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Gert E. de Vries and Karin Metzlaff (Editors).

Remodelling Pectin Structure In Potato

Summary

Pectin is a collection of polysaccharides, which play an important role in controlling the pore size of the plant cell wall, regulating cell-cell adhesion, and providing a source of signalling molecules that elicit a range of cellular responses. Apart from this, pectins are of interest because they are an attractive hydrocolloid for various food applications. The kind and distribution of decorative groups in the pectic molecules largely determines for which application a particular pectin is most suitable. After the extraction of starch from potato tubers, a by-product is obtained, which is relatively rich in pectin. However, the quality of these pectins is poor compared to that from other sources such as citrus and apple. Rather than trying to change the structural characteristics of potato pectin post-harvest, we have embarked on achieving this in the potato plant itself. This paper summarises the structural features of pectin, the distribution of various pectic epitopes in tuber cell walls, the enzymes involved in its biosynthesis and degradation, and strategies employed to alter its fine structure *in planta*.

Introduction

Potato is an important EU crop, not only because it is consumed as such (boiled potatoes) or after processing (French fries, chips, purees), but also because it produces a high-quality starch, which can be used in many industrial applications. In the Netherlands, approximately one third of the potatoes grown are used by the starch industry. After extracting the starch from the potato tubers, substantial amounts of by-products (like fibre and proteins) remain, which have mainly found application in animal feed. However, these by-products contain constituents, which have the potential of generating much higher-value products. In the EC project "Remodelling Pectin Structure in Plants" we have embarked on valorising the fibre fraction. This fraction is a collection of various polysaccharides, which together form the packaging material of the cell contents, i.e. the plant cell wall. Of these, pectin is probably the most interesting polymer because it is a known gelling agent in many food applications [1,2, and references cited]. The suitability of pectin for food applications is governed by many parameters, including its molecular weight, the proportion of smooth versus hairy regions, the degree of methyl- and acetyl-esterification, as well as the distribution of these ester groups along the homogalacturonan backbone (for structural characteristics of the polysaccharides,

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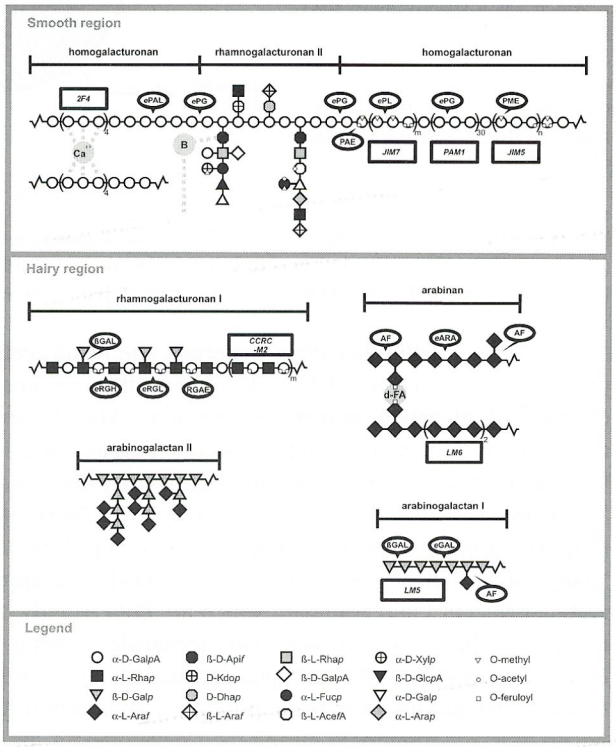
see further) [2,3, and references cited]. For instance, cross-linking of homogalacturonans with Ca^{2+} is promoted when only small amounts of hairs are present, and consequently gels with increased stability can be formed. The primary structure of potato pectin is such that it is of an inferior quality for food applications when compared to, for instance, apple or citrus pectin. In particular, the proportion of hairy regions of potato pectin is too high for good gelling characteristics, and the degree of methylation is too low for use as an emulsifying agent [4]. Thus, adjustments in the potato pectin structure are required to obtain satisfactory gelling properties, and to compete with high-quality pectins.

In our EC project, we have chosen to focus on the hairy regions rather than on, for instance, decoration of the homogalacturonan with ester groups. It is our objective to decrease the proportion of hairy regions *in planta* by genetic modification. In addition to an improvement of the gelling characteristics of potato pectin, we hope that also the starch extraction process will be facilitated (resulting in a higher starch yield). Before discussing the strategies to achieve this, it is important to describe the setting in which these modifications take place. An important part of the project is devoted to characterising wild-type potato tubers in detail, i.e. "What is the chemical fine structure of the polysaccharides?" and "Where are the polysaccharides located in the tuber cell walls?".

Composition and structure of potato tuber walls

Figure 1 summarises the structural features of a number of cell wall polysaccharides, which together form pectin [1,5, and references cited]. Pectins are best described as a collection of various, covalently linked polysaccharides. It has been suggested that these pectic molecules form an independent network, which determines the porosity of the cell wall. Besides the pectin network, the cellulose-xyloglucan network is thought to form the main scaffolding framework of the wall, but this will not be discussed further. Pectins consist of two parts, an essentially unbranched polymer consisting of galacturonic acid residues (homogalacturonan or smooth region), and a polymer composed of alternating rhamnosyl and galacturonosyl residues, which can be substituted with long neutral sidechains (rhamnogalacturonan I with "hairs" or hairy regions). The hairs are mainly composed of galactosyl and/or arabinosyl residues, which are attached to the rhamnosyl residues. They can either be single unit (ß-D-Gal*p*-(1→4)), or polymeric such as arabinogalactan I, and arabinan. Another type of arabinogalactan, arabinogalactan II, is mainly associated with proteins (arabinogalactan proteins). The branching pattern of the hairs is species-dependent. In certain species, the hairs may be cross-linked via ester bonds between diferulic acid residues [6]. It is generally accepted that homogalacturonan (HG) and rhamnogalacturonan I (RG-I) are covalently linked. However, the exact nature of this attachment remains to be determined. HGs can be decorated with ester groups (methyl, acetyl). Stretches of unesterified carboxyl groups of galacturonosyl residues can complex with Ca^{2+}, cross-link different HG molecules, and form gel-like structures [3]. Further, HGs can contain few clusters of 4 different sidechains with very peculiar sugar residues. These sub-structures of HG are referred to as rhamnogalacturonan II (RG-II). Two molecules of RG-II can form a complex with boron (forming a borate-diol ester), which in principle can cross-link two HG molecules [7, and references cited]. Only the apiofuranosyl residues of the 2-*O*-methyl-D-xylose-containing sidechains in each of the subunits of the dimer participate in the cross-linking [7,8]. Certain cations (Ca^{2+}, Pb^{2+}, Sr^{2+}, La^{3+}) promote

Figure 1. Schematic overview of pectic polysaccharide structures. The substitution patterns of the polysaccharides shown in this figure are arbitrary. In potato cell walls arabinan and arabinogalactan carry only very few branches. So far, there is no experimental evidence for the presence of RG-II and arabinogalactan II in potato. In the plant cell wall these structures can be covalently linked (see text), and together they are often referred to as pectin. Pectic polysaccharides can be cross-linked by Ca^{2+}, boron, and diferulic acid residues. These cross-links are indicated by Ca^{2+}, B, and d-FA in gray circles, respectively. Points of attack of various pectin-degrading enzymes are indicated in circles. Monoclonal antibodies raised against various pectic structures are indicated in boxes. Each box spans a number of sugar residues corresponding approximately to the epitope recognized by the antibody. • Homogalacturonan (HG) is composed of (1→4)-α-D-Gal*p*A residues, which can be esterified at the *O*-6 position (methyl) as well as at the *O*-2 and/or *O*-3 positions (acetyl). The distribution of ester groups can either be blockwise or *at random*, which determines the properties of the HG to a large extent. The backbone of rhamnogalacturonan II (RG-II) is composed of approximately 9 (1→4)-α-D-Gal*p*A residues. To this, four complex (but highly conserved among various plant species) sidechains are attached, the exact positioning of which is still unknown. Rhamnogalacturonan I (RG-I) is composed of a backbone with (1→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A repeating units. The Rha*p* units can be substituted with ß-D-Gal*p*-(1→4) residues, whereas the Gal*p*A residues can carry acetyl groups on *O*-2 and/or *O*-3. Arabinan has a backbone of (1→5)-α-L-Ara*f* residues with [α-L-Ara*f*-(1→3)]$_n$ (n=1,2) and/or α-L-Ara*f*-(1→2) sidechains. Arabinogalactan I is composed of a (1→4)-ß-D-Gal*p* backbone, which can be branched with single unit α-L-Ara*f*-(1→3) sidechains. Arabinogalactan II has a backbone of (1→3)-ß-D-Gal*p* residues, which can carry α-L-Ara*f*-(1→6)-[ß-D-Gal*p*-(1→6)]$_n$ sidechains (n=1,2,3). The Gal*p* residues in the sidechains can be branched with α-L-Ara*f*-(1→3) residues.



dimer formation *in vitro* in a concentration- and pH-dependent manner [7,9]. Both Ca^{2+} and borate ester cross-linking play an important role to retain pectins in the cell wall. Boron in particular seems to be required to control the pore size of the cell wall *in vivo* [10].

In order to obtain reliable information on the native polysaccharide composition of potato tuber walls, it is important to consider the presence of endogenous enzymes and huge amounts of starch (up to 20 times more than cell wall material). Endogenous enzymes should be inactivated at the very beginning of the cell wall isolation procedure. Notably, the pectin methylesterase (PME) activity is so large that all methyl groups could be removed from HG within a very short time span. Starch should be removed without extracting cell wall polysaccharides, because it interferes with sugar analysis and fractionation procedures. A method has been developed which effectively deals with these prerequisites. Figure 2 illustrates the abundance of various polysaccharide structures in the wild-type potato tuber cell wall. Pectin (according to the definition given above) comprises more than 55% of the cell wall polysaccharides, which emphasises that they form a very important part of the wall.

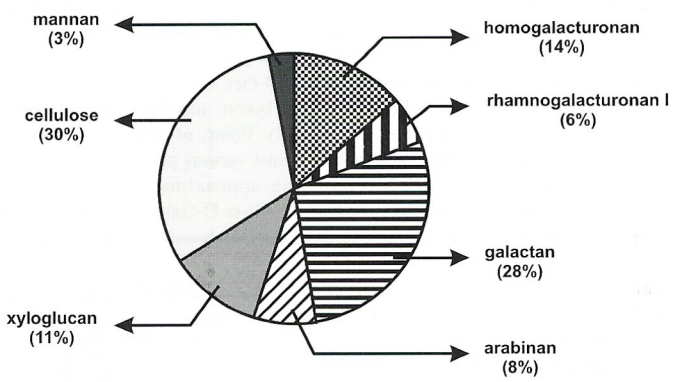


Figure 2. Polysaccharide composition of the potato cell wall (% w/w). The proportion of the various polysaccharides was estimated using monosaccharide-composition data of starch-free cell wall preparation, structural information data from the literature and the following assumptions: (i) all rhamnose is part of RG-I, which has a strictly alternating Rha-GalA backbone; (ii) about 50% of the rhamnosyl units carry a single unit galactosyl sidechain [4,11]; (iii) the remaining (non RG-I) GalA forms the HG; (iv) all xylose is part of xyloglucan (minor amounts of xylan are neglected); (v) potato xyloglucan has a XXGG branching pattern, with c. 50% unsubstituted Xyl and equal amounts of arabinosylated and galactosylated Xyl; [12]; (vi) the remainder of Glc is atributed to the cellulose fraction; (vii) galactan and arabinan are mainly present as unbranched polymers [4]; (viii) methyl groups, which can be present on HG, and acetyl groups, which can be present on HG, RG-I, and xyloglucan [12] have not been taken into account in the estimation; (ix) the presence of RG-II has not been considered.

In comparison with other plant materials, the potato cell wall is extremely rich in hairy regions [4]. It has a lower HG to RG-I ratio than for instance apple or beet pectin, which is clearly a disadvantage in many applications. Also the amount and nature of the hairs differs considerably among these three species. Potato has a large amount of (arabino)galactan I hairs and a much smaller amount of arabinan hairs; potato hairs are hardly branched [4]. Sugar beet is rich in heavily branched arabinan hairs, and poor in galactan-containing hairs [13]. The amount of hairs is more or less comparable to that of potato. Apple RG-I is less "hairy" than that of potato or sugar beet [14]. The apple hairs contain similar amounts of arabinogalactan I and arabinan, which are both heavily branched. Currently, a more detailed analysis of the potato cell wall polysaccharides is being conducted, in order to establish the branching patterns of the wall polysaccharides more precisely, and also to map the distribution of ester groups over these polymers. In addition to this, the cell wall composition of potato tubers is monitored during their development. We will try to relate possible modifications in the wall to the presence of endogenous enzymes.

Next to the chemical characterisation discussed above, the tuber walls are also being studied microscopically [15, and references cited]. Over the years, a number of monoclonal antibodies (mAbs) have been generated, which recognise different, specific pectic structures in the wall. The epitopes recognised by a selection of mAbs are summarised in figure 1. JIM5 and JIM7 recognise low- and high-methylester epitopes of HG, respectively. The epitopes bound by these mAbs are not defined, i.e. it is unknown how many sugar residues they comprise, whether a certain distribution of methylester groups is required, and whether acetylation interferes with recognition. For certain mAbs, such as PAM1 (not used in our studies) and 2F4, this is better documented. PAM1 can bind HG blocks of more than approximately 30 GalA residues [16]. It has been suggested by these authors that JIM5 recognises shorter stretches of contiguous GalA residues. 2F4 can bind unesterified oligogalacturonides of at least 9 GalA residues which have dimerized through Ca^{2+} [17]. When Ca^{2+} was replaced by other divalent cations such as Mg^{2+} the recognition of HG by 2F4 was prevented. CCRC-M2 binds RG-I

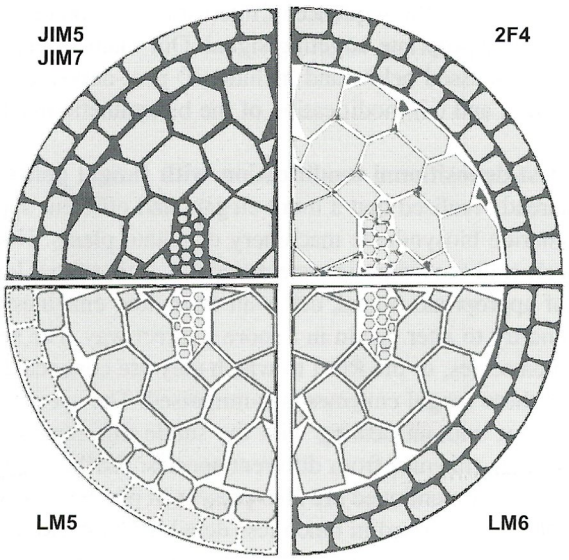


Figure 3. A summary diagram illustrating how pectic epitopes are distributed in potato tuber cell walls. Each quarter segment represents a tuber section labelled with mAbs JIM 5, JIM 7, 2F4, LM5 and LM6. The outermost line represents the periderm, the cortex proper is shown as two layers of light gray cuboidal cells and the storage parenchyma and vascular tissue in the perimedullary region as large and small light gray polyhedral cells respectively. The cell wall is the space between the cells and gold labelling is shown in dark gray. Both mAbs JIM 5 and JIM 7 label the entire wall throughout the tuber. The mAb 2F4 labels the entire wall of the cortical cells, but only the middle lamella at corners in the perimedullary region. Only the primary wall is labelled with mAb LM5 and in the cortex there is a labelling gradient, in cortical walls the middle lamella as well as the primary wall labels with mAb LM6, but not in the perimedullary region. Secondarily thickened walls of xylem do not label with any mAb. From [15], with permission from *Physiologia Plantarum*.

from sycamore, but not much is known about the actual epitope [18]. Our studies suggest that it does not detect potato RG-I. Therefore, mAbs are now being raised against defined oligomeric RG-I fragments within the project. LM5 recognises stretches of more than three ß-(1→4)-linked galactosyl residues [15, and references cited]. It is uncertain to what extent arabinosylation can affect binding. LM6 recognises an epitope of six α-(1→5)-linked arabinosyl residues. Branched arabinans like that from sugar beet are not bound, but after debranching they are. CCRC-R1 (not used in our studies) detects de-esterified RG-II [19]. This mAb has been used to show that RG-II is distributed over the entire wall of suspension-cultured sycamore cells. Only RG-II close to the plasma membrane appears to be unesterified. The antibodies were used in combination with immunogold-labelling to localise the various epitopes in the potato tuber walls [15]. Fig. 3 summarises the results of these studies schematically. Each polysaccharide occupies specific regions of the wall, in a tissue-specific manner. Similar experiments will be done with selected transgenic potato tubers, as well as with tubers from various developmental stages, in order to investigate whether the pectic polysaccharides are differently distributed in these walls.

Altering the cell wall

The main objective of our EC project is to increase the ratio of smooth to hairy regions in potato pectin, in order to obtain a higher-value pectin in potato fibre. Genetic techniques (antisense technology, heterologous expression) are being used to realise these modifications *in planta*. Little is known about the function of the different pectic polymers in the wall, and therefore it is difficult to predict the consequences of down-regulating the amount of hairs for the well-being of the potato plant beforehand. In this respect, it is important to have a collection of signal sequences available, for instance to direct the expression to a particular location within the cell (transit peptides), or to a particular tissue (tissue-specific promoters). In our

experiments the sequence of interest is often preceded by a tuber-specific promoter, followed by an appropriate targeting signal. Our main strategies to alter the structure of potato pectin are discussed below and include: (i) post-depositional modification by expression of fungal genes, and (ii) modification of the biosynthetic machinery.

Post-depositional modification with fungal genes. At the time the project started, it was already realised that a more elegant and efficient approach would be to modify the polysaccharide biosynthetic machinery of potato plants. However, only very few genes involved in cell wall biosynthesis were described at that time. Instead, it was decided to select a number of appropriate fungal, cell wall-degrading enzymes from the large collection of candidates, and try to alter pectin in a more indirect way. The selected enzymes degrade either the hairs themselves, or the RG-I to which they are connected. In Fig. 1 the site of action of a number of these fungal enzymes is summarised. The specificity of the various enzymes is elaborated below, without dealing with the subtle differences in characteristics that may exist among similar enzymes from different species. Furthermore, it should be realised that many of the activities mentioned are also present in plants; however, the corresponding enzymes are usually characterised in much less detail. RG-I is cleaved by two different enzymes, which generate oligosaccharides with 4 or 6 sugar residues in the backbone [20, and references cited]. *Endo*-rhamnogalacturonan hydrolase (*e*RGH) splits the backbone at the non-reducing side of a rhamnosyl residue, whereas *endo*-rhamnogalacturonan lyase (*e*RGL) cleaves at its reducing side. Another important difference between the two enzymes is that *e*RGL produces reaction products with an unsaturated GalA residue (double bond between C_4-C_5), whereas *e*RGH does not. *e*RGL requires longer stretches of RG-I for cleavage than *e*RGH (respectively 12 and 9 glycosyl residues). Both enzymes can cleave an RG-I backbone, which is decorated with single unit galactosyl sidechains. However, the presence of sidechains can influence the catalytic efficiency of *e*RGH and *e*RGL. Accessory enzymes are required for efficient RG-I degradation. Deacetylation of the RG-I backbone by rhamnogalacturonan acetyl esterase (RGAE) enhances the action of both *e*RGH and *e*RGL. Similar observations have been made in HG degradation, where *endo*-polygalacturonase (*e*PG) or *endo*-pectate lyase (*e*PAL) act synergistically with pectin methylesterase (PME) and presumably also pectin acetylesterase (PAE). In contrast with *e*PG and *e*PAL, *endo*-pectin lyase (*e*PL) requires a high degree of methylation to be active [1]. Depending on their degree of branching, the hairs are best degraded by a combination of enzymes [21]. Most *endo*-galactanases (*e*GAL) and *endo*-arabinanases (*e*ARA) are not very tolerant to sidechains. An arabinofuranosidase (AF) is needed to linearise the substrate, and enhance the action of the *endo*-acting enzymes. ß-Galactosidase (ßGAL) can degrade the arabinogalactan I hairs from the non-reducing end. It is unknown whether these enzymes can by-pass an arabinosyl branch point, although there is some preliminary evidence that a ßGAL from *Cicer arietinum* is capable of doing this. Further experiments to confirm this are being performed.

A number of single (*e*GAL, *e*ARA, *e*RGH, *e*RGL, *e*PG) and double transformants (*e*RGH + RGAE, en *e*RGL + RGAE) have already been generated within our EC project. The introduction of *e*GAL or *e*ARA in potato aims at a "shave" of the corresponding hairs. Introduction of *e*RGH or *e*RGL is expected to have an even larger impact on the wall, because it removes all hairs (arabinan and galactan) including the RG-I to which they are attached. The presence of RGAE in the double transformants will presumably increase RG-I degradation, and a larger

effect is anticipated. The plants transformed with *e*PG serve as a kind of control. Here, the consequences of a reduced amount of smooth regions for the potato tuber will be studied. The endogenous PME(s) may act synergistically with the heterologous *e*PG.

All transgenic plants looked normal except those in which the *e*ARA was introduced. The *e*ARA plants show early senescense, and do not form any tubers. A new generation of *e*ARA transformants is currently being made in which the more tightly regulated, tuber-specific patatin promoter is used (instead of the granule-bound starch synthase promoter), in combination with various targeting sequences (vacuole, ER, Golgi). In case of vacuole or ER targeting, *e*ARA and its substrate will meet only during processing of the tubers. With Golgi targeting of *e*ARA the arabinans might be degraded at the place where they are synthesised.

The other transformants are analysed in a step-wise manner, starting with demonstration of transcription from the transgene, gene product accumulation and authenticity, and finally determination of effects on wall composition and architecture in selected transformants. Within the tubers of one series of transformants, the amount of RNA corresponding to the heterologous gene varied considerably. However, in all cases a number of plants with a high transcription of the introduced gene could be selected. For the *e*RGL transformants the RNA data corresponded very well to *e*RGL activity in tuber extracts. Surprisingly no rhamnogalacturonan-degrading activity was found in the *e*RGH transformants. The reason for this is unknown; possibly *e*RGH is incorrectly processed in the tuber after translation. Quite a few of the remaining genes were successfully expressed in the potato plants. Sugar analysis of isolated cell walls from these transformants indeed indicates an altered phenotype. A number of high expressors of the genes mentioned above have been propagated *in vitro*, and are now grown in the greenhouse in order to obtain more potato tuber material. The cell walls of these transgenic plants will be analysed in detail for modifications in composition, architecture, and targeting of the enzymes.

Modification of the biosynthetic machinery. In principle, four different levels of polysaccharide biosynthesis can be distinguished in plants: (i) maintenance of the pool of nucleotide sugars or other precursors, (ii) polymerisation of a particular backbone, (iii) decoration of these backbones with various substituents (glycosyl residues, methyl- and acetyl groups), and (iv) incorporation of the polymers into the cell wall. At this moment only very few genes directly involved in pectin biosynthesis are known [22, see further]. Many efforts to find "pectin synthases" follow the long and laborious route of purifying a protein of interest, digestion of this protein combined with N-terminal sequencing, preparing probes based on the obtained sequences, and finally screening a cDNA library with these probes. However, the "synthases" are often membrane-bound or part of a protein complex, and upon detergent solubilisation or purification they can lose their activity. Enzymes, which are currently under investigation, include methyltransferases, HG synthase or galacturonosyl transferase, and galactan synthase. Methyltransferases transfer a methyl group from S-adenosyl methionine (SAM) to the C-6 of a galacturonosyl residue [23-25]. It seems likely that there are several methyltransferases in one plant species, (at least) one for HG and two for RG-II. In flax, two pectin methyltransferases with different molecular weight and properties have already been described [24,25]. A galacturonosyl transferase from tobacco catalyses the transfer of a GalA residue from UDP-GalA to the non-reducing terminus of oligogalacturonides with a degree

of polymerisation greater than nine [26]. In contrast to the membrane-bound galacturonosyl transferase, the solubilised one displays a distributive mode of action (only one residue at a time is added to an acceptor molecule). It has been suggested that this is related to the dissociation of the protein complex. The lack of processivity may also be related to the nature of the acceptor substrate [27].

As part of our EC project, galactan biosynthesis is studied using flax as a model system. In flax there seem to be at least two different galactosyl transferases. One of these catalyses the processive addition of galactosyl residues to a nascent galactan chain, and may be a true galactan synthase. The other shows a more distributive mode of action, and may be involved in the attachment of galactosyl residues to a RG-I backbone. This hypothesis is currently being tested using defined oligosaccharide acceptor substrates generated with *e*RGH. In parallel with these experiments, the enzymes are purified to homogeneity in order to obtain sequence information. The obtained sequences will be used to clone the potato homologue, and subsequently inhibit the formation of hairs by antisense technology.

Most of the plant biosynthetic genes that have been found to date belong to the group of enzymes that are involved in nucleotide sugar conversions [28,29, and references cited]. In all cases they have been recognised as such because a microbial counterpart had been described. In potato, a GDP-D-mannose pyrophosphorylase has been identified, which converts D-mannose-1P to GDP-D-mannose [24]. The MUR1 gene from *Arabidopsis thaliana* encodes a GDP-D-mannose-4,6-dehydratase, which catalyses the first step in the 3-step conversion of GDP-D-mannose to GDP-L-fucose. Thus, both enzymes are involved in controlling the level of L-fucose, which is a substituent of RG-II. More important for pectin biosynthesis is UDP-D-glucose dehydrogenase (*Glycine max*, *Arabidopsis thaliana*) [28,29], because it plays a key role in maintaining the pool of UDP-D-galacturonic acid. The dehydrogenase converts UDP-D-Glc to UDP-D-GlcA, which is subsequently epimerised to UDP-D-GalA.

The UDP-D-glucose-4-epimerase catalyses the reversible conversion of UDP-D-Glc to UDP-D-Gal. Two (putative) isoforms of this enzyme have been found in *Arabidopsis thaliana*, and one in *Pisum sativum* and *Cyamopsis tetragonoloba*. These epimerases may be very important in the supply of buiding units for galactan hair biosynthesis. According to the current biochemical pathways, UDP-D-Gal can only be formed from UDP-D-Glc, at least when no D-galactose is applied exogenously. In *Arabidopsis thaliana* the UDP-D-Glc-4-epimerase activity has been down-regulated by introducing the gene of one of the isoforms in an antisense orientation [30]. Surprisingly, no decrease in the amount of cell wall galactose was observed. However, when these transgenic plants were grown in a medium containing galactose, an increase in cell wall galactose was found. In the present EC project, two putative UDP-D-Glc-4-epimerases from potato have been cloned. Transgenic potato plants in which the amount of both enzymes, alone and in combination, is down-regulated using antisense technology are currently being prepared. It is expected that "hair growth" can be inhibited to a larger extent than in *Arabidopsis thaliana*, because both isoforms will be antisensed, and because galactan is a much more abundant cell wall polysaccharide in potato than in *Arabidopsis*.

Another interesting gene with respect to pectin biosynthesis is SAM synthethase, because it allows control over the pool of substrate for pectin methyltransferase. The SAM synthethase

has been over-expressed in suspension-cultured flax cells [24]. As a result of this some of the transgenic cell lines produced pectin with a higher degree of methylation, suggesting that the SAM concentration is limiting in the control cell lines.

Another multigene family, which has received a lot of attention with respect to cell wall biosynthesis, is cellulose synthase (CesA; A stands for catalytic subunit) and cellulose synthase-like (Csl) genes [31]. Also in this case, the discovery of the first "plant cell wall synthase" gene was preceded by the characterisation of a number of bacterial cellulose synthases. Alignment of the bacterial enzymes showed a number of highly conserved motifs, which were speculated to be involved in UDP-glucose binding. When it was realised that these motifs were present, the search for a plant cellulose synthase was greatly facilitated. Now, the number of putative cell wall synthases is rapidly increasing. In *Arabidopsis thaliana* 42 CesA or Csl genes have already been found, which can be assigned to 6 different groups. The CesA genes all belong to one group. The biological function of two members of this group has been established by complementation of certain *Arabidopsis* cell wall mutants. RSW1 encodes a primary cell wall cellulose synthase [32], whereas IRX3 is involved in cellulose production in the secondary cell wall [33]. The specificity of the 5 Csl groups remains to be investigated. Because the binding of nucleotide sugars is a probably a common feature of all "plant cell wall synthases", it has been hypothesised that the Csl genes encode other polysaccharide synthases such as xylan synthase, mannan synthase, etc..

In the present EC project we have set out to isolate a number of CesA or Csl sequences for two reasons. (i) The availability of a true potato CesA gene would enable down-regulation of the amount of cellulose using antisense technology. In this way a number of transgenic potato lines could be generated in which the cellulose content in the tubers is reduced to between 0% (wild-type) and 100% (possibly lethal). It is speculated that these plants will compensate this loss by depositing more pectins in their walls. In order to strengthen the wall, these pectins might be cross-linked to a larger extent than those in wild-type potato tubers (see Fig. 1). Such observations have been made for the walls of suspension-cultured tomato cells that have been grown in the presence of 2,6-dichlorobenzonitrile, an inhibitor of cellulose biosynthesis [34]. (ii) In addition to isolating a true cellulose synthase gene, other polysaccharide synthases might be obtained as well. Discovery of a galactan, arabinan or RG-I synthase would of course be preferred with respect to the aim of the project. Inhibition of these genes by antisense technology could increase the proportion of smooth regions in pectin, which would be an elegant alternative for the introduction of the fungal genes discussed previously. So far, seven CesA or Csl genes have been isolated from potato. These are currently introduced in an antisense orientation in potato plants.

Future prospects

From the above it is clear that pectin is an extremely complex component of the cell wall. Although there is a fairly good understanding about the fine structure of the individual polysaccharides, it is obvious that the attachment of the various elements to each other deserves more attention. Considering the complexity of pectin, it is not surprising that there are numerous enzymes involved in its degradation. No doubt the biosynthetic machinery will be equally impressive. Already, a large "toolbox" for pectin remodelling *in planta* is available. Most of

the tools are genes encoding cell wall degrading enzymes. Only a limited number of tools for pectin biosynthesis is known. Of these, the genes involved in the interconversions of nucleotide sugars are most abundant. However, the pathways in which these genes participate are very complex, and in many cases there will be alternative routes by which the effect of down-regulating a certain activity can be by-passed. Also, altering the level of one particular nucleotide sugar in the plant may have an effect on the structure of various polysaccharides (for instance, many polysaccharides contain galactose). Potentially, more specific modifications of the wall polysaccharides can be achieved by down- or up-regulating the activity of synthases or decorative enzymes. Although there are relatively few genes known in this category, it is anticipated that the toolbox will be supplemented with such genes in the near future. The growing number of T-DNA-tagged mutant lines in *Arabidopsis thaliana* in combination with the *Arabidopsis* genome-sequencing project will be very valuable in this respect [29].

A large number of transformants potentially with an altered pectin structure have been generated. Preliminary results with the introgression of heterologous genes look very promising. Larger amounts of these tubers are currently being grown, and will be used for detailed cell wall analysis. Pectins will be extracted and subjected to functional testing in order to investigate whether the desired industrial properties are enhanced. In principle, the tailored hairy region structures may also offer possibilities for new applications.

Finally, the remodelling of pectin will be interesting from a plant-developmental perspective. Recombinant DNA technology provides the possibility of probing the significance of individual polysaccharide structures for the architecture and properties of the cell wall. For this, it is very important that the potato cell wall is characterised in great detail, both at the chemical and the microscopic level, so that the background, in which the cell wall modifications are introduced, is precisely known.

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AUTHOR INDEX