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On the rich pectin repertoire and functional versatility: An emphasis on the overlooked block co-polymers

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ABSTRACT

Pectins are complex structural components of plant cell walls, which can be composed of at least 17 monosaccharides of which α -D-galactopyranosyluronic acid is quantitatively the most important. They are nowadays viewed as multi-block copolymers, containing homogalacturonan, xylogalacturonan, rhamnogalacturonan-I, and rhamnogalacturonan-II structural domains. This overview of pectins is, however, restrictive inasmuch as other block copolymers, namely apiogalacturonan, galacturonogalacturonan, galactogalacturonan, and arabinogalacturonan have also been reported to be integral part of pectins from diverse sources. The review aims at highlighting and updating the extraordinary pectin repertoire and related (bio-)functionalities with a special mention of the overlooked polymers.

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KEYWORDS

The cell wall;
Pectins;
Structural domains;
Functional properties;
Gelling agents.

INTRODUCTION

Pectins are probably the most complex and diversified polysaccharides of the (primary) cell wall of all land plants. They are also the most intriguing and versatile from a functional point of view. The well-known functional properties of pectins are their gelling abilities to which they owe the name 'pectin' in reference to the Greek word 'pektikos', which means to 'congeal, solidify or curdle'.^[1-3] This functionality of pectins is currently exploited in the food industry for the production of jellies, jams, and marmalades. Pectins appear to be also good thickening and stabilizing agents of various food systems such as sauces, desserts and acid milk

products. Certain kinds of pectins and pectin-like polysaccharides from by-products of different plants, such as cacao, cauliflower, chicory, endive, pumpkin, soybean, and more particularly sugar beet, have proved effective emulsifiers of oil-in-water emulsions^[4,5] The so-called modified (citrus) pectins, which are mixtures of galacturonan- and/or galactan-rich pectic fragments, have been reported to be potent inhibitors of galectin-3-mediated cancer cells. *In planta*, pectins are principally structural components fulfilling important biological functions such as promoting seed germination, protecting plants from withering and conferring to them a certain resistance to cold and drought,^[6] and participating in the cell growth and development. *In muro*,

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the mechanical properties and the relative flexibility and strength of the cell wall, its integrity, and porosity are believed to be primarily determined by the pectin matrix polymers,^[7-9] and in association with water, any change in the composition of the pectin components and their relationship one to another can extensively alter the texture and the strength of the cell wall.^[10,11] Pectins are also thought to provide charged surfaces that modulate wall pH and ionic balance, regulate adhesion of cells to one another, and function as signal (or recognition) molecules that alert the plant cells to the presence of symbiotic organisms, pathogens, and insects.^[7,12,13] However, the term 'pectin' is vague enough and a somewhat misleading since it rather implies one macromolecule.^[3,14] Pectins in fact include relatively simple to highly complex polymer structures, all containing essentially the glycosyl residue α -D-galactopyranosyluronic acid (α -D-GalpA). Pectins are also viewed as a group of polysaccharides that have in common a high proportion of α -D-GalpA,^[15] a mixture of heterogeneous, branched and highly hydrated polysaccharides rich in α -D-GalpA that are extracted from the cell wall by Ca^{2+} -chelating agents such as ammonium oxalate, EDTA, EGTA or CDTA,^[12] a family of complex polysaccharides that contain α -D-GalpA^[13] or as a class of molecules defined by the presence of α -D-GalpA^[16]. Pectins are, however, believed to be composed of at least 17 different monosaccharides, containing more than 20 different linkages.^[4,17-19] Studies using enzymes of known specificities to degrade purified cell wall materials (CWMs) or pectins extracted from them have revealed that the different monosaccharides are not randomly distributed in a unique 'giant' pectin macromolecule, but are rather organized in a number of block polymers,^[14,20,21] completely or partly interconnected by as yet unidentified linkages to form the so-called pectin network also referred to as pectin matrix. Over the years, several pectic components have been purified and structurally characterized, allowing pectins to be currently defined as multi-block copolymers, including three-to-four pectic polysaccharide types, viz. unsubstituted homogalacturonan (HG), rhamnogalacturonan-I (RG-I) backbone (branched with different side chains of arabinan, galactan and arabinogalactans-I and II), rhamnogalacturonan-II (RG-II) and/or xylogalacturonan (XGA)^[13,17,18,22]. To

date, the so-called alternating 'hairy' (RG-I) and 'smooth' (HG) regions model^[21] and two challenging models, viz. the 'RG-I backbone' model with HG side chains^[17] and the lately proposed living-thing like model,^[22] in which the previous two have been accounted for, are the three remarkable hypothetical models. It should, however, be underlined that four other pectic polysaccharides, viz. apiogalacturonan (A_p GA), galactogalacturonan (GGA), arabinogalacturonan (A_r GA), and galacturonogalacturonan (GaGA) have been described^[6,24-26], but very often overlooked and omitted from the literature, probably because they seemed not to be amply ubiquitous, compared to the first four. The latter six branched polymers all have in common a (1 \rightarrow 4)-linked α -D-galacturonan backbone, and therefore can be gathered under the generic term of 'substituted galacturonans'^[4,13,16]. Thus, pectins can truly be viewed as a class of highly heterogeneous polysaccharides of at least 8 different block copolymers, from the cell walls and some mucilages and exudates of (higher) plants, some of which form complex (or composite) macromolecules via as yet not clearly elucidated inter-linkages. For example, extracted pectins from apple, citrus, and yellow passion (*Passiflora edulis* f. *flavicarpa*) fruit (YPF) rinds appeared to be complex-like macromolecules, composed chiefly of blocks of HG, followed by RG-I, XGA, and/or RG-II, with probably a minor amount of free GGA in apple pectins^[4,24,27], tragacanthic acid (the main polysaccharide component of gum tragacanth from *Astragalus gummifer*) was reported to include predominantly blocks of XGA and few RG-I^[28], soybean soluble pectin was found to contain mainly stretches of RG-I followed by HG, XGA, and probably GGA^[29,30], and comaruman, an ammonium oxalate (AO)-extracted pectin from the marsh cinquefoil *Comarum palustre* consisted mainly of HG, followed by RG-I and some GaGA blocks^[26]. However, it is worth underlining that it is still a matter of controversy as to the way in which the different structural domains are arranged to form complex pectin(s) *in muro*^[17]. The relative amount, fine structure, and length of each domain may vary widely from source to source, between different cell types, at different stages of cellular development, and even within a single cell wall^[3,14,18]. The fine structures of the pectic polysaccharides govern their biological functions *in-and-*

out of muro^[14,16] Expanding our knowledge of how structurally diversified pectins are proves essential to understanding the functional versatility within the cell wall as well as after extraction and purification. The present review aims at providing an update for the eight possible structural domains of complex pectins with a special attention focused on the four currently overlooked, and yet functionally maybe important, pectic polysaccharides.

THE FOUR COMMON PECTIN STRUCTURAL DOMAINS

Unsubstituted homogalacturonans

Unbranched homogalacturonans (HGs), often referred to as linear (homo)galacturonans, polygalacturonans (PGAs) or 'smooth' regions (SRs) of complex pectins, are the first type of pectic polysaccharides that have been purified from (pectins from) plant cell walls^[31]. They have been shown, by different methods of structural analysis (e.g., methylation analysis, NMR, FTIR, anomeric configuration and optical rotation), to be non-substituted polymeric chains of (1→4)-linked- α -D-GalpA units, partially methylesterified at C-6 position of α -D-GalpA units, irrespective of the origin of plant cell walls, their maturation stage, and physiological behavior. Some of the GalA units of the HG chain can be acetylerified at O-2 and/or O-3 positions with a degree of acetylerification (DAc) amounting over 60%, depending on the source and extraction method^[13,14,32]. The length, the methylesterification degree (DM) and pattern of HG chains may vary widely from source to source and location venue *in muro* (e.g., middle lamella and primary cell wall (PCW)). It has recently been shown that the (native) HG length is likely to be about two-fold shorter in the cell walls of commelinoid-related monocot species than in the cell walls of dicot species at full maturation stage^[4]. Linear HGs are the simplest and the most abundant pectic polymers so far characterized, representing ~50–70% of the cell wall pectins^[14,16,19] HGs as intact as possible, with respect to chain length, are usually isolated as the insoluble products of extracted pectins hydrolyzed under optimized acid conditions^[4,27]. They can account for up to 95% (w/w) of extracted pectins and contain more than 70%

of the whole pectin GalA residues, indicating their overwhelming majority over other structural domains of pectins such as branched RGs-I^[4,14,33]. They seemed to be, however, less profuse (than RG-I domains) in a pectin-like structure from soybean cotyledons^[30] and in strikingly minor amounts in (water-soluble) mucilages from *Arabidopsis thaliana* and *Linum usitatissimum* (flax) seeds^[34,35]. They are (almost) exclusively responsible for the well-known gelling ability of pectins. It is widely believed that high methylesterified (HM) pectins (DM >50) form gels in the presence of sugar (e.g., sucrose) and acid (pH 2.2–3.0), i.e., the so-called HM pectin-sugar-acid-gels ((HM-)SAG) and low methylesterified (LM) pectins (DM < 50) form gels in the presence of divalent cations (e.g., Ca²⁺), commonly referred to as 'calcium gels'. However, it has recently been reported that some (commercial) HM pectins (DM >60%) having sufficiently unesterified GalA residues or block sequences of non-esterified GalA residues, as obtained by treatments with plant pectin-methylesterases (p-PMEs), formed Ca²⁺-mediated gels^[36-39], indicating that the methylesterification pattern (block or non-block wise) is the main determining factor of Ca²⁺-induced gelation of pectins rather than the DM. HGs can also function as biosorbent, owing to a strong cation exchange capacity (CEC), thereby appearing to be potential heavy metal 'scavengers' from, for example, the human body and water and land contaminated with such threatening pollutants^[40]. *In muro*, HG chains are thought to be cross-linked by Ca²⁺, thereby forming gel-like structures, in the middle lamellae, that foster cell-cell adhesion by adjoining different cell walls abut and to function as the glue which consolidates the pectin network of the PCW^[41,42]. They may also determine the water-binding and holding capacity, knowing that water is the main constituent of the cell wall.

Rhamnogalacturonans-I

Unlike HGs, rhamnogalacturonans-I (RGs-I) are compositionally heterogeneous, containing diverse neutral sugar (NS) units, notably α -L-Rhap, β -D-Galp and α -L-Araf in addition to α -D-GalpA. Although (nearly) unbranched RGs-I have been characterized from *A. thaliana* seed mucilage^[43], RGs-I are usually branched, complex polysaccharides with a backbone composed of the repeating diglycosyl [→2)- α -L-Rhap-(1→4)-

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α -D-GalpA-(1 \rightarrow)]_n^{≥1}, which carries, at O-4 (mainly) and/or O-3 (scarcely) positions of α -L-Rhap residues, side chains of single residues to long polymers of different kinds: (1 \rightarrow 5)- α -L-arabinans, (1 \rightarrow 4)- β -D-galactans, arabinogalactans-I (AGs-I), and arabinogalactans-II (AG-II) with a (1 \rightarrow 3)-linked β -D-galactan core. Other would be side chains (residues) are the rare sugar α -L-Galp^[35,44], α -D-Galp^[45], arabinogalactans with (1 \rightarrow 6)-linked β -D-galactan core^[46,47], galactoarabinans^[48,49], linear (1 \rightarrow 2)-linked galactan and even branched (1 \rightarrow 3)-linked rhamnan^[50]. On the other hand, a greater number of Rha over GalA (GalA/Rha ~0.35) has been determined in RGs-I from *L. usitatissimum* and *A. thaliana* seed mucilages with no other linkage group interrupting the expected disaccharide backbone units^[35]. On the basis of the latter data, it can be posited for the existence, in some plant materials, of either unusual RG-I bearing side chains of few Rha units and possibly rhamnan or authentic galacturonorhamnans in which few (1 \rightarrow 4)-linked α -D-GalpA units appear to be inserted into (1 \rightarrow 2)-linked α -L-rhamnan chains. The degree of α -L-Rhap substitution with side chains may amount up to essentially 100%^[51,52]. The GalA units of the backbone are partially acetylated at O-2 and/or O-3 positions and the DA may vary widely from source to source. Also, unusual acetylation at O-3 position of Rhap units has recently been identified in the RG-I domains of okra pectin-like structures^[45]. In contrast, it is widely assumed that the GalA residues of RGs-I are not methyl-esterified, because RGs-I are not degraded under β -eliminative circumstances^[13,19], though methylesterification (up to ~100%) may occur in, for example, apple fruit, tobacco lamina, citrus peel, kidney bean cotyledon, sugar beet pulp, mung bean and flax hypocotyl pectins RG-I regions^[14,53,54]. Albeit long since thought to be homogenous with a \overline{M}_w of ~200 kDa and a backbone containing as many as 100 Rha-GalA repeats^[55], more recent studies have shown that RGs-I are highly heterogeneous in size, not only in the backbone but also in the side chains^[14,56], such an extent that different populations can exist even within homogenous tissues. Moreover, assuming a certain level of homogeneity with respect to source, the \overline{DP} s of the RG-I backbone and polymer (\overline{M}_w ~50–2000 kDa) may vary widely from ~15 to ~400 Rha-GalA repeats and

from 200 to 1000 glycosyl residues per macromolecule, respectively, depending on the origin^[14,53,57]. RGs-I are thus diversified that they are currently viewed as a family of structurally related, often ultra branched heteropolysaccharides present in plant PCWs, mucilages, and exudate gums^[14,43]. RGs-I, as intact as possible, are usually purified by digesting 'intact' CWMs and extracted pectins with highly-purified endopolygalacturonases (EndoPGs) in combination or not with highly-purified exopolygalacturonases (ExoPGs) and fractionating the soluble products by size-exclusion chromatography (SEC) preceded or not by ion-exchange chromatography (IEC) purification^[14,20,58]. They are generally eluted on SEC as the highest (average) molecular-weight (M_w) polysaccharide materials. RG-I polymers are one of the most complex polysaccharides and quantitatively the second structural domains of pectins after the HG domains. However, they are likely to be dominant in (soluble) soybean cotyledon pectins^[28] and overwhelmingly predominant (over HGs) in water-soluble mucilages from *A. thaliana* and *L. usitatissimum* (flax) seeds^[34,35,43]. *In muro*, RGs-I are, for example, believed to hold a central function as a scaffold to which other pectic polymers, such as HGs and RGs-II, are covalently attached as side chains to form the pectin network^[17,41], which governs the cell wall strength, elasticity, flexibility, and mechanical properties. Changes in tissue firmness and tissue softening in ripening fruits are commonly related to degradation (loss) of pectin RG-I galactan and arabinan side chains^[11]. *Out of muro*, certain purified RG-I materials enriched with side chains of (1 \rightarrow 4)- β -D-galactan and/or arabino(1 \rightarrow 3,6)- β -D-galactan proved to be effective immunostimulating, anti-complementary, and anti-cancer (anti-adhesive, anti-proliferative, anti-angiogenic, and anti-metastatic) agents^[42,59,60]. Though RGs-I *per se* are rather poor thickening and typically non-gelling agents, they are assumed to form junction zone-terminating structural elements essential to avoiding micelle formation with ensuing undesirable phenomena, viz. turbidity, syneresis, and precipitation, in HM-SAGs^[52].

Rhamnogalacturonans-II

Among substituted galacturonans (SGs) hitherto characterized, rhamnogalacturonans-II (RGs-II) are the most complex and probably the most ubiquitous. They

have indeed been purified from the cell walls of some lower plants (e.g., ferns, horsetails, and lycopods), almost all higher plants examined so far, wine, and even a crude enzyme preparation from *Aspergillus niger* marketed as Pectinol AC^[14,61,62]. RGs-II are composed of as many as 12 different monosaccharides and ~28–36 individual glycosyl residues, interconnected by more than 20 different glycosidic linkages. This gives rise to highly complex, branched macromolecules having a (1→4)-linked α -D-galacturonan backbone (of \overline{DP} 7–15), partially methylesterified at C-6 position and bearing four well-defined oligosaccharide chains. RGs-II are, therefore, structurally different from RGs-I. Seven out of the different glycosyl residues, namely Apif, 2-O-Me-Fucp, 2-O-Me-Xylp, aceric acid (AceA, 3-C-carboxy-5-deoxy-L-xylose), 2-keto-3-deoxy-D-manno-octulosonic acid (KdoA), 3-deoxy-D-lyxo-heptulosaric acid (DhA), and α -L-Galp are rarely found in natural substances, the first six of which are considered to be specific to RGs-II, thereby representing their diagnostic glycosyl residues. RGs-II are usually purified from 'intact' CWMs and extracted pectins by EndoPGs (+Exo-PGs)/IEC-SEC procedures^[14,61,63]. Less expensive liquefying crude enzyme preparations with a wide range of pectinolytic and hemicellulasic activities, such as Driselases, Pectinases, and Rapidases have alternatively been utilized to solubilize RGs-II from plant tissues^[61,64,65] owing to a high level of resistance to all known polysaccharide-degrading enzyme preparations except for the glycanases-rich cell-free extract of *Penicillium daleae*^[66]. On appropriate SEC columns, RGs-II elute as intermediate M_w materials between RGs-I and oligogalacturonides (from HG domains degraded by EndoPGs (+ExoPGs)). Solubilized RGs-II can be in the form of borate-ester-linked dimers (dRGs-II) and/or (borate-ester-free) monomers (mRGs-II). In case both forms are present in enzymatic digests, the SEC chromatogram shows, in M_w decreasing order, separate peaks of RG-I, dRG-II, mRG-II, and oligoGalA materials^[14,67]. Both mRG-II and dRG-II have the same (or very similar) glycosyl residue and linkage compositions, but are differentiated by the boron content and the M_w values, which are of ~5.0 and 10.0 kDa, respectively. A widely held view is that RGs-II are predominantly present in the cell wall as dimers^[13,55,62,68], which is supported by later work^[14,69,70]. Also, it is a

common belief that RGs-II are structurally conserved throughout the plant kingdom to such an extent they are viewed as a unique macromolecule. It is worth underlining, however, that some intravariations in the backbone length and DM of wine RGs-II (\overline{DP} 8–15) and intervariations in whole RG-II macromolecules have been reported^[67,71] which could result in significant differences in functional properties, more particularly in bioactivity^[68]. RGs-II can account for between 0.5% and 8% (w/w) of the pectin-rich PCW of dicot and non-graminaceous monocot species and less than 0.1% (w/w) of the pectin-poor PCW of commelinoid-related monocot species^[14,62,72], and hence are quantitatively minor cell wall polysaccharides. They are thought to account for 10–11% (w/w) of (cell wall) pectins^[73]. However, the RG-II content of pectins from CWMs of different monocot and dicot species is likely to be less than 5%^[14]. The RG-II fraction represents *ca.* 1.5% (w/w) of Pectinol AC and 20% (w/w) of alcohol-precipitated polysaccharides from red wine^[64,71], thereby being a major polysaccharide component of this fermented beverage. Although usually present in minor amounts in the PCWs of plants, the ubiquity of RGs-II allows to think that they should play special role(s) *in muro*. Immunocytochemical studies have also shown that RGs-II are enriched near the plasma membrane, but almost absent from the middle lamellae. It has, however, been suggested that RGs-II could be attached to a scaffold RG-I structure in the pectin matrix of the PCW^[17,41]. It is believed that RG-II dimer formation is required for the formation of a three-dimensional pectin network *in muro* contributing to the mechanical properties of the PCW^[62]. They are also required for normal growth and development of plants in the sense that changes in the wall properties and pore size that results from decreased borate cross-linking of pectin RG-II chains, may lead to many of the symptoms associated with boron deficiency in plants^[62,74]. Also, pollen tube growth and elongation in *A. thaliana* has been found to be conditioned by the synthesis of the RG-II diagnostic glycosyl residue KdoA^[75]. *Out of muro*, RGs-II are somewhat in quest of functionality, though they may also be effective exchanger of heavy metal cations such as Pb²⁺ and Cu²⁺, thereby appearing as potential reducing agents of the levels of toxic cations in soil and water^[68] and possibly in human organs con-

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taminated with such threatening chemical elements at the genesis of some cancer types. A RG-II-containing pectin-like structure (blupleuran) from *Bupleurum falcatum* has been found to exhibit efficacious anti-ulcer activities^[76], which might be at least partly due to the presence of RG-II. While RGs-II have been identified as the immunologically active components (of water soluble polysaccharides) in the leaves of *Panax ginseng*, RG-II containing extracts from other plant sources have exhibited no pharmacological activity, implying that further investigations are required before assigning a definite role to RGs-II for which some slight structural variations could explain the observed significant differences in terms of bioactivity^[68].

Xylogalacturonans

Xylogalacturonans (XGAs) are the second well-enough known type of SGs. The overall structure consists of an interior chain of (1→4)-linked α -D-galacturonan, partially substituted at O-3 position by single non-reducing β -D-Xylp residues and possibly with longer side chains (\overline{DP} 2–8) of 1→2/1→3/1→4/1→2,3/1→2,4/1→3,4-linked β -D-xylans^[14,50]. Substitution at O-2 position of α -D-GalpA, by β -D-Xylp is not common, but it has exclusively been suggested to occur in the XGA domain of zosterine (or zosteran), which is an AO-solubilized pectin from marine sea grass plants^[77] and together with substitution at O-3 position in XGA from cultured-carrot cells^[78]. The β -D-xylan side chains of XGAs are often substituted with α -L-Araf, α -L-Fucp and β -D-Galp units and the latter two, in turn, can be substituted at O-4 and O-4/O-6, respectively, by β -D-GlcpA units^[6], though pure XGA materials have been reported from a pectin-like structure from soybean CWM^[79]. The degree of substitution of the galacturonan backbone by β -D-Xylp units (i.e., Xyl/GalA molar ratio) and its DM may vary widely from ~20 to ~100% and from ~40 to ~90%, respectively, depending on the source^[14,55]. In contrast, it is not known with certainty if the XGA backbone is acetyl-esterified. Also, the \overline{DP} of the XGA backbone varies widely from 21 to possibly 120 GalA units. The isolation methods of XGAs include various mild chemical (alkaline or acid)^[29,80] and enzymatic^[15,24,79] conditions, which may at least partly explain the wide range of \overline{DP} above-specified, inasmuch as chemical procedures are

not totally selective for glycosidic linkages. On the other hand, the existence of XGAs in the cell walls of plants can be evidenced by treating pectins extracted from them with Endo-xylogalacturonan hydrolase (XGH), which releases XGA oligomers of different \overline{DP} s^[19,81]. XGAs are heavily susceptible to Exo-PGs but hardly to Endo-PGs, though an Endo-PG accommodating a xylosylated GalA residue of the backbone just following the hydrolysis site has recently been reported^[82]. XGAs are usually purified from the walls of reproductive plant tissues, and are therefore thought to have a specialized function associated with storage tissues of reproductive organs^[13,55]. A relation has also been suggested between expression of the LM8 epitope (against XGA) and the subsequent onset of regeneration in wheat callus culture^[83]. On the other hand, XGAs have been identified in the walls of non-reproductive organs (roots, stems, and leaves) of diverse plants^[50,81], thus suggesting a larger array of functionalities, notably contribution to plant (cell wall) resistance to phytopathogens. *Out of muro*, XGAs remain polysaccharides in quest of function. Polymers of XGA are believed to be present in the walls of a restricted number of plants^[13,55], but nowadays this view seems to be outdated, inasmuch as the list of (the walls of) plants containing them is ceaselessly extending. They have indeed been identified, for example, in the cell walls of pine pollen, kidney beans, red beans, soybeans, Japanese radish, onion bulbs, cotton seeds, potato tubers, chestnut bran, pea hulls, *A. thaliana* stem and (young and mature) leaves, mulberry leaves, root caps of wheat, pears, apples, citrus, grapes, watermelons, YPF fruits, and even in tree exudates such gum tragacanth from *A. gummifer*^[4,14,55,84]. This shows that XGAs are rather ubiquitous contrary to previous assumption.

THE CURRENTLY OVERLOOKED PECTIC POLYSACCHARIDES

Apiogalacturonans

Apiose-substituted galacturonans, commonly referred to as apiogalacturonans (A_pGAs), have originally been isolated and partially characterized from CWM of the aquatic monocot (duckweed) *Lemna minor*^[85], though this polysaccharide kind had long since

been suspected in zosteran, a pectin from the cell walls of the eel-grass *Zostera marina*^[86]. By treating CWM of *L. minor* with 0.5% aqueous AO, two A_p GA fragments have been reported^[85], one of which was composed exclusively of D-GalpA and D-Apif residues (25–28%) and the other contained additionally D-Xylp (three-fold lower than D-Apif) and D-Galp. By different structural studies including PAH, autohydrolysis, methylation analysis and Smith (periodate) degradation, it has been shown that A_p GAs possess a linear (1→4)-linked α -D-galacturonan backbone, partly substituted at O-3 position with single non-reducing β -D-Apif residues (Figure 1a). The galacturonan backbone of A_p GAs is partially methylesterified with a generally low DM (15–30%). By chemical treatments of CWM of *L. minor* under conditions (0.5% aqueous AO, pH 6.2, 22 °C or 70 °C, 3 h) similar to those originally used^[85], followed by IEC fractionations, five homogenous A_p GA fractions (regarding the charge density), with an Api content ranging from 7.9 to 38.1% have been reported^[87]. Further structural studies of the most representative fraction by PAH (pH 4.5, 100 °C, 3 h), pectinase-degradation, proton magnetic resonance and molecular optical rotation have confirmed the (1→4)-linked α -D-galacturonan backbone structure, but indicated substitution at O-2/O-3 positions by ‘apiobioside’ (β -D-Apif-(1→3’)- β -D-Apif-(1→) side chains rather than by single non-reducing β -D-Apif residues^[88]. In lieu of apiobiose side chains, the side chains of the A_p GA domains of lemnan, an AO-extracted pectin from CWM of *L. minor*, were found, by PAH, pectinase-digestion, and NMR studies, to be formed of single and (1→5)-linked D-Apif residues attached to O-2 and O-3-positions of D-GalA residues of the galacturonan backbone^[89]. In contrast to the previous reports, the treatment of CWM of *L. minor* with 0.7–1% aqueous AO (70 °C, 3–5 h) did not directly afford A_p GA fractions, but rather lemnan encompassing at least HG and RG-I domains in addition to the A_p GA domain as revealed by the presence, in the lemnan, of glycosyl residues other than GalpA and Apif, namely Galp, Araf, Xylp and trace amounts of Glcp after complete hydrolysis with 0.5–1 M H₂SO₄, at 95 °C for 16 h^[89]. Similar results have been obtained by extraction of CWMs of three species of the Zosteraceae family (*Z. marina*, *Z. pacifica*, and *Phyllospadix*) with 1% aqueous AO (70 °C, 3–5 h)

whereby A_p GA (with a DM20–22%) accounting for ~25% of zosteran have been reported^[77]. These reports, taken together, suggest that A_p GAs are probably present in the cell walls of these aquatic monocotyledonous species, not only as free polysaccharide elements, but also as multi-block copolymers of complex pectins, i.e., larger pectin macromolecules including additionally at least (unsubstituted) HG and branched RG-I domains. Long homogenous A_p GA polymers have been released from lemnan and zosteran by treating them with highly-purified Endo-PGs or partially-purified commercial pectinase preparations, suggesting that native A_p GAs could have a considerably high apiosylation level that protects them from degradation by pectinases. A_p GAs with a high Api content are indeed pectinase-resistant, whereas A_p GAs with a low Api content and extensively de-branched A_p GAs are heavily susceptible to pectinases^[77,87]. That the apiosyl units are evenly, randomly, or cluster-like distributed over the galacturonan core of (intact) A_p GAs is not known with certainty, though the remarkable extent of resistance to Endo-PGs more favorably suggests a random-like distribution. A_p GAs can completely be debranched off their apiosyl side chains by PAH (0.1 M HCl, 100 °C, 30 min or 0.5 M H₂SO₄, 95 °C, 5 h), and almost totally by autohydrolysis (Amberlite IR-120 (H⁺) resin-treated water solution refluxed for 4 h), thus furnishing the galacturonan backbone without apparent degradation^[77,87]. This might be greatly facilitated by the high acid-lability of the glycosidic linkages involving D-Apif residues. Digestion of zosteran (M_w ~ 40–90 kDa) with commercial (*A. niger*) pectinases (37 °C, 48 h) afforded ‘pure’ A_p GA (\overline{M}_w ~ 22 kDa), as ethanol-precipitate, which structural studies (PAH, periodate oxidation and methylation analysis) showed that GalA residues of the A_p GA backbone were substituted, at O-2 and/or O-3 positions by Apif residues with an Api/GalA molar ratio of ~5:4^[77]. The A_p GA domain of zosteran would then be made up of ~65 α -D-GalA units in the backbone, partially substituted with ~80 β -D-Apif residues in single and (1→5)-linked D-Apif residues attached to the O-2 and O-3-positions of D-GalA residues of the backbone. This value of \overline{DP} of the A_p GA backbone is comparable to that of the unsubstituted HG domains of pectins from CWMs of miscellaneous commelinoid-related monocot species^[4]. In contrast to

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zosteran and lemnan, A_p GAs are totally soluble in water with a great salt concentration (up to ~ 2 M NaCl), which would be promoted by the high Api content^[87]. This property is traditionally related to the ability that these aquatic monocots have to grow and proliferate in sea water. To date, no CWMs from 'land' plants have been reported to contain A_p GAs, suggesting that they may be restricted to these aquatic monocotyledonous plants. Zosteran (1–2% aqueous solutions) and A_p GA (2% aqueous solution) from it form dense-and-stable gels and considerably less stable gels, respectively, in the presence of acid (pH 2.0–3.0), at 20 °C, and after 24–40 h of storage^[90]. Addition of sucrose (up to a 25% concentration of it in the solution) has shortened the gelling time^[90], but apparently not the strength of the gels formed, indicating that A_p GAs are rather poor gel-forming agents under low sugar-acid gel conditions. They might, however, prove to be effective gelling agents in the presence of Ca^{2+} , considering the remarkably low DM. On the other hand, they could be potent thickening or stabilizing agents.

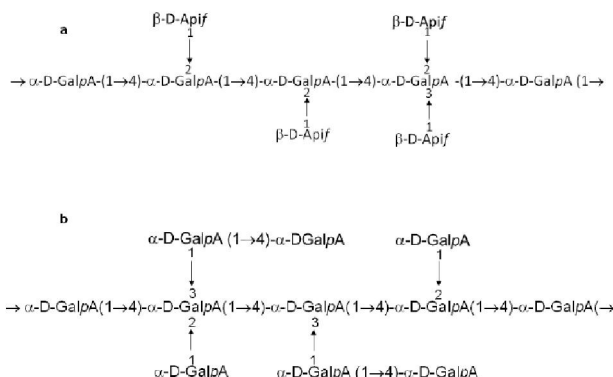


Figure 1 : Schematic representations of the structural features of (a) apiogalacturonans (A_p GAs) and (b) galacturonogalacturonans (GaGAs).

Galacturonogalacturonans

Galacturonogalacturonans (GaGAs) or GalA-substituted galacturonans have been reported as block copolymer of complex pectin only recently^[26,91,92], though a precursory moiety had been long since suggested in alkali-solubilized pectin from CWM of mountain pine pollen^[80]. GaGAs have indeed been shown to be building blocks of comaruman, an AO-extracted pectin from CWM of the marsh cinquefoil *Comarum palustre*^[26,91]. The GaGA domains of comaruman have been released by PAH and Endo-PG digestion of the pectin, indicat-

ing that GaGAs are recalcitrant to Endo-PGs. Structural studies with the help of AFM and methylation analysis have revealed that GaGAs (Figure 1b) are (1→4)-linked α -D-galacturonans bearing at O-2 and/or O-3 positions of some of the α -D-GalpA residues of the main chain, single non reducing α -D-GalpA residues and/or relatively long side chains of (1→4)-linked and 1→3/1→3,4-linked α -D-GalpA residues^[26,91]. Pectins which have been extracted by various chemical agents (CDTA, Na_2CO_3 , HCl) from CWMs of tomato fruit and sugar beet roots have also been shown, by AFM studies, to contain GaGA blocks with long polymeric side chains^[93,94]. These GaGA blocks have lately been isolated by controlled-acid hydrolysis (CAH; 0.1 M HCl, 80 °C) of pectins as 72-h acid-resistant branched polymers^[95] via undetermined branching site(s) on the GaGA backbone. Furthermore, Endo-PG digestion of pectins extracted from leek CWM afforded only 85% of the GalA residues contained in 72 h-CAH-resistant HG material from the same pectic acid, suggesting that Endo-PG-resistant galacturonan fraction made up of 15% of the GalA residues might correspond to extensively substituted GaGAs with side chains of single and/or relatively short GalA residues, considering that the 72 h-CAH-resistant HG material was homogenous in size, eluting as a single, symmetrical, and narrow peak polymer^[14]. This is rather different from the report on GaGA domains with long PGA side chains in Na_2CO_3 -extracted tomato pectin^[93,95]. GaGAs are virtually not degraded by CAH (0.1 M HCl, 80 °C) after 24 h and commence to be broken down after 72 h from their branches under conditions which cleave off linkages involving exclusively GalA residues^[95]. Therefore, it may be that (lower amounts of) GaGAs with long PGA side chains than (linear) HGs, possibly present in the leek pectic acid, have been split off from the branching points, thereby providing only homogenous HG samples after 72-h in our studies^[14]. These data suggest that the length of the backbone of GaGAs can be as long as that of linear HGs. In contrast, the side chains of GaGAs may vary from single GalA residues to polymeric linear and/or sparsely branched PGAs. Since GaGAs are hardly susceptible to Endo-PGs, contrary to HGs, they can be purified from tomato pectins by treating the 72-h CAH-resistant products with Endo-PGs and probably from other complex pectins containing them by

digestion with the enzymatic combination [rhamnogalacturonan hydrolase (RG-ase), endo-(1→5)- α -L-arabinanase (Endo-A), endo-(1→4)- β -D-galactanase (Endo-G), α -L-arabinofuranosidase (α -Ara-ase) and β -D-galactosidase (β -Gal-ase)] shown to be effective on the RG-I regions of hot water-extracted soybean pectin, commercial (hot acid-extracted) citrus pectin, and cold alkali-extracted YPF pectins followed by Endo-PGs^[4,14,30]. Until now, a limited number of plant cell walls have been reported to contain GaGAs. This does not, however, preclude the possibility that GaGAs could be widespread in plants, owing to the phylogenical distance existing among sources that have been found to contain them. Comaruman as well as its galacturonan (GaGA and HG) backbone fragments possess anti-inflammatory properties and are capable of inhibiting adhesion of human neutrophils to fibronectin, and diminishing PMA-initiated generation of radical oxygen species (ROS) in neutrophils^[96].

Galactogalacturonans

Galactogalacturonans (GGAs) are galactose-substituted galacturonans having an interior chain of (1→4)-linked α -D-galacturonan, branched with side chains of single β -D-Galp residues and/or 1→3/1→4/1→6/1→3,6-linked β -D-Galp residues (Figure 2a). The galacturonan backbone of GGAs can be partially methylesterified at C-6 of GalA residues with sometimes a very high DM^[24]. GGAs appeared to be block copolymers of complex pectin-like structures in the exudates (gum Karaya) from *Sterculia* species such as *S. caudata*, *S. setigera*, and *S. urens*^[28], zosterine^[6], acidic polysaccharide from soya sauce^[29], pectin-like fraction from lupin cotyledons^[97], an AO-extracted pectin (named panaxan) and pectin-like structure, both from *Panax ginseng* C. A. Meyer^[6,98]. On the other hand, a separate population of GGAs, accounting for ~5% of apple pectic substances, has been observed in pectin-lyase/pectate-lyase-resistant 'hairy regions' (HRs) of apple pectins and are also hardly degradable by Endo-G^[24]. A similar polymer may be present in the cell walls of potato, onion, and radish tissues^[24]. The structural characteristics of side chains and their branching sites on the interior chain (backbone) vary from source to source. For example, the structure of GGA from red ginseng acidic polysaccharides (RGAP)

was shown to be a linear chain of (1→4)-linked α -D-galacturonan carrying, at the reducing end, a single unit of reducing Galp via its O-6 position^[98], whereas in GGA from zosterine^[6], and complex acidic polysaccharide from soya sauce^[29], side chains of 1→3,6-linked and linear (1→4)-linked β -D-galactan, respectively, were connected, via O-3 position of GalA residues, to the galacturonan core of GGA blocks. In GGA block of the complex pectin-like structure of exudate (Karaya) gums from *Sterculia* species, the side chains occurred at O-2 position of GalA residues of the interior chain as single non-reducing Galp units^[28]. It is worth underlining, however, that GalA-containing galactans from Jeol (*Odina wodier*, Roxb) and cholla (*Opuntia fulgida*) gums, in which single non-reducing GalpA and Galp units appeared to be attached, via O-3 and/or O-4 positions, to an interior chain of (1→6)- β -D-galactan should not be confused with GGAs from some gums and mucilages^[28]. The galactan side chains of GGAs are often terminated by single (non-reducing) α -L-Araf residues and/or 1→3/1→5-linked- α -L-arabinans, thereby giving rise to arabinose-containing GGAs or arabino-galacto-galacturonans. In earlier work, D-Gal- and L-Ara-containing complex macromolecules comprising a framework of (1→4)-linked α -D-galacturonan, which carries at O-2 and/or O-3 positions of GalA residues numerous branches of D-Galp and L-Araf residues have indeed been characterized from mucilages of *Plantago psyllium* seeds and from complex pectin from CWM of the bark of *Amabilis fir*^[99,100]. The isolation methods of GGAs include both chemical (mild-alkali hydrolysis and PAH) and enzymatic (pectin-lyase/pectate-lyase, commercial pectinase, and highly-purified Endo-PG) procedures^[6,24,29,97,98]. The fact that digestion of these extracted pectins with pectinases affords 'intact' GGA blocks strongly suggests that they are resistant to pectinases, an ability which may be conferred by a high level of galactosylation. Though the exact *in mero* location of GGAs to the different cell wall compartments is not known, it is possible that this pectic polysaccharide type is part of pectins present in the middle lamella^[24], and probably attached to HG, inasmuch as they are released by treating (extracted) pectins with the above-mentioned HG-degrading enzymes. They may then contribute to cells adhesion by adjoining different cell

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walls abut. GGAs have been so far identified as part of pectins, exudate gums and mucilages from few plants. Nevertheless, the fact that they occur in very distinct plant materials allows to forward that they may be widespread in plants. *Out of muro*, GGA could be potent immunostimulating, complement-fixing and antitumor agents. Certain GGA core chains have, indeed, been found to enhance immunomodulating tumoricidal activities of natural killer cells and nitric oxide (NO) production of macrophage^[98], thereby appearing to be potential health benefit macromolecules.

Arabinogalacturonans

Arabinogalacturonans (A_rGAs) are arabinose-substituted galacturonans which have a (1→4)-linked α-D-galacturonan core branched, via O-3 position and/or terminal reducing point, by single (nonreducing) or multiple side-stubs of α-L-Araf residues (Figure 2b). This eighth pectic polysaccharide type has been characterized from complex pectins from miscellaneous plant materials such as apples^[25], field-bean (*Dolichos lablab*) hulls^[46], *Plantago psyllium* seeds^[99], *Amabilis* fir bark^[100], *Plantago major* leaves^[101], alfalfa (lucerne) leaves and stems^[6,102], and carnation (*Dianthus caryophyllus*) roots^[103]. The presence of A_rGAs in plant cell walls has been evidenced by immunolocalisation with antibodies to A_rGAs, generated by polyclonal antisera produced by addition of a complete adjuvant^[104]. A_rGAs are probably distinct block copolymers of the HRs of pectins^[101,102] and therefore they could participate in the reinforcement of the pectin network of the PCW. Though the number of plant cell walls shown to

contain A_rGAs seems to be restricted, the possibility that they are widespread enough cannot be overlooked, owing to their presence in distinct plant sources. Purified A_rGAs from the HRs of pectins from *P. major* exhibited efficient anti-complementary activities, and therefore could be a potential health benefit product.

CONCLUDING REMARKS

Pectins are a class of extremely diversified polysaccharides present in the cell walls of most plants, some tree exudates and plant material mucilages. In general, cell wall pectins are believed to be, at least partly, in the form of multi-block copolymers of homogalacturonan, rhamnogalacturonan-I, rhamnogalacturonan-II and to a lesser extent, xylogalacturonan. However, apiogalacturonan, galacturonogalacturonan, galacto-galacturonan, and arabinogalacturonan, which are currently overlooked and hardly mentioned in the literature, also occur as multi-block copolymers of complex pectins from various plants. This suggests that pectins may be structurally much more composite *in muro* than imagined so far, encompassing at least eight different building blocks. This exceptional structural diversity of pectins is likely to be related to their functional versatility *in-and-out-of muro*. There may therefore still a long way to go to completely exhaust the rich pectin repertoire and comprehensively elucidate the fine structure(s) of pectins present within the cell wall, a consequence of which could be a better understanding of their large array of functionalities and possibly the finding of new application domains.

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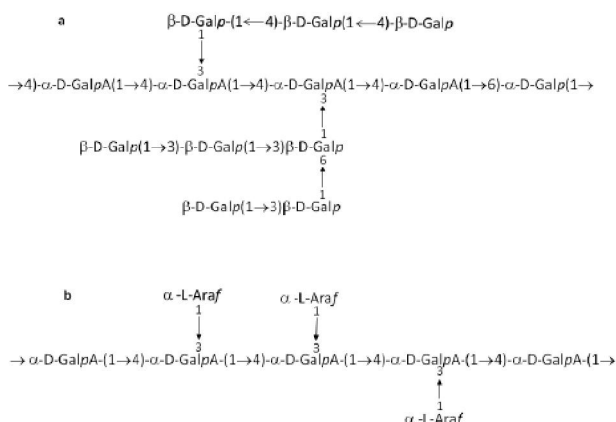


Figure 2 : Schematic representations of the structural features of (a) galactogalacturonans (GGAs) and (b) arabinogalacturonans (A_rGAs).

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