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Solvent effects on the elasticity of polysaccharide molecules in disordered and ordered states by single-molecule force spectroscopy

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Abstract

Single-molecule force spectroscopy, especially as implemented on an atomic force microscopy (AFM) platform is unique in its ability to apply small ($F < 10$ pN) and large ($F > 1000$ pN) stretching forces to individual polymer chains and in this way examines their elasticity and also reports force-induced conformational transitions in whole polymers and in their building blocks. In this paper, we briefly review recent applications of single-molecule force spectroscopy to the study of polysaccharides elasticity. We provide examples illustrating AFM measurements of solvent effects on the hydrogen bonding and the elasticity of individual polysaccharides and how molecular dynamics simulation can aid the interpretation of AFM results. We also discuss the use of single-molecule force spectroscopy in exploring ordered secondary structures of individual polysaccharide chains and their multi-strand complexes.

Keywords: Polysaccharide; Single-molecule force spectroscopy; AFM (Atomic force microscopy)

1. Introduction

The development of laser trapping techniques in the 1970s and surface probe microscopy scanning tunneling microscopy (STM and AFM) atomic force microscopy, in the 1980s revolutionized polymer research in that it allowed not only visualization but also mechanical manipulations of individual polymer molecules. The uniqueness of atomic force microscopy, among these techniques is in its capability of applying very large stretching forces to individual polymers, and in this way measuring their elastic properties not only in the entropic regime characterized by the freely jointed chain (FJC)-like behavior but also in various enthalpic regimes characterized by large deviations from the FJC-type behavior, where force-induced conformational transitions of whole polymer chains and their components are triggered. These types of stretching measurements of polymers evolved into single-molecule force spectroscopy, which examines the relationship between a polymer length (end-to-end distance) and tension. Among biopolymers studied with the use of AFM-based single-molecule force spectroscopy, polysaccharides revealed a particular wealth of conformational behaviors that could be controlled by external forces [1–11]. For example, AFM stretching measurements captured forced chair-to-boat transitions of the pyranose ring in α-1,4 and α-1,6-linked glucose-based polysaccharides such as amylose, and dextran [2,4,7,12], chair-to-inverted chair transitions of α-D-galactose in such polysaccharides as pectin [8] and λ-carrageenan [10,11] and forced rotations around the C5–C6 bond of D-glucose in β-1,6-linked pustulan [3].

Because polysaccharides have an abundance of hydroxyl, acidic and charged groups they are prone to form regular/ordered structures such as single or multiple helices that are stabilized by hydrogen bonds, ionic bridges and also van der Waals interactions. AFM measurements proved invaluable in determining fingerprints of helical structures generated by polysaccharides such as carrageenans [13] Curdlan [14], and Xanthan [9] and even differentiated between their ordered and random coil structures. Consistent with previous measurements of the bulk properties of these polysaccharides, single molecule studies with the AFM also determined that their structures are profoundly affected by solvents and ionic conditions of the solution. The scope of this paper is to briefly review the recent progress in elucidating solvent effects on the hydrogen bonding and the elasticity of individual polysaccharide molecules and their complexes

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(multiple helices) obtained primarily by AFM-based single-molecule force spectroscopy and molecular dynamics simulations.

2. Materials and methods

2.1. Materials

Several polysaccharides were used in this study: amylose (type III, from potato, Sigma-Aldrich), pectin (from citrus, Sigma), pustulan (from lichen, Carbomer, San Diego, CA), dextran (Pharmacia/Pfizer, Peapack, NJ). Amylose triacetate was kindly provided by Prof. Yasuhiro Takahashi of Osaka University, Japan. Polysaccharides were first dissolved in proper solvent to form a solution at a concentration of 0.02–5%. To adsorb polysaccharide molecules onto a substrate, a drop of solution was placed on a clean glass substrate and dried. The sample was then rinsed intensely with water to remove most of the molecules and leave only these, which adsorbed tightly to the substrate [9]. The sample was placed in the AFM and proper solvent (such as water (dielectric constant, $\varepsilon = 80$), dimethyl sulfoxide (DMSO) ($\varepsilon = 47.2$), hexadecane ($\varepsilon = 2.0$), or dimethyl carbonate (DMC, $\varepsilon = 3.1$)) was injected into the liquid chamber.

2.2. Single molecule force spectroscopy

Force spectroscopy measurements were carried out on a home-made AFM instrument. This AFM was equipped with an AFM detector head from the MultiMode AFM (Veeco, USA) and a single-axis piezoelectric actuator with an integrated strain gauge sensor from Physik Instrumente (Germany). The spring constant of each Si_3N_4 cantilever (Veeco) was calibrated using the energy equipartition theorem [15]. To pick up a single polysaccharide molecule for stretching measurements, the AFM tip was pressed against the substrate at a force of 1–40 nN for several seconds and subsequently withdrawn.

2.3. Steered molecular dynamic simulations

SMD simulations [1,3,4,6] of amylose, pustlan and dextran and data analysis were carried out with the programs NAMD2 [16], XPLOR [17], VMD [18] and a new CHARMM-based carbohydrate solution force field [19]. Briefly, AFM stretching measurements on individual polymers are modeled in the SMD protocol by fixing one terminal of the polymer and applying an external harmonic potential to the other terminal. The center of this harmonic potential moves at a constant velocity in the stretch direction simulating the movement of the AFM cantilever. For details on these simulations refer to [4].

2.3.1. Normalization of force–extension curves

Because the contour lengths of each polysaccharide fragment, which is picked up randomly by the AFM tip, is different, force–extension curves obtained by the AFM need to be normalized to compare the results. Briefly, this extension normalization procedure is based on the property of the FJC-model that predicts chain extension at any force to be proportional to its contour length. Thus, by assuming that the extension of a polysaccharide chain, at a given force that is common to all the recordings, is equal to one, we can eliminate the dependence of the shape of the force curve on the particular value of the contour length. Details are available in our previous papers [1,6]. In addition, for these polysaccharides for which we carried out SMD simulations we can use the SMD results to normalize the chain extension with respect to a single sugar ring. This is done by measuring, from the simulation, an average projection of the distance between the consecutive glycosidic oxygen atoms on the direction of the force at the highest stretching forces ($\sim$3000 pN), at which conformational transitions (if any) of the sugar rings have already completed [1]. For example, the average distance between the oxygen atom # 4 on the first ring and the oxygen atom #1 on the 10th ring of an amylose fragment, projected on the direction of a SMD force of 3270 pN was determined to be 55.7 Å, and this length was found to be independent on the dielectric constant of the solvent used in the simulation. This result gives an average extension of amylose at $F = 3270$ pN to be 5.57 Å per ring, which then can be used to normalize the experimental force curves (assuming that the stretching force of 3270 pN or greater was achieved experimentally). This normalization is very convenient because it reports the elastic properties of polysaccharide chains 'per ring' and can be used to compare the results obtained on different fragments, under different (solvent) conditions. Details can be found in the supporting material to Ref. [1].

2.4. Other methods

For more information on the materials and methods used by other authors whose results are discussed in this review, the reader is referred to the original papers.

3. Results and discussion

3.1. Amylose elasticity in high and low dielectric constant solvents

Amylose is a linear 1→4-linked α-D-glucan (Fig. 1(a), inset). The force–extension curves of amylose obtained by AFM in water (dielectric constant, $\varepsilon = 80$) display a characteristic plateau feature at about 280 pN (see Fig. 1(a), black curve) [2]. Thus, the elasticity of individual amylose chains deviates strongly from the entropic elasticity expected of simple FJC-type polymers. This plateau feature was attributed to the force-induced chair-to-boat transitions of the glucopyranose rings, which lengthen the contour length of amylose by about 17% [2].

The close proximity of hydroxyl groups from neighboring rings in amylose implies that they should be able to form hydrogen bonds (H-bonds). In water these bonds will not be very strong because of the competition of glucose hydroxyls to form hydrogen bonds with water molecules. However, we expected that solvents with a very low dielectric constant should stabilize these inter-residue H-bonds. To investigate if