Investigation of the effects of fine structure on the nanomechanical properties of pectin

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Pectin is an important structural polysaccharide found in the cell walls of all land plants. While in detail its composition and its organization *in muro* are complex, it is predominantly a copolymer of galacturonic acid and its methylesterified counterpart. Previous single-molecule stretching studies carried out on a sparsely methylesterified pectin sample indicated the importance of force-induced conformational transitions of the pyranose ring during extension, and the possible biological role of such transitions was discussed. More heavily methylesterified samples are better biomimetic models of the polymeric components as found in the plant cell wall, in particular being less restricted by the shackles of the significant intermolecular interactions expected to constrain the behavior of bare galacturonic acid sequences. Density functional theory calculations revealed that upon extending galacturonic acid monomers, whether methylesterified or not, the initial ($^4C_1$) chair structure is transformed to a ($^3S_5$) skew boat and that subsequently upon further elongation, via an intermediate inverted skew boat ($^5S_3$), the inverted chair ($^1C_4$) is reached. Experimentally, the force-extension curve of highly methylesterified pectin was found to be solvent dependent in the same manner as the unesterified sample, indicating that minimal changes in the strength of interring hydrogen bonding result from such a substitution, and finally, as only subtle changes in the force-extension behavior of pectin resulted from changes in the degree of methylesterification, previous speculations about the role of force-induced transformations *in vivo* are supported.

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I. INTRODUCTION

Single molecule force spectroscopy using the atomic force microscope has given access to an unprecedented level of information regarding the stress response of a host of biopolymers [1–4]. Studies of polysaccharide stretching have yielded particularly interesting data, the interpretation of which requires the marriage of statistical-mechanical theories of polymer physics to the complexities afforded by possible force-induced rearrangements or even conformational transitions of the constituent sugar rings. Such monomer transitions during stretching, from classical chair forms of the pyranose ring to more elongated arrangements, function as molecular sacrificial bonds [5], increasing the polymer's contour length and thus producing characteristic deviations, or "clicks," in the slope of the force-extension curve. Indeed, many curves exhibiting features of this type have been measured [6–12], and while accounting for their detailed origin is still a work in progress [13,14], the fact that they are observed in polysaccharides in which the rings are axially linked and are completely absent in equatorially linked analogs, gives great credence to the idea that axial bonds facilitate these transitions by acting as molecular levers. Thus it is central to the interpretation of polysaccharide force-extension curves to understand that those polymers possessing only equatorial glycosidic linkages such as cellulose (1*e*-4*e*) do not have the possibility of undergoing stretching-driven conformational transitions, while those with one axial and one equatorial bond, such as amylose (1*a*-4*e*) or dextran (1*a*-6*e*) can undergo one conformational transformation, and those with both bonds axial, such as pectin, two [15].

Pectin is one of the few polysaccharides in which the chain linkages at both sides of the constituent pyranose rings are axial (1*a*-4*a*), but nevertheless it has received comparatively little attention since an initial study of its single chain stretching behavior was reported almost a decade ago [8], despite the fact that it is an important structural polysaccharide. It is found in the cell walls of all land plants and although this is a complex biological matrix in which hemicelluloses, pectins, cellulose, proteins, and lignin all play a role in determining structure and properties, it is known that the pectin component has considerable mechanical utility within the cell wall [16,17]. Pectin is extracted commercially from lemon peel and apple pomace and consists mainly of 1,4 linked α-*D*-galacturonate residues (typically $\sim$90%). While this dominant part of the pectin, referred to as homogalacturonan, is a linear and relatively stiff anionic polymer [18], the sugar residue also naturally occurs in a substituted, methylesterified form. Pectins are usually characterized by this degree of methylesterification (DM) and their network formation capabilities are related to this value, with DM $<50\%$ pectins forming ion-induced networks *in vitro*. Interestingly this structural feature is known to be modified *in vivo* as the plant engineers its constituent polymers, eliciting desired mechanical changes in order to facilitate varied physiological processes.

It has been speculated that the force-induced conformational transitions in pectin molecules, proposed to explain the origin of two clicks observed in the single molecule force-extension curves, may have biological significance. This could involve acting as a sensor with a signaling role [8], or as a compliant element possessing extra elastic extensibility, directly contributing to the control and maintenance of the

mechanical properties of the cell wall [11]. In this context it should be noted that the fine structure of many polysaccharides, particularly those fulfilling a structural role, can be modified spatially and temporally by the orchestrated action of specific enzymes, and that the nature, amount, and pattern of substituents thus generated is known to significantly modify the intermolecular interactions of the polymer. It particular, it is clear from *in vitro* studies that the degree and pattern of methylesterification modulates calcium binding and the propensity for intermolecular hydrogen bonding in pectin systems [19,20]. It is therefore interesting to consider the effect that such modifications might have on the nanomechanical behavior of the single chains and thereby their implications for the proposed mechanisms by which force-induced conformational transitions might be functional *in muro*.

In fact it has been predicted in a molecular dynamics study that sugar ring substituents could significantly alter the force-extension behavior of single chains [21] and experimentally observed that methylcarboxylation has a large effect on the force extension curve of dextran [6]. In addition, it would be expected that substituent groups could extensively modify interring hydrogen bonding and in that way may also have a significant effect on the nanomechnics of single chains. Indeed, recent studies have shown that the importance, and even the strength, of interring hydrogen bonds might be assessed by carrying out single chain stretching studies in solvents of different dielectric constant [22,23].

In this paper we specifically investigate the consequences of the degree of methylesterification for the single molecule stretching behavior of pectin. After first observing the behavior of polygalacturonic acid, a completely unsubstituted chain, we address this question in the spirit of the work described above, first using density functional theory (DFT) calculations to investigate the effects of methylesterification upon the intrinsic stabilities of different ring conformers. Full force-extension curves are subsequently predicted using the results of these calculations by employing an extensible wormlike chain (EWLC) model, incorporating the concept of an equilibrium of states to account for the conformational transformations [24–26], and these are compared with the experimental results. Secondly, experiments were performed in solvents of low dielectric constant in order to address the significance of interring hydrogen bonding in highly substituted samples. Finally we report the results of measurements carried out on a series of pectin samples of known methylester contents.

II. EXPERIMENTAL DETAILS

A. Materials

Pectin samples derived from lemon peel were kindly supplied by CP Kelco ApS, Denmark. These samples were manufactured from the same highly methylesterified mother pectin extraction. Their DM (31% and 78%, respectively) was controlled via treatment with a pectin methylesterase (PME) of fungal origin [39]. This enzyme is believed to generate intrachain distributions of methylesterification that are close to random. Polygalcturonic acid (DM$\sim$0) and highly methylesterified ($\sim$90%) samples were purchased from Sigma-Aldrich. The sample average degree of methylesterification and also the width of the intermolecular DM distribution of each sample were experimentally determined by capillary electrophoresis as previously described [27–29]. An estimate of the width of this distribution of DM among polymers is particularly important when it is being sampled one chain at a time [30].

B. Atomic force microscopy (AFM)

In a typical AFM experiment, a single molecule was attached to a substrate and an AFM tip. As the tip and substrate separation is increased, the molecule straightens and stretches, with the force applied to the molecule determined from the deflection of the flexible AFM cantilever. The samples were prepared by applying 20 μl of 0.01 wt % solutions in deionized H_2O to clean glass disks, which were then dried at 11.3% relatively humidity overnight. This was then extensively rinsed with deionized H_2O leaving only the tightly bound molecules on the surface. After drying the sample was mounted in the AFM and the liquid cell filled with the appropriate solvent just prior to the force curve measurements. Force-distance curves were recorded by pulling the molecules at 0.5–4 μm s^{-1} using a scanning probe microscope (Veeco Nanoscope E) with a Si AFM tip calibrated using the thermal excitation method. We performed our AFM experiments in deionized water, 0.1*M* sodium phosphate buffer, or in hexadecane.

C. Calculations

DFT calculations were performed on the α-*D*-galacturonic acid anion and a methylesterified analog using the B3LYP/6–311++G^{**} basis set, implemented in PC-GAMESS. These were carried out either on a single Intel P4 computer in a LINUX environment or finally on a 14 node cluster on the New Zealand Super Computer (NZSC) Weta Workshop facility, using Intel Dual Xeon processors. MPI was used to parallelize the code. The anion was studied as most experimental stretches were performed at *p*H values where the α-*D*-galacturonic acid residue was in its dissociated form. The initial structure of each monomer was optimized in the $^4C_1$ conformer. To simulate the stretching behavior the constrained separation of the glycosidic oxygen atoms was increased monotonically, reoptimizing the structure at each length to determine the relative energy and ring conformation at each stage of the extension process. Care was needed when optimizing the structure of the α-*D*-galacturonic acid anion in its inverted chair form due to the tendency of the ring hydroxyl at *C*(4) to form stabilizing hydrogen bonds with the carboxyl oxygen ion. However, as such a stabilization is not possible in the polymer, owing to the presence of the glycosidic bond, the position of the hydroxyl proton was constrained such that the distance to the carboxylate ion was not in the hydrogen-bonding regime ($\sim$0.21 nm). The preliminary geometry optimization was carried out at the B3LYP/6–31G^* level and was continued using the higher order 6–311++G^{**} basis. The convergence

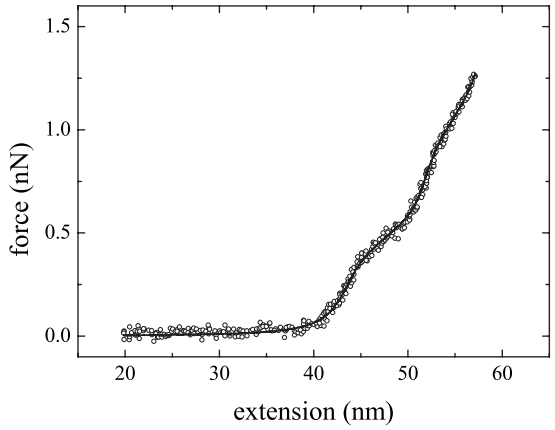

FIG. 1. Force extension curves of polygalacturonic acid (DM =0%) in 0.1*M* sodium phosphate buffer, *p*H=6.0. The line is a simulation using the model described by Haverkamp *et al.* [25] with the ring length values calculated by our DFT calculations for the *α-D*-galacturonic acid anion. Simulation parameters: l_p=1 nm, N_{total}=100 rings, chair length=0.4592 nm, boat length=0.5176 nm, inverted chair length=0.5576 nm, Φ=20 nN, ΔG_{01}=16.8 kJ mol^{-1}, and ΔG_{02}=25.4 kJ mol^{-1}.

criteria energy differences between cycles of optimization were less than 1×10^{-6} hartree and that for the gradient was set to be less than 1×10^{-4} a.u., as mentioned in previous works [31,32]. The calculated energies and length differences between each of the conformers was then used, in combination with values of polymeric persistence length and specific stiffness, to generate a full predicted force-extension curve [25].

III. RESULTS AND DISCUSSION

A. Stretching polygalacturonic acid

As the previously reported work on pectin [8] was carried out with a sample possessing an average degree of methylesterification of 9%, we began by stretching unmethylesterified pectin (polygalacturonic acid) in order to establish a baseline for the study of the effect of methylesterification. In aqueous solution, the force-extension curve revealed one clear "click" at 400–500 pN, similar to that found in the previously reported work on pectin, in which this feature was assigned to a $^4C_1$ to boat transformation (Fig. 1). A second proposed transformation in the previous work, assigned to a conformational transition from boat to $^1C_4$ inverted chair was less clear here (~1000 pN) but in the same force range as previously found [8]. Simulations confirm that a second transformation of this nature would be expected to yield a less defined plateau feature due both to its position relative to the rapidly rising enthalpic part of the curve and to the process of populating the final conformer state through the intermediate. Such a feature will only become clearly visible as a second distinct transformation when the energy difference between the second and final conformational states becomes large enough so that the second state is fully populated before the final state begins to have a significant population. That is, the boats should be stable over a small force range so that the contour length does not change significantly, allowing the chain to behave as a standard EWLC, prior to any later boat to inverted chair transformation at even higher forces. The result of a simulation is also shown in the figure, clearly indicating that the EWLC model modified to take account of force induced conformational transitions, describes the experimental data well. The lengths of the three conformers were fixed at those given by our DFT calculations for the *α-D*-galacturonic acid anion, which will be discussed further in due course. The persistence length, in line with Kuhn lengths from previous work [8], is found to be of the order of 1 nm. This is substantially less than that measured by scattering techniques [33], and in this context it is interesting to note that while generally statistical mechanical models fit a host of single polysaccharide stretching data rather well, including those without clicks, the Kuhn or persistence lengths extracted are often shorter than would be expected; with even less than a single sugar ring being reported [34]. Recent work examining the rescaling of persistence length with anchoring deflections or kinking may shed some light on this problem [35,36].

As described in the Introduction, in order to address the question, "how might the nanomechanical properties of single pectin molecules change as a function of the degree of methylesterification?" we will concentrate on two basic hypotheses. First that the polymer stretching properties might be altered by changes to the inherent conformational energy landscape of the monomer building blocks, and second that induced changes in the degree of interring hydrogen bonding may play a role in modifying the force-extension behavior.

B. Effect of substituents on the conformational energy landscape of monomers

DFT calculations have proven to be a valuable tool for studying single ring conformational energy landscapes and have been used to provide insight into conformational changes that may be force-induced and hence aid the interpretation of single polysaccharide stretching experiments. Therefore we have investigated whether the conformational energy landscape of pectin monomers differs significantly upon substitution at *C*6 by performing DFT calculations of the *α-D*-galacturonic acid anion and a methylesterified analog.

Our calculations show that the initial $^4C_1$ conformer lengths are similar for the *α-D*-galacturonic acid anion and for methoxyl-galactose (0.4592 and 0.4560 nm, respectively), in agreement with fiber x-ray diffraction [37]. In addition, the results confirm that at least three stable states are indeed possible during the extension of both the *α-D*-galacturonic acid anion and the methylesterified analog (Fig. 2). During simulated extensions, when the glycosidic oxygen atoms are constrained to be around 0.52 nm apart, skew boat structures ($^3S_5$) were found to be the most stable conformations in both cases, rather than symmetrical boats [8], consistent with knowledge based on cyclohexane and *α-D*-glucose [38]. The lengths of these skew boats, as determined from the DFT calculations, for both the galacturonic acid anion and the methylesterifed sugar, are given in Table

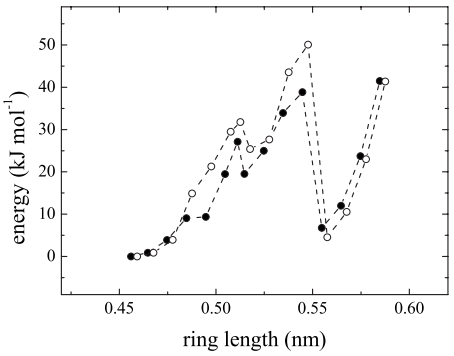

FIG. 2. Energy profile of the α-*D*-galacturonic acid anion and methoxyl-galactose during elongation of the *O*1-*O*4 distance using the B3LYP/6-311++G** level of theory. ○: α-*D*-galacturonic acid anion and ●: methoxyl-galactose.

I. The energy differences between these $^4C_1$ and $^3S_5$ conformers were found to be 25 and 19 kJ mol^{-1}, respectively, similar to that found by others for cyclohexane [38]. Moreover, bearing in mind that solvent and finite temperature considerations are absent in the DFT, these values are similar to that extracted from the experimental data (~17 kJ mol^{-1}) for the unesterified molecule (Fig. 1).

At still longer imposed extensions, when the glycosidic oxygens are separated by around 0.56 nm, inverted chair conformers emerge as the most stable ring structures. It is informative to observe the conformational pathway of these latter transitions. In the course of the extensions, the stretched monomers transform from chairs ($^4C_1$) to skew boats ($^3S_5$) as described (Fig. 3). However, upon further stretching, the monomers transform to inverted skew boat ($^5S_3$) intermediates, rather than directly to the inverted chair as proposed previously [8]. Only after further extension do the molecules transform to the final inverted chair ($^1C_4$) conformation. This intermediate inverted skew boat is not a stable conformer during the extension process, and thus we do not expect this to be significantly populated at equilibrium.

While in fact there is significant general precedent for the relative stability of inverted chairs compared to boat structures, it is also significantly more stable in our calculations than the skew boat structure was *at the smaller extension* (Fig. 2). This raises a difficult question of how it can account for the second transition observed in the AFM stretching experiments (Fig. 1 [8]) given that the predicted force required to extend the ring to the skew boat would be greater

TABLE I. Length of α-*D*-galacturonic acid anion and methoxyl-galactose during elongation of the *O*1−*O*4 distance using the B3LYP/6-311++G** level of theory.

	α-*D*-galacturonic acid anion /nm	Methoxyl-galactose / nm
Chair ($^4C_1$)	0.4592	0.4560
Skew boat ($^3S_5$)	0.5176	0.5147
Inverted chair ($^1C_4$)	0.5576	0.5547

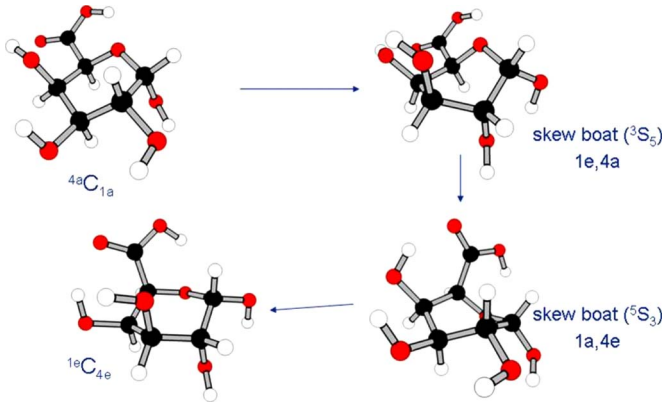

FIG. 3. (Color online) Conformer pathway during the elongation of the α-*D*-galacturonic acid along the *O*1-*O*4 vector using the B3LYP/6-311++G** basis set. Four distinct conformers are observed, three of which are stable ($^4C_1$, $^3S_5$, and $^1C_4$), and one unstable intermediate state ($^5S_3$).

than that required to obtain a greater extension ending in an inverted chair. The consequence of this would be that as soon as a ring is stretched enough to populate the skew boat state it would immediately reduce the force acting on it by elongating further and dropping into the inverted chair, which is significantly less strained regardless of its more extended conformation. This is clearly demonstrated by taking the three-state equilibrium model [25] at face value and using the calculated DFT conformer lengths and energy differences to generate a force-extension curve (Fig. 4). Evidently this results in an indistinguishable single click (centered at 77 pN), very different from the experimental result, where only the chair and inverted chairs are significantly populated during the extension process. This suggests that either (i) the inverted chair is significantly destabilized in the actual poly-

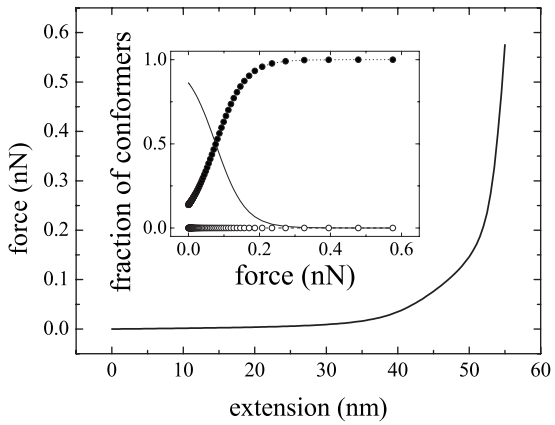

FIG. 4. Simulated force-extension curve for polygalacturonic acid using parameters obtained by DFT calculations of the α-*D*-galacturonic acid anion using the B3LYP/6-311++G** basis set. Inset: The fraction of conformers as a function of applied force, solid line: chair, open circles --: boat, filled circles ···: inverted chair. Simulation parameters: l_p=1 nm, N_{total}=100 rings, chair length=0.4592 nm, boat length=0.5176 nm, inverted chair length =0.5576 nm, Φ=20 nN, ΔG_{01}=25.4 kJ mol^{-1}, and ΔG_{02}=−20.85 kJ mol^{-1}.

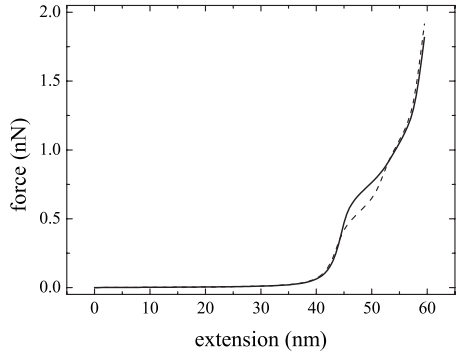

FIG. 5. Simulated force-extension curves for polygalacturonic acid (solid line) and a methylesterified analog (dashed line); using conformer lengths and chair-boat energy differences obtained by DFT calculations of the α-*D*-galacturonic acid anion and a methylesterified version using the B3LYP/6-311++G** basis set. The boat to inverted chair energy differences are set at 25 kJ mol^{-1} based on experimental evidence. The clicks ($^4C_1$ to $^3S_5$ and $^3S_5$ to $^1C_4$) are centered at 722 and 1038 pN for polygalacturonic acid and 552 and 1038 pN for methylesterified polygalacturonic acid. Simulation parameters: l_p=1 nm, N_{total}=100 rings, conformer lengths from Table I, Φ=20 nN, ΔG_{01}=25.4 kJ mol^{-1} (polygal.), ΔG_{01}=19.5 kJ mol^{-1} (polymeth.), and ΔG_{02}=25 kJ mol^{-1}.

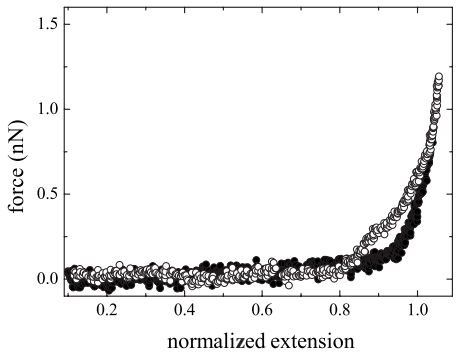

FIG. 6. Force-extension curves of highly methylesterified pectin (DM=90%) in water (○) and hexadecane (●). The extension is normalized to the length at 1 nN.

meric system as opposed to the monomer, owing to interaction effects neglected in the DFT, (ii) the conformational states involved in the second transition are not in equilibrium so that the transition barriers have a significant role to play, or (iii) that the second transition originates predominantly from a change in some other molecular feature or a secondary structure motif. Improving our high force, large extension, measurements to clearly observe the second transition remains a primary part of ongoing work, so that its dependence on rate might be examined.

For the purposes of calculating what effect the differences obtained in the DFT calculations would have for the force-extension curves of completely methylesterified or unesterified pectin, the energy differences of the skew boats to inverted chair conformations has been assumed to be 25 kJ mol^{-1}, in line with the fitting of experimental data (Fig. 1). Curves have then been calculated using the EWLC taking into account a three-state equilibrium model, as described previously. A small but clear difference is observed in the force required for the chair to boat transformation depending on whether the polymer is methylesterified or not (Fig. 5). The model we have employed here is able to predict the form of the force-extension curves from DFT rather well compared with previously reported attempts, including the values of the forces of the first click (500–700 pN simulated vs 400–500 pN experimental). At this stage we conclude that DFT calculations indicate that methylesterification does influence the conformational energy landscape of the monomers, albeit by a reasonably small amount, and therefore may result in a small change in the force-extension behavior of polymers built from these monomers, with the conformational transitions occurring at slightly lower forces in the methylesterified form.

C. Effect of substituents on the interring hydrogen bonding

Clearly the calculations detailed above are single ring calculations and cannot capture the possibility of interring hydrogen bonding. Extensive previous work clearly showed that amylose and pectin display solvent dependent force-extension behavior [22,23], with changes in the solvent dielectric constant modulating the strength of interring H bonding. Full substitution of the amylose ring hydroxyls by acetyl groups resulted in solvent independent force-extension behavior, strongly supporting the hypothesis that some substitutions of the sugar ring can effect its force-induced conformational transformations though modifications of interring hydrogen bonding.

If carboxyl side groups in pectin act as hydrogen bond donors, forming bonds with neighboring ring oxygen atoms [23], then we might expect that by studying a highly methylesterified (DM=90%) sample, we would remove the ability for such bonds to exist and thereby be able to clearly differentiate substituted from unsubstituted samples by their single molecule stretching behavior in low dielectric strength solvents, where interring hydrogen bonding is maximized. However, stretching highly methylesterified (DM=90%) pectin in water and hexadecane, essentially reproduced the result obtained from experiments that used a sample with a low degree of methylesterification, reported previously (Fig. 6); that is, a significant strengthening of hydrogen bonds was found in hexadecane. As the largely methylesterified pectin sample still exhibited solvent dependent force-extension behavior, it is unlikely that differences in interring hydrogen bonding would be sufficient to modify the single molecule stretching behavior between samples with differing degrees of methylesterification. It should be noted that the result obtained at low dielectric constant shows that there are strong hydrogen bonds in both samples, and by implication that, as the bonding is not changed by methylesterification, that the carboxyl group acts as a H-bond acceptor, not donor.

D. Experimental force-extension curves for pectins of varying degrees of methylesterification

Single pectin chains sampled from distributions with average DM values of 31%, 78%, and 90% have been stretched in water (Figs. 7(a)–7(c), respectively). It should be noted

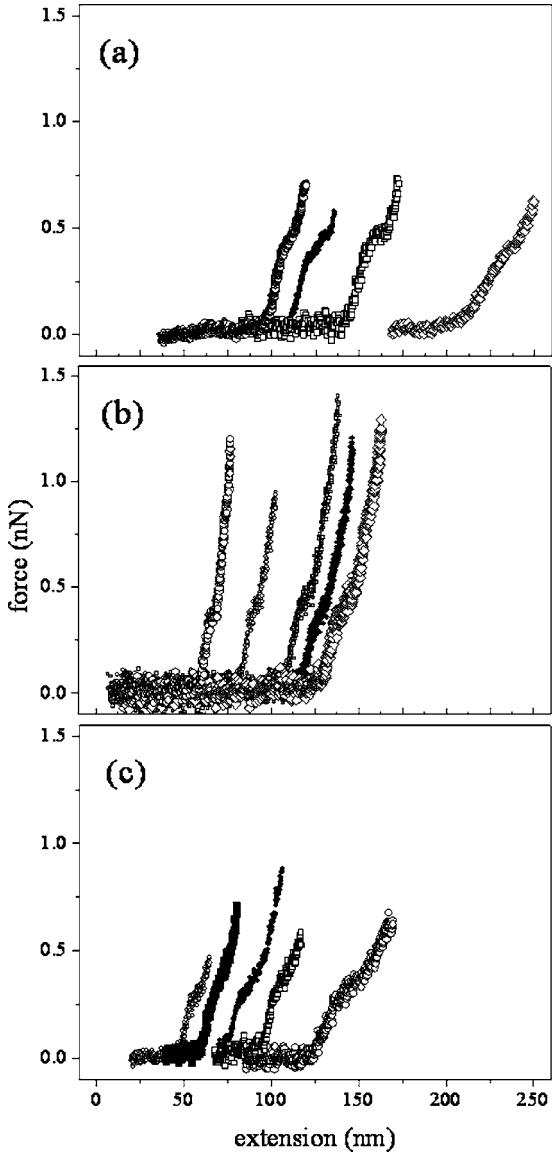

FIG. 7. Force-extension curves of pectin with various degrees of methylesterification in water. (a) 31% DM, (b) 78% DM, and (c) 90% DM.

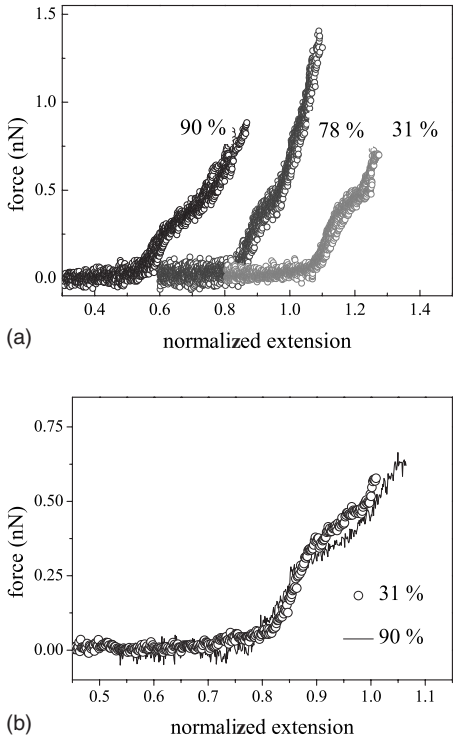

FIG. 8. (a) Concatenated normalized force-extension curves of pectin with various degrees of methylesterification measured in water. The extension was normalized based on the length at 600 pN and the 31% DM and 90% DM curves were offset by 0.25 for ease of viewing. (b) Interpolations of the concatenated normalized data sets from the 31% and 90% DM samples, showing a small (50–100 pN) difference in the force activating the first conformational transition of the pyranose ring.

that these methylesterification values have been obtained by methodologies that measure sample-average properties; that is, polysaccharide samples are heterogeneous with respect to fine structure and not every polymer chain in the 31% methylesterified sample will have 31% of its galacturonic acid groups methylesterified. However, we have measured the intermolecular distributions in addition to the average values using an electrophoretic technique that separates the chains on the basis of their charge density [27–29]. The distributions were found to be approximately Gaussian with full widths at half height of around 10%, so that the polymers stretched from the 31% and 90% DM samples are indeed still likely to have significantly different DM values. In fact, the data will show that the variation of force-extension behavior is generally small, so that differences in repeat experiments originating from the heterogeneity in a single sample will be minor.

The three datasets normalize by length reasonably well, providing good evidence that the data indeed represent single molecule stretches. Unfortunately it proved difficult to obtain good data in the region of the second transition, which is of great interest as discussed earlier, and improving our data in that area remains a focus of further work. It is clear that the data show that, in water, the methylesterification, which naturally varies in biological context, does not influence the force-extension behavior of single pectin molecules by a particularly large amount, as has been found with alternative substitutions of other polysaccharides. Nevertheless, by concatenation of the normalized data sets and a subsequent interpolation, an indication that the most methylesterified sample (90%) displays a click at a lower force than the less substituted sample (30%) can be found (Fig. 8), which is in line with the prediction of the DFT calculations described above.

These data, obtained by experiments carried out on more highly methylesterified substrates than have been investigated previously, show that only subtle changes in the force-extension behavior of pectin result as the degree of methylesterification is modified. This fact is crucial in a biological context, where in particular the "clicks" observed in the single molecule stretching behavior may have a functional role *in vivo*. Bare galacturonic acid groups in the cell wall are considerably more likely to be involved in intermolecular

interactions, particularly those mediated by calcium, and conversely lengths of pectic polymers that are free to exhibit single polysaccharide force-extension behavior in response to stresses are most likely to be significantly methylesterified. Here we show that these fine structures also exhibit similar behavior to that found with previously studied low DM samples, so that the speculations regarding its biological function remain valid. In the future the effects of other naturally occurring fine structure variations in pectin, such as the acetylation found at *C*2 and *C*3 particularly in sugar beet pectins, will be studied. In addition, we aim to improve our measurements at high forces so that the second click and its equilibrium nature can be investigated in greater detail.

IV. CONCLUSIONS

Using the results of DFT calculations regarding the conformational space of individual sugar rings in order to generate an extension-dependent contour length and incorporating this into standard statistical mechanical models of chain behavior captures a good deal of the phenomenology of the single molecule stretching behavior of pectin. On extending galacturonic acid monomers the initial ($^4C_1$) chair structure is transformed to a ($^3S_5$) skew boat and upon further elongation, via an intermediate inverted skew boat ($^5S_3$), the inverted chair ($^1C_4$) is populated. The glycosidic oxygen distances of all forms, the energy difference of the first step, and furthermore the prediction of the effects of methylesterification are all in reasonable agreement with experiment. However, the predicted stability of the inverted chair is problematic in terms of explaining the observed stretching behavior using an equilibrium model. The force-extension curve of highly methylesterified pectin is solvent dependent in a similar vein to the unesterified sample, with interring hydrogen bonding a significant determinant of stretch behavior in solvents with low dielectric constants. In such hydrogen bonding the carboxyl oxygen acts as a proton acceptor, not donor. As only subtle changes in the force-extension behavior of pectin result as the degree of methylesterification is modified, previous speculations about the role of force-induced transformations in low DM polymers hold for more methylesterified analogs which are more likely to behave in this way *in vivo*.

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