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Chapter 7

Probing the Interaction Between Cellulose and Cellulase with a Nanomechanical Sensor

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/50285

1. Introduction

1.1. Cellulose and cellulose biomass

A cellulose molecule is a linear polymer of D-anhydroglucopyranose units linked by β-1, 4-glucosidic bonds (Figure 1). On its reducing end, a cellulose molecule has an unsubstituted hemiacetal. On its non-reducing end, it has a hydroxyl group.

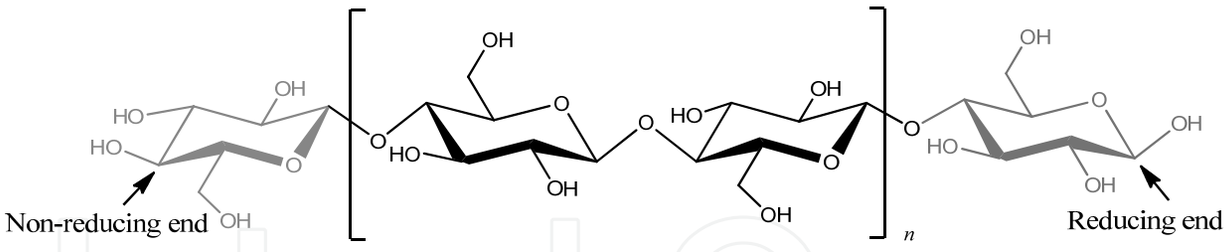


Figure 1. Molecular structure of a cellulose molecule.

Cellulose is the skeleton structure of almost all green plants. It is particularly abundant in non-food plants like trees and grasses, which typically have 40-60% cellulose, 20-40% hemicellulose, and 10-25% lignin (Lynd et al., 2002; Yang et al., 2007). There are four major polymorphs of cellulose: I, II, III, and IV. Cellulose I, often found in native cellulose, contains allomorphs I_α (bacteria and algae) and I_β (higher plants) (Kontturi et al., 2006; Pérez & Samain, 2010). Cellulose I, when treated with a concentrated alkaline solution, turns into cellulose II, a thermodynamically more stable crystalline form than cellulose I. Cellulose III_I can be obtained when cellulose microcrystal is subjected to supercritical ammonia. The structure of another allomorph of cellulose III, III_{II} is still being debated. Cellulose IV_I and IV_{II} are formed when cellulose III is heated in glycerol at 260°C (Peter, 2001).

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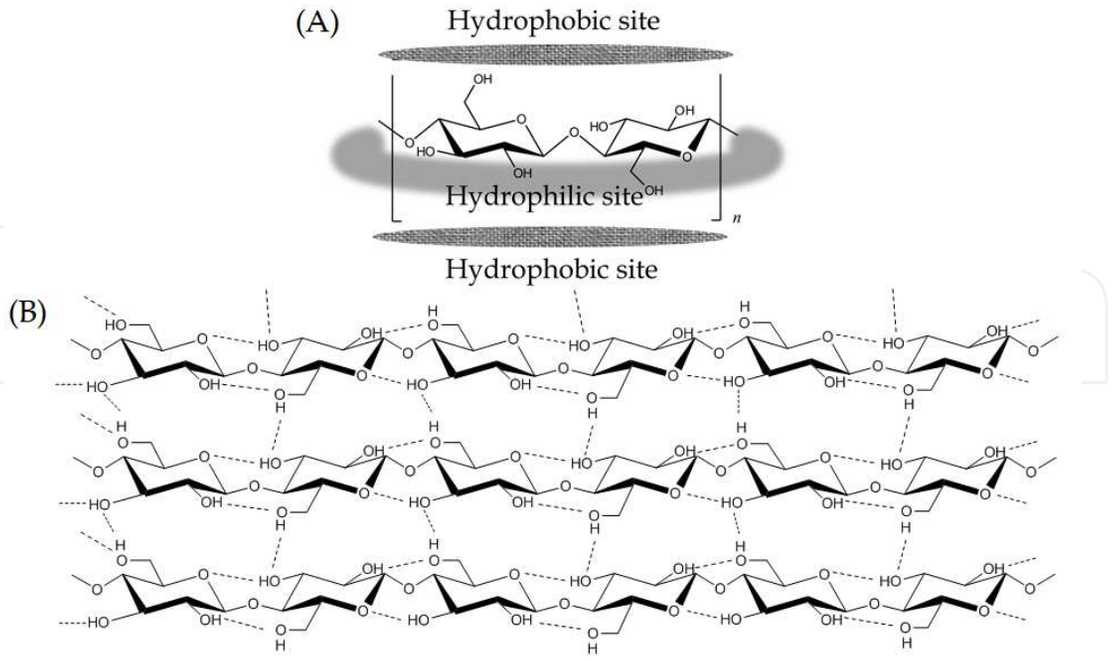


Figure 2. (A) Hydrophilic and hydrophobic sites of cellulose. (B) Schematic drawing of the intrasheet hydrogen-bonding network in cellulose I_α.

Various noncovalent interactions such as hydrogen bonding and van der Waals interactions are present in the ultrastructure of cellulose. While the OH-O hydrogen bonding is mostly responsible for cellulose intrasheet interactions, both the weaker CH-O hydrogen bonding and van der Waals interactions contribute to cellulose intersheet interactions (Li Q. & Renneckar, 2011). Figure 2 shows the arrangement of the intrasheet hydrogen bonding network in cellulose I_α and the resulting hydrophilic and hydrophobic sites of the ring plane (Brown & Saxena, 2007). Overall, because of these noncovalent interactions, cellulose chains aggregate into various forms of ultrastructure, which do not melt or dissolve in any common solvents. Such aggregation prevents the potential cleavage sites (i.e., glycosidic bonds) of a cellulose chain from being accessed by cellulase.

1.2. Degradation of cellulose in biomass conversion

The biomass conversion is the key step to produce biofuel from cellulosic biomass. Such conversion is often accomplished either through biochemical methods or thermochemical methods, where the polysaccharides in cellulosic biomass are hydrolyzed by biochemical agents such as cellulase enzyme, or by thermal treatment such as gasification to produce simple sugars that are fermentable to produce biofuel products (Dwivedi et al., 2009). For biochemical methods (Gray et al., 2006), cellulases are usually employed to convert the solid cellulosic biomass into glucose or small sugar polymers that can be readily fermented with microorganism to produce ethanol. Compared to thermochemical methods which often require a large amount of acid and energy, biochemical methods are more environmentally friendly and economically feasible because of their better conversion efficiencies and milder operating conditions. By far the enzyme-based biochemical methods are considered as the most promising technologies for biomass conversion. However, because of biomass

recalcitrance and high cost of cellulase in biomass conversion, the current process for biofuel production is not yet a viable option for the large-scale production (Dwivedi et al., 2009). Much research and developmental efforts have been dedicated to the improvement of the efficiency of cellulase in biomass conversion. One feasible approach is through the incorporation of new features (mutations) into cellulase that accelerate key steps (e.g., rate limiting step) of the enzymatic process. This approach requires a comprehensive mechanistic understanding of cellulose hydrolysis by cellulase.

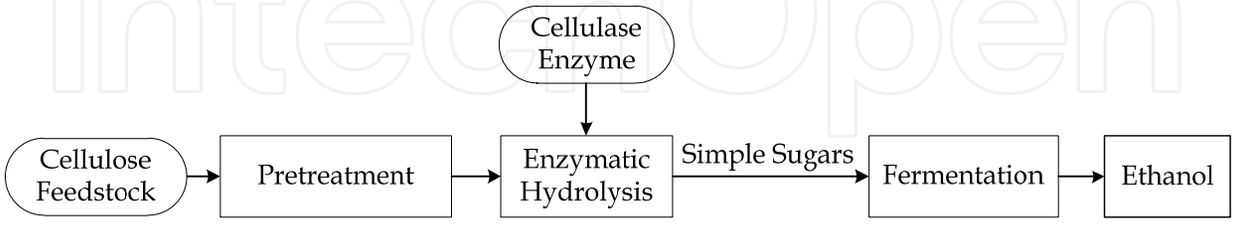


Figure 3. The process of biomass conversion through the enzyme-based biochemical method.

1.3. Cellulase and enzymatic hydrolysis of cellulose

Cellulase (Lynd et al., 2002; Mosier et al., 1999; Wilson & Irwin, 1999), like all glycosyl hydrolase enzymes found in bacteria, fungi, plants and some invertebrate animals, breaks down β-1, 4-glycosidic bonds of cellulose through general acid/base catalysis. There are mainly three kinds of cellulases: exo-β-1, 4-D-glucanase, endo-β-1, 4-D-glucanase and β-D-glucosidase. Each enzyme alone cannot hydrolyze the complex crystalline cellulose efficiently but working synergistically with other types of cellulases can increase the rate of hydrolysis significantly (Dwivedi et al., 2009; Lynd et al., 2002).

- Exo-β-1, 4-D-glucanase can access individual cellulose chains from the exposed reducing end or non-reducing end and cleave two to four glucose units at a time to produce tetrasaccharides or disaccharides (i.e., cellobioses) (Figure 4).
- Endo-β-1, 4-D-glucanase breaks internal glycosidic bonds of individual cellulose chains to disrupt the network structure of cellulose and expose individual polysaccharide chains (Figure 4).
- β-D-Glucosidase or cellobiase hydrolyzes cellobiose to release D-glucose units.

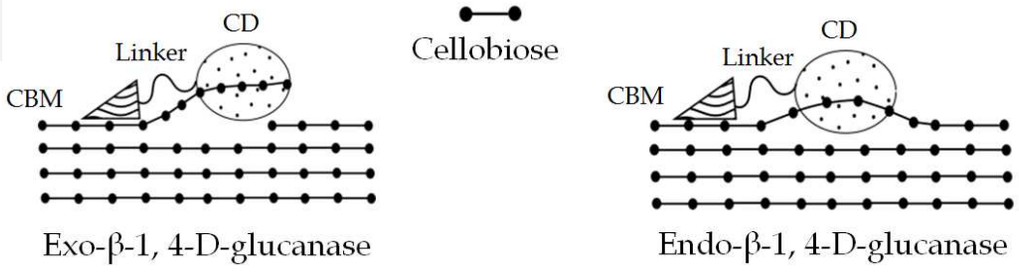


Figure 4. Schematic illustration of cellulose hydrolysis by exo-β-1, 4-D-glucanase and endo-β-1, 4-D-glucanase.

Most of fungal cellulases have a domain like structure that contains a catalytic domain (CD) and a carbohydrate binding module (CBM) (Martin, 2000). These two domains are

connected by a peptide linker, which is known to maintain the separation between the CD and the CBM (Figure 5). The CD contains the enzyme active site that is responsible for cellulose hydrolysis. The CBM is a contiguous amino acid sequence that anchors the CD onto the surface of cellulose through hydrogen bonding and van der Waals interactions (Boraston et al., 2004; Guillén et al., 2010). Cellulases can be grouped into families according to sequence similarities of their amino acid residues within their CDs and CBMs.

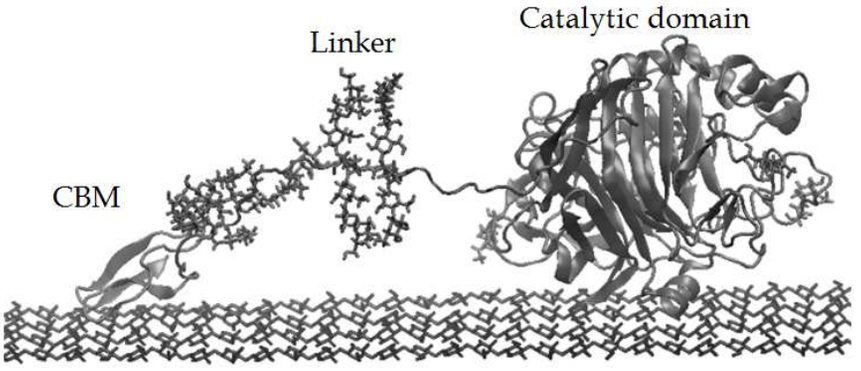


Figure 5. The domain like structure of the cellulase cellobiohydrolase I (CBH I) that is bound to cellulose Iβ microfibril. (Zhong et al., 2008)

Cellulose hydrolysis by cellulase is a multi-step process (Chundawat et al., 2011) that is initiated with the binding of cellulase (E) onto the surface of cellulose (S), shown in Figure 6. After this "initial binding" step, a single cellulose chain will be separated from the cellulose aggregate by cellulase and pulled into the active site of cellulase. This is the "decrystallization" step which forms a pseudo-Michaelis complex (E*S). E*S will then undergo "hydrolytic cleavage" to produce cellobiose as the product (P).

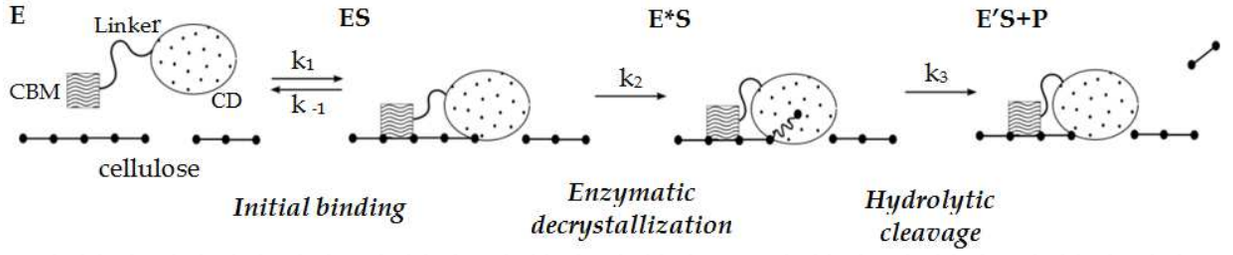


Figure 6. Mechanism of cellulose hydrolysis by cellulase.

For cellulose hydrolysis, the substrate cellulose is water insoluble and resistant to the attack by biological agents. This makes the formation of E*S much more difficult compared to those formed with soluble substrates.

1.4. Knowledge gap

The initial binding (step 1 in Figure 6) has been extensively studied over the years. A Langmuir equation is widely used as a simplified mechanistic model to describe the formation of ES resulting from the initial interaction between the cellulase and the cellulose, which often reaches steady-state within half an hour (Lynd et al., 2002; Zhang Y. H. & Lynd, 2004). Other

equilibrium binding models and dynamic binding models have also been proposed to account for the complexities of the binding process including the partially irreversible adsorption of cellulase, multiple types of adsorption site, and so on (Lynd et al., 2002).

For cellulose hydrolysis, most recent studies have been focused on elucidation of the mechanism of the hydrolytic cleavage reaction (step 3 in Figure 6) (Divne et al., 1998; Li Y. et al., 2007; Parsiegla et al., 2008). Some of the key amino acid residues involved in cleavage reactions have been identified. The distinctions between endo- and exo-glucanases, and between retention and inversion for the stereo configuration of the products have been made after years of biochemical and biophysical studies. The majority of studies on the hydrolytic cleavage step were done by measuring the concentration of the sugars (P) released during cleavage of soluble cellodextrins or insoluble cellulose.

Meanwhile, very little success has been achieved in obtaining fundamental knowledge of the enzymatic decrystallization reaction in step 2 (DOE/SC-0095, 2006). In particular, the importance of enzymatic decrystallization was largely unnoticed until very recently (Chundawat et al., 2011; DOE/SC-0095, 2006; Wilson, 2009). Several pieces of biochemical and physical evidence have indicated the presence of such an activity. Back to 1997, Wilson and his coworker showed that cleavage of the β-1, 4-glycosidic linkage in crystalline cellulose is not the rate-limiting step for *T. fusca* endoglucanase E2 (Zhang S. & Wilson, 1997). They speculated that the binding of a cellulose chain from a microfibril into the active site of a cellulase is the rate-limiting step for degradation of crystalline cellulose (Wilson, 2009). Lee and coworkers found indentations and paths on the surface of cotton fibers that had been treated with a cellulase that was incapable of hydrolytic cleavage (Lee et al., 2000). The evidence suggests that such surface modifications on cellulose are likely caused by the decrystallization activity of cellulase.

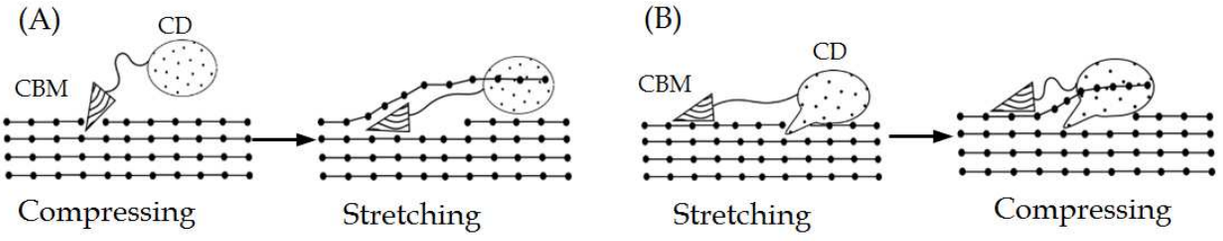


Figure 7. Mechanistic models of enzymatic decrystallization. (A) The CBM model: the CBM serves as a wedge to assist the release of a single cellulose chain. (B) The CD model: the protrusion of the CD domain serves as a wedge to assist the release of a single cellulose chain.

The mechanistic model for enzymatic decrystallization proposed by Reilly and coworkers is shown in Figure 7A (Mulakala & Reilly, 2005). In this model, the CBM is inserted under a cellulose chain like a wedge to separate the chain from the cellulose network. Then the released cellulose chain is pulled into the active site of the CD along the top face of the CBM to achieve the decrystallization. In this model, the CBM is essential to enzymatic decrystallization of cellulose. Numerous biochemical studies, however, have shown that in the absence of the CBM, the CD domain alone still retains 20~50% of the hydrolytic activity on crystalline cellulose (Reinikainen et al., 1992; Srisodsuk et al., 1993; Van Tilbeurgh et al.,

1986). To resolve this discrepancy, we propose an alternative mechanistic model, where a wedge-like structure at the bottom of the CD can be inserted under the cellulose chain to lift it into the active site of the CD (Figure 7B). Since decrystallization by cellulase has been speculated to be the rate limiting step for the degradation of crystalline cellulose (DOE/SC-0095, 2006; Wilson, 2009), understanding the mechanism of this enzymatic activity becomes essential to a comprehensive understanding of cellulose hydrolysis by cellulase.

2. Innovative approach and microcantilever

2.1. Existing technologies and their technical limitations

The conventional approaches to study the cellulose hydrolysis by cellulase are based on the measurement of the concentration of glucose or other simple sugars that are produced in the hydrolytic process. These approaches are not suitable for studying the decrystallization process because no new product is formed and released from this process. Spectroscopic techniques such as Fourier transform infrared spectroscopy (Fengel et al., 1995), Raman spectroscopy (Schenzel et al., 2005), and x-ray photoelectron spectroscopy (Ahola et al., 2008; Fardim et al., 2005) have been used to study the structural change of cellulose fibers. All these techniques focus on the global variations of cellulose. Since cellulose decrystallization only occurs on the outer layer within a relative small region of the surface of cellulose, these techniques are not sensitive enough for such study. Quartz crystal microbalance has been used to study the enzymatic hydrolysis of cellulose (Ahola et al., 2008; Rojas Orlando et al., 2007; Turon et al., 2008), however, its suitability for studying decrystallization has not yet been demonstrated.

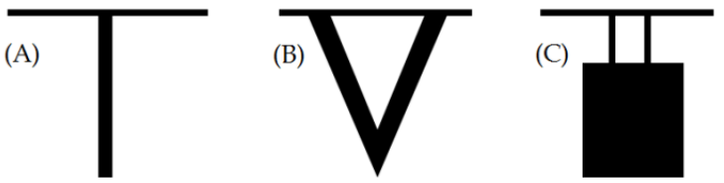


Figure 8. Various shapes of AFM probes.

Atomic force microscopy (AFM) has been used to examine changes of surface morphology of cellulose. Typical size of AFM probe is 200 μm x 40 μm x 1 μm (length x width x thickness) with various shapes (Figure 8). Lee and coworkers used the tapping mode AFM to exam the effects of three different cellulases on the surface of cotton fibers (Lee et al., 2000). Li and coworkers used the AFM to detect structural changes of cellulose microfibril fragments caused by sonication (Li Q. & Renneckar, 2011). With this powerful surface imaging tool, we can investigate the surface change caused by enzymatic activities. However, AFM imaging is mostly limited to the surface analysis at discrete time points. Additionally a high quality image requires that the surface area of cellulose be prepared prior to imaging. Any extensive handling may potentially alter the surface properties and delay the timely analysis under the assay conditions. Therefore, AFM imaging is not an ideal approach for analyzing the dynamic impact of cellulase on cellulose. Both scanning electron microscopy and transmission electron microscopy have similar limitations. This has led to the development of the microcantilever technique for cellulase study.

2.2. Microcantilever and its applications

The microcantilever is a highly sensitive nanomechanical sensor that originates from a micro-fabricated AFM probe. Typical dimensions of a microcantilever, which are similar to AFM probes, are 200 μm long, 1 μm thick, and 20 μm wide (Goeders et al., 2008; Lavrik et al., 2004). The microcantilever is capable of detecting minute changes in interaction energy between individual molecules in the thin film of a polymer coating (cellulose, protein, DNA, or polymer brush) in the form of a measurable bending (10^{-6} to 10^{-12} m) of the microcantilever (Moulin et al., 1999; Mukhopadhyay et al., 2005; Shu et al., 2005; Yan et al., 2006; Zhao et al., 2010; Zhou et al., 2006). The microcantilever bending can be measured based on the deflection of a laser beam reflecting from the tip of the microcantilever in the AFM (Figure 9). Overall, the microcantilever detection has high specificity, high sensitivity, and quick response.

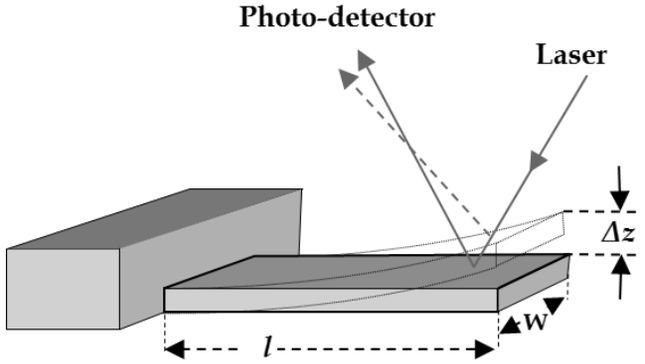


Figure 9. Working scheme of the microcantilever.

Dynamic mode (Vashist, 2007): When an analyte is adsorbed on an oscillating microcantilever, the microcantilever will oscillate at a lower frequency. The difference in frequency can be used to measure properties of adsorbates, such as viscosity and density, etc.

Static mode (Vashist, 2007): Adsorbates tend to induce a significant change in surface stress of a microcantilever, which results in a deflection of the microcantilever (Lavrik et al., 2004; Shuttleworth, 1950). The surface stress and the deflection of the microcantilever are quantitatively related (Yan et al., 2006):

$$\Delta z = \frac{3(1-\nu)l^2}{Et^2}\Delta\sigma \tag{1}$$

Where Δz is the deflection of the microcantilever at the end of the microcantilever, ν and E are Poisson's ratio and Young's modulus of the microcantilever, t and l are the thickness and length of the microcantilever, and $\Delta\sigma$ is the differential stress on the microcantilever. Using the static mode microcantilever sensor, Ji and coworkers investigated the conformational change of calmodulin (Yan et al., 2006). Sauers and coworkers successfully detected 2-mercaptoethanol using a gold-coated microcantilever (Datskos & Sauers, 1999). Zhou and coworkers successfully demonstrated the use of the microcantilever bending as a means to probe changes in internal structure of polymer brushes in response to changes in pH and electrolyte concentration (Zhou et al., 2006).

2.3. Microcantilever in cellulose study

In cellulose, the interaction energy arises from noncovalent interactions (hydrogen bonding and van der Waals interactions) between tightly packed cellulose chains. To release cellulose chains, surface-adsorbed cellulase must break up noncovalent interactions between cellulose chains through decrystallization, which results in a change in overall interaction energy in the cellulose. If cellulose is deposited onto a microcantilever, such dynamic change in interaction energy will be transduced from the cellulose coating into the microcantilever, and will result in a measurable bending of the microcantilever. Thus, the unique link between the enzymatic decrystallization and the resulting energy alteration in cellulose measured by the microcantilever bending has provided a novel strategy to experimentally examine this unusual enzymatic activity.

Such adsorbate-induced bending often occurs in the presence of a specific interaction (e.g., decrystallization) between an adsorbate (e.g., cellulase) and a substrate (e.g., cellulose) that can alter the internal energy of the substrate. Since a mere adsorption such as the initial binding of cellulase on cellulose has a very minimum impact on the internal energy of cellulose, it will not induce a measurable bending even though the surface-bound cellulase changes the overall gravity (mass) on the microcantilever. Therefore the microcantilever bending can be attributed primarily to enzymatic actions (e.g., enzymatic decrystallization and hydrolytic cleavage) after the initial binding. Because the extent of bending is linearly proportional to the effective concentration of adsorbed species on the microcantilever (Berger et al., 1997; Desikan et al., 2006; Velanki & Ji, 2006), the real-time measurement of the microcantilever bending will reveal the kinetics for enzymatic actions including enzymatic decrystallization by cellulase. To our knowledge, this is the first use of a nanomechanical sensor to study mechanistic enzymology and heterogeneous enzymatic catalysis that involves a solid substrate (e.g., cellulose).

3. Investigation of the interaction between cellulase and cellulose

3.1. Materials and methods

Materials: Microcrystalline cellulose, dimethyl sulfoxide (DMSO), and *N*-methylmorpholine -*N*-oxide (NMMO) were purchased from Sigma-Aldrich (St. Louis, MO). Polyvinylamine (PVAM) was purchased from BASF (Florham Park, NJ). The microcantilevers (200 μm × 25 μm × 2 μm, 0.1 N/m) were home-made.

Preparation of the cellulose-coated microcantilever (Zhao et al., 2010): The cellulose II model surface was prepared on the front side of a microcantilever that was made of SiO_2. The surface of the microcantilever was first treated with UV ozone for 20 min. It was then immersed in 0.22% PVAM for 60 min followed by rinsing with water. Both the front side and the back side of the microcantilever were coated with PVAM. A suspension of 0.5 mg of microcrystalline cellulose powder in 25 mL of 50% NMMO was heated while stirring until a transparent brown solution was obtained. While still warm, DMSO was added to afford a cellulose solution with a final concentration of 1%. This solution was first evenly applied onto the surface of the front side of the PVAM-coated microcantilever and then allowed to

sit for about 1 h. Afterwards a drop of water was added to form the cellulose film. The resulting cellulose-coated microcantilever was soaked in water for additional 4 h, during which the water was replaced every 30 min. Finally the cellulose-coated microcantilever was incubated in an oven at 80°C for 1 h to complete the surface coating.

AFM imaging (Zhao et al., 2010): To characterize the coverage, morphology, roughness, and thickness of surface coatings, AFM imaging was performed using a Nanoscope IIIa multimode scanning probe microscope from Digital Instruments, Inc. The samples were scanned in contact mode in air using silicon nitride cantilevers (MLCT) manufactured by Veeco (Camarillo, CA), with a nominal spring constant of 0.05 N/m. Images were obtained from at least three different surface areas of the sample with a typical size of 5 μm × 5 μm. The images of both height and deflection modes were captured and the surface morphology was analyzed using the image-processing software.

Microcantilever measurement: All the experiments were performed using a modified commercial Nanoscope III scanning probe microscope (Digital Instruments/Veeco, Santa Barbara, CA). The cellulose-coated microcantilever was mounted with the coating facing down in a liquid cell of the AFM with a volume of 50 μL. The solutions were introduced through injection. The cellulose-coated microcantilever was usually allowed to equilibrate in the assay buffer (25 mM sodium acetate, pH 5.5) for at least 2 h prior to any addition. The enzyme solution was prepared in the same buffer 30 min prior to use. Each assay was run against a reference to allow the subtraction of the background signal and to control for the bulk solvent effect. A desktop PC, running programs written in LabView (National Instruments, Austin, TX), was used to record the microcantilever deflection signal from the AFM via a data acquisition board with a maximum data acquisition rate of 300 kHz. The deflection measurement was monitored using a 5 mW laser diode with a wavelength of 680 nm, and a split position sensitive detector. During a measurement, 100,000 data points were taken every 30 seconds at a rate of 100 kHz. The bending of the microcantilever was obtained by simply averaging the data points.

3.2. Preparation of a cellulose-coated microcantilever

The surface of a microcantilever was coated with a model film of cellulose II having a thickness between 10 to 20 nm. It is generally agreed that the natural substrate is often too complicated to be useful for detailed characterizations of cellulases (Kontturi et al., 2006). Various cellulose model surfaces have therefore been developed in the past decade (Kontturi et al., 2006) and utilized extensively in a variety of studies including the investigation of the interaction between cellulose and cellulase. Among all the model surfaces, cellulose II has been used most often and there is much technical information available about it. In addition, the surface of cellulose II is easy to prepare and characterize. All of these make the cellulose II film an ideal model surface for the current study.

For attaching cellulose to the surface of a microcantilever, an anchoring layer of PVAM was first prepared (Figure 10A). The cellulose dissolved in a hot mixture of NMMO and DMSO was then deposited onto the top of the anchoring layer (Falt et al., 2003; Zhao et al., 2010). The cellulose coating on the microcantilever was characterized with AFM imaging and the

results of the modification of the surface topography after each coating step were shown in the Figures 10B to D. A typical PVAM-coated surface is shown as a layer of small oval particles in Figure 10C. The smoothness and thickness of the coating can be adjusted by changing the coating time. The cellulose II model film on the microcantilever exhibits a cement-like characteristic with a cover of thick, short fibers (Figure 10D).

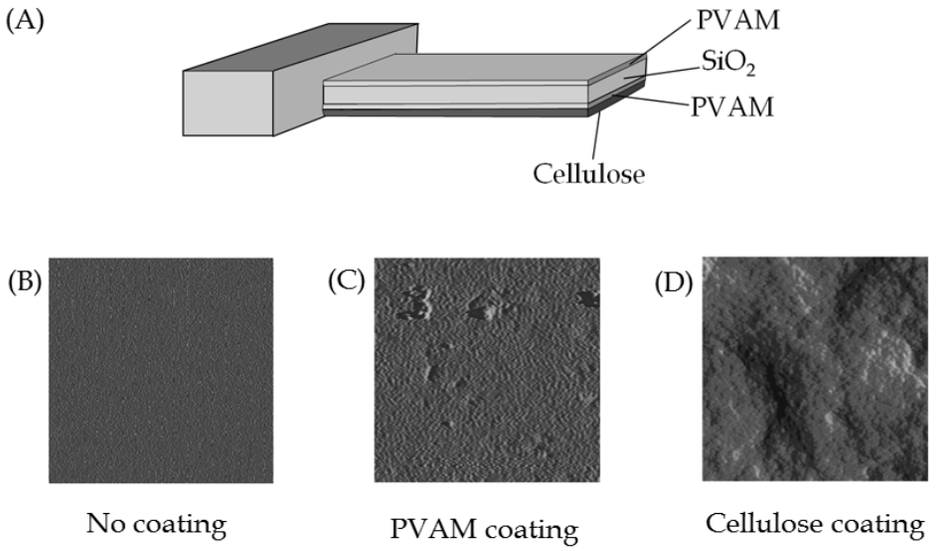


Figure 10. Surface coating on the microcantilever (Zhao et al., 2010). (A) The coating scheme. (B) to (D) AFM images of surface coatings (5 μm × 5 μm).

3.3. Examination of morphological and structural changes of cellulose surface

The microcantilever coated with cellulose II was treated with a successive exposure of different water/salt solutions. The result of bending of the microcantilever is presented in Figure 11 (Zhao et al., 2010). In the experiment, the cellulose-coated microcantilever was allowed to equilibrate in water for 2 h to achieve a stable baseline. The bending of the microcantilever was measured based on the deflection of the microcantilever at its apex. The measurement was initiated with the injection of water and a constant bending was observed during the first 45 min. The level of bending remained virtually constant even after the injection of a solution of 0.1 M NaCl. Upon the subsequent treatment of higher concentrations of NaCl (0.5 and 1 M), a continuous rise in bending was detected at a pace of roughly 1 nm/min and a cumulative bending of more than 100 nm was observed. To compensate for bulk effects of the buffer and the salt, we measured the differential bending, termed simply ''bending'' hereafter, which is defined as the difference in deflection of the microcantilever with the cellulose coating and without the cellulose coating.

The observed bending of the microcantilever can be attributed to the change in interaction energy within the outlayer of the cellulose surface. It has been shown previously that a cellulose model film undergoes a change in internal charge density when exposed to an electrolyte solution. And the magnitude of the change in charge density depends on the concentration of the electrolyte (Ahola et al., 2008; Freudenberg et al., 2007; Tammelin et al., 2006). Such change likely alters the intermolecular repulsion among cellulose molecules,

which leads to the change in interaction energy. When the cellulose is deposited onto one side of the microcantilever (Figure 10A), the change in the interaction energy in the cellulose coating can exert a differential mechanical stress between the opposite surfaces of the microcantilever, leading to the continuous bending of the microcantilever (Figure 11A). Meanwhile, the change in interaction energy also causes the change in surface morphology of the cellulose coating as indicated by the image shown in Figure 11D.

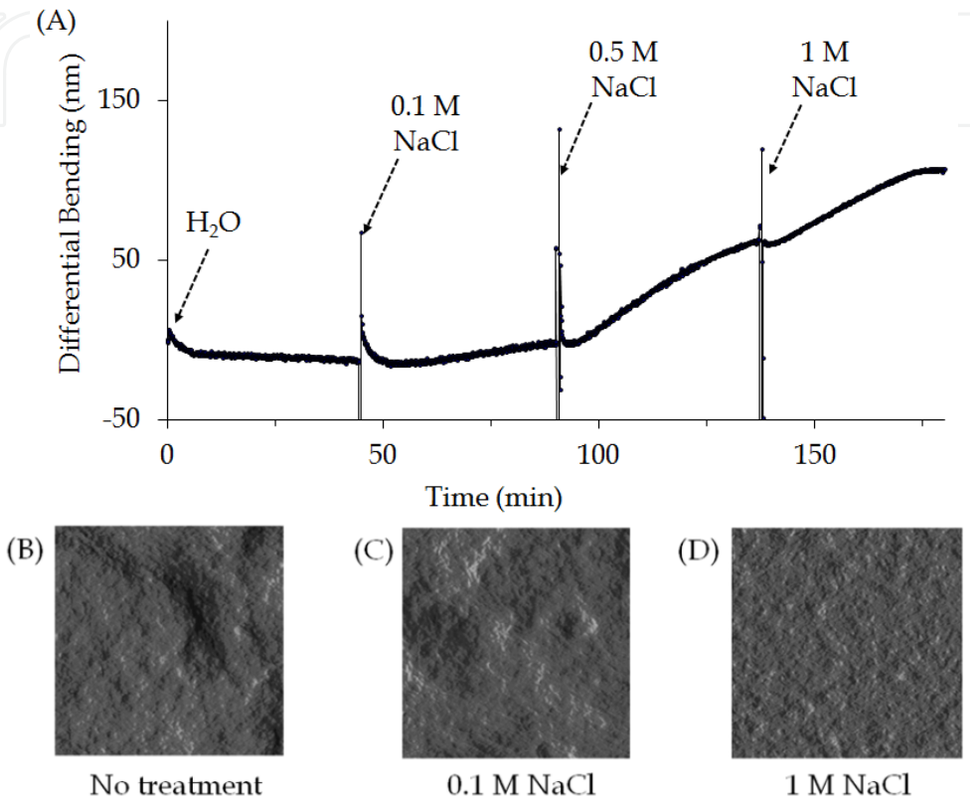


Figure 11. The morphological changes of the cellulose coating monitored by means of the microcantilever technique (Zhao et al., 2010). (A) The bending of the microcantilever increased with the increasing NaCl concentration. (B) AFM image of the surface of the cellulose coating on the microcantilever. (C) AFM image of the cellulose surface (as shown in (B)) after being treated with 0.1 M NaCl. (D) The cellulose surface (as shown in (C)) after being treated with 1.0 M NaCl. Mean roughness: 2.59 nm with a Z range of 25 nm.

Overall, this study validates that the microcantilever technique is highly sensitive and specific in detecting real-time changes in interaction energy in the surface layer of the cellulose coating on the microcantilever. So, it is feasible of using the microcantilever technique to monitor the dynamic change in interaction energy in the surface layer of the cellulose caused by enzymatic decrystallization. The microcantilever bending also correlates well with the change in molecular structure of the surface region of the cellulose film.

3.4. Detection of the enzymatic decrystallization by cellulase on cellulose

The cellulose coating on a microcantilever was treated with the cellulase CBH I (cellobiohydrolase I, k_d = 1 μM, mw = 66 kD), an exoglucanase from *Trichoderma reesei* in 25

mM sodium acetate buffer, pH 5.5 at 25°C. Immediately after the addition of 0.15 μM of CBH I, a bell-shaped bending curve was obtained (Figure 12A), which implies that the cellulase is capable of inducing bending of the cellulose-coated microcantilever.

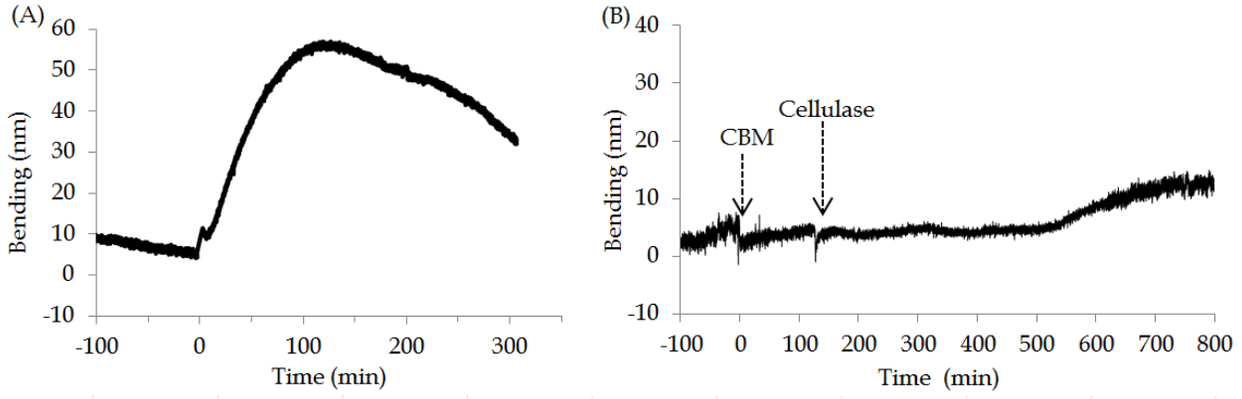


Figure 12. (A) The progress curves of cellulase actions on cellulose measured by the microcantilever sensor. (B) The progress curves of CBM and cellulase actions on cellulose measured by the microcantilever sensor.

Next, the cellulose coating was treated with 0.9 μM of the carbohydrate binding module (CBM, k_d = 0.6 μM, mw = 17 kD) from *Clostridium cellulovorans*, the domain that anchors cellulase to cellulose at 25°C. Figure 12B clearly shows that exposing the cellulose to the CBM does not generate any measurable bending in microcantilever over the course of 120 min. Over the same time frame of the previous experiment, CBH I that contains both CD and CBM domains did induce bending, as shown in Figure 12A. Notably, more weight was probably adsorbed onto the surface of the cellulose coating in the presence of the CBM than in the presence of CBH I due to the difference in protein concentration used in each experiment. This result confirmed that the change in mass (gravity) due to the initial binding of the CBM on the surface of the cellulose does not generate any bending in microcantilever, which was fully expected for a mere protein binding (physisorption) in liquid media. Thus, the cellulase-induced bending shown in Figure 12A was not caused by the initial binding between the cellulase (via the CBM of the cellulase) and the cellulose coating. This bending can therefore be attributed to the result of the cellulase actions that occurred after its initial binding on cellulose. Such actions may include enzymatic decrystallization and/or subsequent hydrolytic cleavage (Figure 6). After the addition of the CBM, the subsequent addition of CBH I (cellulase) did not generate any bending until approximately 6 h later (Figure 12B). This result verified that the CBM was indeed bound to the surface of the cellulose and the cellulose-bound CBM prevented the subsequent binding of CBH I to the cellulose. We believe that the bending in microcantilever beginning after 500 min was due to a slow displacement of the cellulose-bound CBM by CBH I.

4. Conclusion

These studies have demonstrated that a nanomechanical sensor in microcantilever is capable of detecting the interaction between cellulase and cellulose in real time. More specifically,

this technique can be used to probe the dynamic process of the enzymatic decrystallization of cellulose by cellulase. The bending of the microcantilever is likely a result of the change in interaction energy within the cellulose caused by the interaction between cellulase and cellulose (e.g., enzymatic decrystallization), not by the adsorption of cellulase onto cellulose. The innovative microcantilever sensor approach will be used to determine the kinetics of the enzymatic decrystallization by cellulase.

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Acknowledgement

This work was supported in part by a grant from the National Science Foundation (NSF) CBET-0843921, Drexel University Career Development award, and subcontract XCO-4-33099-01 from the National Renewable Energy Laboratory funded by the U.S. DOE Office of the Biomass Program.

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