Chapter 2
Pectin Structure

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2.1 Introduction

Pectin is a natural constituent of all terrestrial plants that is particularly abundant—together with hemicelluloses, cellulose, and low amounts of structural proteins—in the primary cell walls of eudicotyledons and non-graminaceous monocotyledons (Carpita and Gibeaut 1993). Once extracted from citrus peel or apple pomace, commercial pectin is widely used as gelling, thickening, stabilising and emulsifying agent in various food products such as jams, acidic milk drinks, ice creams, or salad dressings, and it is well agreed that the fine structure of pectin deeply affects its functionality and applicability (Willats et al. 2006). The existence of a "jelly" in tamarind extract was discovered more than two centuries ago by the French pharmacist and chemist Louis-Nicolas Vauquelin (1790). The word "pectin" from the Greek πηκτός ("pêktós", which means "thick") was first used in 1825 by Braconnot, who had resumed Vauquelin's work (Braconnot 1825a, b). Smolenski in 1923 was the first scientist to describe pectin as a polymer of galacturonic acid (GalA) and Kertesz (1951) defined pectin as a hetero-polysaccharide containing mainly partly methylesterified GalA together with some neutral sugars. In the 80s, the work of de Vries and co-workers was instrumental in showing that neutral sugars were present as side-chains arranged in blocks in so called "hairy regions" while >90% of the GalA residues could be isolated as chains comprising solely GalA (de Vries et al. 1981, 1982, 1983). Nowadays, it is widely accepted that pectin is a heterogonous macromolecule composed of interlinked distinct domains, the relative amount and structure of which vary according to the botanical origin, the organs and cell types considered, the stages of cellular development, and the precise location within the cell wall (Voragen et al. 2009). On top of this biological diversity, pectin structure varies broadly depending on the extraction

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method used. Different pectin populations that are more or less strongly anchored in the cell wall co-exist and a high number of extraction steps is needed to extract all pectin (Broxterman 2018).

Two main families of pectin structural elements are usually considered: galacturonans and rhamnogalacturonan I (RG-I). Galacturonans are made of a backbone of α-(1,4)-linked D-galacturonic acid (GalA) residues. This galacturonan backbone may be unbranched (homogalacturonan) or decorated with more or less complex side-chains. The backbone of RG-I is made of the diglycosyl repeating unit [2-α-L-Rha-(1,4)-α-D-GalA-(1] (Lau et al. 1985). Rhamnose (Rha) residues are ramified at *O*-4 (mainly) and *O*-3 (scarcely) positions with single to polymeric neutral sugar side chains that include arabinose (Ara) and galactose (Gal) residues in various combinations (Lau et al. 1985; Colquhoun et al. 1990). Four main types of polymeric side-chains are usually envisioned, arabinans, galactans, type I arabinogalactans (AG-I) and type II arabinogalactans (AG-II) (Yapo 2011).

As has been briefly described above, pectin is an extremely complex polysaccharide composed of as many as eighteen distinct monosaccharides connected to each other through twenty different linkages. In addition, several of these monosaccharides can be chemically modified by *O*-ether or *O*-ester groups. Because of polydispersity and polymolecularity, analyses on whole macromolecules are usually not sufficient to reveal all the structural details of pectin. For structural investigation, pectin is thereby commonly degraded into oligosaccharides by chemicals or enzymes. Enzymatic digestion is usually preferred since it offers additional information thanks to the specificity of cleaving. Degradation enzymes are, however, not available for all linkages. Degradation products are further fractionated usually by chromatographic means. The isolated structural elements thereby recovered are in the analytical range of a broad set of techniques (Schols and Voragen 2002) and very valuable structural information has been obtained by chemical analyses and NMR in the 90s. This information has however been restricted to major oligosaccharides that could be purified in quantities large enough to allow full structural characterisation. In the past fifteen years, significant developments have led to a re-emergence of the use of mass spectrometry (MS) for the structural characterisation of oligosaccharides (Dong et al. 2018). This review includes improvements that have been achieved in separation techniques (ultra-performance liquid chromatography (UPLC), capillary electrophoresis, ion mobility) and developments in activation approaches using electron, cation or photon interactions that are much more efficient than classical fragmentation methods (i.e., low energy collision induced (or activated) dissociation (LE-CI(A)D) for the determination of the fine structure of oligosaccharides. Very recent works have been conducted on instrument development for the structural characterisation of oligosaccharides based on ion activation (Ropartz et al. 2014, 2016, 2017), on the coupling of MS and infrared ion spectroscopy (Schindler et al. 2017; Mucha et al. 2018) and on high resolution ion mobility (Ujma et al. 2019; Ropartz et al. 2019). These approaches allow to shed new light on complex structures and mixtures of oligosaccharides, as can be found in pectin.

2.2 Galacturonans

2.2.1 Homogalacturonans

Homogalacturonan (HG) also known as the "smooth region" of pectin, is a linear homopolymer of α-(1,4)-linked D-galacturonic acid (GalA) residues that can be methyl and acetyl-esterified (Fig. 2.1). It usually accounts for approximately 60% of the total pectin amount (Mohnen 2008; Atmodjo et al. 2013) but there are some exceptions—sugar beet or soybean pectin for instance—in which the relative amount of HG is much lower (Voragen et al. 1995).

HG have been specifically isolated from several plant sources by exploiting the susceptibility to acid hydrolysis of the different glycosidic linkages (Thibault et al. 1993; Yapo et al. 2007; Ralet and Thibault 2009; Round et al. 2010) or by enzymatic means (Bonnin et al. 2002; Hellin et al. 2005). Depending on the method used for (1) pectin extraction, (2) HG isolation and (3) molar mass determination, the average degree of polymerisation (DP) ranges between 72 and 300. Ralet and Thibault (2009) isolated HG from pectin samples sequentially extracted from different plant sources: pineapple flesh (*Ananas comosus*, *Bromeliaceae*), leek (*Allium porrum*, *Alliaceae*), cucumber (*Cucumis sativus*, *Cucurbitaceae*), sugar beet root (*Beta vulgaris*, *Amaranthaceae*), fennel bulb (*Foeniculum vulgare*, *Apiaceae*), and lemon albedo (*Citrus medica*, *Rutaceae*) and showed that pectin samples encompassed various amounts of HG domains of very similar DP (70–100) and of low polydispersity, confirming the hypothesis of a HG length periodicity (Thibault et al. 1993).

2.2.1.1 Methyl-esterification

In HG, GalA units are usually partially methyl-esterified at *C*-6 (Fig. 2.1) and not only the degree of methyl esterification (i.e., the number of moles of methanol per 100 moles of GalA), but also the distribution of non-esterified GalA residues on HG segments are key features for pectin functional properties (Willats et al. 2006). Numerous investigations have thereby been devoted to understand the peculiar methyl-ester distribution patterns and their functional implications. The development of methods allowing characterising and quantifying degradation products

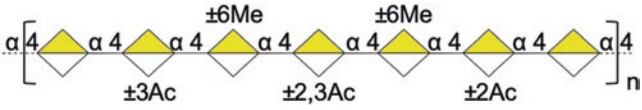

Fig. 2.1 Structure of homogalacturonans according to the symbol nomenclature for glycans (SNFG) introduced by Varki et al. (2015). Homogalacturonan is the dominant feature of pectin. It consists of partly methyl-esterified and, in some plant species, partly acetyl-esterified α-(1,4)-linked D-galacturonopyranosyl chains with a degree of polymerisation 100–300. The amount and distribution of methyl- and acetyl-esters onto homogalacturonans is a key factor for pectin functionality

obtained by treating well-defined pectin samples with HG-degrading enzymes was instrumental in providing such information. The concept of *degree of blockiness* calculated from the amount of oligogalacturonates released quantified by high-performance anion-exchange chromatography or by capillary electrophoresis, has been developed. This concept allowed differentiating pectin encompassing HG domains exhibiting subtle differences in methylesterification patterns (Guillotin et al. 2005; Daas et al. 2000; Limberg et al. 2000; Ström et al. 2007; Ngouemazong et al. 2011; Ralet et al. 2012). Parallel electrospray ionisation multistage mass spectrometry (ESI-MS^n) may be also used to provide information about the location of methyl-esterified GalA residues in oligogalacturonates of DP 3–10 (Körner et al. 1999; van Alebeek et al. 2000; Quéméner et al. 2003; Ralet et al. 2009, 2012). Recent advances in mass spectrometry and separation techniques allowed exploring complex mixtures and characterising oligosaccharides of higher DP (identification up to DP14, structural characterization up to DP9) (Ropartz et al. 2014). Model HGs were degraded by a pectin lyase and digestion products were characterised (Ropartz et al. 2014). The structure of each isomer was stated based on its methylation pattern. These approaches can bring different types of information: (1) the specificity of the enzyme and the tolerance of each subsite to the occurrence of methyl-esters can be stated, and (2) the structure of large oligosaccharides can be revealed, giving insights into parts of the polymers that are recalcitrant to enzymatic digestion.

2.2.1.2 Acetyl-Esterification

In few plant species, GalA residues in HG domains are partially acetyl-esterified at *O*-2 and/or *O*-3 (Rombouts and Thibault 1986; Ishii 1997; Needs et al. 1998; Perrone et al. 2002) (Fig. 2.1) and this has a strong negative impact on gelation (Pippen et al. 1950; Kohn and Furda 1968; Kohn and Malovikova 1978; Renard and Jarvis 1999; Oosterveld et al. 2000a; Ralet et al. 2003). The distribution of acetyl groups onto HG segments has been particularly studied in sugar beet. Keenan et al. (1985) showed by nuclear magnetic resonance (NMR) that acetyl groups could be attached on any of the available ring positions (*O*-2 and *O*-3) of GalA residues. Ralet et al. (2005, 2008) and Remoroza et al. (2014) used combinations of pectin-degrading enzymes to generate partly methyl esterified and acetyl esterified oligogalacturonates that were further separated by chromatographic means and analysed by ESI-MS^n. The large variety of oligogalacturonates that were identified and quantified revealed that (1) the occurrence of *O*-2 and *O*-3 acetyl esterification in roughly similar amounts, (2) the absence of 2,3-di-*O*-acetylation, and (3) the scarcity of GalA residues that are both methyl- and acetyl-esterified (Ralet et al. 2005). Pectins with high degree of methyl esterification (HM) having different patterns of ester distribution could be also discriminated (Remoroza et al. 2014). A blockwise distribution of acetyl groups was evidenced (Ralet et al. 2008) and, in commercially-extracted sugar beet pectin, blocks of (1) non-esterified, (2) partly methyl- and acetyl-esterified, and (3) highly methyl- and acetyl-esterified GalA residues were

identified (Remoroza et al. 2014). The balance between intra-chain heterogeneity (i.e., the different blocks that are present within a single macromolecule) and inter-chain heterogeneity (some pectin molecules are very highly acetyl-esterified and others are not) is however very difficult to appraise.

2.2.2 *Galacturonans Substituted with More or Less Complex Side-Chains*

HG can be more or less heavily substituted at *O*-2 and/or *O*-3 by monomers or dimers of apiose or xylose leading to apiogalacturonan (AGA, Fig. 2.2a) or xylogalacturonan (XGA, Fig. 2.2b), respectively. It can also be substituted with complex side-chains to form rhamnogalacturonan II (RG-II, Fig. 2.2c).

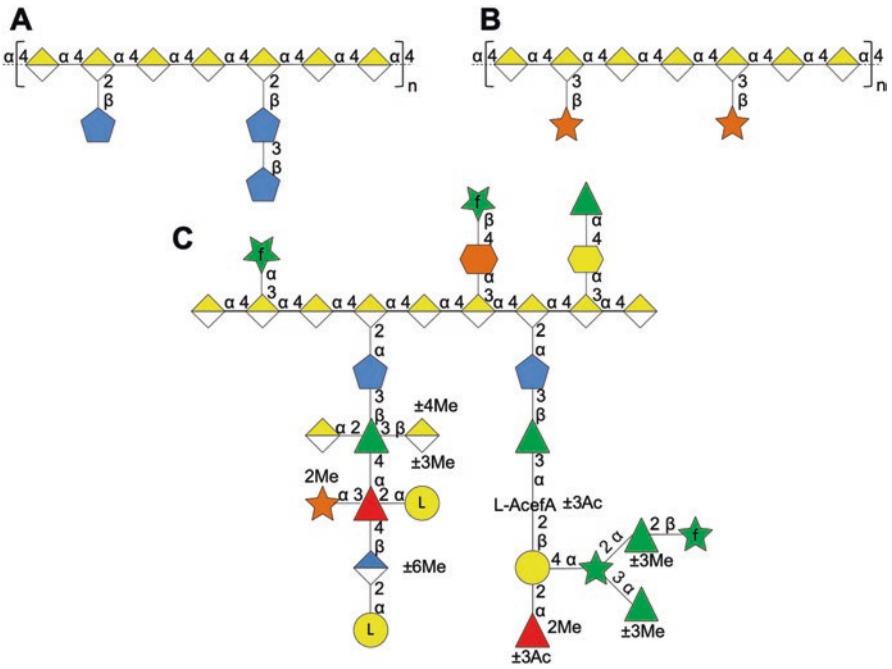

Fig. 2.2 Structure of the side-chains of homogalacturonans (SNFG). (**a**) apiogalacturonan, (**b**) xylogalacturonan, (**c**) rhamnogalacturonan-II. More or less complex side-chains can be attached to homogalacturonan. Rhamnogalacturonan-II is a key substituted galacturonan made of a short backbone branched by up to 5 side-chains varying in complexity from single arabinose unit to highly heterogeneous nonasaccharides

2.2.2.1 Apiogalacturonan

Apiogalacturonan (AGA, Fig. 2.2a) is restricted to some aquatic plants, the duckweeds (*Lemnoideae*) and the marine seagrasses (*Zosteraceae*) (Hart and Kindel 1970; Ovodov et al. 1971; Longland et al. 1989; Gloaguen et al. 2010; Avci et al. 2018). In AGA, β-D-apiofuranosyl residues are linked to *O*-2 and/or *O*-3 of GalA residues as monomers or as the dimer [β-D-Api*f*-(1,3′)-β-D-Api*f*-(1)]. The degree of substitution of HG by apiose varies from 25 to 80% (Hart and Kindel 1970; Ovodov et al. 1971). AGA can represent up to 20% of non-cellulosic cell wall polysaccharides in the green fronds of giant duckweeds (Longland et al. 1989).

2.2.2.2 Xylogalacturonan

Xylogalacturonan (XGA, Fig. 2.2b), an HG substituted solely at *O*-3 by xylose monomers or dimers, has been identified in several plant samples among which duckweeds (Hart and Kindel 1970), apple and watermelon fruits (Schols et al. 1995a; Zandleven et al. 2006; Mort et al. 2008), potato tuber (Zandleven et al. 2006), pea hulls (Le Goff et al. 2001) and *Arabidopsis thaliana* leaves and stalks (Zandleven et al. 2007). Dimeric side chains of xylose have been shown to contain 1,4-linked xylose residues (Zandleven et al. 2006) but 1,2-linked and 1,3-linked xylose residues have also been identified (Le Goff et al. 2001; Nakamura et al. 2002). Methylesterification of XGA has been reported in apple (Schols et al. 1995a). Both the ratio of Xyl monomers and dimers and the degree of substitution of GalA by Xyl vary according to plant samples. A recent study highlighted that the diversification of the *Lemnoideae* was accompanied by a reduction in the abundance of cell wall AGA and an increase in XGA (Avci et al. 2018).

2.2.2.3 Rhamnogalacturonan II

Rhamnogalacturonan II (RG-II, Fig. 2.2c) is a far more complex substituted-HG than AGA or XGA since it encompasses thirteen different sugars and twenty one distinct glycosidic linkages arranged as a backbone formed by nine partially methylesterified GalA residues (from none to three) (Ishii and Kaneko 1998) substituted by different side chains termed A–F (Ndeh et al. 2017).

Large Side-Chains

Side-chains A and B encompass six to nine sugars and are both bound to the HG backbone through a (2,1) β-D-apiofuranose. Although the structure of RG-II is considered as highly conserved throughout the plant kingdom, some heterogeneity may occur within these side-chains. Side-chain A was first described by Stevenson et al. (1988) in *Ficus sycomorus* as an oligosaccharide composed of eight different monosaccharides (Fig. 2.2c). The heterogeneity in the structure of side-chain A comes firstly from the α-L-Gal*p* residue that can be replaced by an α-L-Fuc*p*, not only in

the *Arabidopsis thaliana* mutant *mur1* (Reuhs et al. 2004), but also in the wild-type plants (Pabst et al. 2013). Additionally, side-chain A contains three uronic acids, one α-D-GalA*p*, one β-D-GalA*p* and one β-D-GlcA*p*, that can be methyl-esterified and/or methyl-etherified (Pabst et al. 2013; Buffetto et al. 2014). Side-chain A is involved in RG-II dimerisation through a boron covalent linkage *via* a di-ester bond between two β-D-apiofuranose units (*O*-2 and *O*-3). The three uronic acids residues are able to chelate cations and methyl-esterification that can occur on the β-D-GlcA*p* unit may modulate this chelation property. Side-chain B is particularly heterogeneous (Table 2.1).

Table 2.1 Structural variability of side-chain B in rhamnogalacturonan-II

		Glycosyl substitutions			Chemical modifications			
					Acetyl		Methylether	
Reference	Plant origin	(2-1) α-L-Rha*p*	(2-1) β-L-Ara*f*	(3-1) α-L-Rha*p*	3-α-L-Ace*f*	2-*O*-Me-α-D-Fuc*p*	(2-1) α-L-Rha*p*	(3-1) α-L-Rha*p*
Spellman et al. (1983)	*Acer pseudoplatunus*	X	–	–	–	–	–	–
Thomas et al. (1989)	*Oryza sativa*	X	–	–	–	–	–	–
Whitcombe et al. (1995)	*Acer pseudoplatunus*	X	X/–	–	X/–	X/–	–	–
Kaneko et al. (1997)	*Phyllostachys edulis*	X	–	–	–	–	–	–
Shin et al. (1998)	*Panax ginseng*	X	X/–	X/–	X/–	X/–	–	–
Ishii and Kaneko (1998)	*Beta vulgaris*	–	–	–	X/–	X/–	–	–
Vidal et al. (2000)	Red wine	X	X/–	a	X/–	X/–	–	–
Glushka et al. (2003)	*Arabidopsis thaliana*	X	X	X	X/–	X/–	–	–
Matsunaga et al. (2004)	Lycophyte PteridophyteBryophytes	X	X/–	X/–	X/–	X/–	X/–	X/–
Reuhs et al. (2004)	*Arabidopsis thaliana*	X	–	–	–	–	–	–
Seveno et al. (2009)	*Arabidopsis thaliana*	X/–	–	–	–	–	–	–
Pabst et al. (2013)	*Arabidopsis thaliana*	X	X/–	X/–	–	–	–	–
Buffetto et al. (2014)	*Vitis vinifera* Merlot	X	X/–	X/–	X/–	X/–	–	–
Sun et al. (2019)	*Panax ginseng*	X	X/–	X/–	X/–	X/–	–	–

X detected, – not detected

[a]Additional rhamnose was detected but not localised

It was first described in *Ficus sycomorus* as a heptasaccharide, the main chain being made of a β-D-Api*f*-(3,1)-β-L-Rha*p*-(3,1)-α-L-Aceric acid-(2,1)-β-D-Gal*p*-(2,1)-2-*O*-Me-α-L-Fuc*p*. The Gal*p* residue was substituted by a (4,1)-α-L-Ara*p* unit itself substituted by a (2,1)-α-l-Rha*p* residue (Spellman et al. 1983). Depending on the botanical origin, the stage of development and the organ considered, the number of constitutive sugar residues may vary. Hexa- to nonasaccharides were observed based on the substitution of the laterally branched α-L-Ara*p* residue (Fig. 2.2). Whitcombe et al. have described an octasaccharide with a (2,1)-β-L-Ara*f* linked to the laterally branched additional α-L-Rha*p* unit. These authors also described for the first time the occurrence of two acetylation sites on the α-L-Aceric acid and on the 2-*O*-Me α-D-Fuc*p*. Finally, in 1998, Shin et al. described in *Panax ginseng* a nonasaccharide resulting from a second α-l-Rha*p* unit branched at *C*-3 of the β-L-Ara*p* residue. It is difficult to conclude about the existence of the low DP species in vivo, as α-L-Rha*p* residues being very labile according to the extraction conditions and the analytical methods used. As for side-chain A, 2-*O*-Me-α-D-Fuc*p* can be replaced by 2-*O*-Me-L-Gal*p* in the *Arabidopsis thaliana mur1* mutant (Reuhs et al. 2004). In lycophytes, pteridophytes and bryophytes, α-L-Rha*p* units can be methyl-etherified at *O*-3 (Matsunaga et al. 2004).

Short Side-Chains

Side chain E is composed of a unique residue of α-L-Ara*f* linked to a D-GalA*p* at *O*-3 (Buffetto et al. 2014). This modification is termed side chain F when it occurs on a D-GalA unit that is also substituted by side-chain A. Side-chains C and D are dimers that are branched at *O*-3 of a D-GalA units. They are composed of a α-3-deoxy-D-manno-2-octulosonic acid (4,1) α-L-Rha*p* and an α-3-deoxy-D-*lyxo*-2-heptulosonic acid (5,1) β-L-Ara*f*, respectively. In the fractionation scheme used by Buffetto et al. (2014), these side chains are co-eluted with longer ones (co-elution of A and D and of B and C) (Ropartz 2015).

2.3 Rhamnogalacturonan I

RG-I was pictured as a long sequence of alternating L-Rha and D-GalA residues (Fig. 2.3a), Rha residues being substituted with a variety of L-arabinosyl- and D-galactosyl-containing side-chains (O'Neill et al. 1990, Fig. 2.3b–d). Fucose, glucuronic acid and 4-*O*-methyl glucuronic acid residues may also be present in small amounts. RG-I usually accounts for 20–35% of the total pectin amount (Mohnen 2008) but can, for certain plant sources such as soybean, make up to 75% of pectic polysaccharides (Voragen et al. 2009).

2.3.1 Rhamnogalacturonan I Backbone

The backbone of RG-I is made of the alternating diglycosyl repeating unit [2-α-L-Rhap-(1,4)-α-D-GalAp-(1] (McNeil et al. 1980, 1984; Lau et al. 1985, Fig. 2.3a).

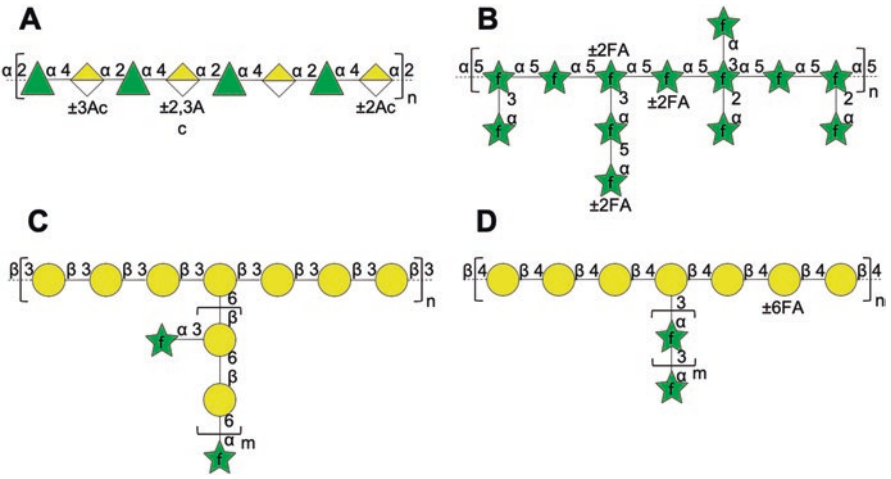

Fig. 2.3 Structure of Rhamnogalacturonan I (SNFG). (**a**) Backbone, (**b**) Arabinans, (**c**) Type I arabinogalactans, (**d**) Type II arabinogalactans. Rhamnogalacturonan I is the second major element of pectin. Its backbone is made of strictly alternating galacturonic acid and rhamnose residues, the latter being branched by arabinose- and galactose-containing side-chains of diverse complexity

Whole RG-I samples isolated from various plant sources usually exhibit Rha/GalA ratios < 1 (Schols and Voragen 1994; Cornuault et al. 2015; Buffetto et al. 2015). Values very close to 1 have however been reported (Konno et al. 1986; Yapo et al. 2007; Ralet and Thibault 2009) and the series of homologous oligomers recovered after controlled acid hydrolysis of pectin samples from apple, sugar beet and citrus all presented strictly alternating sequences (Renard et al. 1995). The presence of RG-I domains in which single Rha residues alternate with two or three consecutive GalA residues is therefore rather implausible. Unlike HG domains, RG-I backbone seems to exhibit a DP that varies depending on the plant source.

An average DP of up to 300 repeats has been estimated for RG-I isolated from suspension-cultured sycamore cell walls (McNeil et al. 1984). Ralet and Thibault (2009) reported values of 60 Rha-GalA repeats for RG-I isolated from citrus albedo and 120 for RG-I isolated from sugar-beet. Acetylation is much more frequent in RG-I than in HG, and RG-I domains isolated from numerous plant species appeared highly acetylated at *O*-2 or *O*-3 of the GalA units (Komalavilas and Mort 1989; Lerouge et al. 1993; Schols and Voragen 1994; Normand et al. 2010; Remoroza et al. 2012). Ishii showed that some GalA residues were 2,3-di-*O*-acetylated in RG-I oligosaccharides isolated from potato tuber and bamboo shoot cell walls (Ishii 1997). In okra, unusual acetylation at *O*-3 of Rha residues has been also shown (Sengkhamparn et al. 2009). Finally, to our knowledge, no evidence for methylesterification of the RG-I domain has ever been reported.

2.3.2 Rhamnogalacturonan I Side-Chains

RG-I is also known as the pectin "hairy region" since GalA but mostly Rha residues are substituted by different side-chains varying in their length and composition. GalA residues typically are not substituted and only one study showed that approximately 2% of the GalA residues in the RG-I backbone were substituted at *O*-3 by single β-D-glucuronic acid residues (Renard et al. 1999). In contrast, 20–80% of the Rha residues are substituted at *O*-4 with side-chains in which Ara and Gal predominate (Ridley et al. 2001). The proportion of side-chains, their composition, length and degree of branching vary enormously not only depending on plant sources, organs and tissues (Lerouge et al. 1993) but also on developmental stage (Willats et al. 2001) and on the isolation method used (Schols et al. 1995b). For instance, arabinan-rich pectins are particularly abundant in apple, sugar-beet and carrot while type I arabinogalactan-rich pectins are commonly found in citrus, lupin and potato and type II arabinogalactan-rich pectins have been found in ginseng (Sun et al. 2019). Further, galactan and arabinan epitopes can occur in different zones of organs and even at distinct locations within a single developing organ (Willats et al. 2001). Finally, for a given cell wall material, pectins that were more difficult to extract were more substituted with side-chains than the more easily extractable fractions (Schols et al. 1995b). Side-chains consisting in single Gal units have been reported for RG-I oligomers that have been isolated without using any side-chain degrading enzymes (Schols et al. 1995b; Oosterveld et al. 2000b; Sun et al. 2019). In okra, specifically, α-Gal substitutions were identified (Sengkhamparn et al. 2009). Polymeric side-chains commonly consist in arabinans and type I and II (arabino)-galactans.

2.3.2.1 Arabinans

Arabinans are made of a backbone of α-(1,5)-linked L-Ara*f* residues that are substituted at *O*-2 and/or *O*-3 with single α-L-Ara*f* units or with short chains of α-(1,3)-linked L-Ara*f* units (Yapo 2011, Fig. 2.3b). Sugar-beet arabinans have been particularly studied over the years. They consist of a long backbone of up to sixty or seventy Ara residues (Oosterveld et al. 2002) one third of which being branched at *O*-3, mainly with single Ara*f* units (Guillon and Thibault 1989). The presence of minor amounts of double branching at *O*-2 and *O*-3 has been further evidenced (Westphal et al. 2010). Occasionally, arabinans substituted with single Gal*p* units or with up to four or five β-(1,4)-linked Gal*p* residues have been isolated in soybean, sugar-beet or potato (Sakamoto and Sakai 1995; Nakamura et al. 2002; Øbro et al. 2004). Arabinans branched to a much lower extent than in sugar-beet have been also observed in ginseng root and in potato tuber (Sun et al. 2019; Øbro et al. 2004).

2.3.2.2 Type I (Arabino)-Galactans

Type I (arabino)-galactans encompass a backbone of β-(1,4)-linked D-Gal*p* residues that may be lowly substituted at *O*-3 by β-D-Gal*p* or α-L-Ara*f* single units or by short α-(1,5)-linked L-Ara*f* chains (Aspinall et al. 1967; Morita 1965a, b, Fig. 2.3c). Variants of this basic structure have been reported such as (1) termination of the main chain at *O*-6 with a Gal*p* residue (Lau et al. 1987), (2) termination of the main chain with an Ara*p* residue at *O*-4 or a Fuc residue at *O*-2 (Huisman et al. 2001; O'Neill et al. 1990), (3) insertion of a single Ara*f* or a short α-(1,5)-linked L-Ara*f* chain within the β-(1,4)-linked D-Gal*p* main chain (Huisman et al. 2001; Buffetto et al. 2015), and (4) β-(1,3) galactosyl interruption of the main chain (Hinz et al. 2005). An average chain length in the range of 45–50 was estimated in soybean (Huisman et al. 2001).

2.3.2.3 Type II Arabinogalactans

Type II arabinogalactans are highly branched side-chains characterised by a backbone of β-(1,3)-linked D-Gal*p* residues substituted at *O*-6 with single Gal*p* residues or with short chains of β-(1,6)-linked D-Gal*p* residues, which can in turn be substituted at *O*-3, *O*-4 and/or *O*-6, by Ara-containing chains (Yapo 2011, Fig. 2.3d). Type II arabinogalactans have been recently identified in ginseng (Sun et al. 2019) and it has been shown that the (1,3)-linked galactan backbone was substituted, mainly at *O*-6 but also at *O*-4, (1) with short (1,6)-linked galactan chains terminated by single Ara*f* units, and (2) directly with single Ara*f* residues.

2.3.2.4 Ferulic Acid

In species from the *Amaranthaceae* family such as sugar-beet and spinach RG-I side-chains are esterified by ferulic acid residues (Ishii and Tobita 1993; Colquhoun et al. 1994; Levigne et al. 2004; Quéméner and Ralet 2004). Ferulic acid moieties are mainly ester-linked to *O*-2 of Ara residues from the α-(1,5)-linked L-Ara*f* arabinan backbone and to *O*-6 of Gal residues from the β-(1,4)-linked D-Gal*p* type I galactan backbone but a more peripheral location at *O*-5 of Ara residues from the α-(1,5)-linked L-Ara*f* arabinan backbone has been also identified. Ferulic acid residues can undergo *in vivo* oxidative coupling reactions to form dehydrodimers, thereby covalently cross-linking the polysaccharides they esterify (Fry 1986). Such cross-links have been identified in sugar-beet pectin (Ralet et al. 2005).

2.3.3 Side-Chain Intra- and Inter-Molecular Distribution

Treatment of "hairy regions" with rhamnogalacturonan hydrolase allowed to evidence that the different types of side-chains were not randomly distributed over the RG-I backbone. Indeed, incubating de-esterified apple or ginseng pectin with rhamnogalacturonan hydrolase released RG-I oligosaccharides based on a $GalA_2Rha_2$ and a $GalA_3Rha_3$ backbone (Schols and Voragen 1994; Sun et al. 2019). Most of the RG-I oligomers recovered contained no side-chains and single unit Gal side-chains even though single units or short Ara chains were also detected in ginseng (Sun et al. 2019). Since Rha-GalA oligosaccharides substituted solely by Ara residues were identified (O'Neill et al. 1990; Sun et al. 2019), a direct linkage between Ara and Rha is plausible although a single Gal unit or short type I galactan anchor seem to be usually necessary for Ara and arabinan branching onto RG-I backbone (Buffetto et al. 2015). Next to these lowly branched rhamnogalacturonan hydrolase-degradable regions, highly branched arabinan- and type II arabinogalactan-rich regions that are not rhamnogalacturonan hydrolase-degradable have been identified (Schols and Voragen 1994; Sun et al. 2019). It has been hypothesised that these side-chains are flexible and long enough to wrap around the RG-I backbone, hindering enzymatic breakdown of the RG-I backbone by rhamnogalacturonan hydrolase (Willats et al. 2001; Voragen et al. 2009). The intra- and inter-molecular distribution of the different domains (lowly and highly branched) is extremely difficult to characterise and virtually nothing is presently known about the specific location of individual side-chains along the backbone. The co-existence of different "hairstyles" within a single pectin molecule remains speculative (Voragen et al. 2009).

2.4 Connection Between Pectin Domains

It is now fully admitted that pectin is a highly complex macromolecule in which several domains are covalently linked to each other. It is however particularly challenging to accommodate all available structural information into a universal model structure. Currently, two models are under debate (1) the *smooth* and *hairy* regions model established by de Vries et al. (1981) and updated by Schols and Voragen (1996) (Fig. 2.4) and (2) the RG-I backbone model proposed by Vincken et al. (2003) (Fig. 2.5).

The smooth and hairy regions model proposes that HG domains alternate with "hairy" regions, the latter including three subunits: subunit I: XGA; subunit II: stubs of RG-I backbone substituted by arabinan side-chains (or AG-II, Sun et al. 2019); and subunit III: rather short RG-I stretches (four or six residues) that are unsubstituted or substituted by Gal single units (Schols and Voragen 1996). In contrast, the RG-I backbone model positions HG and XGA as side-chains of RG-I (Vincken et al. 2003). Coenen et al. (2007) are, according to our knowledge, the

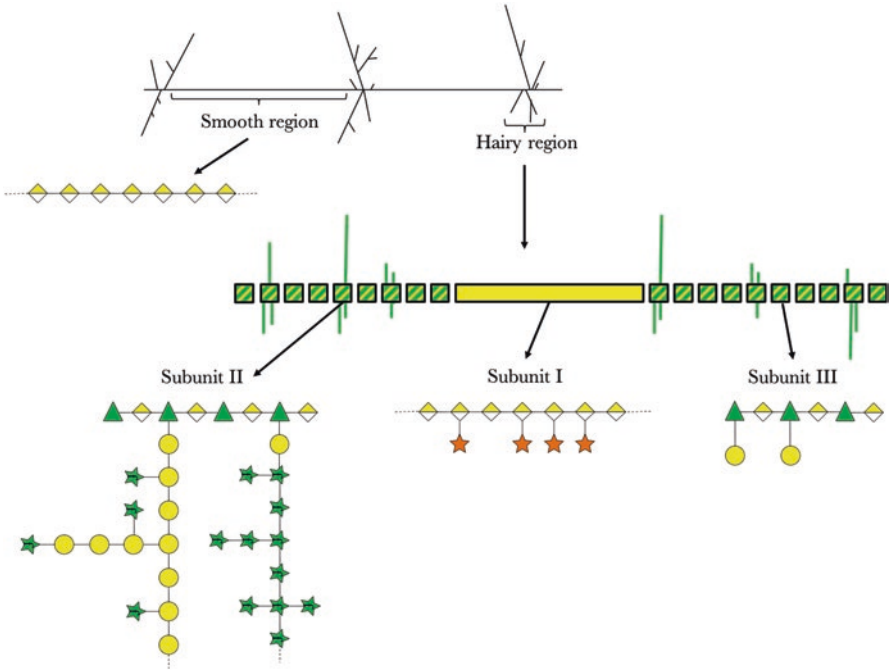

Fig. 2.4 Pectin smooth and hairy region model. The smooth region consists of homogalacturonan. The hairy region consists of three types of subunits: subunit I made of xylogalacturonan, subunit II made of arabinan- and type II arabinogalactan-rich RG-I and subunit III made of lowly substituted RG-I short stretches. Adapted from Schols and Voragen (1996)

only group succeeding in isolating and characterising chimeric oligosaccharides with a HG/XGA segment linked to a RG-I backbone one. A GalA trimer and a Xyl-substituted GalA trimer covalently linked to a short RG-I moiety were identified and H^1-NMR unambiguously showed that the GalA trimer was α-(1,2)-linked to the RG-I moiety.

MS^n experiments further showed that the RG-I moiety was always located at the reducing end of the chimeric oligosaccharides. These results are undoubtedly in favour of the "smooth and hairy regions" model even though this model does not fulfil all the experimental results obtained so far. In particular, it has been calculated that there are in average seventeen HG domains for one RG-I domain in lemon acid-extracted pectin and eight HG domains for one RG-I domain in sugar beet acid-extracted pectin taking into account (1) the partitioning of GalA between HG and RG-I domains, and (2) the length of these domains (Ralet and Thibault 2009). A similar "excess of GalA" was noticed by Yapo et al. (2007) and by Coenen et al. (2007). It is clear that there is a huge surplus of HG domains to build up a pectin macromolecule following the "smooth and hairy regions" model; the "RG-I backbone" model shall therefore not be excluded irrevocably (Coenen et al. 2007).

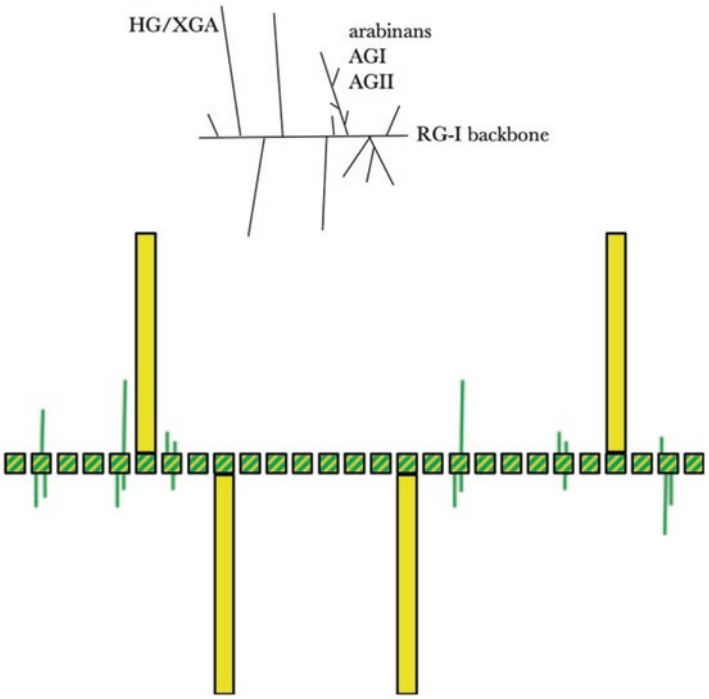

Fig. 2.5 Pectin RG-I backbone model. Homogalacturonan and xylogalacturonan (yellow) together with arabinans, and arabinogalactans (green) are side-chains of the RG-I backbone

2.5 Conclusions

Pectin is a highly complex macromolecule exhibiting a high degree of intra- and inter-molecular heterogeneity. When extracting pectin from a given plant sample, different populations that were located in specific tissues or even in specific spots within a single cell wall are co-extracted and mixed. Studying pectin fine structure implies to be able to master extraction conditions and implement adequate chemical and enzymatic tools to generate oligomers that can be separated and fully analysed. These can further be used to generate probes such as monoclonal antibodies to locate specific motifs *in muro*. To date, complete deconstruction of pectin into analysable oligomers has not been achieved and non-degradable fractions whose structure is unclear always remain. These fractions, encompassing in particular GalA surplus, should be targeted for getting a better understanding of the architecture of pectin macromolecules.

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