Model for stretching elastic biopolymers which exhibit conformational transformations

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We derive an expression that represents the physical behavior of a polysaccharide molecule as it is stretched from the entropic region, through one or more ring conformational transformations, into the Hookean regime. The model adapts existing models in order to accommodate one or more force-induced conformational transformations of the glycan rings and is based on the concept of equilibrium between the clicked (longer conformers) and unclicked states. This equilibrium is determined by the Gibbs energy difference between these two states which is perturbed in favor of the clicked states by the force applied to the molecule. The derived expression is used to generate force-extension curves for model polymers and can illustrate the effect of the Gibbs energy for each transformation on the shape of these curves. It is also used to fit the force-extension curves of polysaccharides to obtain the Gibbs energy differences between the conformers. Good agreement was found between this model and experimental data on carboxymethylamylose, dextran, alginate, and pectin.

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I. INTRODUCTION

Techniques for single-molecule stretching using the atomic force microscope (AFM) [1,2] or optical tweezers [3] are now well established. These techniques have been applied to a variety of biopolymers including proteins and polysaccharides. The results of these experiments are often analyzed using statistical mechanical models of polymer extensibility. The wormlike chain (WLC) model is one that successfully represents the low-force region of the force curve where entropic unraveling of the polymer chain takes place. This model has been further developed to the extensible wormlike chain (EWLC) [4] which accounts for the Hookean extension of a polymer chain at higher forces. Other models have been developed [5].

One important group of biomaterials to which these models have been applied is the polysaccharides. Polysaccharides are ubiquitous in nature, and evidence has recently been obtained to indicate they have a more widespread mechanical function in biology than was previously recognized [6,7].

In addition to the entropic and Hookean mechanisms of chain elasticity certain polysaccharides possess an additional mechanism for generating extra elastic extensibility involving a conformational change that can occur under tension. These conformational changes originate from the structure of the pyranose ring which can exist in varying conformers of different lengths. Transformations between such conformers may, for example, be from one chair form to another (for example, from $^4C_1$ to $^1C_4$), from a chair to a boat form (e.g., $^4C_1$ to $^{1,4}B$), or from chair to boat to chair (e.g., $^4C_1$ to $^{1,4}B$ to $^1C_4$) where the conformation which is present when no tension is applied is the thermodynamically favored form [8–11]. Pyranose-based polymers with a 1-4 glycosidic linkage with one axial and one equatorial bond, such as amylose (1*a*-4*e*) and dermochondan sulfate (1*a*-4*e*), can undergo one main conformational transformation. Those with a 1-4 linkage but with both bonds axial, such as pectin (1*a*-4*a*), undergo at least two conformational transformation. Conversely those with solely equatorial linkages, such as cellulose (1*e*-4*e*) do not have such possibilities. Intermediate states may also exist such as a skewed boat form. In this paper we refer to all these force-induced transformations from a shorter to a longer conformation as "clicks."

It has recently been demonstrated that molecules capable of exhibiting this form of elasticity are found in tissues required to be especially elastic [7]. This suggests that this form of elasticity may have a functional role in controlling the macroscopic properties of important biomaterials.

Biopolymers, including polysaccharides, are also technologically important materials in the food industry and increasingly in manufacturing. Hence understanding the nanomechanics of pyranose ring transformations and their impact on the force-extension curve is important not only in the biophysics of natural materials but also in attempts to synthesize smart materials using soft-nanotechnology.

Monte Carlo methods have previously been used to model the force-extension behavior of molecules that exhibit conformational transformations [12]. The extension by AFM of clicking polysaccharides was found to be an equilibrium process, except at very high pulling rates, in contrast to proteins, that are more commonly in the nonequilibrium region. However, despite being identified, the state of equilibrium of the polysaccharide clicks under typical experimental conditions does not appear to have been exploited. A proposal which contains some similar concepts to this has been suggested for poly(ethylene-glycol) but has not been developed completely [13].

In this paper we derive an expression that describes the force-extension curve of a polysaccharide molecule as it is stretched from the entropic region, through one or more conformational transformations, into the Hookean region, based on this equilibrium concept. We show that this model can be used for simulating force-extension behavior of complex polysaccharides and fits experimental data well to enable the easy extraction of a range of meaningful parameters.

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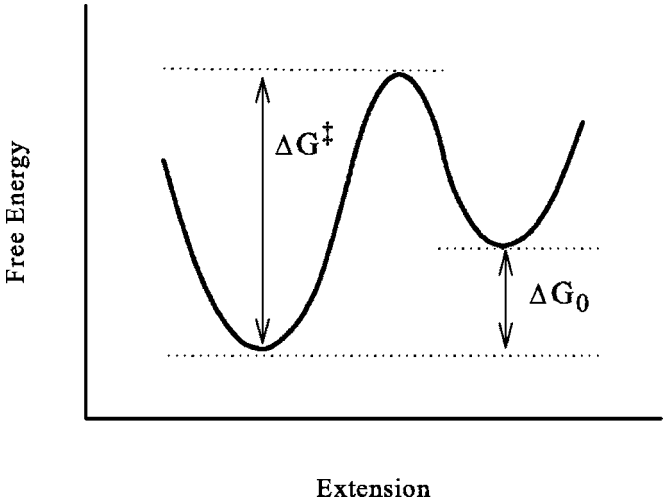


FIG. 1. Gibbs energy barrier $\Delta G^{\ddagger}$ and energy difference between the unclicked (shorter) and clicked (longer) states, ΔG_0.

II. THEORETICAL BASIS

A. Single-click model

To develop the model we consider a polymer consisting of glycan rings, some of which are able to exist in two different conformers that have different lengths. These two conformers may have different thermodynamic energies and may have an energy barrier for the transition between these two states (Fig. 1).

It has previously been shown, by using a Monte Carlo simulation, that at extension rates used in AFM stretching experiments (typically around 500 nm s^{-1}) the molecule maintains an equilibrium between the clicked and unclicked states throughout the stretch [12]. This has also been assumed on the basis of the analogy with the conformational kinetics of cyclohexane [14]. In other words, the height of the energy barrier between the clicked and unclicked states, $\Delta G^{\ddagger}$, does not influence the shape of the force curve. It was calculated that it is not until extension rates approach 1 cm s^{-1} that a significant departure from equilibrium conditions is observed. We could also describe this by saying that the shape of the force curve is not time dependent under experimental conditions.

We therefore use this notion of an equilibrium state being maintained during the extension process as the basis for our thinking. The number of clicked or unclicked sugar rings in the molecule is given by the Gibbs equation

$$\Delta G_0 = -k_B T \ln K, \tag{1}$$

where ΔG_0 is the energy difference between the clicked and unclicked states for one sugar ring (notice we say nothing about the transition between these two states), k_B is the Boltzmann constant, and T is the temperature. The equilibrium constant K is the ratio of the number of clicked to unclicked sugar rings.

If a force is applied to the ends of the molecule, this equilibrium is perturbed favor of the clicked form. This force gradually increases from zero to a large force (nN) over the entire range of the stretching; however, the region where the clicking takes place is only over small portion of this range. The effect of the force can be considered to lower the Gibbs energy difference between the unclicked and clicked states. This lowering of energy will be quantized such that for each click that takes place the applied force will contribute an amount of energy $F\Delta x$ where F is the applied stretching force and Δx is the increase in length of one unit of the molecule (one sugar ring) along the axis of stretching as a result of the conformational transformation. This Δx is the difference between the length of an unclicked and a clicked (X_c) sugar ring.

The effective Gibbs energy, or Gibbs energy under an applied force, ΔG_F, for the transformation between the clicked and unclicked polymers is therefore modified by this energy,

$$\Delta G_F = \Delta G_0 - F\Delta x. \tag{2}$$

We do not have to use Δx as a fitting parameter as this length can be obtained by other means [9].

Now we can calculate the equilibrium constant (the ratio of clicked to unclicked states in the molecule) under a stretching force K_F to be

$$K_F = \exp\left(-\frac{\Delta G_F}{k_B T}\right) = \exp\left(-\frac{\Delta G_0 - F\Delta x}{k_B T}\right). \tag{3}$$

We then consider the fraction of the sugar rings that have clicked, f_c, under any applied force. This can be related to the equilibrium constant thus

$$f_c = \frac{K_F}{1 + K_F}. \tag{4}$$

Combining Eqs. (2) and (3) and rearranging gives

$$f_c = \left[1 + \exp\left(\frac{\Delta G_0 - F\Delta x}{k_B T}\right)\right]^{-1}. \tag{5}$$

It is now possible to incorporate this fraction into any of the existing polymer models that incorporate the concept of a contour length l_c—for example, the freely jointed chain (FJC) model or the EWLC model [4]. For each ring that clicks, the contour length can be considered to increase. We can therefore adapt the EWLC model by substituting an expression for l_c. We will use the term l_e, the effective contour length, for this substitution.

The effective contour length depends on the total number of clickable rings, N, the fraction of clicked rings (at the applied force), and the difference in the lengths, Δx, of the unclicked, X_u, and clicked polysaccharide units in the form

$$l_e = N[X_u + f_c \Delta x]. \tag{6}$$

If we want to relate this to the original contour length, we have $l_c = NX_u$. Substituting the expression for f_c [Eq. (5)] we obtain

$$l_e = NX_u + \Delta x\left[1 + \exp\left(\frac{\Delta G_0 - F\Delta x}{k_B T}\right)\right]^{-1}. \tag{7}$$

This term l_e is then incorporated into the EWLC model in place of l_c, which was previously a constant, to give

$$F = \frac{k_B T}{l_p}\left[\frac{1}{4}\left(1 - \frac{l}{l_e} + \frac{F}{\Phi}\right)^{-2} + \frac{l}{l_e} - \frac{F}{\Phi} - \frac{1}{4}\right]. \quad (8)$$

This expression may be readily used to simulate force extension curves for clicking polysaccharides or fit experimental data and extract N (or l_c), ΔG_0, the persistence length l_p, and the modulus of elasticity, Φ.

So far we have assumed every sugar ring within the molecule is able to undertake an extending conformational change. However, many polysaccharide molecules are made up of a mixture of monomers configured such that some may click and others may not. For example, dermochondan sulfate can contain a mixture of D-glucuronate and L-iduronate, of which only the L-iduronate can undergo force induce conformational transformations [7]. Therefore f_c can be modified by multiplying by the proportion of sugar rings that may undergo this conformational change in the molecule and different lengths can be used for Δx.

B. Double-click model

With previous attempts at modeling the force-extension curves for single-molecule stretching it has been very difficult to extend the models to more than one type of conformational transformation within a molecule [14]. However, it is a relatively simple exercise to extend the model described in this work to two or more clicks. It is also possible to take into account various arrangements that may exist in a heteropolymer but for brevity we do not include all of these permutations here. We imagine three states: (i) where the sugar ring is in it lowest energy form (e.g., starting at $^4C_1$), (ii) where either one end of the ring or the other has clicked (e.g., to $^{1,4}B$), and (iii) where both ends of the ring have clicked (e.g., to $^1C_4$).

The total number of sugar rings in the molecule, N, is therefore made up of the number of unclicked, N_u, the number with one clicked, N_{c1}, and the number with both ends clicked, N_{c2}:

$$N = N_u + N_{c1} + N_{c2}. \quad (9)$$

These states are in equilibrium with each other with equilibrium constants K_{1F} and K_{2F} for the first and second clicks under an applied force:

$$N_u = N_{c1} = N_{c2}. \quad (10)$$

The effective contour length can then be represented as a function of the fractions of rings in each of these states if we take Δx_1 and to be the extension of each unit of the molecule to the intermediate state, Δx_2 the extension from the intermediate state to the final doubly clicked state, and X as the length of the unclicked sugar ring:

$$l_e = NX + (N_{c1} + N_{c2})\Delta x_1 + N_{c2}\Delta x_2 \quad (11)$$

or, in terms of fractions clicked,

$$l_e = N[X + (f_{c1} + f_{c2})\Delta x_1 + f_{c2}\Delta x_2], \quad (12)$$

where the fraction in each state (f_{c1} clicked through to the intermediate form, f_{c1} to the fully extended form) is given by

$$f_{c1} = \frac{K_{1F}K_{2F}}{1 + K_{1F} + K_{1F}K_{2F}}, \quad (13)$$

$$f_{c1} = \frac{K_{1F}}{1 + K_{1F} + K_{1F}K_{2F}}. \quad (14)$$

This results in the expression for the effective contour length:

$$l_e = N\left[X + K_{1F}\frac{(1 + K_{2F})\Delta x_1 + K_{2F}\Delta x_2}{1 + K_{1F} + K_{1F}K_{2F}}\right], \quad (15)$$

where

$$K_{1F} = \exp\left(-\frac{\Delta G_{0_1} - F\Delta x_1}{k_B T}\right) \quad (16)$$

$$K_{2F} = \exp\left(-\frac{\Delta G_{0_2} - F\Delta x_2}{k_B T}\right). \quad (17)$$

ΔG_{0_1} is the Gibbs energy differences for the first click and ΔG_{0_2} is for the second click. Extending the model to include three or more clicks is possible with similar reasoning to that used to develop the two-click model.

III. EXPERIMENTAL METHODS

Force-distance curves were recorded by pulling polysaccharide molecules using a scanning probe microscope (Veeco Nanoscope E) with a Si AFM tip (Ultrasharp CSG11, NT MDT Co, Moscow, Russia, tip radius ca. 10 nm) [7]. An extension rate of 500 nm s^{-1} was used with an indentation force of 5 nN and no waiting time during maximum indentation force and no waiting time between pulls. The samples were prepared by applying 50 μl of 0.01% solutions in H_2O to a clean glass disk, which was then dried at 11.3% relative humidity, then extensively rinsed with H_2O, before mounting in the AFM and filling the cell with water just prior to the force curve measurements. The force curves selected for analysis were those which contained a single extension event or well-separated multiple events with extension forces of at least 1 nN. The spring constant of each cantilever was determined with an Asylum Research MFP-3D scanning probe microscope using the built in software which calculates the force constant from the resonant frequency and the Q value. This method is reported to give an absolute accuracy of only 20% [15]; however, we found multiple calibrations of each cantilever indicated a precision of 6%–12% (at 95% confidence intervals). The cantilevers used had spring constants in the range 60–80 pN/nm.

Carboxymethylamylose (CMA) was purchased from Sigma and used without further purification. Alginate (MW 150 kDa) was supplied by Kurt I. Draget from the Department of Biotechnology, Norwegian University of Science and Technology and originated from FMC. The alginate had a sample average guluronic acid content of 70% as determined by NMR. Dextran of MW $\approx$ 2 000 000 produced by *Leuconostoc mesenteriodes*, strain No. B-512, was purchased from Sigma.

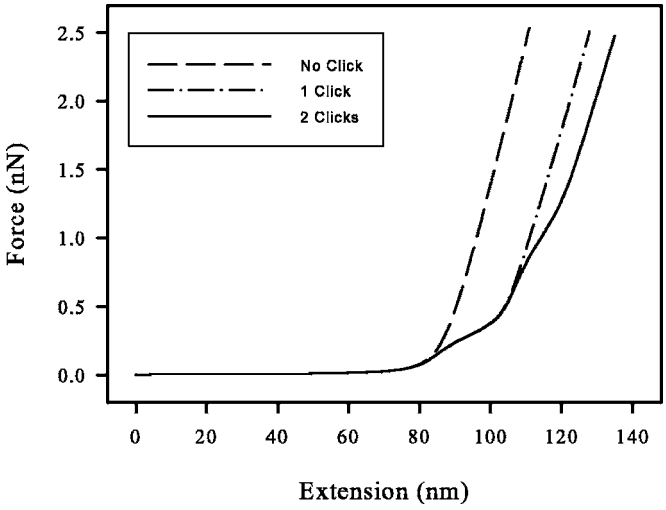


FIG. 2. Simulated force-extension curves of polysaccharides with zero, one, or two clicks. Parameters use are $l_p=1$ nm, $N_{total}=200$, $\Delta x_1=0.07$, $\Delta x_2=0.03$, $\Phi=10$ nN, $\Delta G_{0_1}=12.05$ kJ mol^{-1}, $\Delta G_{0_2}=18.07$ kJ mol^{-1}, and temperature=298 K.

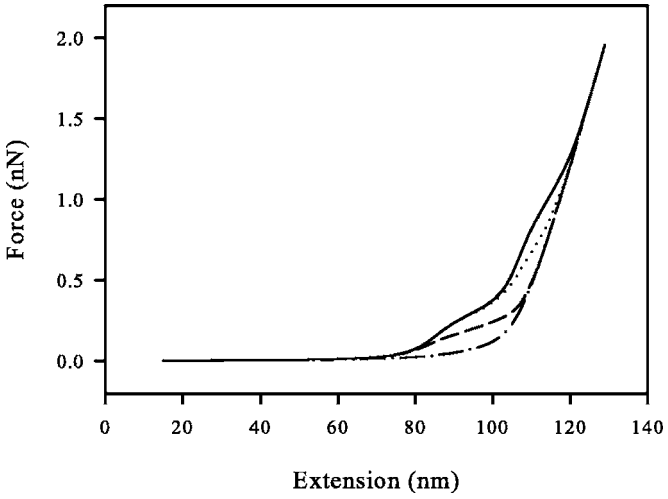


FIG. 3. Simulated force extension curves for a two click polysaccharide with varying ΔG_0. Solid line: $\Delta G_{0_2}>\Delta G_{0_1}$. Dotted line: $\Delta G_{0_2}=\Delta G_{0_1}$. Dashed line: $\Delta G_{0_2}=0$. Dot-dashed line: $\Delta G_{0_2}=-\Delta G_{0_1}$.

In all AFM force measurements there are problems with both the zero-force base line and the zero-distance position. In our normal fitting procedure we include, if necessary, a parameter to effect a base line shift. Base line and zero position can have a significant effect on l_p and Φ although they are not so important for ΔG_0 and Δx since these latter parameters are more influenced by the differences in force and distance rather than their absolute values. We can therefore have a higher degree of confidence in the ΔG_0 and Δx, which are the main focus of this paper, and a lesser confidence in l_p and Φ, for which we do not quote values.

IV. RESULTS AND DISCUSSION

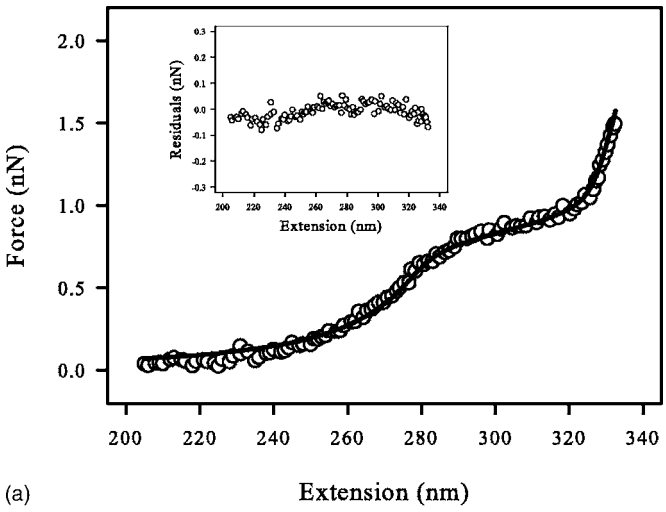
Using the single- and double-click models it has been possible to generate force-extension curves for a range of model polysaccharides. In Fig. 2 we illustrate the predicted behavior for polysaccharides that contain no clicks, one click, or two clicks. For each of the models the curves are coincident up until the point at which the click (or extra click) occurs.

The greatest strength of these simulations, however, is with the complex range of force-extension curves that are possible with a two-click polysaccharide (Fig. 3). With a two-click polysaccharide the form of the force-extension curve depends on the relative magnitudes of the Gibbs energy for the first and second conformational transformations. For a polysaccharide with $\Delta G_{0_2}>\Delta G_{0_1}$ the two conformational transformations will be separated on the force-extension curve with the two inflections visible and it may be possible to unequivocally determine the Gibbs energy and Δx for each of these transformations. For a polysaccharide with $\Delta G_{0_2}\approx\Delta G_{0_1}$ the inflection in the force-extension curve due to the first click will be preserved; however, the second click will be difficult to distinguish. If $\Delta G_{0_2}=0$, the second click will coincide with the first click with the effect that there will be only one inflection in the force-extension curve, however with a Δx, which is the sum of the Δx for each of the transformations. This inflection will occur at a lower force than would be the case if the second click did not exist. Therefore, while in error a one-click model could be fitted to the data, with a good statistical fit, the parameters obtained would give a ΔG_0 which is too small, and a Δx which is too large to be physically reasonable. For a polysaccharide where $\Delta G_{0_2}\leqslant-\Delta G_{0_1}$ no inflection in the force-extension curve is present and the force-extension curve appears like that of a nonclicking polymer.

These simulations enable the force-extension curves to be predicted for polysaccharides which contain two conformational transformations. It also enables information to be obtained on the Gibbs energy for each of the transformations. However, it will be readily appreciated that, where the Gibbs energy of the second transformation is small, there will not be two inflections easily distinguishable in the experimental force-extension curves. The use of this model to fit experimental data therefore depends on some knowledge of the structure of the molecule being stretched in order to be able to apply the correct one-click or two-click model. This knowledge of the structure preferably includes knowledge of the range of values of Δx, ϕ, and l_p that can be considered physically reasonable. Without knowledge of the molecular structure experimental data which show only one inflection could be attributed to either a one-click molecule or to a two-click molecule with a ΔG_{0_2} which is small or negative. With these considerations in mind we have applied these models to experimental data from two molecules known to have a single click and two known to have a double click.

To illustrate the utility of the single click model to AFM data, we have successfully fitted force-distance data we obtained from stretching dextran in water [Fig. 4(a)] and CMA in water [Fig. 4(b)].

For the fit to dextran we used the single-click model with parameters which have previously been applied in Monte



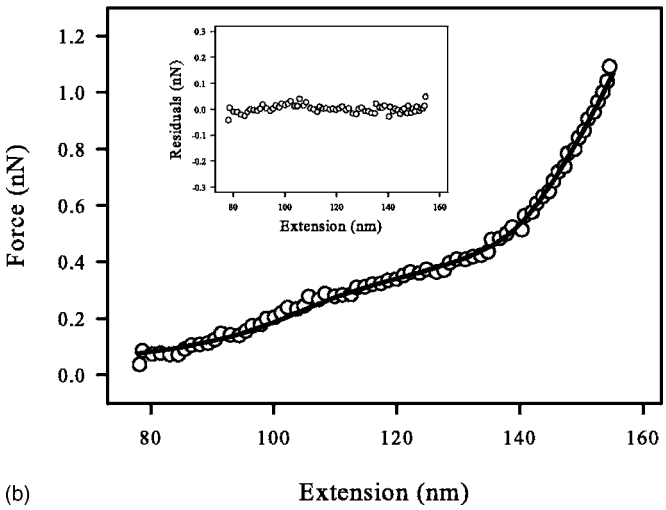
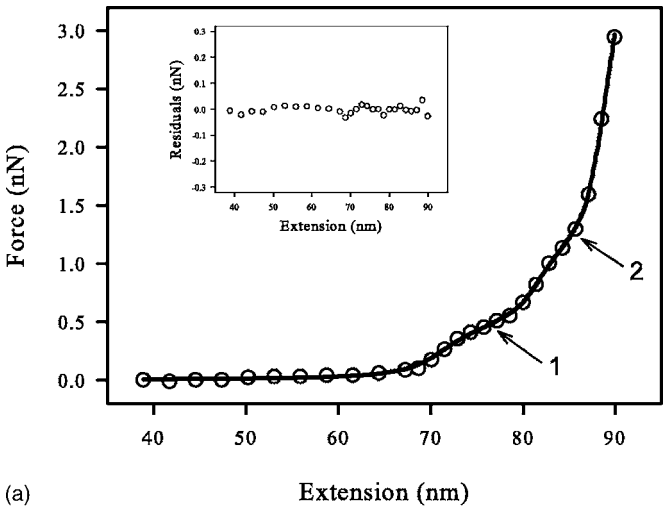


FIG. 4. Force-extension curves (a) of dextran in H_2O and (b) of CMA in H_2O. Open circles are experimental data; the solid line is the fitted model.



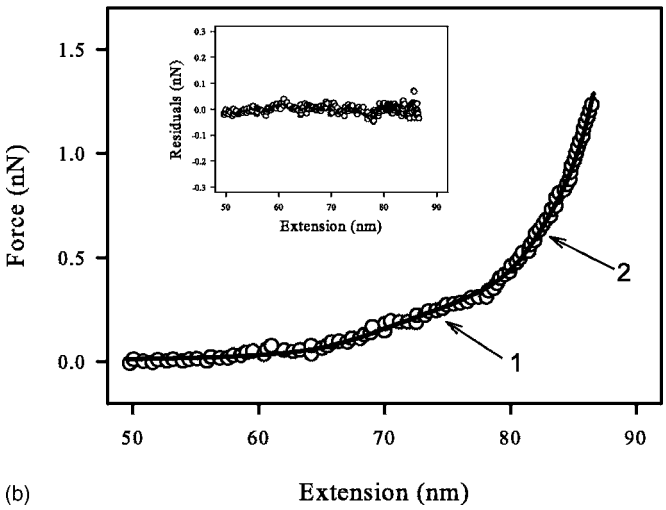


FIG. 5. Force extension curves of (a) pectin and (b) alginate (70% guluronic acid). Open circles are experimental data while the solid lines are the fitted model for two clicks. For clarity, arrows mark the midpoints of the first (1) and second (2) force-induced transformations as calculated by $\Delta G_0/\Delta x$. The midpoint of these transformations for pectin as 450 pN and 1250 pN and for alginate 240 pN and 630 pN.

Carlo simulations of dextran [12]. Using these values we fixed the three parameters $X_u=0.5$ nm, $\Delta x=0.065$ nm, and $\Delta G_0=33.1$ kJ mol^{-1} with only N allowed to vary. The fit very closely follows our experimental data with $r^2=0.9951$ and 0.9972 and, more importantly, with residuals that appear random [Fig. 4(a)]. As expected, comparing our model with the previous Monte Carlo simulation shows it to be identical (not shown), confirming that this function can simulate force extension curves containing conformational transformations without the need for Monte Carlo calculations. The fact that the fit using the parameters from Rief *et al.* [12] coincides so well with our independently obtained data is surprising since we believe the uncertainties in the absolute force calibration of the cantilevers to be 20% [15].

For CMA Δx was fixed at 0.0875 nm based on *ab initio*–optimized structures for α-D-glucose [8]. The fit then gave $\Delta G_0=18\pm4$ kJ mol^{-1} with $r^2=0.9972$ and with residuals that appear random [Fig. 4(b)]. This Gibbs energy for the conformational transformation is in line with 12–20 kJ mol^{-1} quoted by other studies [16,17]. The uncertainty in the absolute value is estimated to be around 20% due to uncertainty in the force calibration of the cantilever [15].

To illustrate the utility of the second model we used experimental data we have obtained for alginate (70% guluronic acid, 30% mannuronic acid) and data for pectin obtained from the literature [10] and fitted these to our model (Fig. 5). For the alginate the best fit to the data gives ΔG_0 of 7.9 ± 1.6 and 8.4 ± 1.8 kJ mol^{-1} for the first and second conformational transformations, respectively, and for pectin ΔG_0 of 12 ± 2 and 17 ± 3 kJ mol^{-1}. For alginate we used an initial chair length of 0.435 nm as given by XRD [18] and for pectin we used an initial chair length of 0.452 nm as given by DFT calculations [10]. In both fits we started with Δx values from DFT calculations [19] and allowed them to vary only within narrow constraints (±0.015 nm). For alginate the best fit gave $\Delta x_1=0.0548$ nm and $\Delta x_2=0.0223$ nm and for pectin $\Delta x_1=0.0449$ and $\Delta x_2=0.0228$ nm. The fits give an r^2

for alginate and pectin of 0.9943 and 0.9996, respectively, but more importantly have residuals that appear to be random. These two polysaccharides illustrate the behavior we have demonstrated by simulation in Fig. 3 where the pectin is an example of $\Delta G_{0_2} > \Delta G_{0_1}$ and alginate is an example of $\Delta G_{0_2} \approx \Delta G_{0_1}$.

The parameters we are interested in, ΔG_0 and Δx, are quite insensitive to typical thermal noise. We tried adding random 20 pN noise to simulated curves and then used our fitting to confirm that the original parameters can be recovered.

We have tried fitting a number (>30) of experimental curves to determine the variability in parameters obtained by the method we describe and find ΔG_0 and Δx to be reproducible to within 5%–10% (depending on the data set). However, noise could decrease this precision for very small ΔG_0 or very small Δx.

The extracted parameters from these models are sensitive both to the data and to the way the fit is carried out. The primary requirement for reliable parameter extraction is that the data extends well into the elastic region (>1 nN) so that force-induced transformations (particularly the second) are not mistaken for the Hookean region.

Although we can obtain l_p and Φ for all the fits, we feel that it would be misleading to provide numbers for these. l_p is very sensitive to noise, to base line corrections, and to an accurate determination of the zero-distance position. Φ requires force curves to extend to sufficiently high force, and not all of the curves displayed go to sufficient force to give values in which we would have confidence. Therefore when fitting these models to experimental data it is normally a good idea to apply some constraints to l_p and Φ to keep them within physically realistic values. The values of l_p and Φ are, of course, not the main focus of this work.

The largest uncertainly in ΔG_0 for the molecules we are interested in is due to the accuracy of the cantilever calibration, which is not related to our data fitting procedure. This uncertainty has been cited as being of the order of 20% [15], although this value is greater than the apparent precision with which we are able to calibrate the cantilevers which is around 6%–12% (95% confidence intervals).

An important requirement for the data analysis is that it be possible to discriminate between no-click, single-click, and double-click models. The ability to do this depends on the quality of the data and ΔG_0 and Δx for the polysaccharide, as we have detailed. It is possible to do a comparison between the models, which we show in Fig. 6 for pectin which does contain two well-separated clicks and for which we have good data to high force. The quality of the fit is well represented by the distribution of the residuals which clearly show the inflections in the curve which have not been fitted (Fig. 6, insets). It is clear with these data that it is possible to discriminate between the models. The alginate data may be similarly analyzed.

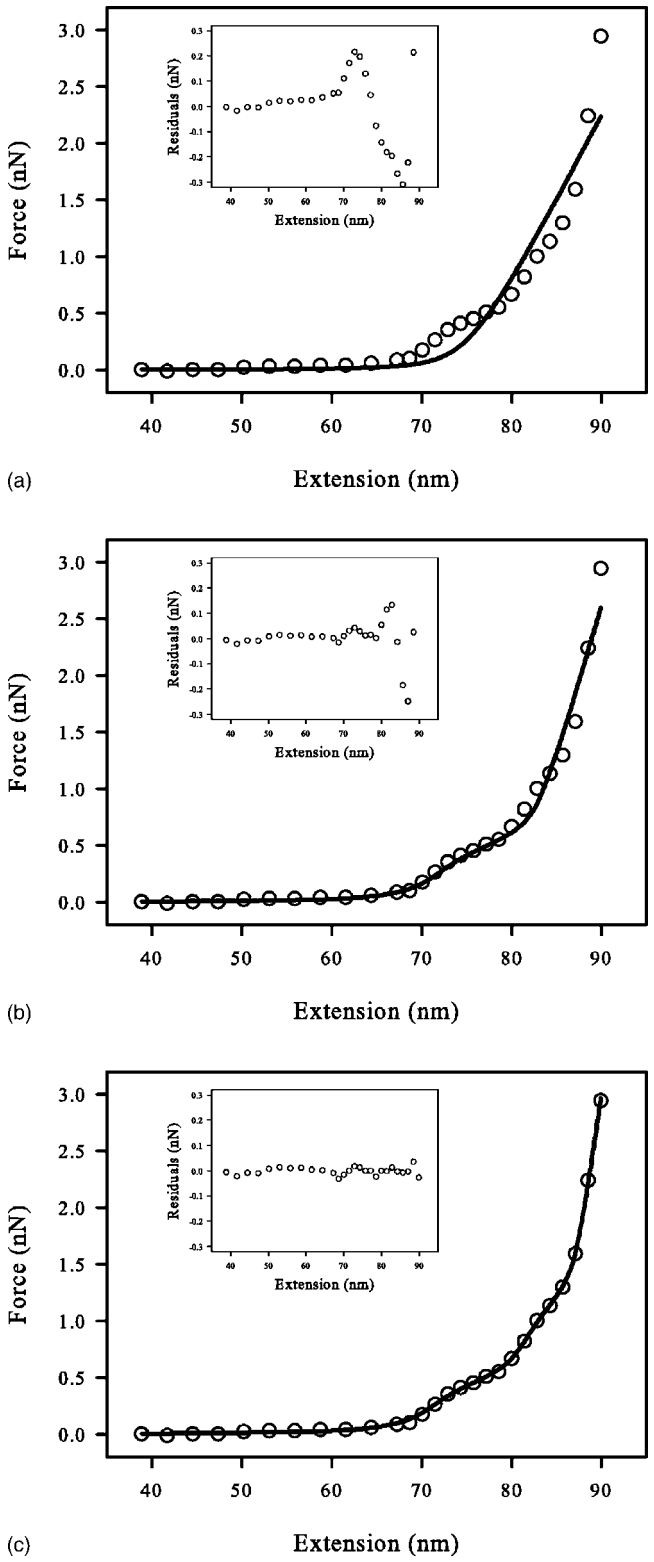


FIG. 6. Force extension curve of pectin fitted with the (a) no-click model, (b) one-click model, and (c) two-click model. Residuals are shown in the inset.

V. CONCLUSIONS

We have derived an expression that describes the force-distance behavior of a polysaccharide molecule as it is stretched from the entropic region, through one or more ring conformational transformations, into the Hookean regime. The model adapts existing models in order to accommodate force-induced conformational transformations of the glycan

rings and is based on the concept of equilibrium between the clicked and unclicked states. This equilibrium is determined by the Gibbs energy difference between these two states which is perturbed in favor of the clicked state by the force applied to the molecule. We have used the derived expressions to generate force-extension curves for model polymers which then illustrate the effect of the Gibbs energy for each transformation on the shape of these curves. We have also used the expressions to fit the force-extension curves of polysaccharides to obtain the Gibbs energy differences between the conformers. With suitable experimental data it is possible to discriminate between zero, one, and two click molecules. Good agreement was found when the expression was fitted to experimental data for CMA and it was possible to easily extract numerical values for the Gibbs energy difference between the conformers. The model was expanded to a system with two conformation transformations and was fitted to experimental data for alginate and pectin to obtain the Gibbs energy for both transformations in each molecule.

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