Carbohydrate Research 344 (2009) 1872–1878

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

An array of possibilities for pectin

Iben Sørensen, Henriette Lodberg Pedersen, William G. T. Willats *

Department of Biology, University of Copenhagen, Ole Maaløes Vej 5, 2200 København N, Denmark

ARTICLE INFO

Article history:
Received 19 August 2008
Received in revised form 2 December 2008
Accepted 3 December 2008
Available online 13 December 2008

Keywords:
Pectin
Microarray
Antibodies
Enzymes

ABSTRACT

Pectins are a major component of plant cell walls and have numerous roles in plant growth and development. Extracted pectins are widely used as functional food ingredients in products including ice creams, jams, jellies and milk drinks. Although all are based on a galacturonan-rich backbone, pectins are an immensely diverse family of polysaccharides, the functional properties of which are dictated by their fine structures. Understanding the biological roles of pectins and optimizing their industrial usage requires a detailed knowledge of their diversity and spatial and temporal distributions, and microarray technology is a promising tool for high throughput pectin analysis. This article discusses the technical aspects of the production of pectin microarrays and explores their current and potential future uses in the context of recent work in the field.

1. Introduction

Microarrays for the high throughput analysis of nucleotides were first described in 1995,[1] and soon after this technology was adapted for peptides.[2] Producing carbohydrate microarrays proved to be rather challenging—not least because the immense chemical and structural diversity of glycans made it difficult to develop a 'catch-all' procedure for immobilization onto slides or membranes. The first carbohydrate microarrays were produced in 2002 and in one study the arrayed glycans were pectins.[3–5] This may seem like a case of a technological sledgehammer being used to crack a food ingredient nut, but brief consideration of the remarkable complexity and diversity of pectins reveals why this sophisticated technology was developed for their analysis.

Pectins are ubiquitous components of the cell walls of land plants and many green algae.[6] They have key roles in the life of plants, and have a multitude of uses as functional food ingredients, the many splendours of which were reviewed by Pilnik.[7] The word 'pectin' in fact describes a large family of related polysaccharides that are all based on a galacturonan-rich backbone. Three major pectic polymers are generally recognized, un-branched homogalacturonan (HG) and the complex branched domains rhamnogalacturonan I (RGI) and rhamnogalacturonan II (RGII). HG is a linear homopolymer of (1→4)-α-linked-D-galacturonic acid (GalA) that is thought to generally contain some 100–200 GalA residues.[6] Galacturonic acid residues in HG can be methyl esterified at the C-6 carboxyl and acetylated at C-3 and C-2. The degree of methyl esterification (DE) and the distribution patterns of methyl esters are important in determining the functional properties of HG, particularly with respect to gel formation.[6,7] In pectins with a high DE, junction zones are formed by the cross-linking of HG chains by hydrogen bridges and by hydrophobic forces between methoxyl groups, both promoted by high sugar concentration and low pH. This type of gelation underlies many of the commercial uses of pectins, for example, in jams and jellies.[8] In pectins with a low DE, junction zones are formed by calcium cross-linking between free carboxyl groups, and this to a large extent mediates HG functionality in plants, particularly with respect to the cell-to-cell adhesion and the control of cell-wall porosity.[9,10] GalA residues in HG may also be substituted with xylose residues to produce xylogalacturonan (XGA) or with apiose to produce apiogalacturonan (AGA).[11–14] XGA appears to be quite widespread in the plant kingdom, and XGAs with different structures have been isolated and characterized.[11] In contrast, AGA has been found in certain duckweeds but does not appear to be generally abundant.[13,14] RGI consists of the disaccharide (1→2)-α-L-rhamnose-(1→4)-α-D-galacturonic acid and is generally thought to be glycosidically attached to HG. In most cases, 20–80% of the rhamnose residues in RGI are substituted at C-4 with side chains that can vary from a single to 50 or more glycosyl residues.[6] Side chain composition is highly variable but a common structural feature is arabinogalactan domains rich in (1→4)-β-linked galactosyl residues and (1→5)-α-L-arabinosyl residues.[6,8] In some species a small number of GalA residues in the RGI backbone are substituted with single glucuronic acid residues. Despite its name, RGII is a highly branched domain based not on rhamnogalacturonan but rather on a short HG backbone of around 9 GalA residues.[15,16] Unlike RGI, RGII is highly conserved and contains four heteropolymeric side chains of known and consistent lengths. These highly complex side chains contain eleven

* Corresponding author. Tel.: +45 35322132.
E-mail address: willats@bio.ku.dk (W.G.T. Willats).

0008-6215/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2008.12.008

different sugars including apiose, aceric acid and 2-keto-3-deoxy-D-manno-octulosonic acid (KDO).[15,16]

Pectin structure and biosynthesis has recently been reviewed by Mohnen[10] and elsewhere in this issue, but the above brief description serves to illustrate the immense complexity of the pectic matrix. Equally important though is the fact that the fine structures and relative amounts of pectic domains vary widely not just between plant species but also between organs, within tissues and even within different micro-domains of the same cell-wall. This diversity provides a wide range of functional properties that are reflected by the numerous roles of pectin *in planta*, and also by diverse industrial applications. In all cases, pectin functionality is intimately related to fine structure and there is therefore considerable interest amongst plant scientists and pectin producers to develop sophisticated techniques for pectin analysis, including pectin microarrays.

2. The construction of pectin microarrays

The three basic components of pectin microarrays are a set of target molecules (pectins), a set of probes to detect the targets (e.g., antibodies) and a surface onto which the targets are arrayed (slides or membranes). In all pectin microarrays to date, these components are arranged in a format that is essentially based on probe capture. In other words, the pectins are immobilized onto a surface and then exposed to probes in a solution. Binding of the probes to arrayed pectins is then detected by means of a fluorescent or colourimetric tag, usually coupled to a secondary antibody. This is analogous to antibody-capture enzyme-linked immunosorbent assays (ELISAs) and contrasts with most nucleotide microarrays and some protein microarrays in which the probes (oligonucleotides or antibodies) are immobilized and the targets added in solution. An important distinction is between microarrays that are constructed using samples of well characterized and relatively pure pectins ('defined glycan arrays), and arrays that are constructed from pectins extracted from plant tissues as complex mixtures ('extracted glycan arrays) (Fig. 1). These two approaches use essentially the same technologies but have rather different uses. Defined glycan microarrays are applicable when there is a requirement to analyze interactions between specific pectins and ligands or enzymes and where a detailed knowledge of defined structural features (such as DE or side chain composition) is required. In contrast, extracted glycan microarrays are used when there is a requirement for the high throughput analysis of pectins in biological samples. For example, this kind of microarray can be used to compare pectin composition in a range of different plant species, or to compare mutant and wild type plants.

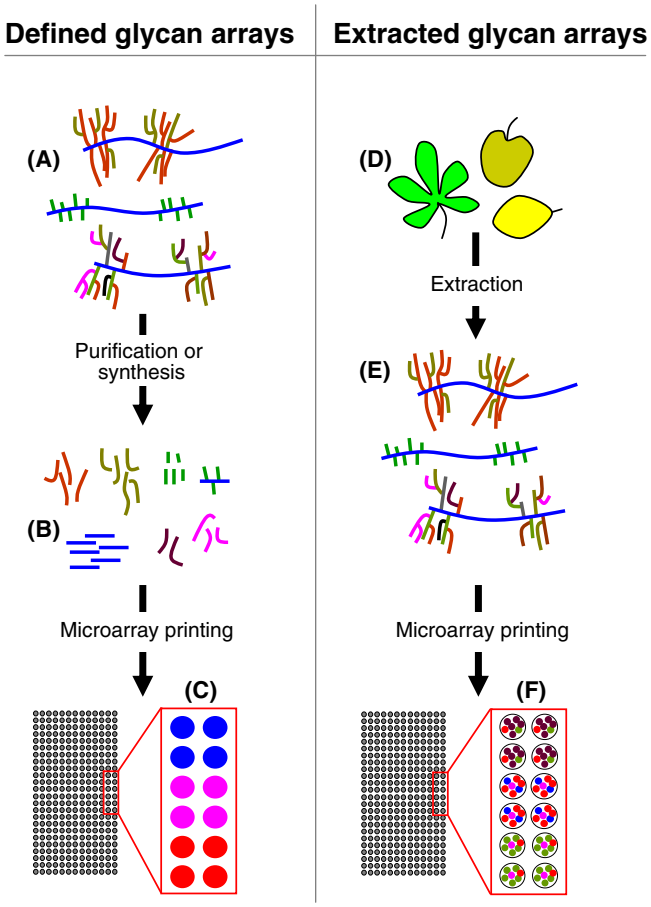

Figure 1. Two approaches for the production of pectin array. (A and B), 'Defined glycan arrays are constructed from pectic oligo-or polysaccharides obtained either by enzymatic fragmentation of large pectic polymers followed by purification, or by chemical synthesis. If non-covalent coupling is used, then oligosaccharides can be coupled to a carrier molecule such as BSA to aid immobilization (see Fig. 2). (C) when samples are printed as microarrays, the resulting spots each contain a relatively pure population of pectin domains, or if chemical synthesis is used, an absolutely pure population. Such microarrays can be used for antibody or enzyme screening. 'Extracted glycan arrays are produced by first making a crude, inclusive extract of pectic polymers from a source material of interest, for example, different plant organs (D). The extractions which contain a variety of pectic oligo- and polysaccharides (E) are printed, and the resulting microarrays contain spots that each contain a complex mixture of cell-wall glycans, but are enriched in pectins (F). This type of microarray can be used to assess the relative abundance of pectic epitopes in a large number of plant materials.

3. Sourcing of pectin samples

In the case of defined glycan microarrays, pectins are obtained by purification from various types of plant cell-wall material, usually by a combination of chemical and enzymatic treatments, and the methods used depend on the specific end use of the microarrays. However, the sourcing of well-defined samples is currently a major limitation to producing pectin microarrays. Although there are many well-established methods for the enzymatic fragmentation of pectins and subsequent fractionation into structural domains, the fact remains that it is technically challenging and time consuming to produce milligram amounts of pure pectin fragments. One powerful approach is exemplified by the glycan microarrays produced by Feizi and coworkers.[17,18] Oligosaccharides are coupled to lipids to ensure that they can be readily immobilized onto nitrocellulose. Importantly though, a deconvolution step is used such that aliquots of the oligosaccharides are subjected to structural characterization by mass spectrometry. So far this approach has not been applied to pectins, but it may in the future be an important new method of producing microarrays of well-characterized pectic structures. The synthesis of target molecules is well established in the production of nucleotide and protein microarrays but the synthesis of complex glycans is notoriously difficult. Nevertheless, Clausen et al. produced a series of short oligogalacturides (OGAs) by chemical synthesis that were substituted with methyl esters attached at known positions along the short backbone,[19,20] and Guillaumie et al. demonstrated the feasibility of enzymatic synthesis of OGAs.[21]

The sourcing of samples for extracted glycan microarrays presents a completely different set of challenges because the aim is not to obtain pure pectins but rather to obtain a mixture that represents as far as possible the entire pectin population of a particular biological sample. This is achieved by the extraction with solvents that are conventionally used for pectin solubilization, such as hot water or calcium chelators. The use of extracted glycan

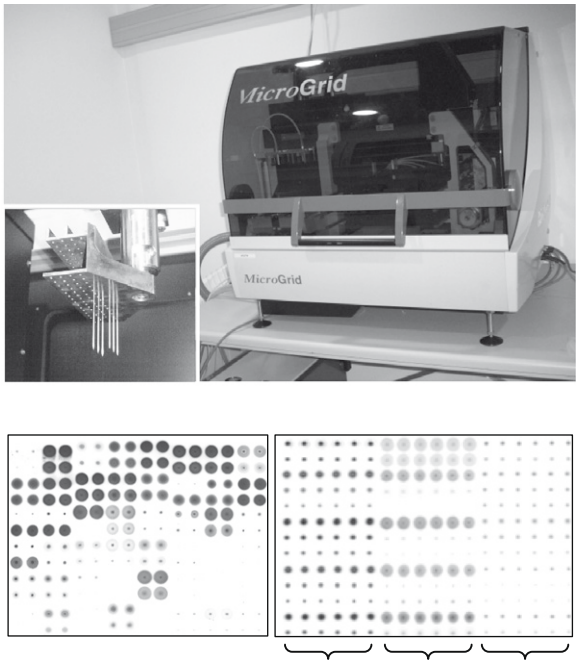

Figure 2. (A) A robotic microarray printer (MicroGrid II, Genomic Solutions, Ann Arbor, MI, USA) and inset, a print head with six metal split pins fitted. (B) An example of a defined glycan microarray on which a series of cell-wall polysaccharides, including pectins, have been spotted. The microarray was probed with an mAb with specificity for HG. (C) An example of an extracted glycan microarray on which cell-wall polymers extracted from various *Arabidopsis thaliana* organs using CDTA (c), NaOH (n) and Cadoxen (ca) have been spotted. The microarray was probed with an mAb with specificity for (1→4)-β-D-galactan. (D) High magnification images of spots from pectin microarrays printed with split or solid pins. In both cases partially methyl esterified lime pectin was spotted in duplicate at a concentration of 0.5 mg/mL (top) or 0.1 mg/mL. The microarrays were probed with an anti-HG mAb. Note that the use of split pins produces a ring (particularly evident at the lower concentration) which results from migration of HG away from the centre of the spot after pin contact. (E) A sample of chemically synthesized partially methyl esterified hexagalacturonate ($[GalA]_6$) and the same sample coupled to bovine serum albumin (BSA) by reductive amination ($[GalA]_6$-BSA) were spotted onto nitrocellulose membrane. The microarray was probed with an mAb with specificity for the hexagalacturonate sample. Note that coupling to BSA is an effective method of achieving immobilization onto nitrocellulose. Antibody binding to the microarrays shown was detected using secondary antibodies conjugated to alkaline phosphatase, and microarrays were developed using a 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium-based substrate. (F) An example of output from an extracted glycan microarray. The heatmap shows the relative levels of pectic epitopes (listed on top row) in a range of plant species (listed left). Samples were extracted from leaves (L), stems (S) and roots (R) with CDTA. The microarrays were produced and quantified as described by Moller et al.[37] The heatmap in which colour intensity is correlated to mean spot signals was created using heatmapper software (http://bar.utoronto.ca/ntools/cgi-bin/ntools_heatmapper_plus.cgi). The mAbs used bind to partially methyl esterified HG with a relatively low DE (JIM5); Partially methyl esterified HG with a relatively high DE (JIM7); xylogalacturonan (XGA, LM8); (1→4)-β-D-galactan (LM5); (1-5)-α-L-arabinan (LM6).

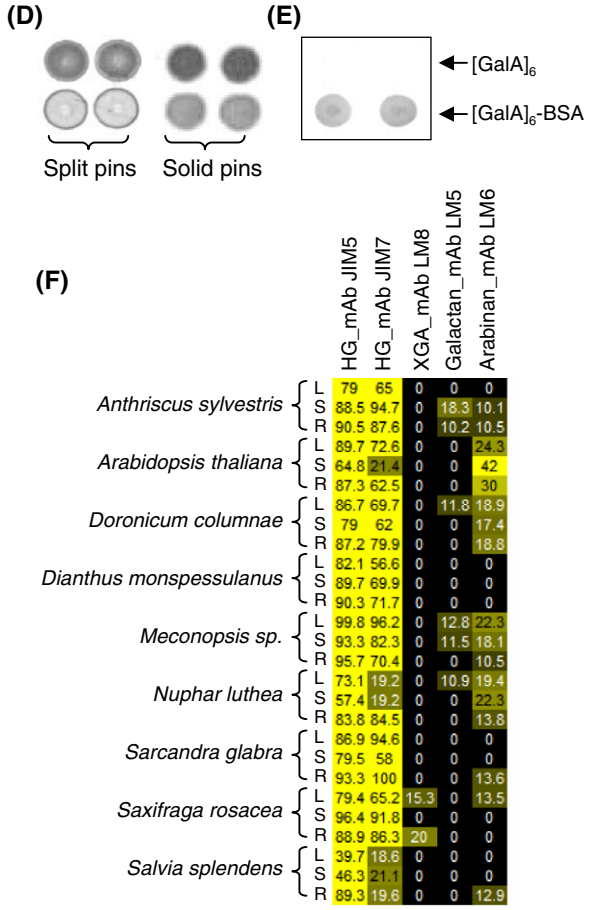

Fig. 2 (*continued*)

microarrays is exemplified by the Comprehensive Microarray Polymer Profiling (CoMPP) technique described below.

4. Printing and immobilization

Pectin microarrays are printed using robot microarray printers that take samples from a multi-well source plate (usually 96 or 384 wells) and deposit a small volume (usually nano-or picolitre amounts) onto the surface of a slide or membrane (Fig. 2). All pectin microarrays to date have been made using pin-based microarray technology, in which either solid or split fine metal pins are used. Split pins usually incorporate a small reservoir (much like the nib of a fountain pen) and this, to some extent, defines the amount of liquid that is taken from the source plate and subsequently spotted. Nevertheless, pin-based-technology does not allow spot volumes to be precisely controlled, especially since pectins typically form rather viscous solutions and the amount of liquid adhering to the outside of pins cannot easily be regulated. An alternative microarray technology based on piezo-electric inkjet technology has recently been developed (http://www.arrayjet.co.uk/), and initial studies suggest that this is an effective method of producing pectin microarrays with consistent spot morphologies (data not shown).

Whatever the printing technology used, pectins can be immobilized either covalently or non-covalently onto slides or membranes. Covalent attachment can be achieved by several linking chemistries, and these have been reviewed by Larsen et al.[22] and Horlacher and Seeberger.[23] Covalent attachment has the advantages that the amount of immobilized molecules can be controlled to some extent, and so can the orientation of the arrayed molecules. However, it has the disadvantage of introducing another step into the microarray procedure which can reduce throughput. Non-covalent attachment has the advantage that samples can be directly spotted, even if they are complex mixtures obtained from plant materials. As with covalent attachment, several different methods for non-covalent attachment have been developed. The first pectin microarrays were made from lime pectins printed directly onto slides made from modified polystyrene.[4] This surface, known as 'MaxiSorp', is identical to that used in the production of many microtitre plates that are widely used for pectin ELISAs and therefore has well-characterized properties with respect to

pectin immobilization. However, most pectin microarrays are currently produced on nitrocellulose membrane which has the advantages of low cost, easy availability and very high binding capacity because of its three-dimensional micro-porous structure. When pectins are printed onto nitrocellulose, spots are generally larger than if glass or plastic slides are used. However, this has the advantages that if microarrays are developed using a colourmetric (rather than fluorescent) system, the quality of microarrays can be assessed by eye, and microarrays can be scanned using a standard flat bed scanner. This allows microarrays to be processed by non-experts using standard laboratory equipment.

When pectins are printed onto nitrocellulose using split pins, the components of the spotted solution migrate away from the point of contact and are separated in a micro-scale chromatographic fashion. The same process occurs on a larger scale when pectins are applied to nitrocellulose in dot-blot assays and the effect has been well characterized.[24] The extent of migration of each pectin molecule is affected by the degree of branching, size and presumably also charge. For example, the migration of highly branched RGI domains is limited, and probing with anti-side chain monoclonal antibodies (mAbs) with specificity for arabinan or galactan therefore typically produces small spots. In contrast, un-branched HG is relatively mobile, and probing with anti-HG mAbs typically produces a ring-shaped spot. Since solutions obtained by extraction from plant tissues contain a mixture of pectic domains with a variety of properties, the result is that each spot is composed of a series of concentric rings. This complex spot morphology is problematic in terms of spot signal quantification, but is also information-rich since each spot is in effect a micro-chromatogram. So far this information has not been utilized, but this may in the future be useful for assessing differences in pectin structure between sets of biological samples. Post-spotting separation is largely avoided if solid rather than split pins are used, or when pectic oligosaccharides are coupled to a protein carrier (Fig. 2D and E). One limitation of non-covalent attachment is that it is generally not effective for immobilizing small oligosaccharides of less than around 5–10 sugar residues. In order to overcome this, we have recently produced microarrays made from oligosaccharides coupled by reductive amination to bovine serum albumin (BSA) (Fig. 2E**).**

5. Detection of microarrayed pectins

The development of pectin microarrays has been possible because a relatively large panel of anti-pectin mAbs has been developed over the last two decades. The repertoire of mAbs available and their specificities have been reviewed by Knox,[25–27] but it is nevertheless useful to consider some general points. Most anti-pectin mAbs are highly specific for certain epitopes that generally contain 3–8 sugar residues. However, the extent to which epitopes have been identified varies widely and this is related to the procedure used for antibody production. Many anti-pectin mAbs have been produced using an immunogen containing a mixture of polysaccharides, and specificities were established by subsequent screening against a selection of oligo- and polysaccharides. This process creates the possibility that several mAbs with different specificities can be produced at the same time, but the disadvantage that retrospectively assigning specificities can be time consuming and may be impossible if appropriate well-defined oligo- and polysaccharides are not available. Nevertheless, this approach has been extended recently by the use of microarrays for high throughput retrospective screening (see also Section 6). An alternative strategy is to immunize with a single oligosaccharide coupled to protein to yield an immunogenic neo-glycoprotein. For example, this procedure has been applied to produce highly specific mAbs against pectic arabinan and galactan.[28,29]

All hybridoma-based antibody production is limited by the requirement for immunogenicity, and it has proven to be difficult to obtain mAbs to certain pectic epitopes, even after immunization with neo-glycoproteins. Phage display antibody technology is a possible route to overcome this problem, since using this technique antibodies are produced entirely in vitro, and antibodies against HG and RGII have been produced using this approach.[30,31] The anti-HG mAb PAM1 binds to a long, probably conformational, epitope consisting of stretches of non-esterified HG, and this specificity is distinct from the anti-HG mAbs produced by hybridoma technology. This demonstrates that phage display can be used to extend the repertoire of anti-pectin mAbs but additional methods are also emerging. One promising approach developed by Mats Ohlin and colleagues[32] uses carbohydrate binding modules (CBMs) as a scaffold onto which diversity is built by mutagenesis. The resulting highly diverse libraries contain around 1.6×10^6 variant CBMs, and using appropriate screening methods it is possible to generate CBMs with specificities against a wide range of target glycans. This method has been successfully used to generate probes against xyloglucan and may well be a valuable tool for the production of anti-pectin probes. Whatever the type of probe used, the binding to arrayed samples must be detectable and this is accomplished using either fluorescent or enzyme-linked tags, coupled directly to the mAbs or CBMs or via secondary antibodies. In both cases, spot signals can be quantified using microarray analysis software of the type also used to quantify nucleotide or protein microarrays, for example, ImaGene 6.0 (BioDiscovery, El Segundo, USA). Once quantified, large pectin microarray data sets can be presented in various ways. For example, scatter plots provide an overview of the level of variance between two sets of samples, and heatmaps are a convenient way of presenting values for each sample in large data sets (Fig. 2F).

6. Defined glycan microarrays for antibody screening

Microarrays are ideally suited for the high throughput analysis of antibody specificities and are particularly useful for assigning specificities to a large panel of mAbs with partially defined specificities. This approach is illustrated by one study in which antibodies were produced following immunization with a crude plant cell-wall extract containing polysaccharides extracted with the calcium chelator diamino-cyclo-hexane-tetra-acetic-acid (CDTA).[33] After initial screening to determine binding to plant material *per se*, a custom-made microarray of cell-wall polysaccharides was used to rapidly obtain information about mAb binding. An innovative aspect of this work is that cluster analysis was used to compare the binding profiles of the newly produced mAbs with the profiles for previously described and well-characterized mAbs, and this enabled mAbs specificities to be assigned to antigen classes. For example, one mAb (designated LM13) had a binding profile that closely clustered with that of two mAbs, LM5 and LM6, with known specificities for (1→4)-β-D-galactan and (1→5)-α-L-arabinan, respectively and LM13 was subsequently demonstrated to bind to a novel arabinan epitope.[33] So far, pectin microrrays have not been used to their full capacity for antibody screening. Most screens to date have involved tens of antibodies tested against microarrays consisting of tens of potential targets. In fact, using pectin microarray technology in its current state, it would be feasible to screen thousands of antibodies against thousands of samples within a reasonable time frame (~2 weeks). The limiting factors are lack of well-defined pectin targets and, to a lesser extent, a lack of antibodies to screen. Nevertheless, there are a large number of cell-wall-directed mAbs for which specificities have not been defined, and microarrays are likely to have a role in assigning their specificities.

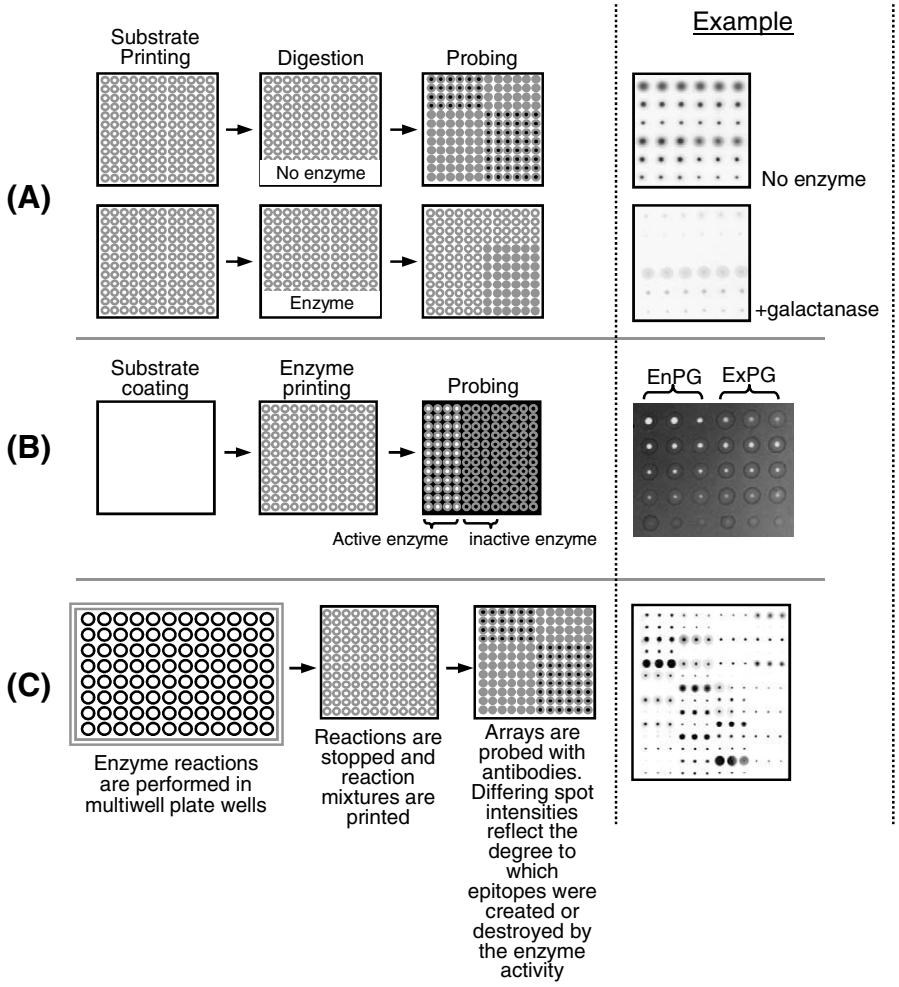

Figure 3. Pectin microarrays for enzyme screening. Pectin microarrays have been used for the analysis of pectinolytic enzymes in three different ways. (A) with printed substrate microarrays, enzymes are used to digest a panel of pectin substrates post spotting in situ, and the effects of enzyme activity are monitored by a decrease in antibody binding as epitopes are modified or destroyed. The example (right) shows a microarray of lime pectin samples that were incubated with galactanase (bottom) or buffer (top). The effect of the enzyme digestion was revealed by probing with a monoclonal antibody with specificity for (1→4)-β-D-galactan. (**B**), with printed enzyme microarrays, a whole slide or membrane is coated with a single substrate, and if probed with a substrate-specific mAb the entire surface is developed and produces a fluorescent or colourimetric signal. However, if enzymes are spotted onto the substrate prior to probing, they act upon the substrate and probing then reveals blank areas in the substrate coating where epitopes have been degraded. In the example (right), the whole membrane was coated with lime pectin prior to spotting with either endopolygalacturonase (EnPG) or exopolygalacturonase (ExPG). The effect of the enzyme digestion was revealed by probing the microarray with an mAb with specificity for HG epitopes within the coating substrate. (C) A third strategy is based on digestion in solution rather than in situ, and small scale enzyme reactions are performed in wells of microtitre or multi-assay plates. Aliquots of the enzyme/substrate solution are then spotted as microarrays and again, the effects of enzyme activity are assessed by changes in antibody binding to the arrayed samples. In the example shown (right), the effects of pectin lyase digestion on lime pectins with different DEs are revealed by the different spot signals obtained after probing with an mAb with specificity for HG.

7. Defined glycan microarrays for pectinolytic enzyme analysis

Pectins are modified *in muro* by a large number of pectinolytic enzymes, and this post-synthesis modification enables the functionality of pectin polymers to be fine-tuned in response to prevailing functional requirements. Plant pathogenic microbes also produce a battery of pectin degrading enzymes in order to breach the cell-wall defensive barrier. Many of these plant and microbial enzymes have been recruited by pectin producers to modify and improve the functional properties of pectins, or in certain cases to remove or degrade pectins during food production.[7,8] The number of pectin-active enzymes available in nature with potential industrial uses is huge, but most remain unused because their activities have not been characterized in detail. The repertoire of potentially useful enzymes is further expanded when one considers the scope for improvement by mutagenesis.

Glycan microarrays have been shown to be of value for characterizing enzyme activities,[34,35] and pectin microarrays have an important role to play in the high throughput screening of pectinolytic enzymes. Pectin microarrays can be used for this purpose in at least three different ways (Fig. 3). First, enzymes can be used to digest a panel of pectin substrates in situ, and the effects of enzyme activity are monitored by a decrease in antibody binding as epitopes are modified or destroyed (Fig. 3A). The feasibility of this approach was demonstrated by the simultaneous digestion of a range of lime pectins by pectin lyase, and the consequent change in the binding of the anti-HG mAbs.[36] This method is most applicable where there is a requirement to screen a limited number of enzymes against a large number of substrates. A second strategy is to spot not substrates but enzymes (Fig. 3B). In this approach a whole slide or membrane is coated with a single substrate, and if probed with a substrate-specific mAb the entire surface is

developed and produces a fluorescent or colourimetric signal. However, if enzymes are spotted onto the substrate prior to probing, and they act upon the substrate, probing then reveals blank areas in the substrate coating where epitopes have been degraded. This strategy is most applicable where there is a need to screen a large number of enzymes against a limited number of substrates. A third strategy is based on digestion in solution rather than in situ, and small scale enzyme reactions are performed in wells of a microtitre or multi-assay plate (Fig. 3C). Aliquots of the enzyme/substrate solution are then spotted as microarrays and again, the effects of enzyme activity are assessed by changes in antibody binding. This method has the advantages that the digestion environment in terms of temperature, pH and buffer composition can be varied, and that reactions can be followed over time. We have used this strategy to gain insights into the activity of PME from *Erwinia chrysanthemi*. A series of 99 mutated versions (covering seven amino acids) of the PME were created and activities tested against a series of lime pectin samples. The reaction mixtures were printed as microarrays and probed with anti-HG antibodies. The resulting profiles provided new information into the roles of the selected amino acids in determining the activity of this PME (data not shown).

8. Extracted glycan microarrays for analyzing relative abundance of pectic epitopes in plants

Extracted glycan microarrays constructed from pectin-rich extractions from plant materials also have an important role to play in understanding pectin biology. One example of this is the CoMPP (Comprehensive Microarray Polymer Profiling) technique.[37] With CoMPP, cell-wall components are sequentially extracted using a series of solvents that solubilize the major wall polymers. For example, CDTA, NaOH and Cadoxen (cadmium oxide with diaminoethane) are routinely used to yield fractions rich in pectins, hemicelluloses and cellulosic polymers, respectively. Typically, extractions are obtained from a series of different plant materials, for example, a range of different organs, different species or a population of mutant plants. The extractions are spotted (usually in replicate and as series of dilutions) and the resulting microarrays are probed with antibodies or CBMs. The aim of the technique is to rapidly obtain a profile of cell-wall polysaccharide composition. CoMPP is very high throughput and the relative levels of 10–20 polysaccharides in several thousand samples can be assessed in around a week. However, the technique is semi-quantitative because the extractions are not necessarily equally effective for all the samples, and because different mAb and CBM probes have different avidities for their respective epitopes. Therefore, the relative abundance of a certain epitope can be compared between samples, but the abundance of epitopes recognized by different probes cannot be directly compared. Nevertheless, CoMPP is a powerful new tool for the high throughput screening of cell-wall polysaccharides, including pectins in diverse plant materials, and can direct the focus of other lower throughput techniques that provide additional and quantitative information. So far, CoMPP has been used primarily to explore the biological rather than the commercial aspects of pectins. For example, CoMPP was used to characterize cell-wall, including pectin, composition in the model species *Physcomitrella patens* (a moss)[37] and *Selaginella moellendorffi* (a spikemoss, http://wiki.genomics.purdue.edu/index.php/Cell_wall_composition_and_glycosyltransferases_involved_in_cell_wall_formation). CoMPP was also used in a study designed to map cell-wall diversity across the plant kingdom, and one of the findings of this study was that the horsetail species *Equisetum arvense* has highly unusual cell walls that are rich in both (1→3)(1→4)-β-D-glucan and HG.[38] However, CoMPP-based approaches also have considerable potential for the analysis of pectins for commercial applications. For example, it is easy to envisage how CoMPP could be used to rapidly and simultaneously assess the levels of multiple pectic epitopes in large numbers of pectin source samples, such as different batches of lemon and lime pulp. This detailed knowledge may then be useful for refining subsequent processing.

9. Concluding remarks

The value of microarrays for the analysis of pectins has been demonstrated but their full potential has not yet been realized, and the sourcing of well-defined pectins to populate microarrays remains a barrier to progress. However, significant recent progress has been made in the development of efficient chemical coupling methods, and this should enable ever smaller amounts of sample to be reliably immobilized. This is important because a goal for the future will be to integrate nano-scale separation of pectin fractions with parallel structural analysis and microarray construction. The development of more sophisticated and comprehensive pectin microarrays is likely to extend the range of their applications, for example, in the area of pectin–protein interactions.

References

1. Schena, M.; Shalon, D.; Davis, R. W.; Brown, P. O. *Science* **1995**, *270*, 467–470.
2. MacBeath, G.; Schreiber, S. L. *Science* **2000**, *289*, 1760–1763.
3. Fukui, S.; Feizi, T.; Galustian, C.; Lawson, A. M.; Chai, W. *Nat. Biotechnol.* **2002**, *2010*, 1011–1017.
4. Willats, W. G.; Rasmussen, S. E.; Kristensen, T.; Mikkelsen, J. D.; Knox, J. P. *Proteomics* **2002**, *2*, 1666–1671.
5. Wang, D.; Liu, S.; Trummer, B. J.; Deng, C.; Wang, A. *Nat. Biotechnol.* **2002**, *20*, 275–281.
6. Willats, W. G.; McCartney, L.; Mackie, W.; Knox, J. P. *Plant Mol. Biol.* **2001**, *47*, 9–27.
7. Pilnik, W. In *Gums and Stabilizers for the Food Industry*; Phillips, G. O., Williams, P. A., Wedlock, D. J., Eds.; Oxford University Press: Oxford, 1990; pp 313–326.
8. Willats, W. G. T.; Knox, J. P.; Mikkelsen, J. D. *Trends Food Sci. Technol.* **2006**, *17*, 97–104.
9. O'Neill, M.; Albersheim, P.; Darvill, A. In *Methods in Plant Biochemistry 2*; Dey, P. M., Ed.; Academic Press: London, 1990; pp 415–441.
10. Mohnen, D. *Curr. Opin. Plant Biol.* **2008**, *11*, 266–277.
11. Mort, A.; Zheng, Y.; Qiu, F.; Nimtz, M.; Bell-Eunice, G. *Carbohydr. Res.* **2008**, *19*, 1212–1221.
12. Willats, W. G.; McCartney, L.; Steele-King, C. G.; Marcus, S. E.; Mort, A.; Huisman, M.; van Alebeek, G. J.; Schols, H. A.; Voragen, A. G.; Le Goff, A.; Bonnin, E.; Thibault, J. F.; Knox, J. P. *Planta* **2004**, *218*, 673–681.
13. Longland, J. M.; Fry, S. C.; Trewavas, A. J. *Plant Physiol.* **1989**, *90*, 972–976.
14. Hart, D. A.; Kindel, P. K. *Biochem. J.* **1970**, *116*, 569–579.
15. Vidal, S.; Doco, T.; Williams, P.; Pellerin, P.; York, W. S.; O'Neill, M. A. *Carbohydr. Res.* **2000**, *326*, 277–294.
16. O'Neill, M. A.; Warrenfeltz, D.; Kates, K.; Pellerin, P.; Doci, T.; Darvill, A. G.; Albersheim, P. *J. Biol. Chem.* **1996**, *271*, 22923–22930.
17. Feizi, T.; Fazio, F.; Chai, W.; Wong, C. H. *Curr. Opin. Struct. Biol.* **2003**, *13*, 637–645.
18. Feizi, T.; Chai, W. *Nat. Rev. Mol. Cell. Biol.* **2004**, *5*, 582–588.
19. Clausen, M. H.; Madsen, R. *Chemistry* **2003**, *18*, 3821–3832.
20. Clausen, M. H.; Willats, W. G.; Knox, J. P. *Carbohydr. Res.* **2003**, *12*, 797–800.
21. Guillaumie, F.; Sterling, J. D.; Jensen, K. J.; Thomas, O. R.; Mohnen, D. *Carbohydr. Res.* **2003**, *10*, 1951–1960.
22. Larsen, K.; Thygesen, M. B.; Guillaumie, F.; Willats, W. G.; Jensen, K. J. *Carbohydr. Res.* **2006**, *341*, 1209–1234.
23. Horlacher, T.; Seeberger, P. H. *OMICS* **2006**, *10*, 490–498.
24. Willats, W. G.; Knox, J. P. *Anal. Biochem.* **1999**, *268*, 143–146.
25. Knox, J. P. *Curr. Opin. Plant Biol.* **2008**, *11*, 308–313.
26. Knox, J. P. *Int. Rev. Cytol.* **1997**, *171*, 79–120.
27. Knox, J. P. In *Gums and Stabilisers for the Food Industry 13*; Williams, P. A., Phillips, G. O., Eds.; RSC Publishing: Cambridge, 2006; pp 33–40.
28. Willats, W. G.; Marcus, S. E.; Knox, J. P. *Carbohydr. Res.* **1998**, *308*, 149–152.
29. Jones, L.; Seymour, G. B.; Knox, J. P. *Plant Physiol.* **1997**, *113*, 1405–1412.
30. Willats, W. G.; Gilmartin, P. M.; Mikkelsen, J. D.; Knox, J. P. *Plant J.* **1999**, *18*, 57–65.
31. Williams, M. N. V.; Freshour, G.; Darvill, A. G.; Albersheim, P.; Hahn, M. G. *Plant Cell* **1996**, *8*, 673–685.
32. Cicortas Gunnarsson, L.; Montanier, C.; Tunnicliffe, R. B.; Williamson, M. P.; Gilbert, H. J.; Karlsson, E. N.; Ohlin, M. *Biochem. J.* **2007**, *406*, 209–214.

33. Moller, I.; Marcus, S. E.; Haeger, A.; Verhertbruggen, Y.; Verhoef, R. P.; Schols, H. A.; Ulvskov, P.; Mikkelsen, J. D.; Knox, J. P.; Willats, W. G. T. *Glycoconjugate J.* **2007**, *25*, 37–48.
34. Laurent, N.; Voglmeir, J.; Wright, A.; Blackburn, J.; Pham, N. T.; Wong, S. C.; Gaskell, S. J.; Flitsch, S. L. *Chembiochem* **2008**, *9*, 883–887.
35. Shipp, M.; Nadella, R.; Gao, H.; Farkas, V.; Sigrist, H.; Faik, A. *Glycoconjugate J.* **2008**, *25*, 49–58.
36. Øbro, J.; Sørensen, I.; Moller, I.; Skjøt, M.; Mikkelsen, J.; Willats, W. G. T. *Carbohydr. Polym.* **2007**, *70*, 77–81.
37. Moller, I.; Sørensen, I.; Bernal, A. J.; Blaukopf, C.; Lee, K.; Øbro, J.; Pettolino, F.; Roberts, A.; Mikkelsen, J. D.; Knox, J. P.; Bacic, A.; Willats, W. G. *Plant J.* **2007**, *50*, 1118–1128.
38. Sørensen, I.; Pettolino, F. A.; Wilson, S. M.; Doblin, M. S.; Johansen, B.; Bacic, A.; Willats, W. G. *Plant J.* **2008**, *54*, 510–521.