In Situ Studies of a Fungal Polysaccharide

Using MAC Mode AFM

Application Note

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Introduction

In atomic force microscopy (AFM), a sharp tip is scanned over a sample while maintaining a constant interatomic force between atoms on the end of the tip and atoms on the surface of interest (1). Early implementation of AFM employed the contact mode of operation in which the repulsive force experienced by the tip was measured by recording the cantilever deflection. Under ambient conditions, these repulsive forces range from a few tens to hundreds of nanonewtons. The forces between the AFM tip and the sample cause pressures large enough to induce distortion, remove portions of the sample from the substrate, and even damage the tip. By scanning in a liquid, capillary forces are reduced, which decreases the forces between the tip and sample by orders of magnitude. Other forces such as contact pressures, however, can still lead to dulling of the AFM tip and deformation of soft biological samples.

An effective method to reduce destructive lateral forces and to enhance sensitivity and reproducibility involves dynamic oscillation of the AFM tip while imaging. Agilent's MAC Mode AFM was developed (2) to reduce damage-causing vertical interactions between the sample and the AFM tip. In MAC Mode, a cantilever coated with a paramagnetic film is oscillated directly by a solenoid located underneath or above the sample. The oscillation amplitude that is utilized to drive the cantilever in MAC Mode is better controlled and can be much smaller than that used in other dynamic-force AFM modes (3). Compared to other AFM imaging modes, DNA molecules resolution has been greatly improved using MAC Mode AFM (4).

In this application note, we demonstrate the ability to image a (1⟶6)-branched (1⟶3)-linked-ß-D-glucan polysaccharide of fungal origin, in liquid, using MAC Mode AFM. These polysaccharides are widely distributed in plants, fungi, bacteria, and yeast, and serve as structural components of cell walls or as reserve polysaccharides (5). Scleroglucan (6), also known as schizophyllan (7) or lentinan, is a fungal polysaccharide with immunostimulatory properties (8, 9). The rod-like Scleroglucan triple helix structure can be dissociated or denatured in dilute solution into random coil chains (10) and subsequently renatured as linear and circular forms (11), depending on annealing temperature and polymer molecular weight (11-15).

Figure 1a Computer-generated projections of the triple-helical structure of hydrated crystalline curdlan (16), which is a (1⟶3)-ß-D-glucan with no side chains. Each strand of the helix is shown in a different color. A cross-section through the triple-helix structure (**Figure 1b**) reveals the intrahelix hydrogen bonds that stabilize the triple helix. The triangles indicate hydrogen-bonded networks located at successive levels along the (1⟶3)-ß-D-glucan helix axis (16).

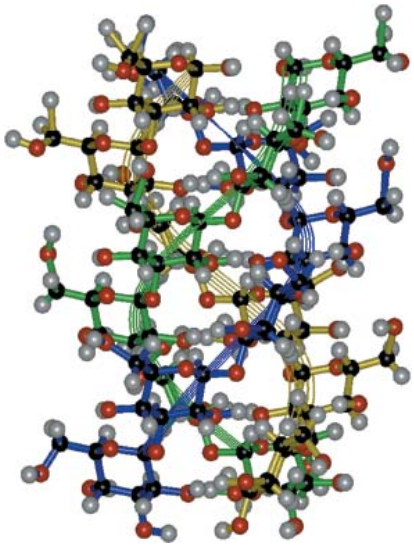

Figure 1a. The accepted triple-helical structure of hydrated crystalline curdlan.

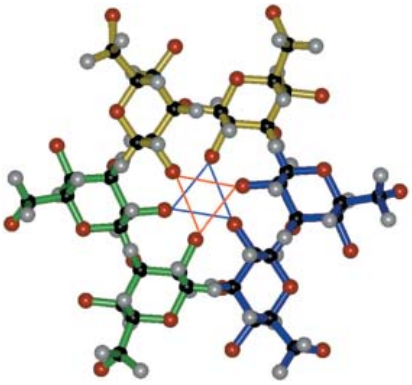

Figure 1b. A cross-section through the triple-helix structure reveals intrahelix hydrogen bonds.

Materials and Methods

Crude scleroglucan powder (Actigum CS 6, Lot No. 26, CECA S.A., Velizy, France) was suspended in aqueous 0.02% sodium azide and stirred overnight at room temperature. The resultant turbid solution was centrifuged to remove cell debris and unsolubilized polymer. Aliquots of the clear supernatant were sonicated (20 kHz, 375 W, 0.5″ tip) at 0 °C for 4 hours, centrifuged again, and filtered through 0.45-µm filters. The resultant clear solution was then fractionally precipitated (17) with acetone to produce fractions of differing mean molar mass. Each fraction was redissolved separately in water, dialyzed exhaustively against distilled water, and recovered by freeze drying. Fractions chosen for study were redissolved in distilled water at 1 mg/mL.

For this study, one of the redissolved scleroglucan fractions was exposed to a denaturation-renaturation procedure:

Aliquots in sealed 10-mL micro-reaction vials were first heated to 160 °C for 15 minutes in a thermostat-controlled oil bath to dissociate the native triple helix (10, 18). The vials were then placed in a thermostat-controlled oil bath at 90°C, annealed at constant temperature for 4 hours, and quenched to room temperature in an ice bath (12, 15).

Results and Discussion

The denatured-renatured polysaccharide solution described was diluted with filtered distilled water to a final polymer concentration of about 10 µg/mL. Aliquots (20 µL) of the diluted solution were drop-deposited onto freshly cleaved mica and allowed to air dry. Approximately 200 µL of n-propanol was placed onto the mica with the dried polysaccharide sample in the liquid cell of an Agilent atomic force microscope. The sample was imaged in MAC Mode with a small multi-purpose scanner. A piezoelectric scanner with a range up to 6.5 µm was utilized for all images. The AFM tips used were MACLevers with a force constant of 2.0 N/m and resonant frequency ω0 of approximately 80 kHz in air. Images were stored as 512 x 512-point arrays and analyzed using Agilent image-processing software.

Figure 2 shows a MAC Mode AFM image of a denatured-renatured scleroglucan sample (described below). Clearly, the image shows some aggregation of the molecules, which probably occurred during drying. The tiny spots in the background are due to impurities in the n-propanol. In air, the mean thickness of the renatured cyclic structures is 15–20% smaller than the mean thickness of the renatured linear structures. Both structures are independent of scleroglucan molecular weight and annealing temperature (15).

Conclusions

These studies have demonstrated that linear and circular polysaccharide molecules can be clearly visualized *in situ* upon using MAC Mode AFM. With its high spatial resolution, MAC Mode offers a great opportunity to identify local structural differences at the surface of the linear and circular forms. Furthermore, easy setup makes MAC Mode AFM an excellent tool for optimizing imaging conditions for biological samples such as polysaccharides in buffered aqueous solutions. Future studies using MAC Mode AFM in conjunction with Agilent's temperature-controlled stage may reveal other interesting aspects of polysaccharide structure.

Acknowledgment

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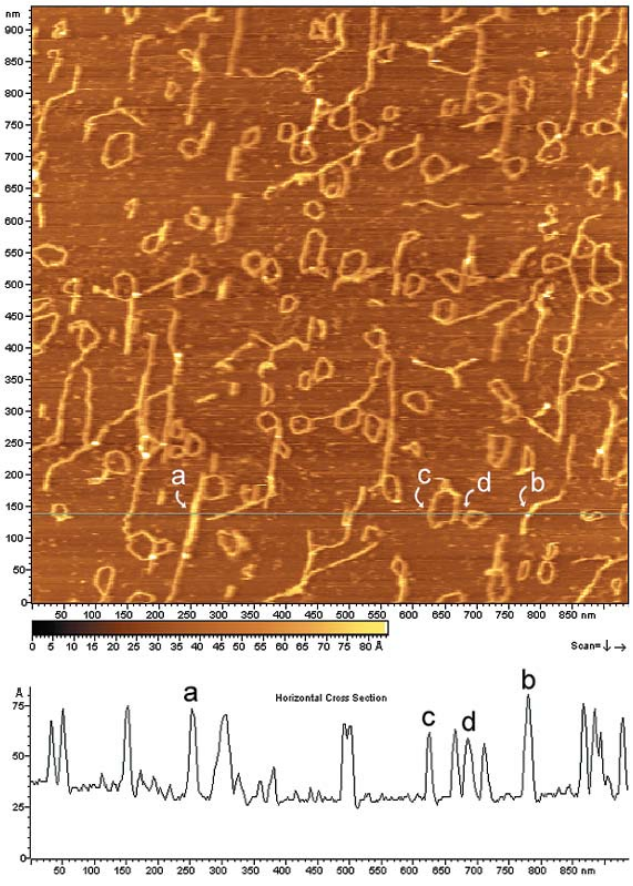

Figure 2. MAC Mode AFM image of renatured linear (a,b) and cyclic structures (c,d).

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