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Carbohydrate Polymers 71 (2008) 640–647
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Atomic force microscopy of tomato and sugar beet pectin molecules

Andrew R. Kirby, Alistair J. MacDougall [1], Victor J. Morris *

Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK

Received 9 February 2007; received in revised form 10 July 2007; accepted 16 July 2007
Available online 26 July 2007

Abstract

Atomic force microscopy has been used to image the structure of pectin molecules isolated from unripe tomato and sugar beet tissue. The tomato pectin molecules were found to be extended stiff chains with a weight average contour length of $L_W = 174$ nm and a number average contour length of $L_N = 132$ nm ($L_W/L_N = 1.32$). A proportion of the pectin molecules (~30%) were found to be branched structures. Chemical analysis of the sugar beet pectin extracts showed that the samples contained protein (8.6%). This protein proved difficult to remove and is believed to be covalently attached to the polysaccharide. Imaging of the extracted pectin revealed largely un-aggregated chains: a small fraction (33%) of which were extended stiff polysaccharide chains and a major fraction (67%) of which were of polysaccharide–protein complexes containing a single protein molecule attached to one end of the polysaccharide chains ('tadpoles'). In addition the sample contained a small number of aggregated structures. The un-aggregated pectin molecules were found to be predominately linear structures with a small fraction (~17%) of branched structures. The branched structures were all in the free polysaccharide fraction and no branched pectin chains were observed in the protein–polysaccharide complexes. Alkali treatment was found to remove the protein. For the alkali-treated, un-aggregated structures the average contour lengths were found to be $L_W = 137$ nm, $L_N = 108$ nm with $L_W/L_N = 1.27$. It is proposed that the 'tadpole' structures contribute to the unusual emulsifying properties of sugar beet pectin.

Keywords: Tomato pectin; Sugar beet pectin; AFM; Atomic force microscopy

1. Introduction

Pectin polysaccharides consist mainly of a predominately linear backbone of galacturonic acid (homogalacturonan) which may contain rhamnogalacturonan 1 (RG1) units (O'Neil and York, 2003; Ridley, O'Neil, & Mohnen, 2001). The homogalacturonan backbone is composed of (1 → 4) linked α-D-galacturonic acid residues (Gal*p*A) in which some of the carbonyl groups are methyl-esterified. Sugar beet pectin is partially O-acetylated. In general, O-acetylation of homogalacturans occurs at C-2 or C-3. RG1 units consist of a repeating disaccharide [→4)-α-D-Gal*p*A-(1 → 2)-α-L-Rha*p*-(1-)] of galacturonic acid and rhamnose (Rha*p*) residues. The backbone Gal*p*A residues may be O-acetylated at C-2 and/or C-3 although there is generally no evidence that these residues contain methyl esters. A proportion of the Rha*p* residues contain neutral sugar sidechains comprising α-L-arabinose (Ara*f*) and/or β-D-galactose (Gal*p*) residues. Generally the Gal*p*A residues are unsubstituted, but it has been reported that there is attachment of single β-D-Glc*p*A residues at C-3 of ~2% of the Gal*p*A residues in the RG1 from sugar beet (Renard, Crepeau, & Thibault, 1999). Sugar beet pectin also contains ferulic acid esters linked at C-2 of the arabinose and at C-6 of the galactose in the neutral sugar sidechains (Fry, 1983; Saulnier & Thibault, 1999). After saponification of sugar beet pulp enzymatic digests dehydrodiferulic acid linkages have been detected and identified as 8–5', 5–5', 8–8' and 8-O-4'. This has been taken to indicate the cross-linking of pectin chains via diferulic acid linkages between neutral sugar sidechains (Saulnier & Thibault, 1999).

* Corresponding author. Tel.: +44 0 1603 255271; fax: +44 0 1603 507723.
E-mail address: vic.morris@bbsrc.ac.uk (V.J. Morris).
[1] Present address: National Starch and Chemical Company, 10 Finderne Avenue, Bridgewater, New Jersey 08807-3300, USA.

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doi:10.1016/j.carbpol.2007.07.014

Commercial extracts of apple and citrus pectin are reported to contain detectable levels of hydroxyproline-rich protein. The quoted protein contents (Kravchenko, Voragen, & Pilnik, 1992) are ~1.6% in apple pectin and ~3–3.3% in citrus pectin. The protein content of sugar beet pectin appears to be sensitive to the details of extraction and purification and has variously been reported at about 2% (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003), 3.7% (Williams et al., 2005) and as high as ~10.4% (Thibault, 1988). It has been proposed that the protein content is responsible for the emulsifying action of sugar beet pectin (Leroux et al., 2003; Williams et al., 2005). Leroux and co-workers (2003) proposed that the protein component is adsorbed at the oil–water interface leaving loops and tails of polysaccharide in the aqueous phase (Fig. 1). It was considered that bridging flocculation could occur through calcium-induced aggregation of pectin chains, and that the acetyl content of the pectin may reduce the calcium sensitivity (Leroux et al., 2003).

Although it has been reported (Ridley, O'Neil, & Mohnen, 2001) that there is no strong evidence for covalent links between pectin and glycoproteins, there are suggestions that such links may indeed occur. Recently it has been implied that arabinogalactan proteins are linked to pectin extracted from hops (Oosterveld, Voragen, & Schols, 2002) or carrots (Immerzeel, Eppink, de Vries, Schols, & Voragen, 2006). Extensins are also considered to form links with pectin molecules, possibly through a type II pectic arabino-3,6-linked galactan (Keegstra, Talmadge, Bauer, & Albersheim, 1973), or a phenolic cross-link from a feruloylated sugar in sugar beet pectin to an amino acid in the extensin protein. Fragments of extensin protein from cell walls of cotton suspension cultures, co-purified with RG1 following trypsin digestion, were found to run independently on an SDS–PAGE gel only after fluoride deglycosylation. This was taken to suggest a covalent association between the RG1 and the extensin protein (Qi, Behrens, West, & Mort, 1995).

Atomic force microscopy (AFM) provides a relatively new technique for characterising polysaccharides and polysaccharide–protein interactions. The technique has been used to image cell wall polysaccharides (Adams, Kroon, Williamson, & Morris, 2003; Adams, Kroon, Williamson, & Morris, 2005; Gunning et al., 2000; Morris, Ring, MacDougall, & Wilson, 2003; Round, MacDougall, Ring, & Morris, 1997; Round, Rigby, Ring, & Morris, 2001) to characterise the contour length distribution of polysaccharides (Ridout, Brownsey, Gunning, & Morris, 1998), to study polysaccharide association (Gunning, Kirby, Ridout, Brownsey, & Morris, 1996) and to detect branching of polysaccharide structures (Adams et al., 2003, 2005; Gunning et al., 2000, 2003; Morris et al., 2003; Ovodova et al., 2006; Round et al., 1997, 2001). Recently it has been shown that AFM can be used to investigate polysaccharide–protein interactions (Adams, Kroon, Williamson, Gilbert, & Morris, 2004; Morris, Gunning, Faulds, Williamson, & Svensson, 2005). This has been used to characterise heterogeneous cell wall polysaccharides (Adams et al., 2004) and to investigate the role of the starch-binding domain in the digestion of crystalline starch by glucoamylase (Morris et al., 2005).

In this article, we have used AFM to visualise the structures of pectin molecules extracted from tomato and sugar beet cell wall material. In particular, we have used the technique to assess suggestions of covalent linkages between protein and polysaccharide, particularly for the sugar beet pectin.

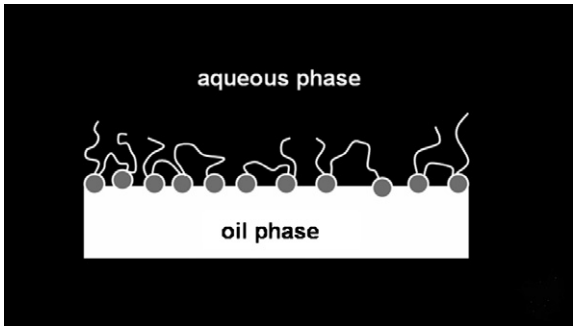

Fig. 1. Schematic illustration of the model for the adsorption of sugar beet pectin at an oil–water interface as proposed by Leroux et al. (2003).

2. Materials and methods

2.1. Extraction of tomato pectin

The preparation of cell walls from unripe tomatoes (*Lycopersicon esculentum*) and the extraction of a CDTA-soluble pectic polysaccharide both used the methods described by MacDougall, Rigby, and Ring (1996). The aqueous extract was stored at 4 °C (with 0.5% by volume $CHCl_3$ as an anti-microbial agent) at a concentration of 1 mg ml^{-1}, until required.

2.2. Extraction of sugar beet pectin

Freshly harvested sugar beet roots (variety Wildcat) were peeled and sliced into 1 cm cubes. About 200 g of tissue were added to a Waring blender (Waring laboratory, Torrington, CT, USA) containing 800 ml phenol saturated with Tris buffer (prepared by equilibration of phenol with 0.5 M Tris HCl pH 8). The blender was run at slow speed for 1 min and at maximum speed for 2 min. Cell wall material (CWM) was recovered on muslin, re-extracted twice with buffer-saturated phenol, and then washed with water 10 times. The pH was adjusted to 6.0 by addition of CH_3CO_2H, and the CWM was ball-milled for 48 h at 120 rpm in distilled water. CWM was recovered by centrifugation at 3500*g*, followed by two washings with four volumes of distilled water. The CWM was stirred overnight in 100 mM NaCl, 50 mM CH_3CO_2Na, pH 4.5, 0.02% NaN_3; CWM was recovered by centrifugation, and washed once with water.

To extract the pectin a suspension of the CWM in 1 litre of distilled water was adjusted to pH 1.5 with HCl, and was stirred for 1 h, followed by centrifugation. CH_3CO_2Na (10 mM) was added to the recovered supernatant and the pH was adjusted to 4.5 with NaOH. The material liberated from the cell wall was dialysed under pressure with distilled water in a circulating system with a 10 K molecular weight cut off membrane (Amicon CH2; H1P10-20 fibre cartridge).

The pectin samples were stored as aqueous solutions in order to avoid possible aggregation induced on freeze-drying, with 0.5% (by volume) $CHCl_3$ added to the aqueous samples as an anti-microbial agent.

2.3. Chemical and physicochemical characterisation of pectin samples

All samples for sugar analysis were freeze-dried and then dispersed in 72% H_2SO_4 for 3 h at room temperature. After dilution to 2N H_2SO_4, samples were hydrolysed for 1 h at 100 °C, prior to colourimetric assay (Blumenkrantz & Asboe-Hansen, 1973) of total uronic acid, and for 2.5 h at 100 °C prior to the assay of neutral sugars by reduction, acetylation and analysis (Blakeney, Harris, Henry, & Stone, 1983; Englyst & Cummings, 1984) by gas-chromatography. The protein contents of the extracted pectins were measured using the Lowry method (Lowry, Roseborough, Farr, & Randall, 1951) with BSA as the protein standard. Viscosities of the tomato pectin were measured in the presence of 100 mM NH_4Cl after adjustment of the pH to 6.5 with ammonia. For the viscosity measurements on the sugar beet pectin, the samples were prepared in 100 mM NaCl, 50 mM CH_3CO_2Na, with the pH adjusted to 4.5. The viscosities were measured using Ubbelholde viscometers at 25 °C. Specific viscosities (η_{sp}) were determined as $(\eta-\eta_O)/\eta_O$, where η and η_O are the measured viscosities for the sample solution and solvent, respectively. The intrinsic viscosity $[\eta]$ was determined by extrapolation of η_{sp}/C against the sample concentration (C).

2.4. Atomic force microscopy

The atomic force microscope used in this study was manufactured by East Coast Scientific Limited (Cambridge, UK). The cantilevers were the short, narrow variety from the 'nanoprobe' range. The quoted spring constants for these cantilevers are 0.38 Nm^{-1}.

Samples were drop-deposited (2–3 μl of 5 μg ml^{-1} solution) from buffer (10 mM NH_4HCO_3) onto freshly cleaved mica and allowed to stand in air before imaging. The samples were than imaged under redistilled butanol. For the sugar beet pectin it was found necessary to drop-deposit the pectin solutions onto freshly cleaved mica at 35 °C, in order to promote even spreading and to reduce molecular aggregation. This temperature was maintained using a heating stage, Babraham Tech^{nix} (Cambridge, UK). After a time interval of a few minutes, the deposit was completely dry and any remaining buffer was sublimed off before imaging.

In later experiments some of the sugar beet pectin sample was treated with alkali (0.2 M KOH, overnight and at room temperature). These samples were than neutralised with 0.2 M HCl, dialysed and diluted into 10 mM NH_4HCO_3 buffer to a concentration of approximately 5 μg ml^{-1}. These samples were drop-deposited onto freshly cleaved mica and imaged under redistilled butanol.

3. Results and discussion

The carbohydrate analysis of the CDTA extract from unripe tomato cell walls is shown in Table 1. The results are similar to those obtained previously (MacDougall, Rigby, & Ring, 1997; MacDougall et al., 1996; Round et al., 2001; Seymour, Colquhoun, DuPont, Parsley, & Selvendran, 1990) and are consistent with data published for CDTA pectin extracts (MacDougall et al., 1996, 1997; Round et al., 2001). The protein levels were <0.5% and the measured intrinsic viscosity was $[\eta] = 810$ ml g^{-1}.

In the case of sugar beet, in order to obtain relatively pure samples of CWM, the cell walls were isolated from fresh sugar beet using a protocol which is effective at removing cellular components. To extract relatively undegraded pectic polysaccharides from the primary plant cell walls, chelating agents (e.g. CDTA) are commonly used. However, in preliminary experiments relatively low yields of pectic polysaccharides were obtained from sugar beet CWM with this treatment. Commercial extraction of pectin from a variety of plant sources is generally carried out with hot water acidified with nitric acid. Therefore, attempts were made to extract pectin from sugar beet cell walls under acidic conditions. However, the extraction was carried out at room temperature, since a high yield of pectin was considered less important than the recovery of the pectin in a relatively un-degraded form.

The pectin extracted under these conditions was obtained in reasonable yield (~6% of the CWM). The composition of this material is given in Table 1 and is similar to the reported composition for pectic polysaccharides isolated previously from sugar beet (Kirby, MacDougall, & Morris, 2006; Leroux et al., 2003; Thibault, 1988; Williams et al., 2005). The level of protein present in the sugar beet pectin extract (Table 1) was high compared to tomato pectin, but was similar to the highest values (~10%) found previously for sugar beet pectin (Thibault, 1988). An attempt was made to further purify the pectic polysaccharides; a sample was passed down a cation ion exchange column (Dowex AG50W x8) at pH 2. This treatment only reduced the protein content to 7%. Only alkali treatment was found to completely remove associated protein. The measured intrinsic viscosity of the sugar beet extract (with associated protein) was found to be 216 ml g^{-1}.

Typical AFM images of tomato pectin molecules are shown in Fig. 2. Both topography (Fig. 2a) and error signal mode (Fig. 2b) images are shown. Examples of branched

Table 1
Characterisation of the pectin samples

Sample	Anhydro sugars (μg per mg dry weight)									Protein
	Gal	Ara	Rha	Glc	Xyl	Fuc	Man	Uronic acid	Total sugars	
Tomato	101	29	7	5	1	1	<1	710	854	<0.5%
Sugar beet	69	226	16	<1	<1	<1	<1	453	764	8.6%

molecules are easier to see in the error mode images. Height measurements on the topography images were used to identify true branched structures using methods discussed in previous publications (Round et al., 1997, 2001).

The results obtained are similar to data reported previously (Round et al., 1997, 2001). However, the quality of the images obtained in this study is better and the images of both linear and branched structures are more convincing. The majority of the molecules are stiff, extended linear chains. The stiff, extended structures suggest that the pectin molecules adopt a helical structure when they adsorb onto the mica substrate. Similar behaviour has been observed for arabinoxylan molecules (Adams et al., 2003., 2004, 2005). Time-dependent observations (Gunning et al., 2000) of the desorption of arabinoxylans from mica into a buffered medium are consistent with a helix-coil transition facilitating this process.

A significant fraction (about 30%) of the pectin chains were observed to be branched and examples of a few typical branched pectin molecules are shown arrowed in Fig. 2b. This agrees with previous reported studies on tomato pectin (Morris et al., 2003; Round et al., 1997, 2001) and recent studies on comaruman (Ovodova et al., 2006) . It has been shown (Morris et al., 2003; Round et al., 2001) that mild acid hydrolysis, under conditions that cleaved neutral sugars, did not actually cleave the branches observed by AFM. Prolonged acid hydrolysis, under conditions that cleaved linkages between galacturonic acid residues, led to a reduction of the contour length of the molecules and shortening of the branches. These studies were taken to suggest that the branches are formed from galacturonic acid residues and linked via a branched galacturonic acid residue (Morris et al., 2003; Round et al., 2001). For the comaruman pectin chemical analysis has been taken to imply that branching occurs at the positions C2, C3 or C2,3 on the galacturonic acid backbone (Ovodova et al., 2006). The total contour length distribution of the pectin molecules is shown in Fig. 3. The weight average (L_W) and number average (L_N) contour lengths were found to be 174 nm and 132 nm, respectively, with ($L_W/L_N = 1.32$). Based on a 3_1 helix (Walkinshaw & Arnott, 1981) with a pitch of 1.34 nm, the equivalent molecular weights are $M_W = 62{,}466$ and $M_N = 47{,}388$ with $M_W/M_N = 1.32$.

The sugar beet pectin molecules proved to be more difficult to image than the tomato pectin. It was found that the deposition conditions had to be more strictly controlled in order to obtain reproducible images. If samples were deposited onto mica at room temperature then the material appeared to collect as large aggregates on the substrate. To obtain uniform spreading of the pectin sample over the entire mica substrate it was necessary to place the mica in a heating stage and to deposit material with the mica held at a temperature of 35 °C. Under these conditions the pectin appeared to spread fairly evenly across the mica.

Typical images of sugar beet pectin are shown in Fig. 4. Such images show that about 33% of the un-aggregated molecules appear as extended, stiff chains. Examples of such structures are labelled 's' in Fig. 4. The larger majority (about 67%) of the molecules were protein–polysaccharide complexes, with the protein attached to one end of the polysaccharide chain: these structures have been termed 'tadpoles'. Examples of such complexes are generically labelled 'c' in Fig. 4a. These complexes exhibit a variety of different forms. In some cases the polysaccharide chain is extended and rigid and examples of such structures are labelled 'c_1' in Fig. 4b. In other cases, the polysaccharide chain is completely (c_2) or partially (c_3) wrapped around the protein. Examples of these structures are shown in Fig. 4b. Fig. 5 shows images of individual pectin–protein

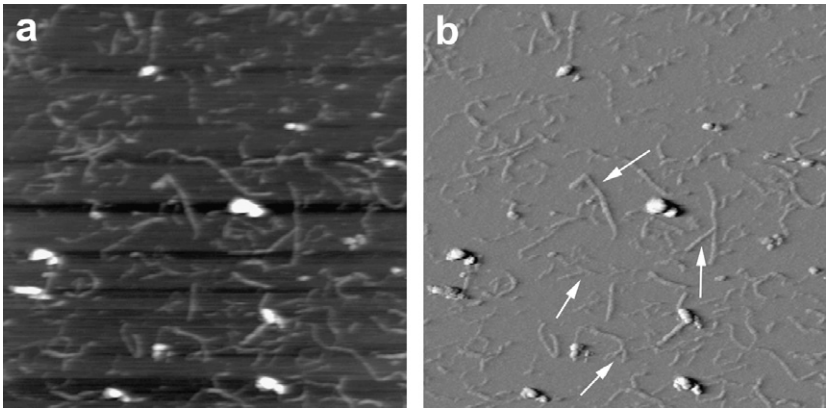

Fig. 2. AFM images of tomato pectin. Image sizes 1 × 1 μm. (a) Topography image. (b) Error signal mode image. The arrows show typical examples of branched pectin molecules.

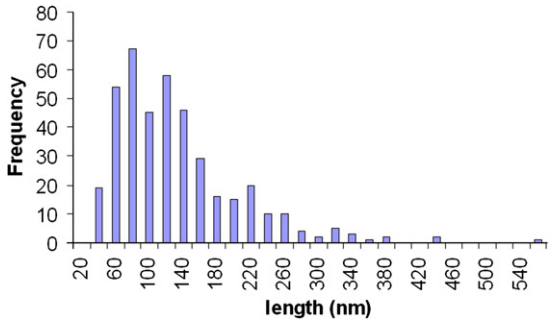

Fig. 3. Histogram of tomato pectin contour lengths.

complexes (tadpoles) illustrating different conformations of the polysaccharides chain. These images provide further support for the idea that the extended pectin chains in contact with the mica adopt a helical conformation: clearly the pectin chains wound around the proteins are more flexible. Probe broadening effects will broaden the images of the pectin chain and the attached protein. The broadening will depend on both the major dimension of the molecule (the radius of the globular protein or the radius of the cylindrical polysaccharide chain) and the radius of curvature of the tip. A more realistic estimate of the size of the objects can be obtained from measurements of the heights of the molecules, although the tip will compress the molecules to some extent. Typically the proteins were found to be ~2.5 nm in height and the pectin chains were found to have heights of ~0.5 nm. For a hemi-spherical tip of radius R and a molecular radius r, the maximum broadened width (w) will be $w \sim 4(rR)^{0.5}$. Thus if the heights are considered as reasonable estimates of the diameter of the molecules then the polysaccharide chains will be more broadened than the protein by a factor of ~2.3.

The images suggest that the complexes contain single proteins attached at the end of each polysaccharide chain. At present it is not known whether the proteins are attached at the reducing or non-reducing ends of the pectin chains. The presence of protein attached to the polysaccharide might account for the need to heat the substrate in order to allow the pectin to spread on the mica. When the sample is drop-deposited onto the mica it will spread across the mica surface. The protein would be expected to concentrate at the air–water interface and, as the solvent evaporates, the complex will be concentrated and the partially denatured protein would be expected to aggregate. This aggregation process which is not seen with the tomato pectin appears to provide preliminary evidence for the self-association of the complexes through protein–protein aggregation. Heating the substrate may evaporate the solvent more uniformly and rapidly, preventing aggregation of the complexes, and thus aid spreading of the pectin on the substrate.

The protein cannot be removed by ion exchange chromatography; suggesting that it is unlikely to be a non-specific ionic complex. However, the protein can be removed by alkali treatment. Fig. 6 shows AFM images of the sample after alkali treatment. The images show stiff extended polysaccharide chains with some freed protein attached to the mica substrate. The fact that free protein is seen in the images suggests that the alkali treatment cleaves the protein–polysaccharide linkage, rather than degrading the protein. The fact that the protein–pectin linkage is alkali-labile would be consistent with an ester linkage and the conditions are sufficiently severe to cleave phenolic linkages. A phenolic linkage could explain why such linkages are seen with sugar beet pectin but not for tomato pectin. However, further studies are needed in order to identify the nature of the linkage and the nature of the protein. Examples of singly branched and multiple branched pectin molecules are indicated by arrows in Fig. 6. The total percentage of branched pectin molecules was found to be about 17%. Interestingly, all the branched pectin appeared to be contained in the fraction free from protein. None of the protein–polysaccharide complexes were found to contain branched pectin chains. The branches are not cleaved

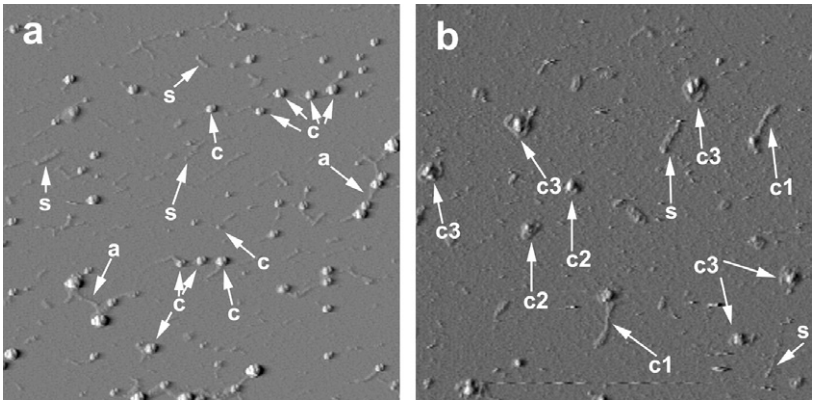

Fig. 4. Error signal mode AFM images of sugar beet pectin. The image sizes are 1 × 1 μm. Examples of individual extended pectin chains are labelled 's' and 'c' indicates protein–polysaccharide complexes. 'Tadpoles': polysaccharide–protein complexes showing individual proteins attached to one end of a pectin chain are labelled 'c1'. Examples of pectin wound tightly 'c2' and loosely 'c3' around the protein are also shown. Aggregates of tadpoles are labelled 'a'.

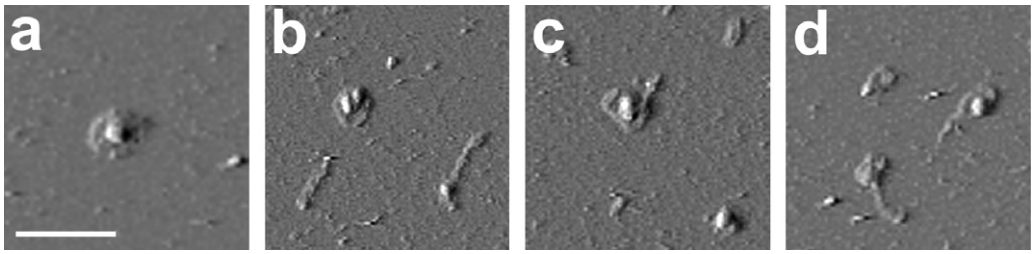

Fig. 5. AFM images of individual protein–polysaccharide complexes showing different conformations of the polysaccharide tail. Error-signal mode images with the scale bar equal to 100 nm. The images correspond to the structures labelled C1–3 in Fig. 4.

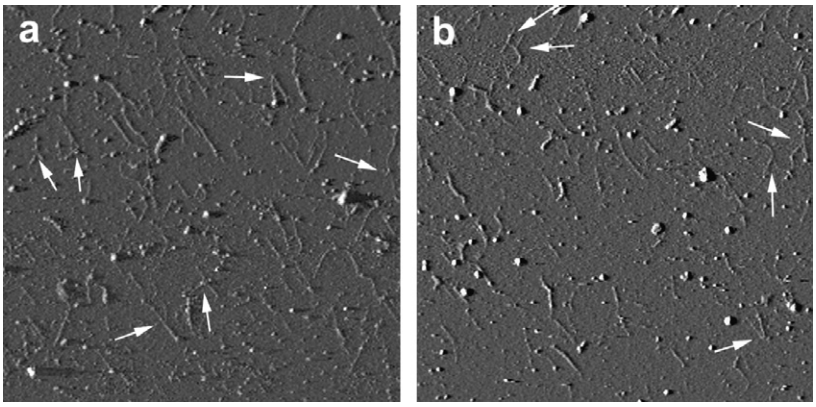

Fig. 6. Error signal mode AFM images of alkali-treated sugar beet pectin. The image sizes are 1×1 μm. Small amounts of protein are present attached to the mica substrate. The protein–polysaccharide complexes have cleaved by the alkali treatment. Arrows indicate examples of branched pectin chains.

by alkali and hence these structures are not aggregates formed by cross-linking of pectin chains through phenolic linkages. By analogy with the data on tomato pectin it is believed that these branched structures represent branching of the polygalacturonic acid backbone.

The contour length distribution for the alkali-treated, un-aggregated molecules is shown in Fig. 7. The data is presented for the alkali-treated material since it would not be possible to measure the chain lengths for the protein–polysaccharide complexes where the chain is wrapped around the protein.

The values of $L_W = 138$ nm, $L_N = 108$ nm with $L_W/L_N = 1.27$ were obtained. If the extended chains adopt the 3_1 helical structure, proposed from modelling of the X-ray fibre diffraction data (Walkinshaw & Arnott, 1981), then the equivalent molecular weights are $M_W = 49{,}542$ and $M_N = 38{,}772$.

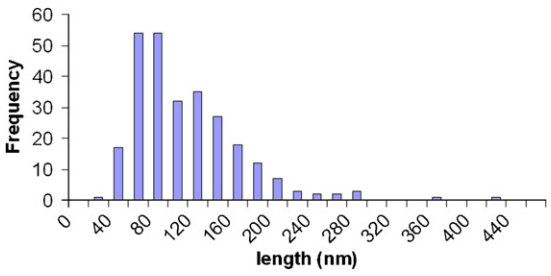

Fig. 7. Histogram of the contour lengths of alkali-treated sugar beet pectin.

The contour length distributions are similar for the tomato and sugar beet pectin. The major difference between the samples is the lower degree of branching and the presence of the protein–polysaccharide complexes in the sugar beet samples. The alkali treatment may lead to some degree of depolymerisation due to β-elimination reactions. Thus the estimated contour lengths may be slightly smaller than for the 'native' material.

The sugar beet pectin samples contained a few aggregates. Two main types of aggregates were observed. One type of aggregate is shown in Figs. 4a and 8a. This type of aggregate seems to consist of 'tadpoles' linked together, presumably through the protein component, into higher molecular weight linear structures. The other type of aggregate structure is shown in Fig. 8b. These are more micelle-like structures, and it is not clear how individual chains are linked in these structures.

It has been proposed (Leroux et al., 2003) that the protein content is responsible for the unusual emulsifying action of sugar beet pectin. Fig. 1 shows the suggested conformation of the sugar beet pectin molecules at the oil–water interface. When this model was proposed, the sites of attachment of protein to the polysaccharide were not known. It was suggested that the protein component adsorbs to the oil–water interface leaving loops and tails of polysaccharide sticking out into the water phase. The loops and tails were considered to prevent coalescence through steric hindrance and to be able to control bridging flocculation through calcium-induced association of the pectin chains.

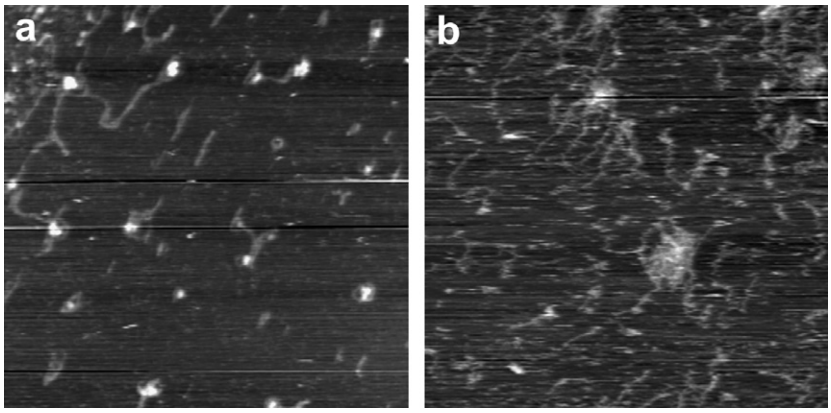

Fig. 8. Topographic AFM images of sugar beet pectin. The images show examples of the types of aggregates seen in the samples. Image sizes 1×1 μm. (a) Examples of linear aggregates of 'tadpoles'. (b) Micellar aggregates.

In a recent study Williams and co-workers (2005) have fractionated beet pectin using hydrophobic affinity chromatography and looked at the relative efficiencies of different fractions as emulsifiers. These studies confirmed that emulsification is influenced by the accessibility of the protein component.

The lower molecular weight 'tadpoles' should absorb more rapidly at the interface but may ultimately be displaced by the larger aggregates of tadpole-like structures. This would be prevented if the protein component of the tadpoles can aggregate at the interface to form an elastic network. The formation of these networks would be particularly important in allowing the proteins to resist competitive displacement by other components (Wilde, Mackie, Husband, Gunning, & Morris, 2004). It remains to be determined whether such interfacial protein–protein aggregation does occur. However, the demonstration of the existence of protein–pectin complexes supports the proposals (Leroux et al., 2003; Williams et al., 2005) that emulsification is determined by the protein content of the sugar beet pectin extracts.

Both the tomato and the sugar beet pectin are extracts from the respective plant cell walls. Thus, the branched structures are likely to be remnants of the original pectin network within the cell wall. For the sugar beet extracts, the nature of the pectin–protein linkages needs to be identified and then considered in models of the pectin network within the cell wall.

The present experiments are consistent with the protein being covalently attached to a fraction (~67%) of the sugar beet pectin chains. The protein component can be removed with alkali treatment suggesting an ester linkage. Further experiments are needed to establish whether this is a phenolic linkage. This would be consistent with previous suggestions that sugar beet pectin may be linked to extensions (Qi et al., 1995) but further studies would be needed to confirm such a proposal. Isolation, purification and characterisation of the protein and the nature of the protein–polysaccharide linkage would help understand the role of the protein within the cell wall.

Possible roles for such protein–polysaccharide complexes could be as primers for synthesis of oligosaccharides and/or polysaccharides, transport mechanisms for oligosaccharides or polysaccharides within the plant tissue, or components involved in cell wall assembly, modification and/or disassembly.

4. Conclusions

The present studies confirm previous reports that pectin molecules extracted from unripe tomato cell walls are not purely linear structures. A fraction (~30%) of the pectin molecules are singly or multiple branched structures. New data has been obtained on the physical structure of sugar beet pectin. A main fraction (~67%) of un-aggregated extracts has been shown, for the first time, to consist of pectin–protein complexes, with the protein attached to one end of the pectin chain. A minor fraction (~33%) consists of free pectin chains. The protein–polysaccharide linkages are alkali-labile. It is suggested that the protein–polysaccharide complexes, termed 'tadpoles', contribute to the unusual emulsifying action of pectin extracts. It has been found that a fraction (~17%) of the sugar beet pectin molecules are branched structures. None of the pectin–protein complexes were observed to contain branched pectin chains, and all of the branched pectin molecules appear to be free of protein.

Acknowledgement

The research reported in this article was supported by the BBSRC through the core grant to the Institute.

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