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Nanomechanical Control of Glucopyranose Rotamers

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The pyranose ring is the fundamental scaffold of many biological structures and a mediator in molecular recognition and adhesive interactions where it is subjected to stresses and deformations such as these that occur, for example, during the lysozyme reaction. However, the mechanics of the pyranose ring are poorly understood. Recent measurements of the elasticity of pyranose polysaccharides with atomic force microscopy (AFM)[1−8] have revealed force-induced chair-boat and chair inversion transitions of the pyranose ring,[2−6] suggesting that conformational analysis of ring structures[9] needs to be expanded to include force-driven transitions.[2,3] Here we use a powerful combination of AFM-based single molecule force spectroscopy[1] with ab initio and molecular dynamics calculations to capture and characterize rotations of the exocyclic group on the glucopyranose ring that are driven by an external force.

The orientation of the primary alcohol group attached to the pyranose ring can be conveniently characterized by the $O_6-C_6-C_5-O_5$ torsion angle (ω-angle, Figure 1a inset). Three stable staggered rotamers are possible for ω: *gt*, *gg*, and *tg* (Figure 1b, inset). However, X-ray and NMR measurements show a significant preference of gluco- and mannopyranosides toward *gt* and *gg* rotamers, which are populated almost equally, with a nearly complete absence of the *tg* rotamer (*gg*/*gt*/*tg*/ 60:40:0).[10] Here, we employ atomic force microscopy to determine the effect of the mechanical force on the orientation of the O_6-C_6 bond about the C_6-C_5 bond on the glucopyranose ring. For our studies we chose a β-1 → 6-linked glucan pustulan,[11] which, in its backbone contains the rotatable C_6-C_5 bonds (Figure 1a inset). The *equatorial* orientation of its glycosidic bonds is of significance to us because according to the model of Marszalek et al.[3] such bonds will not affect the ring conformation upon stretching, and therefore they are ideal for investigating the rotation about the C_5-C_6 bond *in isolation* from other complex conformational transitions, which are promoted by axial linkages.[1−6,12]

For single-molecule stretching measurements, pustulan molecules were adsorbed onto a glass surface from a water solution (0.001−0.1% w/v). They were picked up by the AFM tip (Si_3N_4, Veeco), in water, by pressing the tip against the substrate with forces of 5−40 nN and subsequently stretched. Figure 1a shows a family of force extension curves obtained from single pustulan molecules with various lengths. First, we note that these curves do not follow the freely jointed chain model of entropic elasticity. Second, we determined that their shape is identical on stretching and relaxing the molecules, indicating that the stretching process occurs in equilibrium. Third, we observe that the normalized recordings overlap well, proving that the measurements were obtained on individual molecules (Figure 1b, 16 different curves). Fourth, we

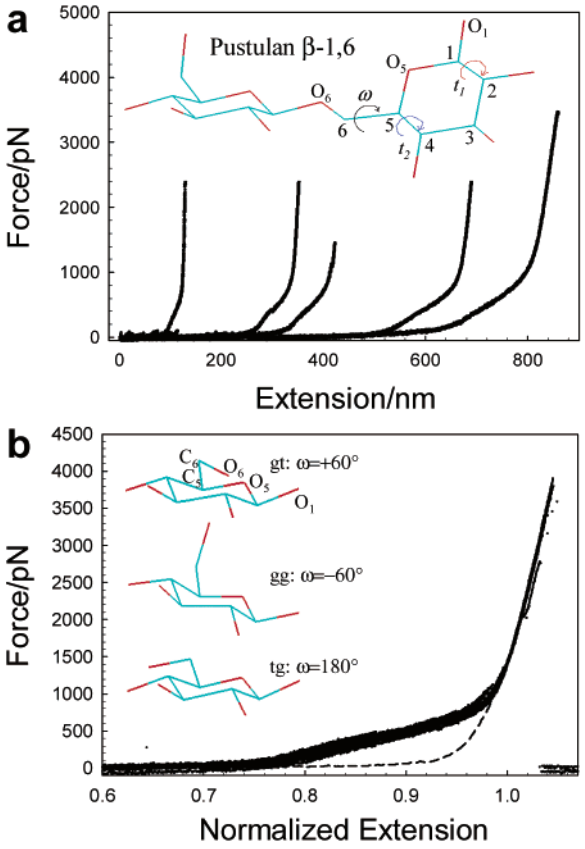

Figure 1. (a) Force spectrograms of single pustulan molecules of various length. (Inset) Structure of pustulan showing two β-D-glucopyranose residues connected by a 1 → 6 linkage. ω, t_1, and t_2 are the dihedral angles defined in the text. (b) Normalized force spectrograms of 16 different pustulan molecules. Force spectrogram of methylcellulose is represented by the dashed line. (Inset) *gt*, *gg*, and *tg* rotamers of β-D-glucopyranose.

note that in the force range of 100−700 pN, the pustulan length increases almost linearly with the force (pustulan behaves as a hookean spring), and its elasticity strongly deviates from that of methylcellulose (dashed line in Figure 1b). This is an interesting observation indicating that the mechanical properties rendered by β-1 → 6 linkages of pustulan are significantly different from those rendered by the "freely rotating" β-1 → 4 linkages of methylcellulose.[3,6] We conclude that the segments in the pustulan chain experience rotational restrictions, and an additional work, numerically equal to the area between the dashed line and the black curves, needs to be done to fully extend the pustulan chain. We propose that this work is used to rotate the aglycone bond (O_6-C_6) about the C_6-C_5 bond, because this rotation contributes to the extension of pustulan but is absent in cellulose. To estimate the possible length gains obtained from such rotations, we carried out the ab initio

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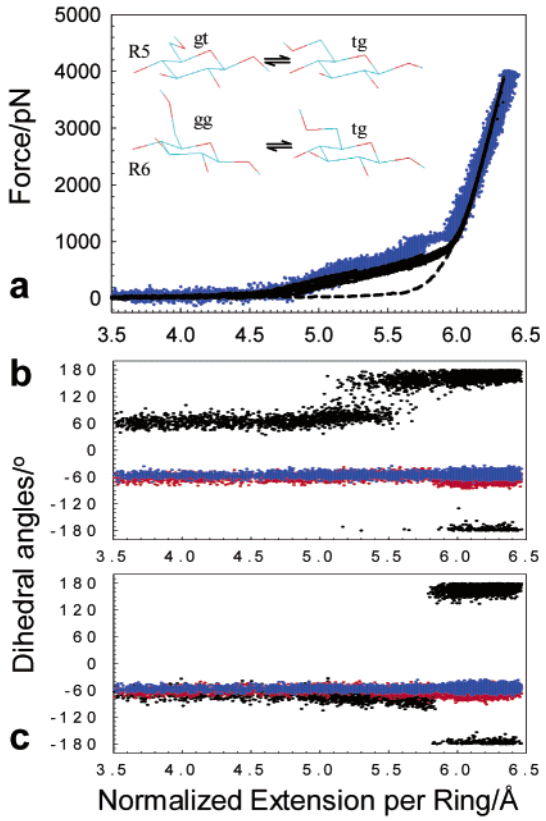

Figure 2. (a) Comparison between force extension curves of pustulan obtained by AFM (black curves) and by a 1 microsecond SMD simulation (blue trace). The renormalized methylcellulose curve is shown as the dashed line. (Inset) Structures of ring 5 (R5) and ring 6 (R6) shortly before and after the conformational transition to the *tg* state obtained from the SMD trajectories shown in (b) and (c), respectively. Trajectories of ω (black trace), t_1 (red trace), and t_2 (blue trace) for R5 (b) and for R6 (c).

quantum mechanical calculation of the O_1-O_6 distance in all three *relaxed* rotamers *gt*, *gg*, and *tg* for β-D-glucopyranose (see Supporting Information). It is interesting that the O_1-O_6 distance is the same for *gt* and *gg* rotamers and that it increases by ~0.92 Å (19%) in the *tg* rotamer. It is significant that the increase in the length of the pustulan chain between 100 and 700 pN measured by AFM is similar (~23%) (Figure 1b). This simple analysis supports our conjecture that the hookean elasticity of pustulan is generated by forced *gt* → *tg* and *gg* → *tg* rotations about the C_5-C_6 bond.

To examine conformational events during the stretching of pustulan, we carried out a 1 microsecond steered molecular dynamics (SMD) simulation[13] of this process on 10 1 → 6-linked β-D-glucose rings (Supporting Information). Figure 2a shows the force extension relationship obtained from this simulation (blue trace) and compares it with the AFM results (black solid lines). We note that the SMD result follows the AFM data very closely. To examine the conformational behavior of the pyranose rings, we monitored trajectories of the three dihedral angles in each ring ω, t_1 and t_2 (Figure 1a, inset). ω reports the rotameric status about the C_6-C_5 bond, while $t_1 = O_1-C_1-C_2-O_2$ and $t_2 = O_5-C_5-C_4-C_3$ are sensitive to changes in the ring conformation (e.g., transition to a boat). We find that torsions t_1 and t_2 remained constant throughout the simulation for *all the rings* (Figure 2b,c). This result is consistent with our prediction that equatorial glycosidic bonds cannot change the conformation of the pyranose rings. Next, we find that all the *gt* and *gg* rotamers flip to the *tg* state, and these transitions generate the unusual hookean elasticity of the chain. *gt* rotamers flip first, and they are followed by *gg* rotamers, which flip to the *tg* state at somewhat higher forces. We also note that the O_6-C_6 bond in *gt* rotamers swings many times between the *gt* and *tg* position before it settles in the stable *tg* state (Figure 2b), while *gg* rotamers flip to the *tg* state rather abruptly (Figure 2c). By calculating the area between the force curves of pustulan and methylcellulose (Figure 2a), we estimate that the work necessary to rotate the O_6-C_6 bond to the *tg* state is on average $w_{rot} = 5.7$ kcal/mol. This work is similar to the work necessary to flip the ring to a boatlike conformation.[2]

In conclusion, we have determined the nanomechanical fingerprint of the rotation of the O_6-C_6 bond about the C_5-C_6 bond on the glucopyranose ring. We have shown that the stretching (or relaxing) of 1 → 6-linked polysaccharides provides a unique means to control the distribution of their *gt*, *gg*, and *tg* rotamers. We speculate that the types of forced conformational transitions we describe here may occur in biological settings involving, for example, interactions between lectins and 1 → 6-linked sugar ligands. Our manipulations of single 1 → 6-linked polysaccharides increase the understanding of the conformational mechanics of the pyranose ring and expand single-molecule mechanochemistry.

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Supporting Information Available: Experimental and calculation details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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