J|A|C|S
COMMUNICATIONS

Published on Web 04/05/2006

Identification of Sugar Isomers by Single-Molecule Force Spectroscopy

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Recent measurements of polysaccharide chains by AFM-based single-molecule force spectroscopy[1] indicated that this purely mechanical technique should find useful applications in carbohydrate research. This is because AFM-obtained force spectrograms register molecular fingerprints of force-driven conformational transitions of the pyranose ring, which are sugar and glycosidic linkage-type specific.[2−7] A rule that equates the number of plateau features in polysaccharides' force spectrograms with the number of axial glycosidic linkages per sugar ring was worked out[3] and was used to identify individual polysaccharides in solution.[4] Here, we hypothesize that force spectroscopy can not only identify the number of axial linkages per ring[3] but also pinpoint their exact location on the pyranose ring; for example, it can discriminate between single axial and equatorial bonds at the C_1 or C_4 position.

To verify this conjecture, we investigated two complementary polysaccharides: β-galactan, a polymer composed of β-1 → 4 linked D-galactose (Figure 1b, inset), which has not been studied by force spectroscopy and amylose, a polymer composed of α-1 → 4 linked D-glucose (Figure 2 inset), which was extensively studied by AFM.[2−5,8] Both polysaccharides have one axial and one equatorial bond in their glycosidic linkages. However, in amylose, the glycosidic bond (C'_1-O_1) is axial and the aglycone bond (O_1-C_4) is equatorial, while in β-galactan, the orientation of these bonds is opposite. We asked a question whether simple mechanical stretching measurements on these polysaccharides can capture these subtle structural differences.

Figure 1a shows a family of force-extension curves of β-galactan molecules with various contour lengths. These force spectrograms were obtained by stretching individual molecules in water in the AFM (for technical details, see Supporting Information). All these curves reveal a distinct *single* plateau feature at a force of 640 ± 20 pN (number of recordings, $n = 12$). After extension normalization (see Supporting Information), these force-extension curves overlap well (Figure 1b), indicating that the stretching measurements were all performed on single polysaccharide molecules and not on their bundles.[5] The presence of a single plateau in these curves is consistent with our previous studies according to which a *single* plateau is a fingerprint of a conformational transition of the pyranose ring, such as the chair-boat transition, which is driven by a *single* axial bond, working as an atomic lever.[3,4]

In Figure 2, we compare the representative force-extension curves of β-galactan (black trace) and amylose (gray trace). We note that, although both curves have similar shapes and overlap well at low normalized extensions (<4.4 Å/ring) and at high extensions (>5.2 Å/ring), they significantly differ at intermediate extensions where the plateau features occur. We conclude that this significant and highly reproducible difference in the plateau level, 640 pN for β-galactan versus 280 pN for amylose,[2,8] must be somehow related to the structural differences between α-D-glucose and β-D-galactose and can be the basis for the mechanical identification of both sugar isomers.

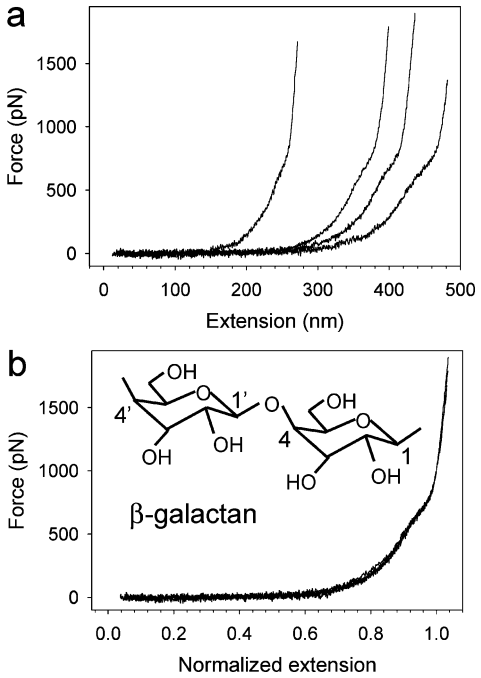

Figure 1. (a) Force spectrograms of single β-galactan molecules of various lengths. (b) Normalized force spectrograms from a. (Inset) Structure of β-galactan showing two β-D-galactose residues connected by a 1 → 4 linkage.

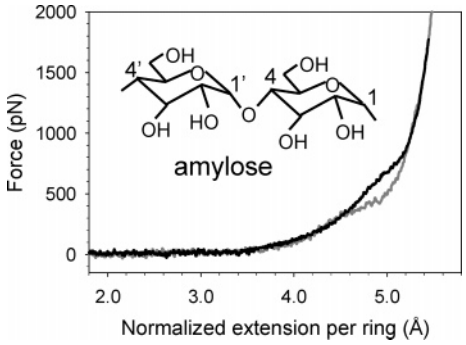

Figure 2. A comparison between normalized force spectrograms of β-galactan (black trace) and amylose[2−5] (gray trace). (Inset) Amylose structure: α-D-glucose residues connected by 1 → 4 linkages.

We and others proposed that the plateau feature in the force-extension curve of amylose represents the lengthening of the distance between consecutive glycosidic oxygen atoms upon the force-induced conformational transition of the α-D-glycopyranose rings from their $^4C_1$ chair conformation to a boat-like conformation.[2−5,8−10] To determine which conformational transitions produce the plateau feature in the force-extension curve of β-galactan and to pinpoint the origin of the differences in the elastic properties of β-galactan and amylose, we simulated forced stretching of single

10.1021/ja058828e CCC: $33.50

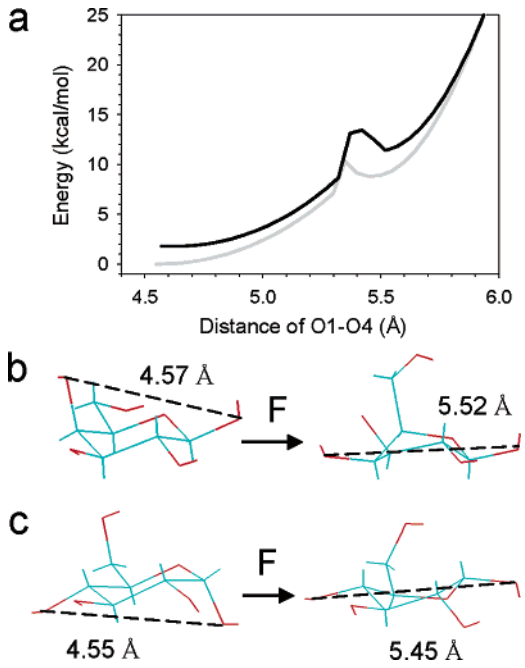

Figure 3. (a) A comparison between energy profiles of β-D-galactose (black trace) and α-D-glucose (gray trace) obtained by DFT geometry optimization with the O_1-O_4 distance constrained and stepwise increasing. (b) Structures of β-galactose before and after the forced chair to an inverted boat transition. (c) Structures of α-glucose before and after the forced chair to a skew-boat transition.

rings of β-D-galactose and α-D-glucose using quantum mechanics-based methods. We constrained the O_1-O_4 distance in these sugars and allowed it to increase in 0.05 Å increments, while carrying out, at each distance, full geometry optimization at the B3LYP/6-311++G** level of theory. The results of these energy profile calculations are shown in Figure 3a. First, we note that the starting structures presumed to be the minimum energy conformers for α-D-glucose[11] and β-D-galactose are $^4C_1$ chairs with the *gg* and *gt* orientation of the exocyclic group, respectively, with β-D-galactose being 1.8 kcal/mol higher in energy than α-D-glucose. Second, while the stretched α-D-glucose flipped to a skew boat with the flagpole hydrogen atoms pointing up (Figure 3c), the stretched β-D-galactose flipped to an inverted boat-like structure (Figure 3b), consistent with the mechanistic notion that the atomic lever C_4-O_4 would exert a torque about the rotation axis (C_3-C_5) to swing the C_4 atom downward. Third, the approximate position of the transition state for β-D-galactose is at the extension ~0.1 Å greater than that for α-D-glucose (Figure 3a). Fourth, the energy difference between the metastable inverted boat and the original chair structure of β-D-galactose is 9.6 kcal/mol, which is 0.8 kcal/mol greater than the energy difference between the $^4C_1$ chair and a skew boat for α-D-glucose (8.8 kcal/mol). By integrating the force-extension curves in Figure 2, it is possible to calculate and compare the work that needs to be done by the external force to fully stretch a single ring in β-D-galactan and in amylose. It is striking that this work is 1.1 kcal/mol greater for β-D-galactan as compared to amylose. While it is necessary to exercise caution when comparing the results of vacuum DFT calculations on monomer sugars with the experimental results obtained on long polysaccharide chains immersed in water, it is possible that the differences in the plateau force and the stretching work between β-D-galactan and amylose captured by the AFM indeed reflect the subtle differences in the conformational mechanics of β-D-galactose and α-D-glucose, captured by the DFT calculations (see Supporting Information for a further discussion on forces as determined by AFM measurements and DFT calculations).

In conclusion, we have investigated the mechanical properties of β-galactan by single-molecule force spectroscopy and identified a unique plateau feature in its force spectrogram that occurs at a force of 640 pN. By comparing the force spectrograms of β-galactan and amylose, we demonstrate that force spectroscopy is able to discriminate between sugar isomers in which axial and equatorial bonds at C_1 and C_4 are swapped. These results further validate and expand the application of force spectroscopy as a fine analytical tool for carbohydrate research.

Acknowledgment. We thank Drs. Weitao Yang and Zhenyu Lu for discussions. This work was supported by a grant from the NSF and by Duke University funds to P.E.M.

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

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JA058828E