Pectic Polysaccharides and Their Functional Properties

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Beda M. Yapo and Dago Gnakri

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Abstract

Since their first discovery in 1790–1825, pectins are still fascinating plant and food scientists who continue to carry out numerous structural as well as functional studies on them. This great interest of scientists for pectins is accounted for by their large spectrum of (bio)functionalities, starting from their natural location in plant cell walls as bioactive components for cell growth, defense, and protection via diverse manufactured food and nonfood products as techno-functional (gelling, emulsifying, film-forming, etc.) agents to terminate in human welfare as health-benefit (prebiotic, anticomplementary, antioxidant, anticancer, etc.) agents. The extraordinary functional versatility of pectins is thought to be intimately related to fine structure. Unfortunately, structurally, pectins are extremely diversified that establishment of structure-function relationship appeared so far a difficult task to go through. On the other hand, the extended structural variability of pectins presages for the finding of new functions hitherto unknown. Nevertheless, for some structurally well-known pectic cobiopolymers such as homogalacturonan, solid evidence for

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structure-related functions, especially gelling properties, has been provided, including new insights very recently.

After a brief introduction on the "pectin structural repertoire," the main sources of industrial pectins will be exposed, followed by a succinct structural description of the different pectic block cobiopolymers, commonly referred to as "pectic polysaccharides." Finally, some remarkable structure-related functions, namely, gelling, emulsifying/emulsion-stabilizing, and antitumor properties of pectins will be revisited in the light of the latest work.

Keywords
Pectins • Sources • Production • Structures • Properties

1 Introduction

Pectic substances (or merely pectins) are a group of extremely diversified polysaccharides from plant cell walls, mucilages, and exudates. No less than 17 different glycosyl residues (Vincken et al. 2003), such as α-D-galactopyranosyluronic acid (α-D-Gal*p*A), α-L-rhamnopyranose (α-L-Rha*p*), β-D-galactopyranose (β-D-Gal*p*), α-L-arabinofuranose (α-L-Ara*f*), β-D-xylopyranose (β-D-Xyl*p*), and β-D-apiofuranose (β-D-Api*f*), to name a few, are believed to be sugar constituents of pectins. Several structural studies using combinatory approaches including enzymatic and chemical fingerprints have revealed, in heterogeneously complex pectic macromolecules, that these glycosyl residues are not randomly distributed, but rather concentrated in different regions, giving rise to various block cobiopolymers. To date, the pectin group may encompass at least eight distinct block cobiopolymers (often referred to as "pectic polysaccharides"), namely, unbranched (or linear) homogalacturonan (HG), rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II), xylogalacturonan (XGA), apiogalacturonan (A_pGA), galacturonogalacturonan (GaGA), galactogalacturonan (GGA), and arabinogalacturonan (A_rGA) (Schols and Voragen 1996; Caffall and Mohnen 2009; Ridley et al. 2001; Yapo 2011a). The latter six are sometimes gathered under the generic terms of "substituted galacturonans." This striking structural diversity renders pectins functionally versatile with multiple biological (plant cell growth, defense, protection against harmful hosts, etc.), techno-functional (gelling, emulsifying/emulsion-stabilizing, thickening, film-forming, etc.), and health-benefit (immunological, prebiotic, anticomplementary, antioxidant, antitumor, etc.) properties. Pectin functionality is, indeed, intimately related to fine structure (Sørensen et al. 2009; Sila et al. 2009).

2 Marketable Pectins: Sources and Production

Most commercial (or industrial) pectins, mainly intended for the preparation of gelling food products, are produced from citrus (lemon, lime, orange, and grapefruit) peel and apple pomace (May 1990; Voragen et al. 1995; Sriamornsak 2003; Srivastava and Malviya 2011), two raw materials from the western juice industry,

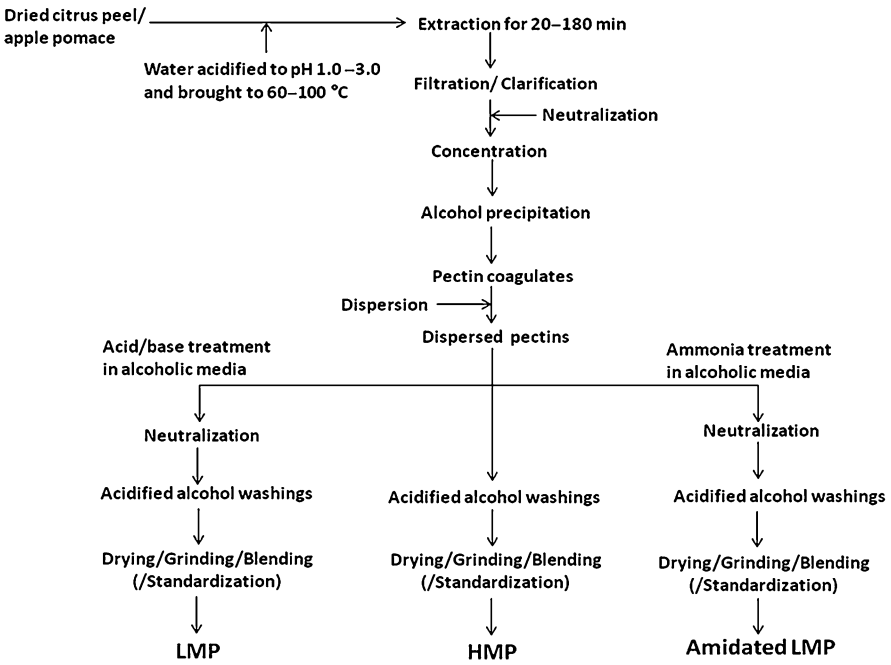

Fig. 1 Simplified scheme for the production of marketable HMP, LMP, and amidated LMP (LMP = low methoxy pectins; HMP = high methoxy pectins)

available in large quantities. The two industrial by-products are pectin-rich sources, with a pectin content of 15–30 % (for citrus peel) and 10–15 % (for apple pomace) on a dry weight basis. Moreover, acid-extracted citrus and apple pectins are usually high methoxy pectins (HMP) with high viscosity-average molecular weight ($\overline{Mv}$ ~100–300 kDa) and galacturonic acid content no less than 65 % on a dry weight basis as required by the international market. These features enable one to prepare good sugar-acid-mediated high methoxy pectin gels (HMP-SAG). In addition (amidated) low methoxy pectins (LMP) with good gelling properties can be produced from initially extracted citrus or apple HMP by partial de-esterification with acid (commonly)/base or with ammonia in alcoholic media (Fig. 1).

It is worth underlining that other commercially viable sources of pectins are sugar beet pulp (for producing pectin emulsifiers), sunflower head residues (for naturally (nonchemically tailored) LMP), and some tropical fruit by-products such as mango peel (for HMP), yellow passion fruit rind (for naturally HMP and LMP), and cashew apple pomace (for naturally LMP) (Schieber et al. 2001; Yapo and Koffi 2014). Passion fruit rind, for example, is used in some emerging countries such as Brazil for manufacturing marketable pectins.

Commercial pectins are usually extracted by acidified hot water under specified extraction conditions kept secret by producers but generally thought to be as follows: dry raw material to solvent ratio 1:35–1:15 (w/v), water acidified with

HNO_3 (or HCl) to pH 1.0–3.0, temperature 60–100 °C, and duration 20–180 min (Fig. 1). The pectin slurry is then filtered, concentrated, and precipitated with alcohol, usually isopropanol. The gelatinous pectin material is then extensively purified by acidified alcohol washings to remove various impurities including pigments and free saccharides of small sizes and salts, thereby converting pectin galacturonates into pectin galacturonic acid residues (the acid form of the pectin product). During this treatment, neutral sugar chains of solubilized pectin polymers are further hydrolyzed, which results in increase of the pectin galacturonic acid content. As pectins are extracted from natural plant materials in a practically unchanged form, they demonstrate different properties depending on the quality of the raw material (Herbstreith 2014a, b). Therefore, quality control and standardization of the pectins are very important criteria. The yield, sugar composition, esterification degree, and functional properties of pectins are influenced by extraction conditions, and therefore optimization is always a prerequisite for every new pectin source that is being investigated for marketing purposes.

To manufacture low-calorie gelling products, LMP which form Ca^{2+}-induced gels are needed. This compels producers to add a demethylesterification step to the production process yielding HMP. Usually, commercial LMP are produced by acid demethylation from primarily extracted citrus or apple HMP. In this case, initially methylesterified pectin GalA residues are randomly demethylated, which gives LMP products with randomly distributed unesterified GalA residues in the pectin chains. Ammonia treatment can also be applied, in which case methyl groups are partially replaced by amide groups, thereby yielding amidated LMP with randomly distributed unesterified GalA residues in the pectin chains. As required by the market, the pectin degree of amidation (DAm) should not exceed 25 %.

"Nonchemical" demethylation may also be performed by using demethylating enzymes, viz., fungal (f-PME) or plant (p-PME) pectin methylesterases. This produces LMP with non-blockwise (by f-PME) or blockwise (by p-PME) distribution of unesterified GalA residues within the pectin chains. More than acid demethylation, enzymatic demethylation is kinetically precisely controlled to produce LMP with the exactly desired degree of methylesterification (or degree of methoxylation, DM) without significant modification of the pectin molecular weight. However, this requires the use of highly purified PME preparations and will obviously add to the cost of production. For all these reasons, no commercial LMP have so far been enzymatically tailored.

Various extraction methods, which are relatively new, such as microwave-assisted extraction (MAE) and ultrasound-assisted (UAE) extraction, have been compared with the conventional acid extraction on a laboratory scale. Most studies showed that MAE or UAE gives higher amount of pectins with often higher viscosity-average molecular weight for considerably shorter extraction duration (Fishman et al. 2003; Wang et al. 2007; Bagherian et al. 2011). In addition, an environmentally friendly aspect of MAE and UAE methods using water solvent instead of dilute acid has also been put forward by some workers (Maran et al. 2013) with respect to generated effluents. On an industrial scale, however, the practicability of these "new methods" remains to be proven.

3 Structural Characteristics of the Different Pectic Cobiopolymers

3.1 Homogalacturonan

Homogalacturonan (HG) is generally the most abundant pectic polysaccharide, accounting for more than 50 % of complex pectins from various sources and up to 90 % of citrus pectins (Ridley et al. 2001; Voragen et al. 2009; Yapo 2011b). HG is an unbranched polymer of 1,4-linked α-D-Gal*p*A residues (Fig. 2), partially methylesterified at *C*-6 position and sometimes acetylesterified at *O*-2 and/or *O*-3 positions. The degree of methylesterification (DM) is the number of α-D-Gal*p*A residues, out of 100, esterified with methyl alcohol groups, and the degree of acetylesterification (DAc) corresponds to the number of α-D-Gal*p*A residues, out of 100, esterified with acetyl groups, assuming one acetylation per α-D-Gal*p*A residue. Depending on the value for DM of pectin HG, low methoxy pectins (LMP, DM <50 %) can be distinguished from high methoxy pectins (HMP, DM ≥50 %). Native HG may be (almost) fully methylesterified and only later partially de-esterified to about 70–80 % by plant PME. HG is renowned for its ability to form gels, a property widely utilized in the food industry and in all likelihood a property that determines some of the functions of (HG-containing complex) pectins in primary cell walls (Albersheim et al. 1996). Both DM and DAc of pectin HG influence its functional properties. The average degree of polymerization ($\overline{DP}$) of individual native HG is not known with certainty, but may be approximately of 50 in cell walls of commelinoid-related monocot species and a twofold higher in cell walls of dicotyledonous species at maturation stage (Voragen et al. 2009; Yapo 2009). The amount and $\overline{DP}$ of pectin HG also affect the pectin functional (especially gelling) properties (Yapo 2009).

3.2 Rhamnogalacturonan-I

Rhamnogalacturonan-I (RG-I) is generally quantitatively the second pectic cobiopolymer, which may represent 10–25 % of complex pectins and up to 45 % of sugar beet pulp, potato tuber, and tobacco leaf pectins (Ridley et al. 2001; Voragen et al. 2009; Yapo 2011a). RG-I is a structurally complex polysaccharide with a [→2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A-(1→]$_{n\geq 1}$ backbone partly branched, at *O*-4 (mainly) and/or *O*-3 positions of α-L-Rha*p* residues, with neutral sugar side chains of various types and sizes, viz., (1 → 5)-α-L-arabinan, (1 → 4)-β-D-galactan, arabinogalactan-I, and galactoarabinan (Fig. 3), and probably also by arabinogalactans-II.

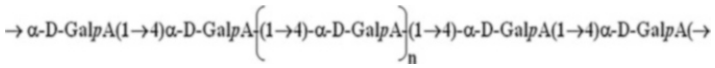

Fig. 2 Schematic representation of homogalacturonan

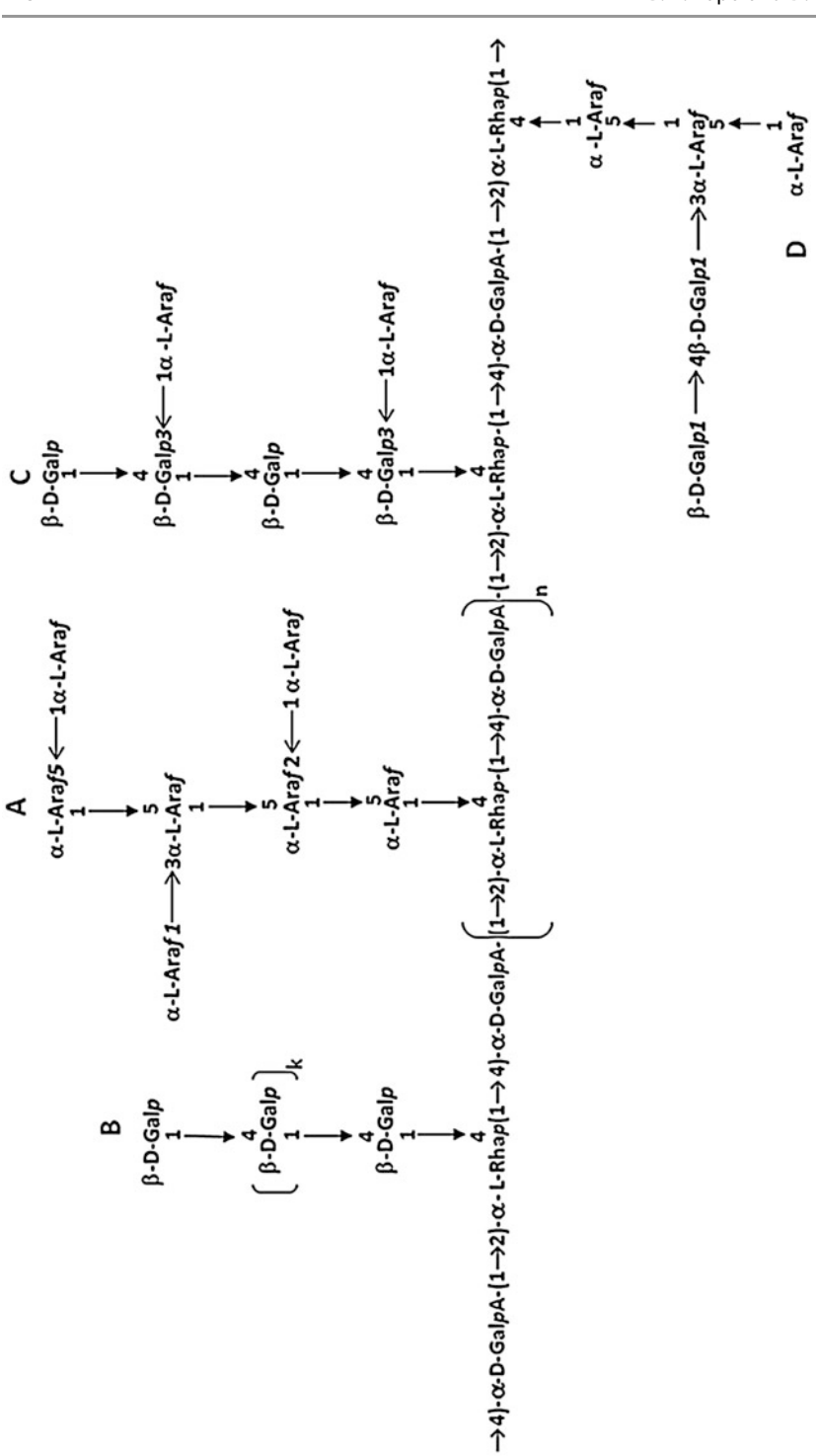

Fig. 3 Schematic representation of rhamnogalacturonan-I (With permission from Yapo (2011a). Copyright Taylor & Francis Group)

The $\overline{DP}$ of the backbone of individual native RG-I is not known with certainty and may be dependent on the polysaccharide origin; for example, ~30–40 diglycosyl repeats for citrus RG-I, 80 repeats for beet RG-I, and as many as 100–200 diglycosyl repeats for RG-I from suspension-cultured sycamore cells (Albersheim et al. 1996; Yapo 2011a). RG-I is structurally extremely varied, depending on plant source, organ, and even on location within the same cell tissue that it is nowadays viewed as a family of molecularly diversified polysaccharides with the same diglycosyl repeating backbone carrying side chains of different types and sizes.

3.3 Rhamnogalacturonan-II

Rhamnogalacturonan-II (RG-II) is undoubtedly the most ubiquitous of substituted galacturonans, being purified from primary cell walls of seedless plants such as ferns (e.g., *Ceratopteris thalictroides*), horsetails (e.g., *Equisetum hyemale*), and lycopods (e.g., *Lycopodium nummularifolium*) and all higher plants hitherto examined for. RG-II is, however, a quantitatively minor block cobiopolymer of complex pectins, accounting hardly for 0.1 % of the pectin-poor primary cell wall of commelinoid-related monocotyledonous species and for 0.5–8.0 % of the pectin-rich primary cell wall of non-commelinoid monocotyledonous and dicotyledonous species (O'Neill et al. 2004; Yapo 2011c). It is also remarkably structurally conserved throughout vascular plants and is believed to exist in the primary cell wall, predominantly as a borate diester cross-linked dimer (dRG-II) rather than as a monomer (mRG-II) (O'Neill et al. 2004). In vitro interconversion of dRG-II into mRG-II or vice versa is now well demonstrated. This pectic cobiopolymer, with an average molecular weight of 5.0–10.0 kDa, has an oligogalacturonan backbone ($\overline{DP}$ 11–15), which is branched with four well-defined side chains (A, B, C, and D) containing six unusual and specific (or diagnostic) sugars, namely, apiose (Api; 3-*C*-(hydroxymethyl)-*β*-D-erythrose), 2-*O*-methyl-fucose (2-*O*-Me-Fuc), 2-*O*-methyl-xylose (2-*O*-Me-Xyl), aceric acid (AceA; 3-*C*-carboxy-5-deoxy-L-xylose), 2-keto-3-deoxy-D-*manno*-octulosonic acid (KdoA), and 3-deoxy-D-*lyxo*-heptulosaric acid (DhaA) (Fig. 4). RG-II is, therefore, structurally different from RG-I in that rhamnose is not present in its backbone, but rather in three (A, B, and C) out of the four authenticated side chains and has only been named so for distinction sake.

3.4 Xylogalacturonan

Xylogalacturonan (XGA) is the second known substituted galacturonan. This pectic cobiopolymer has an (1 → 4)-linked α-D-galacturonan backbone, which is partially substituted at *O*-3 (mainly)/*O*-2 position by single nonreducing β-D-Xyl*p* residues and/or by relatively short 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 (Fig. 5). The degree of substitution by β-D-Xyl*p* residues of the galacturonan backbone can range from approximately 20 % to

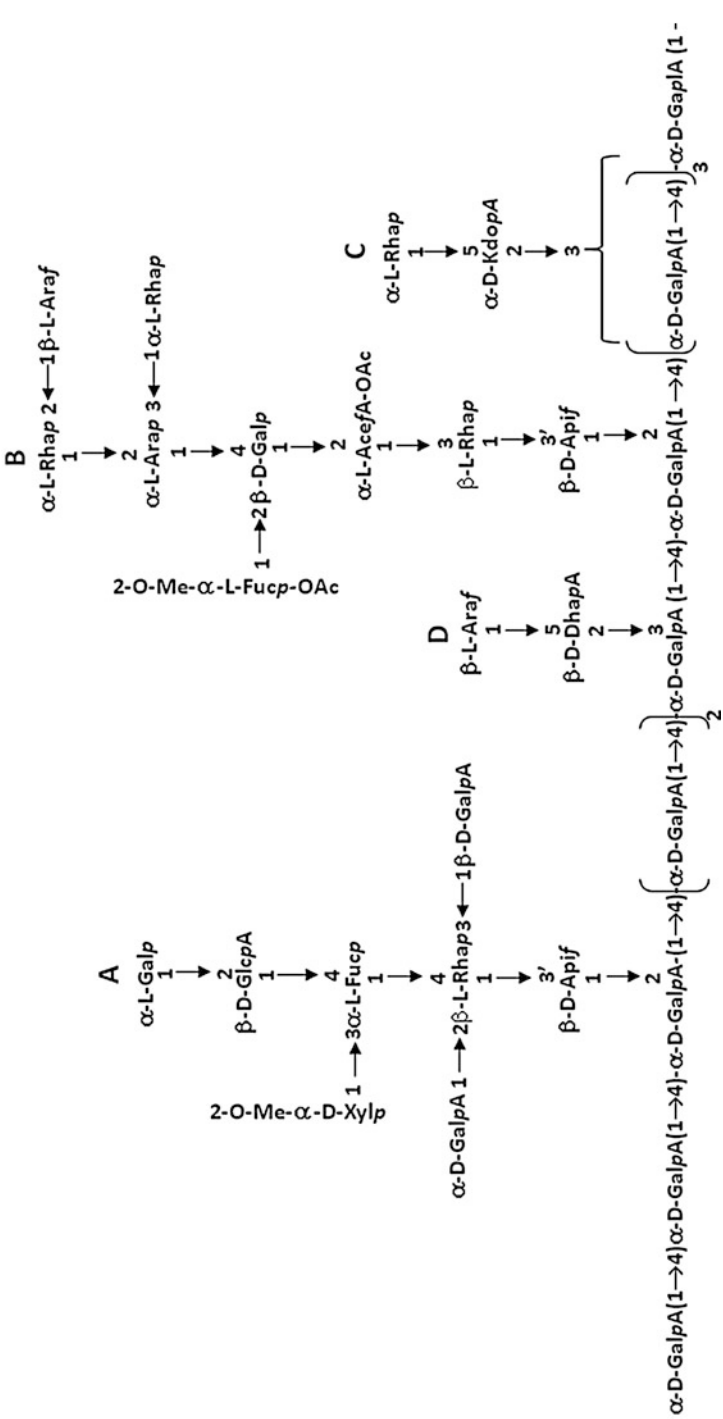

Fig. 4 Schematic representation of rhamnogalacturonan-II (With permission from Yapo (2011c). Copyright Hindawi Publishing Corporation)

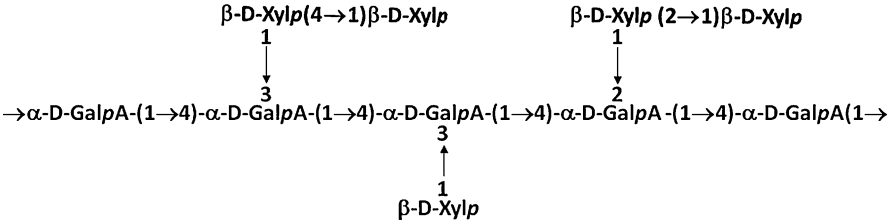

Fig. 5 Schematic representation of xylogalacturonan

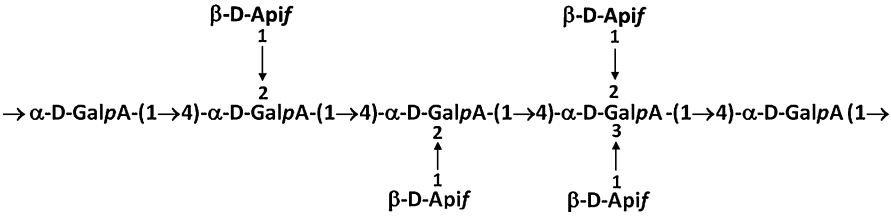

Fig. 6 Schematic representation of apiogalacturonan

100 %, depending on the polysaccharide origin. The $\overline{DP}$ of the galacturonan backbone of XGA is not known with certainty, but may be comparable to that of HG. Furthermore, the β-D-xylan side chains of XGA may be substituted with α-L-Ara*f*, α-L-Fuc*p*, and/or β-D-Gal*p* units. The DM of XGA is dependent on the polysaccharide source and may vary from about 40 to 90 %. In complex apple pectins, XGA is present in the vicinity of RG-I and is therefore an integral part of the so-called pectic hairy regions (Schols and Voragen 1996; Coenen et al. 2007).

3.5 Apiogalacturonan

Apiogalacturonan (A_pGA), an apiose-substituted galacturonan, is a relatively low molecular weight (20–25 kDa) pectic cobiopolymer from the primary cell wall of certain aquatic monocotyledonous species such as the duckweed *Lemna minor* (Lemnoideae) and eelgrasses of the Zosteraceae family (*Zostera marina*, *Z. pacifica*, and *Phyllospadix*). A_pGA has a (1 → 4)-linked α-D-galacturonan backbone, partly substituted at *O*-3 (mainly)/*O*-2 position by single nonreducing β-D-Api*f* (Fig. 6), (1 → 5)-linked β-D-Api*f* residues and/or by "apiobioside" (β-D-Api*f*-(1 → 3′)-β-D-Api*f*-(1→).

The degree of substitution of the galacturonan core may vary from about 5 to 90 %. Moreover, the galacturonan core of A_pGA is partially methylesterified to a usually low DM (15–30 %). The $\overline{DP}$ of the galacturonan backbone of A_pGA is not clearly known, but may be in the order of 65. A_pGA is likely an integral part of

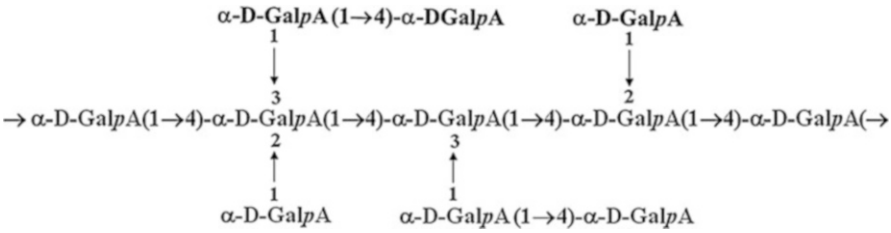

Fig. 7 Schematic representation of galacturonogalacturonan

complex pectins called lemnan and zosteran encompassing additionally blocks of HG and RG-I (Golovchenko et al. 2002). Unlike lemnan and zosteran, A_pGA is soluble in highly salted waters (containing up to 2 M NaCl). This property may be promoted by a high degree of apiosylation of the pectic polysaccharide and is likely related to the ability of these species (Lemnoideae and Zosteraceae) to grow and proliferate in aquatic and marine media. Contrary to zosteran, A_pGA is a rather poor-gelling agent for preparing sugar-acid-mediated gels. It is, however, not known if A_pGA may be a good gelling agent in calcium-mediated gels, considering its rather low DM.

3.6 Other Quantitatively Minor Substituted Galacturonans

Galacturonogalacturonan (GaGA), a galacturonic acid-substituted galacturonan, is a block cobiopolymer of complex pectins isolated from cell wall materials of plant species such as the marsh cinquefoil *Comarum palustre* and *Solanum licopersicum* (tomato) (Ovodova et al. 2006; Round et al. 2010). GaGA has a (1 → 4)-linked α-D-galacturonan backbone, partially substituted at *O*-2 and/or *O*-3 positions by single nonreducing α-D-Gal*p*A residues (Fig. 7) and/or by relatively long side chains of 1 → 3/1 → 4/1 → 3,4-linked α-D-Gal*p*A residues.

Galactogalacturonan (GGA), a galactose-substituted galacturonan, is a block cobiopolymer of various complex pectins such as gum karaya (from *Sterculia* species), zosteran (from *Z. marina*), panaxan (from *Panax ginseng* C. A. Meyer), and soya sauce acidic polysaccharide. GGA has a (1 → 4)-linked α-D-galacturonan backbone, partly substituted by single β-D-Gal*p* residues and/or 1 → 3/1 → 4/1 → 6/1 → 3,6-linked β-D-Gal*p* residues (Fig. 8). The galacturonan backbone of GGA can be highly methylesterified.

Arabinogalacturonan (A_rGA), an arabinose-substituted galacturonan, has 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-Ara*f* residues (Fig. 9). This cobiopolymer has been isolated from complex pectins extracted from miscellaneous plant materials such as apple fruits, field-bean hulls, *Plantago psyllium* seeds, *Plantago major* leaves, *Amabilis* fir bark, alfalfa leaves and stems, and carnation (*Dianthus caryophyllus*) roots.

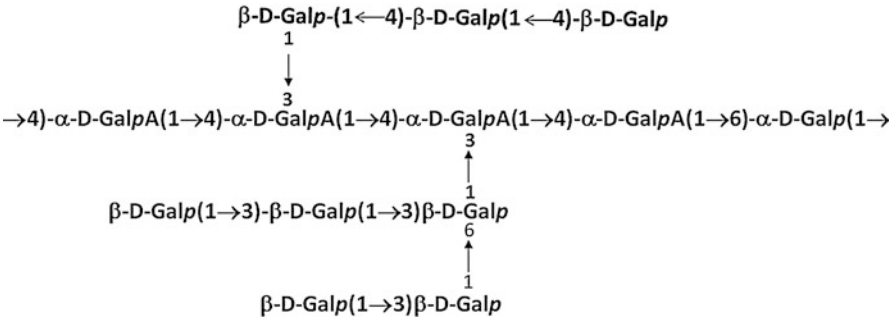

Fig. 8 Schematic representation of galactogalacturonan

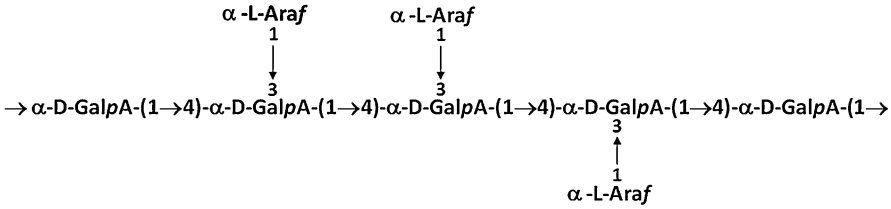

Fig. 9 Schematic representation of arabinogalacturonan

4 Structural Aspect of Complex Pectins

Native pectins in plant cell walls may generally be viewed as multi-polymer complexes which are formed by interconnections of various "pectic polysaccharide" elements, including block cobiopolymers of HG (predominantly), RG-I, and substituted galacturonans such as RG-II and to a lesser extent XGA (Albersheim et al. 1996; Schols and Voragen 1996; O'Neill and York 2003; Ovodov 2009; Voragen et al. 2009; Yapo 2011a). However, the type(s) of substituted galacturonans present in complex pectins may be dependent on the source. For example, in complex pectin fractions from cell wall materials of the marsh cinquefoil *Comarum palustre* and tomato, GaGA and/or RG-II appeared to be attached to HG and RG-I. In complex apple pectins, RG-II and XGA are associated to HG and RG-I. Zosteran and lemnan from *Z. marina* and *L. minor* (respectively) are composed of blocks of A_pGA, HG, and RG-I. In all cases, HG and RG-I elements appeared to be the most widespread in complex pectins, and therefore, most general schemes proposed for (complex) pectin structure included at least the two types of pectic polysaccharides, often referred to as pectin "smooth" (HG) and "hairy" (RG-I) regions. To date, three key hypothetical models exist in the literature. These are (i) the alternating "smooth" and "hairy" regions model (Schols and Voragen 1996; Fig. 10a), (ii) the "RG-I backbone" model (Vincken et al. 2003; Fig. 10b), and (iii) "the living thing-like" model (Yapo 2011b; Fig. 10c).

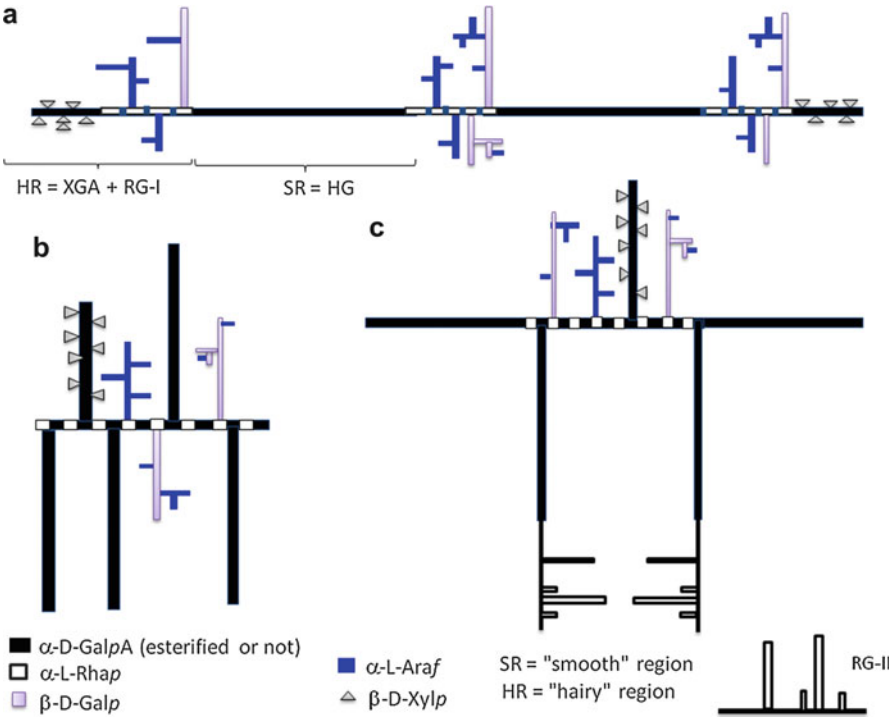

Fig. 10 The key hypothetical models proposed for pectins as multi-block cobiopolymer complexes of HG, RG-I, XGA, and/or RG-II (With permission from Yapo (2011b). Copyright www.Elsevier.com)

5 Functional Properties of Pectins

Pectins are undoubtedly the most functionally versatile biopolymers, with a large spectrum of biological (plant cell growth, defense, protection against harmful hosts, etc.), techno-functional (gelling, emulsifying/emulsion-stabilizing, thickening, film-forming, etc.), and health-benefit (biosorbent, immunomodulating, prebiotic, anticomplementary, antitumor, antioxidant, antimicrobial, etc.) properties. The extraordinary functional versatility of pectins may be related to their high structural variability. The impressive range of activities of pectins has remarkably been reviewed for years by several (groups of) workers (Voragen et al. 1995; Thakur et al. 1997; Sriamornsak 2003; Willats et al. 2006; Ovodov 2009; Sila et al. 2009; Yapo 2011a; Gullón et al. 2013; Espitia et al. 2014). However, new insights into the different mechanisms of action of pectin cobiopolymers are continuously provided for a better understanding of their structure-related properties. Will be dealt with here are the gelling, emulsifying/emulsion-stabilizing, and antitumor properties of pectin multi-block cobiopolymers.

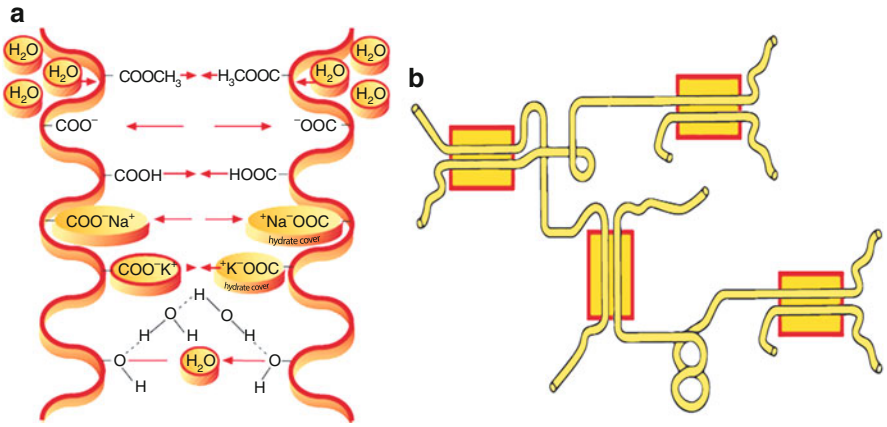

Fig. 11 Schematic representations: (**a**) Gelling mechanisms of high methylester pectins; (**b**) Bonding zones in the gel network. (With permission from Herbstreith and Fox Corporate Group. Copyright www.herbstreith-fox.de)

5.1 Gelling Properties of Pectins

As conveyed by the name per se, "pectin" (*in reference to the Greek word* "*pektikos*," *which means to* "*congeal*, *solidify or curdle*") is a naturally gelling polysaccharide. This oldest functionality of pectins is mainly exploited for manufacturing diverse gelling food products such as marmalades, jams, preserves (for HMP), and low-calorie jellies (for LMP). However, the mechanism of gelation of HMP is different from that of LMP.

HMP form sugar-acid-mediated gels (HMP-SAG). Gelation of HMP requires the presence of 55–65 % soluble sugar (generally sucrose) and acidic conditions (pH 2.0–3.5). Gelation occurs by formation of junction zones between the pectin HG chains, promoted by added cosolutes (sucrose). The soluble sugar added to the dispersed pectin system (intended for gelation), indeed, reduces the water activity and thus fosters pectin chain-to-chain interactions at the expense of pectin-solvent interactions. It may also function as a "coating agent" that aids in strengthening branch points, possibly through cooperative interactions and may influence self-association of pectin chains by "condensing" around them or binding to them (Fishman and Cooke 2009; O'Brien et al. 2009). Acid (low pH condition) prevents deprotonation of unesterified GalA residues of the pectin HG chains, thereby annealing (or at least minimizing) intermolecular electrostatic repulsions. The junction zones formed are then stabilized by interchain hydrogen bonds between protonated carboxyl groups and secondary alcohol groups of the pectin HG chains and by interchain hydrophobic interactions between pectin HG methylester groups (Fig. 11a). Gelation results in formation of vast tridimensional networks in which solvent and cosolutes are all immobilized (Fig. 11b). The gel formed is a thermo-irreversible cohesive system, resisting deformation and showing a stress/strain relationship for small deformation. Studies using atomic force microscopy (AFM)

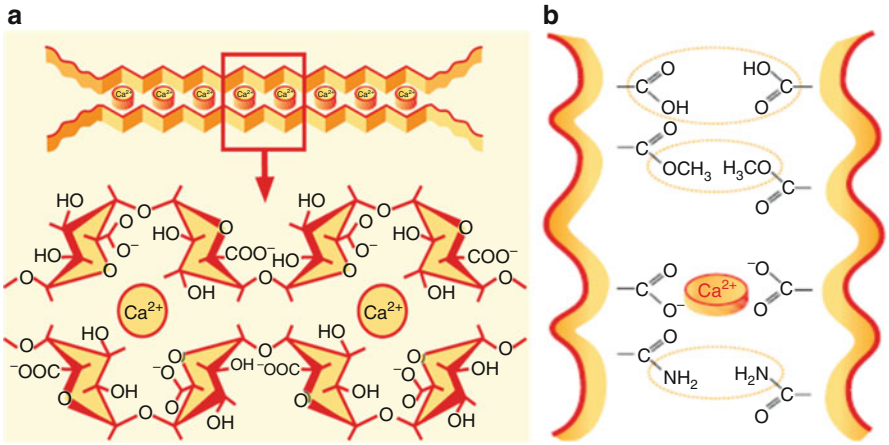

Fig. 12 Schematic representations: (**a**) Gelling mechanism after the "egg-box model" of (non-amidated) low methylester pectins; (**b**) amidated low methylester pectins. (With permission from Herbstreith and Fox KG. Copyright www.herbstreith-fox.de)

have, however, revealed that HMP-SAG comprise partially cross-linked networks, quite deformable in that they are held together by relatively easily disrupted secondary interactions such as hydrogen bonds and van der Waals forces, and this could actually be the basis of the unique ability of pectins to form spreadable gels (Fishman and Cooke 2009). Furthermore, the strength of the gels formed appeared to be correlated to the density of pectin strands and uniformity of pectin (chain) distribution within the gels (Fishman and Cooke 2009).

LMP solutions require, for gel formation, the presence of multivalent cations, especially Ca^{2+}, but not necessarily sugar (sucrose) and low pH conditions. Gelation can indeed occur within a larger pH range (3.0–7.0) whether soluble solids are added or not to the calcium-containing pectin system. This property allows LMP, unlike HMP, to be used in various food systems, such as low-calorie jellies, acidic milk products, yogurts, and desserts. Gelation is believed to result from formation of intermolecular junction zones, via Ca^{2+}-bridges, between unesterified GalA residues of the pectin HG chains, according to the so-called egg-box junction model (Fig. 12a), first posited for polyguluronate-rich alginates (Grant et al. 1973), since (1 → 4)-linked α-L-guluronate is a mirror image of (1 → 4)-linked α-D-galacturonate. The minimum length of sequences of unesterified GalA residues required for formation of stable egg-box junction structures may vary from approximately 6 to 20 residues, corresponding to a minimum array of approximately 3–10 site-bound Ca^{2+} between chains of twofold symmetry (O'Brien et al. 2009; Fraeye et al. 2010; Morris et al. 2011). Once formed, the junction zones are stabilized by van der Waals interactions and hydrogen bonds between protonated carboxyl groups and by electrostatic interactions. Cohesive LMP gels are thermo-reversible, with sometimes appearance of structural changes-related thermal hysteresis after a relatively long period of ageing.

It should be underlined that the ability of LMP to form egg-box-type junction zones does not only depend on its degree of demethylation (100 – DM) but also on intermolecular and intramolecular distribution patterns of unesterified GalA residues of the individual polyelectrolyte chains (Kohn and Luknar 1977; Taylor 1982; Voragen et al. 1995; Fraeye et al. 2010). Thus, the de-esterification pattern, according to the "blocky parameter" known as the absolute degree of blockiness, which represents the ratio of the amount of unesterified (mono, di-, and tri-) GalA residues, liberated by homogenous endopolygalacturonases, to the total amount of (esterified and unesterified) GalA residues within pectin chains, has been found to play a crucial role in the gelling behavior of LMP (Fraeye et al. 2010). In addition, the block size and the block size frequency may actually be the two key factors that govern calcium-mediated gelation of LMP, while stabilization of the gel formed requires junction-zone-terminating structures evolved from pectin RG-I block cobiopolymers (Yapo and Koffi 2013a).

The presence of amide groups improves, under low pH conditions, the gelling ability of LMP (Alonso-Mougan et al. 2002) and the elasticity and stability of the gel formed and decreases its tendency to syneresis by promoting additional hydrogen bonds (Fig. 12b). Thus, amidated LMP are able to form homogenous gels which are less sensitive to calcium ion concentration over a wide range. The presence of acetylester groups in polygalacturonate chains, by contrast, would hinder formation of junction zones, by decreasing the polyelectrolyte affinity for Ca^{2+} ions. This would mainly account for the rather poor gelling ability of amply acetylated pectins from, for example, cauliflower, chicory, cacao pod husk, endive, pumpkin, and sugar beet by-products. Nevertheless, good gelation has been reported for enzymatically deacetylated beet pectins (Oosterveld et al. 2000).

5.2 Emulsifying Properties of Acetylated Pectins

While high acetylation is likely to impair the gelling capability of certain HG-rich pectins, it may, due to the hydrophobic character, at least be partly accountable for the oil-in-water (O/W) emulsifying/emulsion-stabilizing activities of some amply acetylated pectins from various plant by-products such as sugar beet pulp, cacao pod husks, pumpkin pulp, and cauliflower leaves (Dea and Madden 1986; Yapo and Koffi 2013b). On the other hand (almost unacetylated) depolymerized pectins, from citrus, apple, or apricot by-products, containing 1–4 % proteinaceous compounds also appeared able to emulsify O/W systems (Akhtar et al. 2002; Baississe et al. 2010), suggesting that hydrophobic compounds other than acetyl groups might be involved in the pectin-emulsifying activity. Among acetylated pectins, much intention has been focused on (acid-extracted) sugar beet pectin (BP). It is posited that a hydrophobic protein moiety, relatively strongly associated to BP, is responsible for its emulsifying activity, while the hydrophilic polysaccharide part stabilizes the emulsion droplets formed by providing a protective thick layer, which bestows an effective steric stabilization during extended storage (Akhtar et al. 2002). AFM has enabled a group of workers (Kirby et al. 2008) to show

that BP is mainly composed of un-aggregated linear protein-polysaccharide complexes in which a single protein strand is attached to one end of the polysaccharide chains, thereby forming the so-called tadpoles that contribute to its unusually interesting emulsifying properties. However, other studies showed that the freak emulsifying activity of BP cannot only be explained by the presence of tadpole structures but could result from combination of several emulsion-promoting factors, viz., a high acetylation level, medium-sized polymer chains, and high amount neutral sugars that might constitute the interacting zones with protein which confers this activity without the need for pre-depolymerization (Yapo et al. 2007a). It has subsequently been found, in corroboration, that protein is predominantly bound to the neutral side chains of BP (Funami et al. 2011), which shows involvement of branched polysaccharide structures in the emulsifying/emulsion-stabilizing properties of BP. According to the same authors, contribution of BP ferulation might be little to null, in contrast to previous reports (Williams et al. 2005). Furthermore, BP has been found to exhibit, under acidic conditions (pH ~3), a higher emulsifying ability than gum arabic (the hydrocolloid emulsifier so far unequalled), at an approximately sevenfold lower polymer concentration (Nakauma et al. 2008). Because gum arabic is expensive to use in practice and that a rather high gum/oil ratio (~1:1) is required for producing fine stable emulsion droplets (Dickinson 2009), naturally acetylated pectins with demonstrated emulsifying properties may appear to be good alternative to gum arabic, the benchmark hydrocolloid emulsifier.

5.3 Anticancer Properties of Pectins

Among the miscellaneous potential health-benefit effects of pectic polysaccharides, special attention has recently been focused on their antitumor properties. In this connection, the so-called modified citrus pectin (MCP) has gained interest as the (pharmacological) product of choice for investigation. Most studies on the anticancer properties of pectic polysaccharides have indeed been carried out on MCP. Interestingly, the bulk of the studies (Platt and Raz 1992; Eliaz 2002; Nangia-Makker et al. 2002; Glinsky and Raz 2009) showed that MCP could be effective against any of the main four stages (cell adhesion, cell aggregation, angiogenesis, and metastasis) of development of galectin-3-meditated malign tumors (Fig. 13). Nevertheless, the molecular origin of MCP bioactivity has remained unknown hitherto, probably because MCP is in fact a heterogeneous mixture of pectic oligosaccharides (POS) and polysaccharides (PPS), produced by depolymerization of commercial citrus HMP ($\overline{Mv}$ ~100–300 kDa), based on modification of the reaction medium pH and temperature. In addition, preparation methods of MCP products varied widely from workers to workers to such a point that final products appeared considerably different from a molecular point of view.

Depending on the method of preparation, three molecularly different MCP products can be distinguished: polygalacturonan-rich MCP ($\overline{Mw}$ 10–20 kDa), commercially known as PectaSol (MCP_1); galactose-enriched RG-I-like MCP (MCP_2);

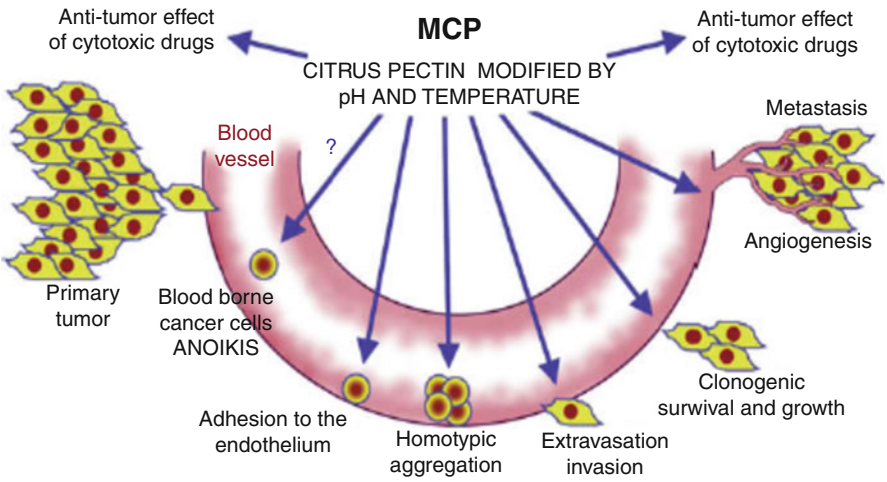

Fig. 13 Schematic representation of critical rate-limiting steps in cancer metastasis, which could be efficiently targeted by MCP. (With permission from Glinsky and Raz (2009). Copyright www.Elsevier.com)

and fractionated citrus pectin powder (MCP_3). The preparation method of PectaSol is not known, for obvious commercial purposes; however, according to the producer, this product belongs to a special class of polysaccharides known as polyuronides with low DM ($\leq$10) and $\overline{Mw}$ (~10–20 kDa). Therefore, MCP_1 is likely produced on the principle of difference in resistance of pectin glycosyl residue linkages to acid agents (Ara*f*-Ara*f* < Ara*f*-Gal*p* $\leq$ Gal*p*-Ara*f* < Gal*p*-Gal*p* < Rha*p*-Gal*p*A $\leq$ Gal*p*A-Rha*p* << Gal*p*A-Gal*p*A) and sounds like a relatively homogenous HG product, as judged from similar data in the literature (Yapo et al. 2007b; Yapo 2009). MCP_2 is obtained by hot alkaline treatment (β-elimination) of commercial citrus pectin followed by mild acid hydrolysis (Platt and Raz 1992; Nangia-Makker et al. 2002) and is classified as a nondigestible galactose-rich polysaccharide fiber of shorter size, readily soluble in water, and better absorbed and utilized by the human body than ordinary high molecular weight citrus HMP. MCP_3 is obtained by treating commercial citrus pectin under neutral pH and high-temperature conditions (Jackson et al. 2007), which causes β-elimination of the pectin HG regions, and is a mixed product of oligogalacturonans and galactose- and/or arabinose-rich-RG-I oligomers of various types. The discrepancy in the three methods adds to the complexity of the problem to solve, thereby reducing chances to unravel, in the short run, the molecular origin of the antitumor properties of MCP.

Furthermore, enzymatically purified okra pod RG-I, carrying short galactan side chains, also exhibited antiproliferative and proapoptotic activities (Vayssade et al. 2010), and hot water-extracted galactan-rich RG-I fractions from various plant materials such as yellow passion fruit rind, banana peel, and tobacco leaf showed efficacious antiproliferative activities in vitro

(Yapo 2011a; Silva et al. 2012). Moreover, specific binding of pectin galactans to galectin-3 has been reported (Gunning et al. 2009). All these recent reports strongly suggest that "modified pectins" may principally exert their antitumor activity, hitherto not clearly understood, at least by binding (via not yet identified linkages) to galectin-3 on the surface on malign cells. Elucidation of this extremely important "pharmacological" of pectins may lie in the use of enzymatically and/or chemically tailored model pectin products that are homogenous in size, in composition, and if possible in charge density.

6 Concluding Remarks

Since their first discovery in 1790–1825 and the correct prediction of Sir Henri Braconnot that pectins would have important functions in all plants and many applications in the art of the "confiseur," these naturally occurring polysaccharides continue to fascinate plant and food scientists who carry out numerous studies on them. However, It is a quite difficult task to deal with the impressive structural diversity and functional versatility of pectins, starting from their natural location within plant cell walls, as bioactive agents for growth, defense, protection of the cells, etc., via their technological properties as gelling, emulsifying/emulsion-stabilizing, film-forming, etc., agents in miscellaneous manufactured food and nonfood products to their physiological and pharmacological effects as health-benefit (biosorbent, immunomodulating, prebiotic, anticomplementary, antitumor, antioxidant, antimicrobial, etc.) agents for the human body. Pectins then represent an outstanding group of heterogeneously complex biopolymers with extraordinary functions, some of which remain to be understood in relation to their fine structure. This fully accounts for the particularly remarkable attention that multidisciplinary scientists should continue to pay to them by developing powerful techniques with the aim of unraveling the intriguing and so far puzzling structures of some pectic cobiopolymers (especially RG-I) and complex native pectins *in muro*, a result of which will surely be a great step toward a full understanding of structure-related functions and improvement of their known properties and finding of new area of applications.

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