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The effect of peptide–pectin interactions on the gelation behaviour of a plant cell wall pectin

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Abstract

The effect of basic peptides on the gelation of a pectin from the cell wall of tomato was examined through the determination of gel stiffness, and swelling behaviour of the gel in water. Poly-L-lysine, poly-L-arginine, and a synthetic peptide, designed to mimic a sequence of basic amino acids found in a plant cell wall extensin, act as crosslinking agents. Circular dichroism studies on the interaction of synthetic extensin peptides with sodium polygalacturonate demonstrated that a conformational change was induced as a result of their complexation. In addition to their effect as crosslinking agents, the polycationic peptides reduced the swelling of the pectin network in water.

Keywords: Pectin; Extensin; Hydroxyproline-rich glycoprotein; Poly-L-lysine; Gelation

1. Introduction

The pectic polysaccharides are major components of the primary cell wall and middle lamella of dicotyledonous food plants.[1] Structurally they consist of a backbone of (1→4)-α-D-galacturonosyl residues interrupted with typically 10% substitution of (1→2)-α-L-rhamnopyranosyl residues. A portion of the rhamnosyl residues are branch points for neutral sugar side-chains. Further structural diversity is obtained from the methyl esterification of a portion of the galacturonosyl residues.[1] They are understood to be predominantly crosslinked in the cell wall through ionic interaction with calcium ions.[2] Studies on a pectin extracted from the cell wall material of unripe tomato, have shown that even at relatively low concentrations (2–4% w/w) of polysaccharide, this ionic interaction was sufficiently strong for gelation to occur.[3] The extent to which these gels swelled in dilute salt solution was governed by the balance between gel stiffness and the affinity of the network for solvent.[4] The gels exhibited swelling behaviour similar to that of other polyelectrolyte networks, in which the affinity for solvent is profoundly affected by the extent of dissociation of the ionisable groups carried by the network, and ionic strength. It was proposed that although a major fraction of the unesterified carboxyl groups in the pectin chain participated in ionic crosslinking, there was a sufficient concentration of 'free' carboxylate to have a substantial effect on the observed swelling behaviour.

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Although a range of studies have examined the interaction of pectins with inorganic cations, relatively little work has been published on the possibility of structurally significant interactions occurring between pectic polysaccharides and organic cationic compounds. The binding of polyamines to plant cell walls has been investigated,[5] and it has been suggested that these compounds may have a direct structural function in the cell wall.[6] Dilute solution studies have also shown that the interaction of basic peptides with polygalacturonate can induce a conformational change.[7,8]

Several different classes of structural proteins have been identified in plant cell walls, and prominent among these are the hydroxyproline-rich plant glycoproteins (HRGPs).[9] HRGPs are basic proteins; they are rich in hydroxyproline and serine, and are glycosylated mainly with arabinose and galactose. In aqueous solution, HRGPs are reported to adopt a rod-like conformation, with a polyproline II helical structure predominating.[10,11] Early hypotheses concerning the structural role of HRGPs concentrated on the possibility that covalent crosslinking through tyrosine residues could lead to the formation of a protein network. There is evidence also for covalent linkage between HRGPs and pectin.[12,13] At the same time there has been consistent speculation that HRGPs form ionic complexes with pectins.[9,14,15]

In this paper, we investigate the proposal that ionic interactions between basic plant cell wall proteins and pectic polysaccharides have the potential to contribute to the assembly of the cell wall network. The materials selected for study were a HRGP from carrot root (wound-induced extensin), and the chelator-extracted pectin from unripe tomato pericarp. The presence of clusters of basic amino acids in the amino acid sequence predicted for carrot extensin[16] appeared to favour ionic interactions with pectin, and synthetic peptides were used to examine these interactions more closely. Their behaviour was compared to that of other basic peptides. The effect of these peptides on the crosslinking and swelling behaviour of pectin gels was examined.

2. Experimental

Preparation of pectin.—Pectin was extracted from purified tomato cell walls with *trans*-1,2,diaminocyclohexane-*N*,*N*,*N*′,*N*′,-tetraacetic acid (CDTA);[3] concentrated pectin solutions were prepared by rotary evaporation of stock solutions of the potassium salt, pH 6.5. The uronic acid and methyl ester content of the pectic polysaccharide were determined as described previously.[17]

Preparation of extensin.—Extensin was purified from carrot phloem parenchyma following the procedure of Stafstrom and Staehelin.[18] Ion-exchange chromatography was carried out on a 1.6×20 cm column of CM Sephadex C50 (Pharmacia) equilibrated in 25 mM Tris–HCl pH 8.0 at rt; the flow rate through the column was 30 mL/h. Carrot proteins were dissolved in 25 mL of buffer and were centrifuged for 15 min at 2500*g* to precipitate insoluble material. After loading the protein solution, the column was washed with buffer until the absorbance at 280 nm dropped to a constant value. The column was then eluted with a NaCl gradient from 0 to 200 mM in 160 mL of buffer. Fractions from the extensin-containing peak were pooled and subjected to diafiltration with 10 vols of water, followed by freeze-drying. The purified protein was redissolved in distilled water at a concentration of 20 mg/mL and stored at -20 °C. Analysis of the amino acid content of the preparation was carried out by the Protein and Nucleic Acid Chemistry Facility, Cambridge University. Sensitivity of the preparation to peroxidative crosslinking was tested by exposure to a partially purified tomato extensin peroxidase.[19] Approximately 60 μg of extensin protein was incubated with extensin peroxidase in the presence of 10 nmol of H_2O_2, in a total volume of 100 μL of 0.1 M NaOAc buffer pH 6.0. The elution profile of this mixture and the separate proteins was determined by gel-filtration using a Pharmacia Superose-12 column, eluted at 0.3 mL/min with 0.1 M

NaOAc, pH 5.0, 0.1 M NaCl, 0.02% sodium azide, and UV detection at 280 nm.

Source and preparation of peptides.—Polyamino acids were obtained from Sigma. For poly-L-lysine the reported average-molecular weights (determined from viscosity) were 1000, 2900, 3900 and 9800 Da, indicating average dps of 6, 14, 19 and 47, respectively. Technical details provided by Sigma indicated that the dp of the 1000 Da material was confirmed by capillary electrophoresis; M_w/M_n (SEC-LALLS) for the 9800 Da material was 1.35. For poly-L-arginine the reported average M_w was 10,800 Da (dp 56) with M_w/M_n 1.1. Peptides were synthesised with a NovaSyn Crystal (Calbiochem–Novabiochem, UK) automatic peptide synthesiser using fluorenylmethoxycarbonyl (Fmoc)–polyamide chemistry, based on the method of Atherton and Sheppard.[20] Fmoc protected, activated amino acids were purchased from Calbiochem–Novabiochem. After synthesis, peptides were cleaved from the polyamide resin support using trifluoroacetic acid containing 5% (v/v) triisopropyl silane (Aldrich Chemical Co.) and 5% (v/v) water as scavengers for the side-chain protecting groups of the individual amino acids. Scavengers were removed by Et_2O extraction from aqueous solutions prior to freeze-drying. Purity was assessed by C_{18} reversed-phase HPLC, and fast-atom bombardment mass spectrometry (FAB-MS). Calcium contamination was removed from synthesised peptides by gel filtration. A 1.6×98 cm column of Biogel P2 ultrafine (Bio-Rad) was equilibrated in glass distilled water and samples, dissolved in 1 mL of water, were loaded on to the column under gravity. The column was eluted with distilled water at 15.5 mL/h at rt. Peptides were monitored by absorbance at 280 nm and calcium by atomic absorbance spectrometry.

Formation of gels.—Gel formation was carried out at 4 °C. To form gels with basic compounds, 350 mg of pectin solution ($\sim 1.5\%$ w/w) were placed in a 2 mL straight-sided (8 mm diameter) polythene microcentrifuge tube, and 12 μL of 5.8% (w/v) HC1 was added to the surface. After 30 min, the basic compound was added in a total volume of 10–20 μL. After 24 h, 30 μL of 1 M NaOAc (pH 8) was added. Gels were removed from the microcentrifuge tubes after a further 24 h, and their physical properties were determined after equilibration to rt. The final pH was between 5.5 and 6.0.

Physical measurements of gels.—The stiffness of the gels was determined as the shear modulus (G') at 200 Hz calculated from the measured velocity of a shear-wave passing through the gel using a Rank pulse shearometer.[21] Gel swelling was measured either from the weight gain after 24 h, or from the change in linear dimensions of gel pieces of approximately $1 \times 1.5 \times 4$ mm. For the latter, gels were formed in 40 mM $CaCl_2$ and equilibrated with 2 mL of 4 mM $CaCl_2$ for 6 h (Stage 0). Gels were transferred to 1 mL of 4 mM $CaCl_2$ containing peptides and incubated for 16 h (Stage 1). The bathing solution was diluted fourfold with distilled water (Stage 2) and this was repeated after two successive periods of 24 h (Stages 3 and 4).

Circular dichroism.—Aqueous solutions of sodium polygalacturonate (0.05% w/w) and peptide (0.01% w/w) were prepared in water pH 5.5–6.0. Spectra were recorded over the range 260–185 nm in a 0.2 cm cell using a JASCO 5-710 spectrophotometer, calibrated using camphor sulphonic acid at 290 nm. The scan speed was 100 nm/min and the reported spectra are the mean of three scans. Data analysis was carried out using SELCON software.

3. Results and discussion

Characterisation of synthesised peptides and extensin.—Two different peptides were synthesised from data for the genomic sequence of carrot extensin.[16] Peptide 1 (seven amino acids) contained the sequence His–His–Tyr–Lys–Tyr–Lys. Peptide 2 (30 amino acids) contained this sequence and the additional sequence His–His–Tyr–Lys–Tyr–Lys–Tyr–Lys (Fig. 1). Both peptides gave single peaks on HPLC. With FAB-MS peptide 1 yielded the predicted molecular ion, $[M + H]^+$, $m/z =$ 974.5. For peptide 2, mass determination was by electrospray-ionisation mass spectrometry (ESI-MS). The major peak, which accounted for over 70% of the material, was measured as 3690.6 ± 0.6 compared to a predicted molecu-

MGRIARGSKM SSLIVSLLVV LVSLNLASET TAKYTYSSPP PPEHSPPPPE
HSPPPPYHYE SPPPPKHSPP PPTPVYKYKS PPPPMHSPPP PYHFESPPPP
KHSPPPPTPV YKYKSPPPPK HSPAP**VHHYK YK**SPPPPTPV YKYKSPPPPK
HSP**APEHHYK YKSPPPPKHF PAPEHHYKYK YKS**PPPPTPV YKYKSPPPPT
PVYKYKSPPP PKHSPAPVHH YKYKSPPPPT PVYKSPPPPE HSPPPPTPVY
KYKSPPPPMH SPPPPTPVYK YKSPPPPMHS PPPPVYSPPP PKHHYSYTSP
PPPHHY

Fig. 1. Amino acid sequence for carrot extensin predicted by the genomic clone pDC5A1. The amino acid sequence given is from the data of Chen and Varner,[16] with the putative signalling sequence underlined. The two peptides synthesised for use in this study are given in bold type. Peptide 1 contains seven residues, and peptide 2 contains 30 residues.

lar weight of 3690. Two additional peaks, which accounted for the remainder of the material, were observed at 3619.6, and 3761.7, and represented the loss and gain of an alanine residue, respectively. This was considered to be due to difficulties experienced in adding the final residue (alanine), and not to be of significance for the interpretation of the experimental data. Peptides were purified by gel filtration to give a calcium content of < 0.01 μg calcium/mg solid.

Extensin was extracted from the cell walls of aerated carrot root slices with calcium chloride.[18] Extensin proteins were purified by ion-exchange chromatography of the trichloroacetic acid-soluble material in these extracts (Fig. 2), with an overall yield of ~ 10 mg from 550 g of carrot phloem parenchyma. In carrots, two distinct forms of extensin (extensin I and II) can be isolated.[18] A restricted set of fractions (6 mg) was therefore pooled (Fig. 2) for use in experiments. When chromatographed by gel filtration (Fig. 3), the purified protein eluted as a symmetrical major peak with a faster running minor peak, which accounted for 7% of the total peak area. A marked decrease in elution time for the major peak was noted on pre-treatment with H_2O_2 in the presence of a partially purified peroxidase known to specifically crosslink extensins (Fig. 3).[19] The molecular weight of the extensin, as determined by SELDI-MS (surface-enhanced laser desorption ionization mass spectrometry), was $\sim 71{,}000$ g/mol. These data are consistent with the presence of a monomeric extensin. The amino acid composition of the purified extensin is given in Table 1, together with the composition of extensin I and II, and

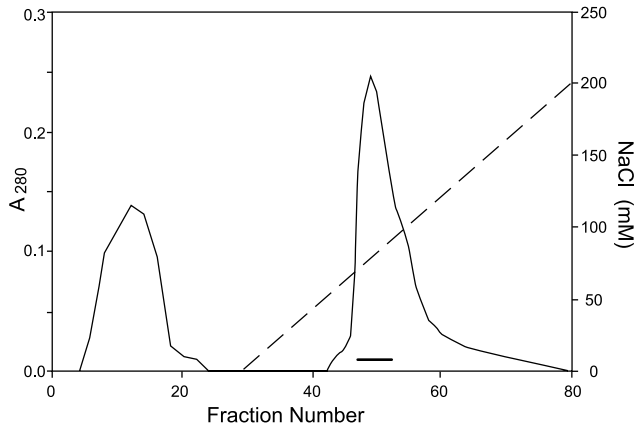

Fig. 2. Ion-exchange chromatogram of partially purified carrot extensin. The dashed line indicates the salt gradient used, and the horizontal bar marks the fractions pooled for use in experiments.

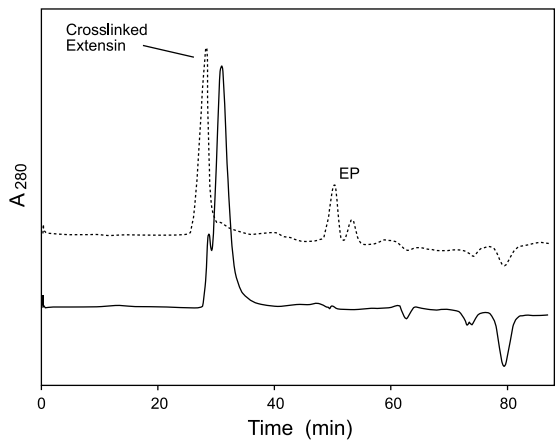

Fig. 3. Gel-filtration profile of carrot extensin, before and after crosslinking with an extensin specific peroxidase. Before crosslinking, solid line; after crosslinking, dotted line; EP, peaks attributable to extensin peroxidase. The void volume of the column determined with Blue Dextran 2000 was 6 mL (retention time ca. 20 min).

Table 1
Amino acid composition of carrot hydroxyproline-rich glycoproteins

Amino acid	Carrot HRGP (mol%)	Extensin 1 [a] (mol%)	Extensin II [a] (mol%)	Genomic clone pDC5A1 [b] (mol%)
Hyp	50.0	46.5	27.5	–
Asx	1.4	1.3	2.9	–
Thr	1.2	1.4	4.1	3.6
Ser	11.9	14.9	16.9	10.9
Glx	0.2	2.6	6.0	2.6
Pro	0.5	0.2	0.0	42.3
Gly	0.2	3.2	7.9	–
Ala	0.4	1.4	3.4	1.4
Cys	nd [c]	0.0	0.0	–
Val	6.0	5.5	3.7	4.0
Met	–	0.2	0.0	1.1
Ile	0.2	0.5	1.0	–
Leu	0.4	0.7	1.2	–
Tyr	4.5	10.5	3.9	12.0
Phe	nd	0.3	0.0	0.7
His	14.5	5.8	10.5	9.5
Lys	7.3	4.7	9.1	11.7
Arg	0.1	0.4	1.7	–
Trp	–	–	–	–

[a] Data from Ref. 31.
[b] Data from Ref. 16.
[c] Not detectable.

predicted data from the carrot genomic clone pDC5A1. The amino acid composition of the material is similar to that of extensin I, and to pDC5A1 bearing in mind the post-translational conversion of proline to hydroxyproline. The low levels of threonine, glycine and alanine suggest that the level of extensin II in the sample is low.

Effect of basic peptides and extensin on pectin gelation.—The pectic polysaccharide used in these studies was of the following composition (mol%): GalA, 83; Gal, 12; Ara, 3.2; Rha, 0.8; Glc, 0.6; Xyl, 0.1; Fuc, 0.1; Man, < 0.1. The degree of methylesterification was 65%.[3] A significant proportion of the unesterified galacturonic acid is presumed to be distributed in a blockwise manner because of the ability of this pectin to form gels in the presence of calcium ions. The pectin had a high intrinsic viscosity (810 mL/g) and gave a broad peak on size-exclusion chromatography.[3] A wide range of different molecular lengths has been observed in a similarly extracted tomato pectin which was examined by atomic-force microscopy.[22] The basic peptides and extensin preparation formed precipitates when mixed with concentrated pectin solutions at neutral pH. This is consistent with the reported extent of interaction for mixtures of pectin and gelatin[23] or pectin and poly-L-lysine.[7] Mixing of the basic peptides and proteins with concentrated solutions of pectin was achieved by initial acidification of the pectin solution to a pH of ~ 2 (50 mM HCl) in order to suppress the charge on the pectin. No measurable change in the level of methyl esterification was observed at these pH values although the pectin did form a weak network structure. The basic components were then allowed to perfuse into this network and the pH adjusted to 5.5–6.0.

Using this procedure it was possible to form elastic gels between pectin and a variety of different basic compounds, including the highly basic peptides, poly-L-lysine (average dp 6 or 19), poly-L-arginine (average dp 56). Fig. 4 shows the effect of the addition of poly-L-arginine (average dp 56) at 0.076, 0.14, 0.28 and 0.43% (w/w) on the appearance of a 1.3% (w/w) pectin gel. These additions correspond to charge ratios for peptide:pectin of 0.22, 0.45, 0.90, and 1.37 respectively. All the gels are free standing. At initial levels of addition, the resulting gel is essentially clear and

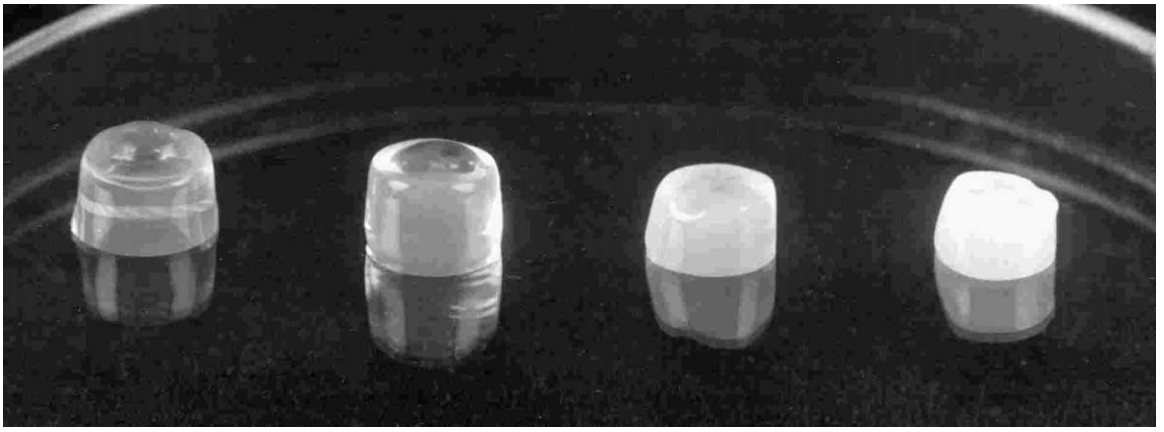

Fig. 4. Appearance of mixed pectin (1.3% w/w) poly-L-arginine gels (as a function of increasing peptide:pectin charge ratio (from left to right: 0.22, 0.45, 0.90, 1.37).

has the ability to recover from static deformation. At higher levels a marked opacity, indicative of substantial polymer aggregation is apparent, coupled with a marked shrinkage and syneresis of the gel. Although peptide 1 (dp 7) formed gels with pectin, peptide 2 (dp 30) did not reliably form gels. In vivo carrot HRGP undergoes post-translational modification. None of the residues in peptide 1 are affected in this way, but peptide 2 contains several proline residues which in the naturally occurring protein are converted to hydroxyproline, with a portion of these being further substituted with tetra-arabinoside side-chains. It is possible that the lack of these features will affect the interaction with pectin. In all cases, gelation could be reversed by leaching the gels under the same acidic conditions used to encourage mixing, demonstrating that the interaction was non-covalent, and reversible, and that gel formation was not due to reductive amination of the pectin.[24] In addition, we found that gels formed between peptide 1 and pectin were readily dispersed in 50 mM CDTA pH 6.5. This observation was of interest because CDTA is commonly used to extract ionically bound pectin from plant cell walls, and it demonstrates that CDTA can disrupt other ionic bonds crosslinking pectins besides those involving inorganic cations.

The shear modulus (G') of the gels formed with peptides or calcium ions was examined as a function of the molar concentration of crosslinking agent (Fig. 5). The most effective crosslinking agent for inducing gelation was poly-L-arginine, followed by poly-L-lysine, peptide 1 and finally calcium ions. These data reflect the different affinities of the crosslinking agent for the pectin chain and suggest that while a calcium crosslink is a cooperative association involving a number of calcium ions, a single multiply charged peptide can also function as a crosslink. However the maximum level of crosslinking in the peptide–pectin gels, as indicated by the shear modulus (~ 1100 N/m^2, Fig. 5), is low compared to the level found in calcium–pectin gels prepared under identical conditions at saturating levels of calcium ions (2500 N/m^2). This suggests that for the peptides there are a limited number of potential crosslinking sites on the pectin backbone. Peptide 1 induced a higher degree of crosslinking than poly-L-lysine dp 6, despite

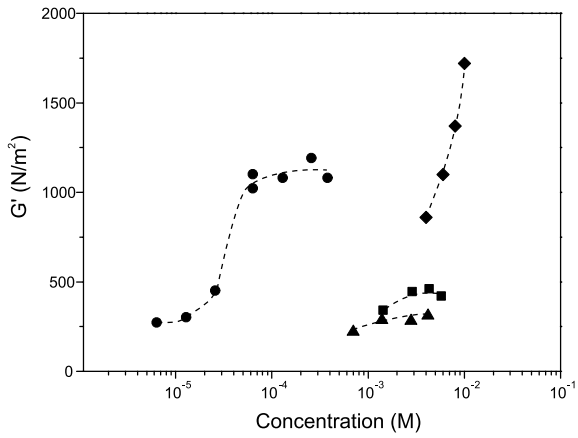

Fig. 5. Shear modulus as a function of the molar concentration of added compound for gels formed with pectin (1.3% w/w) and different cations: ●, poly-L-arginine dp 56; ▲, poly-L-lysine dp 6; ■, peptide 1; ◆, calcium.

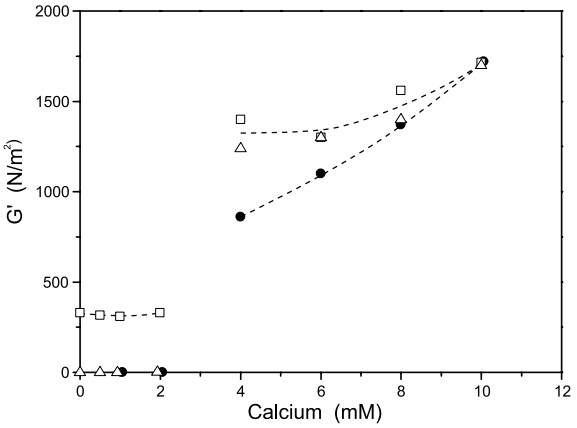

Fig. 6. Shear modulus of pectin gels (1.3% w/w) formed with mixtures of calcium and peptide 1 or peptide 2: ●, no added peptide; □, plus peptide 1; △, plus peptide 2.

Table 2
Stiffness and swelling of gels formed by addition of $CaCl_2$ to pectin pre-treated with carrot extensin

Weight of extensin relative to pectin (%)	Stiffness (G') (N/m^2)	Volume change on swelling (%)
0.2	485	+56
0.5	345	+59
1	365	+58
2	350	+62
5	360	+65
10	280	+74

Extensin was mixed with a 1.3% w/w pectin solution by acidification as described in the Experimental Section. $CaCl_2$ was added after neutralisation to give a concentration of 4 mM. Swelling was carried out by addition of 5 vols of water. Data for each treatment are the averages from the results from two gels.

having roughly half the charge (calculated on the basis that at the pH of the gels histidine residues do not contribute significantly to the charge). The ability of peptide 1, to induce network formation, despite its relatively low-charge density, suggests that non-ionic interactions can also contribute to the formation of the crosslink.

In order to further assess the interaction between peptide 1 or 2 and pectin in the presence of calcium ions, mixtures containing a constant level of the peptide and increasing amounts of calcium were added to the acidified pectin solution (1.3% w/w). The charge ratio of peptide:pectin in these experiments was 0.14 for peptide 1 and 0.11 for peptide 2. After incubation and subsequent neutralisation, the shear modulus of the gels was measured (Fig. 6). These data show that in the absence of added calcium ions, peptide 1 formed gels, whereas peptide 2 did not, and the lowest level at which calcium alone induced gel formation was 4 mM. At low levels of added calcium, addition of either peptide was more effective on a molar basis at increasing crosslinking than calcium alone. This effect disappeared at higher levels of crosslinking. The increased level of crosslinking in the mixtures could be either due to both the peptide and the calcium crosslinking the pectin, or due to the peptide facilitating calcium mediated crosslinking. The absence of any effect of added peptides on the ability of 2 mM calcium to give increased crosslinking, suggests that crosslinking with both calcium and the peptide is occurring in the mixed systems.

Although carrot extensin interacted at neutral pH (forming a precipitate) using the method described above gel formation was not observed at levels of addition up to 10% (w/w) relative to pectin. We examined the effect of pre-incubation of the pectin (1.3% w/w) with extensin on the properties of gels formed after subsequent addition of 4 mM $CaCl_2$ (giving a Ca:pectin charge ratio of 0.5). Under these conditions, maximum crosslinking occurs at 30 mM $CaCl_2$ (a charge ratio of 3.5). The results (Table 2) show that increasing the level of added extensin reduced the shear modulus of the mixed gels. This suggests that extensin is a poor crosslinking agent under these conditions and its interaction with the pectin chain interferes with calcium crosslinking.

Effect of basic peptides and extensin on the swelling behaviour of pectin gels.—An additional physical property of calcium pectin gels is their tendency to swell when placed in dilute salt solutions. In this respect they show behaviour typical of polyelectrolyte networks, with swelling principally driven by an imbalance in the distribution of mobile counter ions between the gel and the surrounding solution, which arises as the result of a Donnan effect. At equilibrium, the osmotic force driving swelling is balanced by an elastic restoring force resulting from the crosslinking of the

polymer network. The swelling pressure can be estimated from the relationship,[25]

$$\pi = RT(ic_2)^2/2wvc_s^*$$

where R is the gas constant, T is the temperature in Kelvin, i is the degree of ionisation multiplied by the valency of the charge on the polymer, c_2 is the concentration of polymer charge expressed as moles of repeating unit, w is the valency factor of the electrolyte, v is the number of cations and anions into which the electrolyte dissociates, and c_s^* is the concentration of strong electrolyte external to the gel. Swelling is influenced by the extent of ionisation of the polymer and the valency of the counterion. Counterions of higher valency reduce the swelling pressure.

The effect of addition of peptides on the swelling of mixed pectin gels prepared with poly-L-lysine dp 6, or poly-L-arginine dp 56 and pectin is shown in Table 3. Although the shear modulus of the gels formed with different amounts of each compound was similar, indicating that there is a similar level of crosslinking of the pectin matrix, the subsequent swelling behaviour showed a marked dependence on the amount of added peptide. At higher levels of added peptide reduced swelling was observed and ultimately, for poly-L-arginine, shrinkage—which is a feature not found in pectin gels formed with equivalent levels of calcium. At swelling equilibrium the osmotic pressure driving swelling and the modulus are equivalent.[26] Variation in the shear modulus at swelling equilibrium (Table 3) therefore reflects the effect of complex formation on the extent of ionisation of the pectin network, and the effect of peptide multivalency on the Donnan equilibrium. Estimates of the extent of ionisation of the pectin network for gels formed with poly-L-arginine at a charge ratio of 0.22 were made, assuming the added peptide is fully complexed with the pectin, but not allowing for loss of pectin from the gel during swelling. Dissociation of less than 50% of the remaining ionisable groups is required to explain the observed swelling modulus. This in turn would be consistent with a blockwise distribution of the charge on the pectin leading to reduced ionisation through counter-ion condensation.[27]

In a related experiment, the effect of peptides on the swelling of calcium–pectin gel networks was observed. Gels were formed in 40 mM $CaCl_2$ and were allowed to reach swelling equilibrium in 4 mM $CaCl_2$; pectin which did not participate in network formation was removed at this stage.[3] The gels were then placed in 4 mM $CaCl_2$ in the presence of different amounts of peptide, and were subjected to a progressively increased swelling pressure by successive dilution of the bathing solution (Fig. 7). The effectiveness of added peptides in reducing swelling broadly reflects

Table 3
Stiffness and swelling of peptide–pectin gels

Peptide	Weight relative to pectin (%)	Charge ratio peptide:pectin	Stiffness (G') (N/m^2)	Volume change on swelling (%)	Stiffness after swelling (G') (N/m^2)
Poly-L-arginine	6	0.22	1020	+28	1825
(average dp 56)	11	0.45	1080	+24	2325
	22	0.90	1190	−19	1640
	34	1.37	1082	−14	1735
Poly-L-lysine	6	0.31	230	+49	265
(average dp 6)	11	0.63	213	+41	425
	22	1.25	264	+24	350
	34	1.90	242	+1	355

Gels were formed as described in the Experimental Section using a pectin solution of 1.3% (w/w). The amount of peptide added is presented both as the weight relative to the pectin and the charge relative to pectin assuming full ionisation of both compounds. Swelling was carried out by placing the gels (approximately 250 mg) in 4 mL of distilled water. Data for each treatment are the averages from two gels.

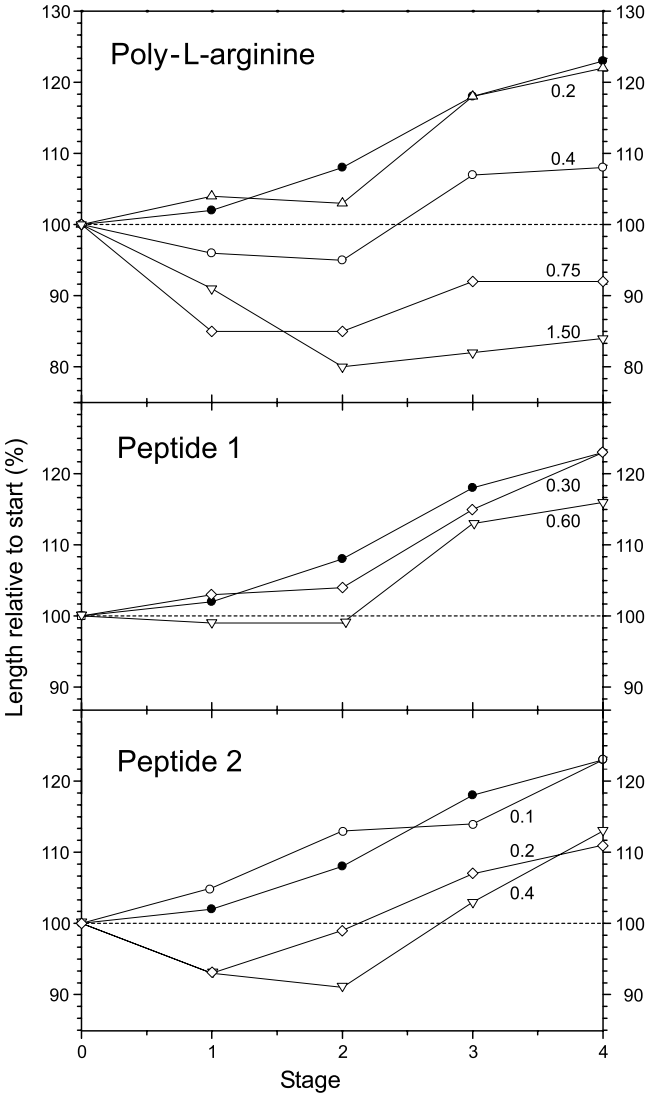

Fig. 7. Swelling of calcium pectin gels on successive dilution after treatment with basic peptides. Stage 0, no addition; Stage 1, addition of peptide; Stages 2, 3 and 4, successive fourfold dilutions of the bathing solution with distilled water. The amounts of peptide added as a proportion of the total pectin present were: ●, no peptide added (control); □, 2; △, 5; ○, 10; ◇, 20; ▽, 40% (w/w). The peptide:pectin charge ratio for each treatment is given on the graph.

the peptide:pectin charge ratio. These data partly reflect the ability of the peptides to crosslink the pectin network, but may also result from suppression of polymer ionisation and the valency of the peptide as described above. These effects of the peptides are of interest because of the potential physiological significance of pectin hydration for plant cell wall behaviour in vivo.[28]

Effect of basic peptides and extensin on the solution conformation of pectin.—In the tomato pectin used in this study, the unesterified residues, which account for 35% of the total galacturonic acid, are believed to be mainly distributed in blocks.[3] The interaction of basic peptides with sequences of unesterified uronic acid residues was probed through examination of the circular dichroism (CD) of dilute aqueous solutions of polygalacturonic acid–peptide mixtures. The change in CD of polygalacturonic acid (0.05% w/w) on addition of calcium ions (5 mM) is shown in Fig. 8(a). The observed change is consistent with previous observations[29] and has been interpreted as a perturbation in the carboxylate absorbance as a result of ion binding. For mixtures of peptides (0.01% w/w) and polygalacturonic acid (0.05% w/w), the observed behaviour is compared with that predicted from summation of the spectra of the separate components. The poly-L-lysine–polygalacturonic acid interaction was examined as a function of dp of the poly-L-lysine. For dps 6, 14 and 19 there was no marked difference in predicted and observed behaviour (Fig. 8(b–d)), indicating that the poly-L-lysine crosslinking of the pectic polysaccharide network does not necessarily induce a conformational change. At the higher dp of 47 (Fig. 8(e)), there was a difference in predicted and observed behaviour indicative of conformational change and consistent with published observation.[7,8] The CD spectrum for peptides 1 and 2 (Fig. 9(a)) were analysed by SELCON curve fitting. The analysis of peptide 1 did not produce reliable results but peptide 2 was fitted at ($P < 0.01$) for a composition of 4% α helix, 46% β sheet, 29% β turn, with 21% unassigned. Fitting for the polyproline II helix, which is likely to be the conformation of the adjacent proline residues, was not included in the analysis and may account for part of the unassigned data.[30] On addition of polygalacturonic acid (0.05% w/w) to peptides 1 and 2 (0.01% w/w) a spectral change is observed (Fig. 9(b,c)). This indicates that complexation of these peptides with polygalacturonic acid induces a conformational change. The evidence for conformational change on mixing peptide 1 and polygalacturonic acid stands in contrast to the data for poly-L-lysine oligomers of similar dp where no spectral change is observed. A specific interaction be-

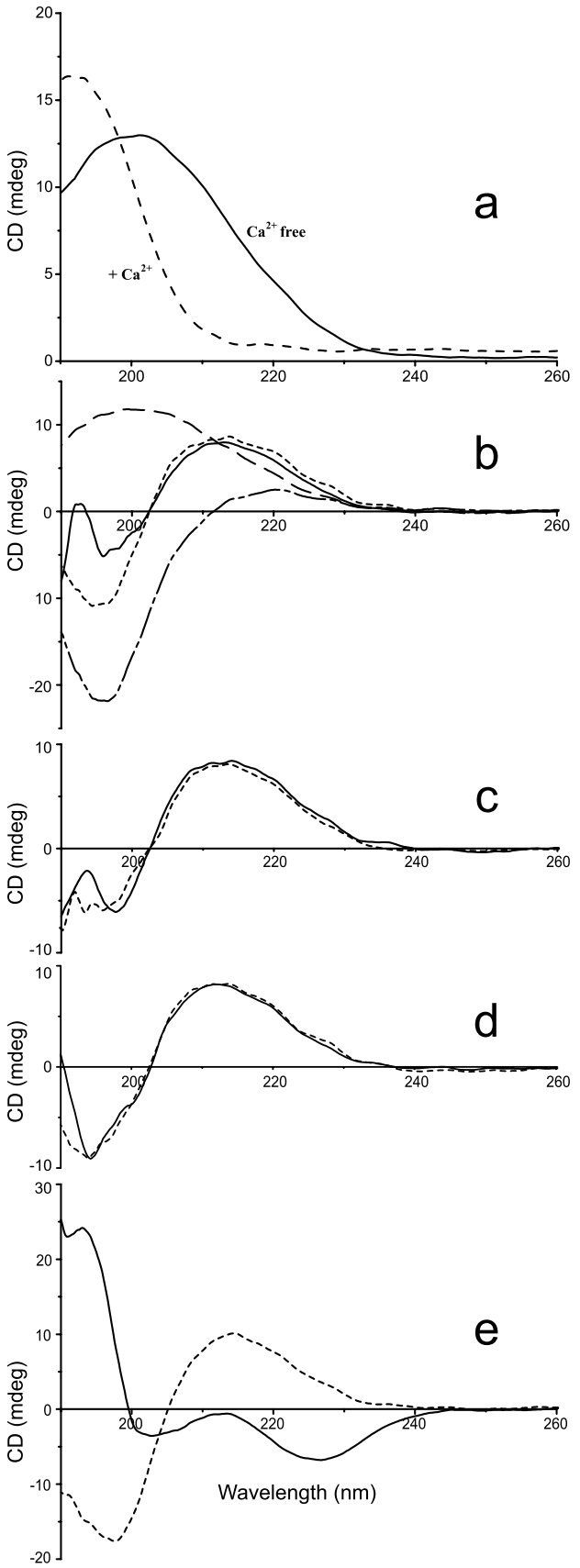

Fig. 8.

tween peptide 1 and 2 and polygalacturonic acid is indicated. It is not possible to tell from these data alone, however, whether the conformational change is in the polygalacturonic acid, the peptide or both.

The conclusion that there is a specific interaction between peptide 1 and polygalacturonic acid is of interest in understanding the role of the peptide motif Tyr–Lys–Tyr–Lys. This has been identified as a potential site for covalent inter- and intramolecular crosslinking through the formation of isodityrosine.[15] The data we have obtained can be interpreted as providing an alternative function for this sequence, or as indicating that isodityrosine formation is likely to be affected by complexation of extensins with pectic polysaccharides.

4. Conclusions

Oligomeric basic peptides have the potential to act as non-covalent crosslinking agents in pectin networks. A basic, oligomeric, fragment of a cell wall extensin can also function in this way. A synergy between calcium-mediated crosslinking and peptide crosslinking was observed under some conditions. In addition to their effect on crosslinking, basic peptides within the pectin network can have a marked effect on the swelling behaviour of the gel. This is partly due to the inclusion of a multivalent counterion, and is also partly a result of specific interactions of the peptide with the pectin chain reducing its effective charge. A plant cell wall carrot extensin does interact non-covalently with pectin, and this interaction can be explained as the outcome of specific binding to the pectin of particular basic amino acid sequences present in the protein. The data support the contention that non-covalent interactions anchor the extensin to the

Fig. 8. CD spectra for 0.05% (w/w) polygalacturonic acid complexed with calcium (a), or 0.01% (w/w) poly-L-lysine, dp 6 (b), dp 14 (c), dp 19 (d), dp 47 (e). For mixtures of polygalacturonic acid and poly-L-lysine the observed spectrum (solid line) is compared with a predicted spectrum obtained from the sum of the two components analysed separately (short dash). For dp 6 (b) the separate spectra for polygalacturonic acid (long dash), and poly-L-lysine (short and long dash) are included.

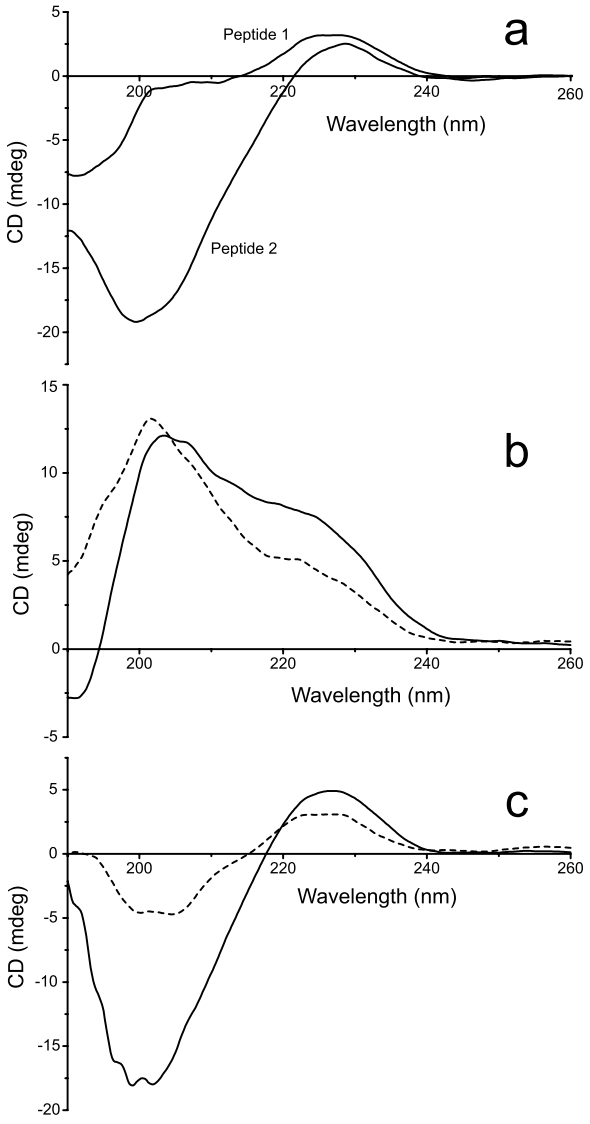

Fig. 9. CD spectra for; (a) peptide 1 and 2; (b) peptide 1 mixed with polygalacturonic acid; (c) peptide 2 mixed with polygalacturonic acid. Peptides were at 0.01% (w/w) and polygalacturonic acid at 0.05% (w/w). For mixtures (b,c) the observed spectrum (solid line) is compared with a predicted spectrum obtained from the sum of the two components analysed separately (short dash).

pectin matrix and contribute to the structural assembly of these proteins in the cell wall.

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