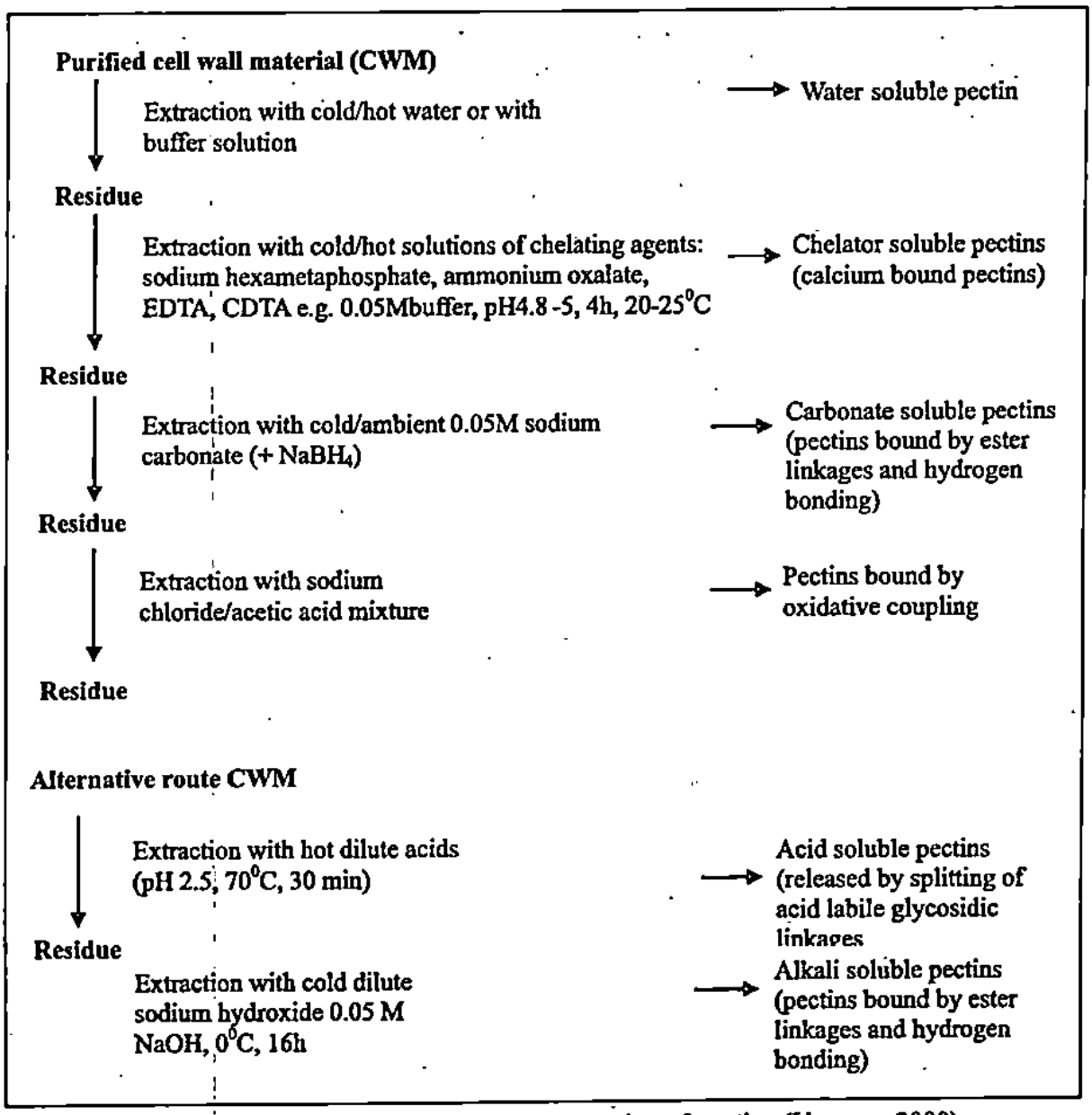

Figure 4. The scheme for laboratory extraction of pectins (Voragen 2000)

6.1. Analytical methods for structural characterization

Some analytical methods can be employed to characterize the pectin samples:

1. Neutral sugars: determined as their alditol acetates by GC-MS (Blakeney 1983; Ros 1998).
2. Linkage composition: established by methylation analysis before and after carboxyl reduction (Kim 1992; Sims 1995). The degree of methyl-esterification (DM) can be worked out from the analyses.
3. The degree of methyl-esterification (DM) and degree of acetylation (DA): HPLC (Voragen 1986) and Fourier Transform Infrared (FTIR) spectroscopy (Bociek 1975).
4. Molecular weight distribution: high-performance siza exclusion chromatography (HPSEC) (Kravtchenko 1992; Corredig 2000).

5. Charge distribution: high-performance anion-exchange chromatography (HPAEC) (Lau 1988; Schols 1989; Voragen 2000; Daas 2001).

6. Various chemical methods and/ or purified pectic enzymes can be employed to achieve specific cleavage of pectin samples, and the fragments obtained can be separated and analysed.

7. SUMMARY

In order to have a better understanding of pectin, combinations of different approaches should be employed. For examples, the use of enzymes and physicochemical analyses to study the structure of pectins extracted from various plant materials, especially citrus albedo tissue; the use of antibodies to map the distribution of pectin in plant cell wall.

The physicochemical analyses of pectin, such as compositional and linkage analyses continue to be important tools in understanding pectins. However, homogenization of cellular structures involved in these methods would result loss of information concerning the spatial and developmental aspects of pectin structure, a fundamental issue that we should be aware of. The use of antibodies to study the occurrence of pectic polymers in relation to cell wall architecture and cell development, therefore, is very valuable to complement the information obtained through physicochemical analyses.

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