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Binding behavior of calcium to polyuronates: Comparison of pectin with alginate

Yapeng Fang [a,*], Saphwan Al-Assaf [a], Glyn O. Phillips [a,b], Katsuyoshi Nishinari [a], Takahiro Funami [c], Peter A. Williams [d]

[a] *Glyn O. Phillips Hydrocolloid Research Center, North East Wales Institute, Plas Coch, Mold Road, Wrexham LL11 2AW, UK*
[b] *Phillips Hydrocolloid Research Ltd., 45 Old Bond Street, London W1S 4AQ, UK*
[c] *Hydrocolloid Laboratory, San-Ei Gen F.F.I., Inc., 1-1-11 Sanwa-cho, Toyonaka, Osaka 561-8588, Japan*
[d] *Centre for Water-Soluble Polymers, North East Wales Institute, Plas Coch, Mold Road, Wrexham LL11 2AW, UK*

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Abstract

For polyuronates, such as pectin and alginate, the ability to bind calcium and to form gels is the basis of their biological functions and technological applications. In a previous paper [Fang, Y. P., Al-Assaf, S., Phillips, G. O., Nishinari, K., Funami, T., Williams, P. A., & Li, L. B. (2007). Multiple steps and critical behaviors of the binding of calcium to alginate. *Journal of Physical Chemistry B*, *111*, 2456–2462], we investigated the binding of calcium to alginate by using isothermal titration calorimetry and viscometry, and proposed a multi-step model which involves several steps: monocomplexation, dimerization, and lateral association. The present study examines two pectins of low and high methoxyl contents, and compares their behavior with alginate. In contrast to alginate, low methoxyl pectin has a less demarked dimerization step, which starts even when the stoichiometry of the egg-box structure is not achieved. Moreover, low methoxyl pectin shows less dimerization, and no significant lateral association. This behavior can be interpreted in terms of the structural features of the pectin. The random distribution of ester and amide groups along the pectin chain introduces much more defects into the formation of egg-box dimers and even hinders the subsequent lateral association of the dimers. The high methoxyl pectin shows a negligible chain–chain association upon binding with calcium, and its behavior can be simply depicted as a conventional polyelectrolyte without strong specific interactions.

Keywords: Binding; Calcium; Pectin; Alginate; Multiple steps; ITC

1. Introduction

Pectin and alginate, both polyuronates, are two characteristic examples of natural polyelectrolytes undergoing chain–chain association and forming hydrogels upon addition of divalent cations (e.g., Ca^{2+}). These properties are responsible for a wide range of applications in the food, pharmaceutical, biotechnological, and pollutant-treating industries (Chen, Hong, Wu, & Wang, 2002; Kohn, 1975; Lim & Sun, 1980; Soonshiong et al., 1992; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Alginate is composed of (1 → 4)-linked β-D-mannuronate (M) and α-L-guluronate (G) units, which are present in the linear macromolecule in homopolymeric blocks of each monomer, together with blocks of alternating sequence (Donati et al., 2005). Pectin is composed of long sequences of partially methyl-esterified (1 → 4)-linked α-D-galacturonate residues (known as 'smooth' region), interrupted by defects of other sugars such as D-xylose, D-glucose, L-rhamnose, L-arabinose, and D-galactose (known as non-gelling 'hairy' region) (Guillotin, 2005). Alginate is characterized by its G/M composition, whereas pectin is usually identified by its degree of ester-

* Corresponding author. Tel.: +44 (0) 1978 29 3330.
E-mail address: y.fang@newi.ac.uk (Y. Fang).

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ification (DE) and in some cases also by its degree of amidation (DA) (Capel, Nicolai, Durand, Boulenguer, & Langendorff, 2006).

Calcium-induced gelation results from specific and strong interactions between calcium ions and guluronate and galacturonate blocks in alginate and pectin, respectively. Alginate–Ca gels are described in terms of the so-called 'egg-box' model. X-ray diffraction data of dehydrated gel specimens shows that the junction zones are formed by the paring of 2/1 helical chains of guluronate sequences, providing cavities which can accommodate calcium ions (Grant, Morris, Rees, Smith, & Thom, 1973; Morris, Rees, Thom, & Boyd, 1978). The egg-box model has been transposed to describe pectin–Ca gels also, but its validity has not been corroborated on the basis of direct structural information (Braccini & Perez, 2001). The analogy is drawn largely because of the strong similarities of the structures and calcium-binding behavior of the two polymers. These can be identified as:

(i) polygalacturonate and polyguluronate are nearly mirror images in structure (Braccini & Perez, 2001);
(ii) a cooperative effect in calcium binding is observed for both polymers at chain lengths above a threshold of ~20 residues (Braccini & Perez, 2001; Braudo, Soshinsky, Yuryev, & Tolstoguzov, 1992);
(iii) large circular dichroism changes occur in the $n - \pi^*$ region of the same type for both polymers (Rees & Welsh, 1977).

Because of this parallel behavior, most previous studies treat pectin and alginate as almost equivalent in their binding and gelation mechanisms with Ca. For example, formation of 'egg-box' dimers followed by lateral association of the dimers was considered as a general structural pathway for alginate and pectin during gelation (Dobies, Kusmia, & Jurga, 2005; Stokke et al., 2000; Yuguchi, Urakawa, Kajiwara, Draget, & Stokke, 2000). Siew et al. proposed the same charge annihilation and reversal mechanism to interpret the binding of Ca to alginate and pectin (Siew, Williams, & Young, 2005). Donati et al. formulated a common theoretical framework incorporating specific interaction and counterion condensation concepts to describe the chain–chain association of alginate and pectin upon binding Ca (Donati, Benegas, Cesaro, & Paoletti, 2006; Donati, Benegas, & Paoletti, 2006; Donati, Cesaro, & Paoletti, 2006). In contrast, only a sparse literature highlighted the dissimilarities in the binding and gelation mechanisms of alginate and pectin with Ca. By molecular modeling, Braccini et al. pointed out significant differences at the level of chain–chain association for Ca-induced alginate and pectin gels (Braccini & Perez, 2001). The conventional 'egg-box' model, proved applicable to alginate gel, but did not represent the most energetically favorable association for pectin gel; pectin–Ca gel is more correctly described as a modified 'egg-box' model, namely, the 'shifted egg-box' model.

Previously, we (Fang et al., 2007) investigated the binding of Ca to alginate using isothermal titration calorimetry (ITC), viscometry, and Ca-selective potentiometry. Structural evolution of alginate chains was found to proceed by a critical multi-step mechanism, including monocomplexation, dimerization, and lateral association steps. Here, we extend the same methodology to the binding of Ca to pectin. Emphasis is placed on identifying and explaining the differences between pectin and alginate with regard to their binding mechanisms with Ca.

2. Experimental

2.1. Materials

Two pectin samples of low and high methoxyl contents, designated as LMP and HMP, respectively, were obtained from Danisco A/S (Denmark). They are sodium type pectins, containing negligible amount of divalent cations. Their relevant molecular characteristics are listed in Table 1. For clarity, the P2000G46 and P170G64 alginate samples that had been used in the previous paper (Fang et al., 2007) were re-named here as low G alginate (LGA) and high G alginate (HGA), respectively. Their molecular characteristics have been reported previously (Fang et al., 2007). The other chemical reagents used in this work were all purchased from Fisher Scientific (UK), and are of analytical grade.

2.2. Sample preparation

Pectin solutions used for ITC and relative viscosity measurements were made in 20 mM acetate buffer at pH 5. A weighed amount of pectin powder was rapidly dispersed into acetate buffer with vigorous stirring, and then heated at 60 °C for 1 h. After cooling to room temperature, pectin solutions were put on to a roller to allow continuous shaking until clear solutions were obtained. $CaCl_2$ solutions were also prepared in the same acetate buffer.

2.3. Isothermal titration calorimetry (ITC)

ITC measurements were conducted on a CSC 4200 isothermal titration calorimeter (Calorimetry Sciences Corpo-

Table 1
Molecular characteristics of pectin samples used

Sample	DE (%)	DA (%)	FGAC (w/w %)	M_w ($\times 10^3$ Da)
LMP	32.3	17.4	46.2	175
HMP	70.1	Negligible	26.1	220

Note: Degree of esterification (DE) and degree of amidation (DA) were measured by near infrared spectroscopy, and are based on the total number of uronate units in pectin. Free galacturonic acid content (FGAC) by weight was determined by using an alkali titration method (Committee on Food Chemicals Codex, 1997), and is based on the weight of dried matter of pectin. Weight average molecular weight (M_w) was measured by GPC-MALS at 25 °C using 0.1 M NaCl as solvent.

rations, USA) at 25 °C. Twenty-four portions of 10 μL of 15 mM $CaCl_2$ solution were stepwise injected into a 1300 μL reaction cell containing 0.1% pectin solutions, with an interval of 1800 s between two successive injections. Blank experiments were also done, in which 15 mM $CaCl_2$ was injected into acetate buffer. A continuous stirring at 297 rpm was maintained throughout the experiments. Binding isotherms were obtained by the integration of each injection peak followed by subtraction with blank experiment, using the BindWorks 3.1 software provided by CSC. The ITC data for the binding of Ca to pectin was compared with that for alginate in the manner of normalized heat per mole of specific-binding sites:

$$q_i^{\mathrm{N}} = \frac{q_i}{Vc_i^{\mathrm{s}}} \tag{1}$$

$$Q_i^{\mathrm{N}} = \sum_i q_i^{\mathrm{N}} \tag{2}$$

where q_i is the heat observed for the ith injection, q_i^{N} the normalized heat for the ith injection, and Q_i^{N} the normalized accumulative heat up to the ith injection. V is the volume of ITC reaction cell (1300 μL). c_i^{s} is the concentration of specific-binding sites in the reaction cell at the ith injection. Note that the specific-binding site refers to guluronate unit and free galacturonic acid unit for alginate and pectin, respectively.

2.4. Relative viscosity measurements

An Ubbelohde-type capillary viscometer was used to measure the changes of relative viscosity of pectin solutions during binding with $CaCl_2$ at 25 °C. To account for the dilution effect involved with the addition of $CaCl_2$ solution, control experiments were also done in which acetate buffer was added into pectin solutions. The detailed procedures had been described in the previous paper (Fang et al., 2007). Relative viscosity η_{r} is expressed as the ratio of the flow times of sample (t_{s}) to solvent (t_0).

$$\eta_{\mathrm{r}} = t_{\mathrm{s}}/t_0 \tag{3}$$

To compare the changes of relative viscosity for pectin and alginate during binding with Ca, we introduced a term of 'normalized relative viscosity', which is defined as follows:

$$\eta_{\mathrm{r}}^{\mathrm{N}} = \frac{\eta_{\mathrm{r}}^{\mathrm{Ca}}}{\eta_{\mathrm{r}}^{\mathrm{C}}} \tag{4}$$

Here, $\eta_{\mathrm{r}}^{\mathrm{Ca}}$ is the relative viscosity measured for the titration of $CaCl_2$ solution into pectin solution (or alginate solution), and $\eta_{\mathrm{r}}^{\mathrm{C}}$ is the relative viscosity measured in the corresponding control experiment.

3. Results

3.1. Isothermal titration calorimetry results

A typical ITC thermogram for the sequential injection of 15 mM $CaCl_2$ into 0.1% LMP solution is displayed in Fig. 1a; the corresponding blank experiment is shown in Fig. 1b in which 15 mM $CaCl_2$ is titrated into acetate buffer solution. The titration of $CaCl_2$ into LMP has a distinctly different ITC pattern from that of the blank experiment. The injection peak first decreases with addition of $CaCl_2$, and then increases; after passing through a hump, the injection peak again decreases. Similar multi-step ITC patterns have also been observed for alginates (LGA and HGA) (Fang et al., 2007). This phenomenon can be attributed to the binding of Ca to low methoxyl pectin rather than any solvent effect.

Fig. 1c shows the binding isotherm obtained by integrating the injection peaks in Fig. 1a followed by subtracting the heat effect of the blank experiment (Fig. 1b). Since free galacturonic acid (FGA) units in pectin are the only binding sites for Ca, it is more relevant to express the binding isotherm as a function of the molar ratio of Ca to FGA (R) (Fig. 1d). Clearly, a turning point for the injection heat to increase is located at $R = 0.25$, which coincides with the position of the critical transition observed for LGA and HGA at a Ca/guluronate ratio of 0.25 (Fang et al., 2007). Similarly, two steps can be assigned for the binding of Ca to LMP based on the turning point (Fig. 1d). Note that for LGA and HGA, the second steps of binding isotherms fit well a model of independent-binding sites (Fang et al., 2007). However, the curve fittings using this model or even other models fail for LMP, making it impossible using this method to determine apparent thermodynamic parameters for the binding of Ca to pectin.

ITC results for the other pectin, HMP, are shown in Fig. 2a and b. Different from that of LMP, the ITC thermogram of HMP binding with Ca exhibits a single-step pattern: the injection peak reduces with addition of $CaCl_2$, and gradually levels off. This is more clearly reflected in the binding isotherm shown in Fig. 2b. The injection heat approaches a plateau at $R \approx 0.5$. This type of behaviors is typical of conventional polyelectrolytes where the binding of counterions is mainly driven by pure electrostatic interaction (Sinn, Dimova, & Antonietti, 2004).

3.2. Relative viscosity results

Fig. 3 shows the changes in the relative viscosities of 0.052% LMP solutions upon titration with 7.5 mM $CaCl_2$ solution or acetate buffer (control experiment). As a result of dilution, the relative viscosity in the control experiment ($\eta_{\mathrm{r}}^{\mathrm{C}}$) has a monotonic decrease during titration of acetate buffer. However, upon titration of $CaCl_2$ ($\eta_{\mathrm{r}}^{\mathrm{Ca}}$), there is first a very slight increase which accelerates at $R \approx 0.25$. This indicates a small growth of overall molecular size at $R < 0.25$, which afterwards intensifies. Consistent with the ITC data, two steps can be identified based on the inflexion at $R = 0.25$. This two-step change of relative viscosity for LMP during binding with Ca is markedly different from those for LGA and HGA, where a third step was also identified (Fang et al., 2007).

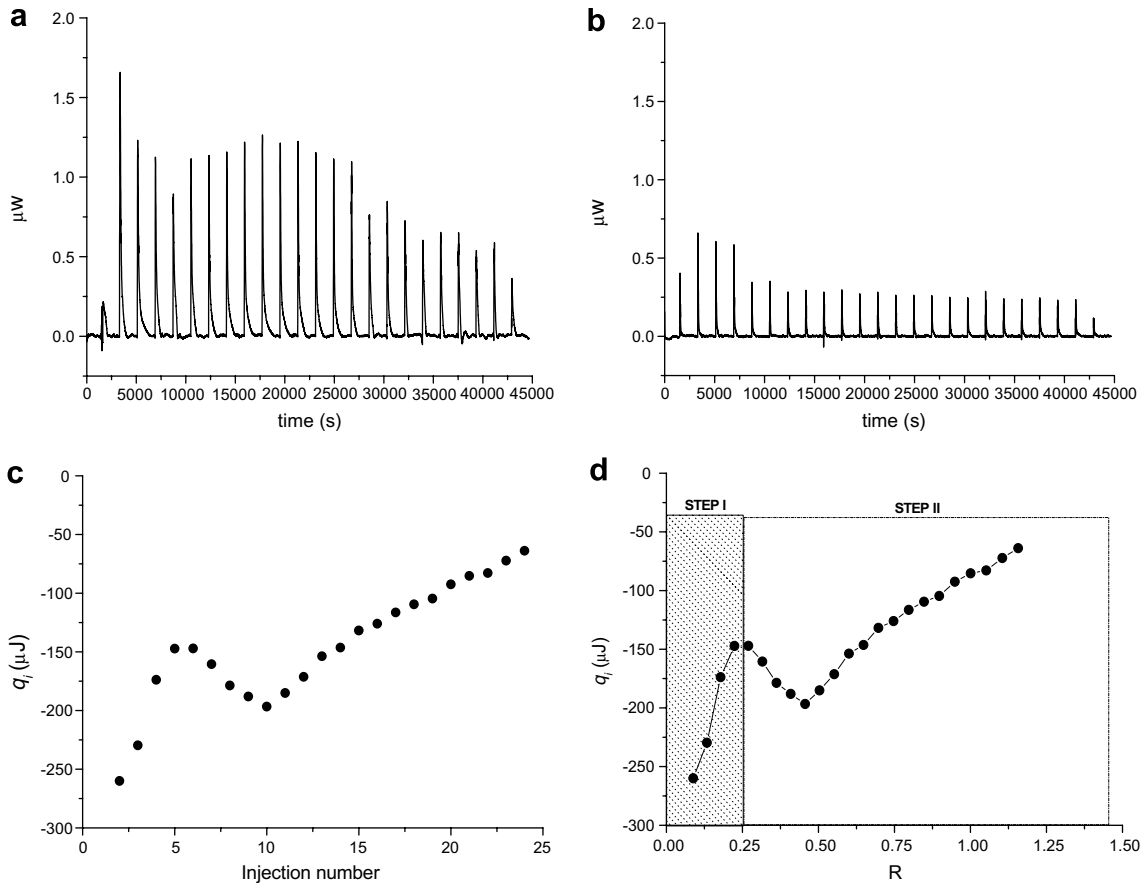

Fig. 1. ITC thermograms obtained for injecting 15 mM $CaCl_2$ into (a) 0.1% LMP and (b) acetate buffer solutions. The corresponding binding isotherm is displayed in (c), in which the first data point is omitted due to an imprecise titration volume at the first injection. The binding isotherm is re-plotted as a function of the molar ratio of Ca to free galacturonic acid unit (R) in (d).

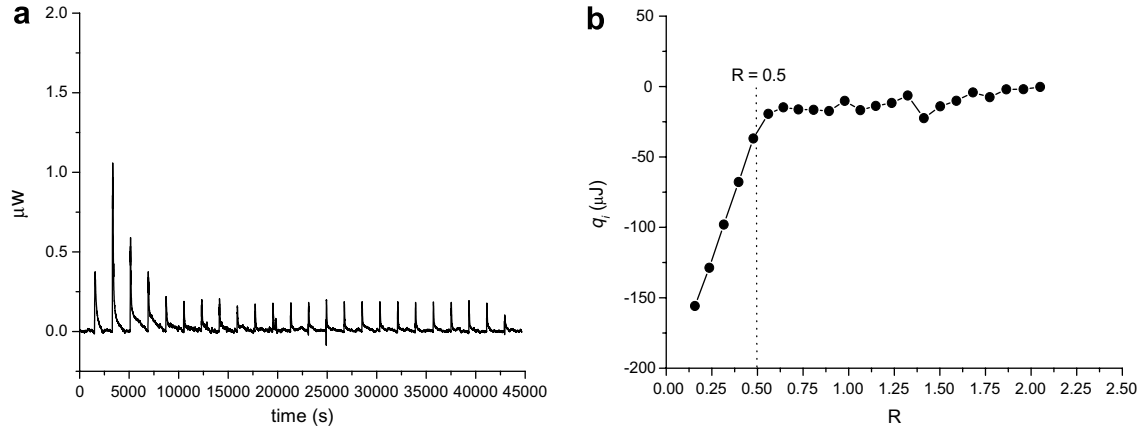

Fig. 2. ITC thermogram obtained for injecting 15 mM $CaCl_2$ into 0.1% HMP solution (a) (see Fig. 1b for blank experiment). The corresponding binding isotherm is displayed in (b), in which the first data point is omitted due to an imprecise titration volume at the first injection. R is defined as the molar ratio of Ca to free galacturonic acid units of pectin.

The relative viscosity during titration of 7.5 mM $CaCl_2$ or acetate buffer into 0.052% HMP solution is shown in Fig. 4. η_r^{Ca} for the titration of $CaCl_2$ almost overlaps with η_r^{C} for the titration of acetate buffer, indicating that no appreciable change of molecular size happens to HMP during binding with Ca.

4. Discussion

4.1. Calcium-binding behaviors of pectins

The two pectin samples investigated here are typical of low and high methoxyl pectins. They have the similar

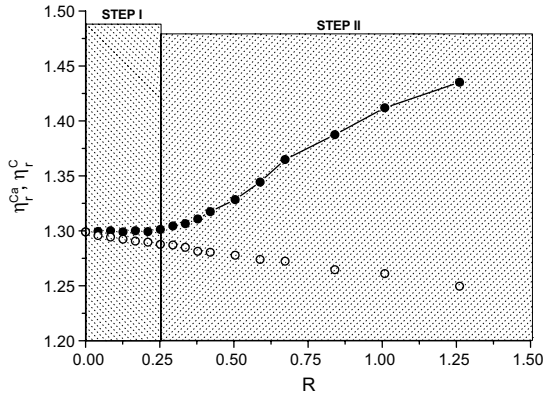

Fig. 3. Changes of relative viscosity with R during the titrations of 7.5 mM $CaCl_2$ solution (η_r^{Ca}, ●) and acetate buffer (η_r^C, ○) into 0.052% LMP solution. R is defined as the molar ratio of Ca to free galacturonic acid units of pectin.

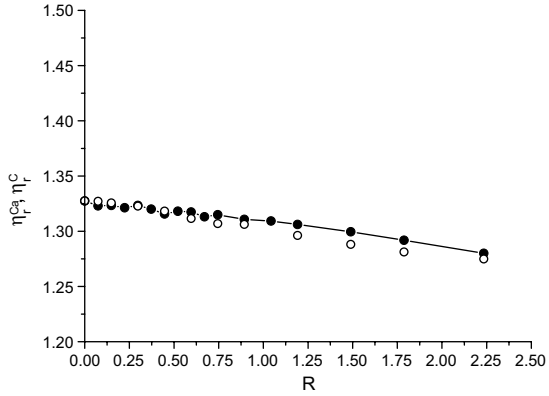

Fig. 4. Changes of relative viscosity with R during the titrations of 7.5 mM $CaCl_2$ solution (η_r^{Ca}, ●) and acetate buffer (η_r^C, ○) into 0.052% HMP solution. R is defined as the molar ratio of Ca to free galacturonic acid units of pectin.

molecular weight, but differing in DE, DA, and FGAC (Table 1). The different values of FGAC represent different numbers of binding sites per chain available for Ca, since FGA in pectin is the only possible binding site. As demonstrated in the ITC and relative viscosity measurements, the two pectins have distinctively different binding behaviors with Ca.

The binding of LMP with Ca is a two-step process, with a boundary located around $R = 0.25$. ITC results show a reduced binding heat in Step I, which subsequently increases in Step II (Fig. 1d). This indicates the formation of a certain new ordered structure in Step II, generating an extra heat effect. The molecular size of pectin also experiences a rapid growth in Step II (Fig. 3). Furthermore, the boundary between Steps I and II ($R = 0.25$) corresponds to the stoichiometry of egg-box dimers constituted of 2/1 helical pectin chains (Donati, Benegas, & Paoletti, 2006). These observations lead us to interpret the binding mechanism of pectin following the same assignments as for alginate (Fang et al., 2007). Step I can be attributed to the formation of monocomplex between Ca ions and free galacturonic acid units of pectin, and Step II to the formation of egg-box dimers through pairing of the monocomplexes. It should be noted that in addition to Steps I and II, the binding of alginate with Ca includes also a third step assigned to lateral association of egg-box dimers. This then is one of the differences between alginate and pectin.

ITC results indicate that the binding of HMP with Ca is more like a single-step process, with the binding isotherm saturating at $R \approx 0.5$ (Fig. 2b). This value coincides with a stoichiometric equivalence based on the valency charge of Ca^{2+} ions and the COO^- groups of pectin. The viscosity measurements reveal a negligible chain–chain association (or crosslinking) of HMP upon addition of Ca (Fig. 4). This would indicate that the binding of Ca is mainly an intramolecular event rather than an intermolecular event. Siew et al. carried out binding studies of Mn^{2+} to alginate and low methoxyl pectin and reported similar values of R (around 0.5) for plateau bindings (Siew et al., 2005). Donati et al. investigated the interaction of Mg^{2+} with polyguluronate (polyME6 as coded by the authors), and they also found that the mixing enthalpy and the fraction of bound ions reach a plateau at $R \approx 0.5$ (Donati, Cesaro et al., 2006). Compared with Ca^{2+}, Mn^{2+}, and Mg^{2+} are less- or non-specific divalent cations to alginate and low methoxyl pectin, and have either poor or no capability of inducing chain–chain association, and thus gelation of the polymers (Donati, Cesaro et al., 2006; Siew et al., 2005). In this respect, the binding of Ca^{2+} to HMP, in relative terms, resembles those of Mn^{2+} and Mg^{2+} to alginate and low methoxyl pectin. HMP can be approximately regarded as a conventional polyelectrolyte, to which the binding of Ca is predominantly controlled by electrostatic or ion-binding interactions rather than by strong specific interactions, with almost no egg-box structures being produced.

4.2. Comparison of pectin with alginate

As previously noted, it has been assumed that the mechanism and structural pathway are the same for alginate and pectin binding with Ca, but this assumption is not based on solid experimental evidence. Previously we showed that the binding of Ca to alginate proceeds via three successive steps, consisting of monocomplexation, dimerization, and later association of dimers (Fang et al., 2007). The first two steps we find now are also applicable to the binding of Ca to LMP. But, important differences do exist between alginate and pectin with regard to their Ca-binding behaviors.

Figs. 5 and 6 compare the heat effect and the change of relative viscosity for the bindings of Ca to LGA, HGA, LMP, and HMP. The heat generated is normalized by per mole of specific-binding sites (G and FGA units for alginate and pectin, respectively), and the relative viscosity is normalized against control experiments. The former characterizes the capability of the polyuronates to form

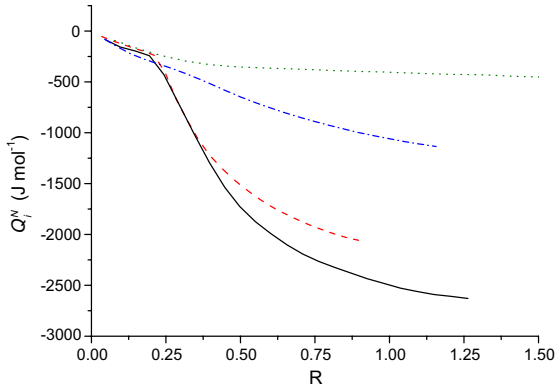

Fig. 5. Comparison of the normalized accumulative heat Q_i^N for the bindings of Ca to different polyuronates: LGA (solid line); HGA (dashed line); LMP (dash dotted line); and HMP (dotted line). Normalization is done on the basis of per mole of specific-binding sites, which refer to guluronate and free galacturonic acid units for alginate and pectin, respectively. R is defined as the molar ratio of Ca to the specific-binding sites. Note that the data for alginate was reproduced from the previous paper (Fang et al., 2007).

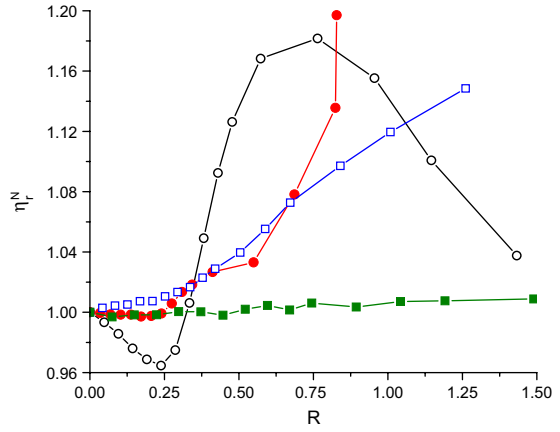

Fig. 6. Comparison of the changes of the normalized relative viscosity η_r^N for the bindings of Ca to different polyuronates: LGA (○); HGA (●); LMP (□); and HMP (■). R is the molar ratio of Ca to guluronate and free galacturonic acid units for alginate and pectin, respectively. The definition of η_r^N has been described in Section 2. Note that the viscosity data for alginate was reproduced from the previous paper (Fang et al., 2007).

ordered structures (i.e., egg-box structure), whereas the latter is an index of relative molecular size change of the polyuronates upon interaction with Ca. As can be seen from Fig. 5, at the initial stage of the binding (monocomplexation step, $R < 0.25$), the normalized accumulative heat Q_i^N is almost the same for the four polyuronates, except LMP differing slightly. This implies that the four polyuronates undergo basically the same extent of monocomplexation. Differences in Q_i^N start to be significant when $R > 0.25$. The final values of Q_i^N are about −2700, −2500, −1100, and −450 J/mol for LGA, HGA, LMP, and HMP, respectively, which follows the order: LGA > HGA ≫ LMP ≫ HMP. This suggests that the two alginates have much greater potential and/or capability of forming egg-box structures than the two pectins.

The comparison of the normalized relative viscosity η_r^N of the four polyuronates reveals two important differences (Fig. 6). Firstly, scrutiny of the initial stage of the binding ($R < 0.25$) shows that η_r^N for LGA, HGA, and HMP are all smaller than unity, and the only exception is LMP which shows $\eta_r^N > 1$. As discussed in our previous paper (Fang et al., 2007), $\eta_r^N < 1$ indicates a reduction in molecular size, and is characteristic of the formation of monocomplexes. The introduction of Ca ions along individual polyuronate chains by monocomplexation decreases chain charge density and screens the intramolecular repulsion between carboxylic groups, leading to the collapse/shrinking of individual polyuronate chains. In contrast, $\eta_r^N > 1$ for LMP at $R < 0.25$ suggests an increase in molecular size, and it totally conflicts with an interpretation based on monocomplex formation. The appreciable increase in molecular size can only arise from an inter-chain crosslinking or dimerization of the LMP chains. This therefore means that the dimerization of LMP is more of a progressive rather than a sharp critical process (Ralet, Dronnet, Buchholt, & Thibault, 2001). The formation of egg-box dimers starts immediately upon addition of Ca to LMP. This is contrasted to LGA and HGA where a critical boundary marks the monocomplexation and dimerization steps, and egg-box dimers emerge only when the stoichiometry ($R = 0.25$) is satisfied. Actually, the slight divergence of Q_i^N of LMP over LGA, HGA, and HMP when $R < 0.25$ (Fig. 5), as pointed out above, supports this conclusion, since dimerization at this stage produces additional heat for LMP.

Secondly, as described in the previous paper, LGA and HGA shows a clear third lateral association step following dimerization at higher Ca concentrations ($R > 0.55$), and this association depends strongly on alginate molecular weight (Fang et al., 2007). The lateral association step can be seen as a more steep increase in η_r^N for HGA and as a fall from maximum in η_r^N for LGA from Fig. 6. In contrast, such a third step can hardly be identified for LMP from the corresponding η_r^N–R curve, where η_r^N shows a smooth increase at an equivalent R rang ($R > 0.55$). This suggests that there is no appreciable lateral association of egg-box dimers for LMP during binding with Ca. As for HMP, neither the dimerization step nor the lateral association step can be discerned in the η_r^N–R curve where η_r^N keeps a value close to unity throughout $R > 0.25$. The binding of Ca to HMP limits to mainly an intramolecular event, i.e., monocomplexation.

The G content of LGA and HGA has been reported to be 46% and 64% (w/w), respectively (Fang et al., 2007). The free galacturonic acid content of LMP and HMP is about 46.2% and 26.1% (w/w), respectively (Table 1). Thus apart from HMP, the other three polyuronates, LGA, HGA, and LMP, have about the same proportion of specific-binding sites available for Ca. What then is responsible for the big differences in their binding behavior? It is highly probable that it is the structural difference between alginate and pectin that holds the answer (Fig. 7). Alginate

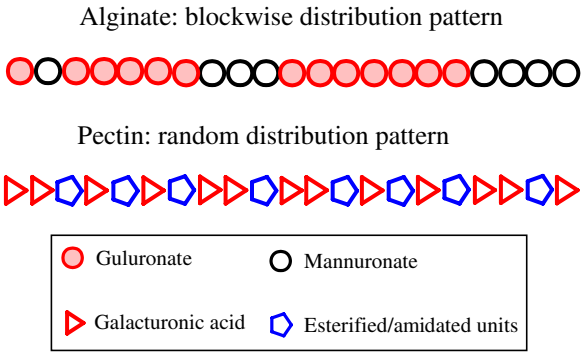

Fig. 7. Schematic illustration of the structural difference of alginate and pectin. Note that in reality the structure of pectin is much more complicated than is illustrated here, which is interrupted by many branched regions (so-called 'hairy' regions).

generally has a blockwise distribution of guluronate binding sites, though the detailed pattern may vary to small extent according to the species of seaweed. Low methoxyl pectin is usually produced by de-esterifying high methoxyl pectin to obtain gelling ability with Ca. The chemical de-esterification and in some cases amidation procedures result in free galacturonic acid residues being distributed quite randomly along pectin chain (Winning, Viereck, Norgaard, Larsen, & Engelsen, 2007). Relative to the blockwise distribution pattern of alginate, the random distribution pattern of pectin would introduce more defects during the formation of egg-box structures, and would also suppress dimerization to some extent. This would lead to a much smaller heat effect observed per mole of specific binding sites for pectin in comparison with alginate (Fig. 5). The random distribution pattern would also account for the absence of an appreciable lateral association step for pectin (i.e., LMP), since such an arrangement of binding sites would create tremendous spatial hindrance, e.g., from ester and amide units, for the subsequent lateral association of egg-box dimers.

The reason why the emergence of egg-box dimers is a critical process for alginate, but a progressive process for pectin, could also be due to their different distribution patterns of binding sites. Dimers are most likely to grow in a 'zippering' mode in alginate, but in a 'dotting' mode in pectin, due to their blockwise and random distribution patterns, respectively. The 'zippering' growth would be most energetically favoured when the stoichiometry of dimers ($R = 0.25$) is satisfied. Therefore, the dimerization in alginate is quite a critical event. In contrast, the 'dotting' growth of pectin would not require the stoichiometry to be strictly met, and egg-box dimers would emerge immediately upon adding Ca. Therefore, dimerization in pectin is more like a progressive event.

It is the main consideration which has been advanced to explain the differences found. This should not rule out other factors, e.g., molecular weight, flexibility of polymer chains. For example, the final value of Q_i^N for LGA is larger than that for HGA (Fig. 5), indicating that the low G content alginate sample has greater potential/capability of forming egg-box structures. This seems anti-intuitive, but interpretable if the effects of molecular weight and chain flexibility are taken into consideration: LGA has significantly higher molecular weight and greater chain flexibility compared with HGA (Fang et al., 2007), so would thus have longer G sequences to form more ordered egg-box structures. It would also have more configuration freedoms to allow polymer chains to take the best spatial positions to form more egg-box structures.

5. Conclusion

The binding behavior and structural evolutions of two typical pectin samples (LMP and HMP) upon addition of Ca have been investigated, and compared with our previous results for alginate (Fang et al., 2007). The different distribution patterns of binding sites, blockwise for alginate and random for pectin, can account for the differences between alginate and pectin with regard to their binding with Ca. LMP shows some similarity with alginate, both manifesting monocomplexation and dimerization steps. LMP, however, is different from alginate in that it has a progressive dimerization step in contrast to the critical behavior of alginate, and no appreciable lateral association of dimers is observed for LMP.

HMP is almost incapable of forming chain–chain associations during binding with Ca. Its behavior is close to a conventional polyelectrolyte without strong specific interactions.

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