New and Notable

Polysaccharide Helices in the Atomic Force Microscope

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Polysaccharides are a ubiquitous class of biopolymers that have been studied relatively little. In the Current Contents database, polysaccharides and carbohydrates are cited only one-tenth as often as DNA and RNA and one-fifteenth as often as proteins. Part of this neglect of polysaccharides comes from the fact that proteins are structurally much more diverse than polysaccharides and nucleic acids carry more information. Still, polysaccharides are a widespread and highly successful class of biological macromolecules. Polysaccharides and oligosaccharides are used not only for energy and energy storage but also for cell recognition and as structural elements in chitin and plant cell walls. In addition to being highly antigenic, polysaccharides function in blood clotting and have many industrial uses (Aspinall, 1983).

Polysaccharide structures are difficult to determine. Unlike proteins and nucleic acids, polysaccharides are sometimes branched and have a variety of different linkages between adjacent monosaccharides. Techniques used to elucidate polysaccharide structures include gas liquid chromatography and mass spectrometry, x-ray diffraction, electron microscopy, nuclear magnetic resonance, and a range of other chemical and enzymatic techniques. A newcomer to the analysis of polysaccharide structure is the atomic force microscope (AFM), which was invented only 9 years ago (Binnig et al., 1986). The AFM maps surfaces by raster scanning a fine tip gently over the surface, giving a three-dimensional profile of the surface that can sometimes reach atomic resolution on hard flat surfaces (Ohnesorge and Binnig, 1993; Rugar and Hansma, 1990). This issue presents new AFM images of the bacterial polysaccharide acetan that show periodicity with the spacing of helix turns along the acetan molecules (Kirby et al., 1995). X-ray diffraction has been used previously to see helix turns in oriented fibers of polysaccharides with regular chemical repeats.

What looks like periodicity and substructure in AFM images is sometimes a tip artifact. There is, for example, an image of ribosomes (Hansma and Hoh, 1994) that seems to show two "subunits" in each ribosome. With careful observation, one sees that the ribosomes all appear to be oriented in the same way, with their small "subunits" on their right sides. Because there was no reason for the ribosomes to be rotationally ordered, one concludes that the ribosomes were imaged with a double tip, with the smaller of the two tips on the right. In contrast, the substructure on the acetan fibers of Kirby and co-workers looks like true helix turns. Fibers running horizontally at the top of Fig. 3 (Kirby et al., 1995) show features—helix turns?—with approximately the same spacing as the features on the vertical acetan fibers in the lower part of the figure. Another indication that substructure is a tip artifact is that one sometimes sees, in addition to the substructure of interest, low contrast substructure with the same periodicity in the background. Kirby's figures show no indication of periodic substructure in the background, which further validates the interpretation that these are high quality images showing helix turns in acetan.

AFM imaging under butanol contributed to the high resolution of these images. Imaging forces are much smaller in fluid than in air (Weisenhorn et al., 1989). A thin fluid film covers samples in air, even at low humidity, and creates a large meniscus force during AFM imaging. In fluid, this meniscus force is absent; and also the buoyancy of many fluids further reduces the force of the AFM tip upon the sample.

Although low imaging forces are important for high resolution imaging, Kirby and co-workers also realized that the minimum force does not necessarily give the best images. Here, an empirical approach is best. When the cantilever deflection and, consequently, the imaging force are too low, the tip does not track the sample well. And clearly when the imaging force is too high, the polysaccharide chains are pushed and damaged. Kirby and co-workers found that imaging forces of 3–4 nN gave the clearest images.

The high quality of the images obtained by Kirby and co-workers are due not only to the imaging conditions but also to the sample preparation method. The sample was prepared in a way that gave close-packed arrays of molecules oriented parallel to one another. As the authors point out the measured width of

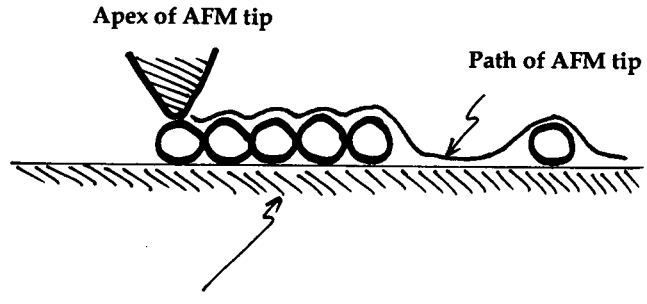

FIGURE 1 When the AFM tip scans a close packed array of molecules (*left*), the true widths of the molecules can be measured. Isolated molecules (*right*) appear broader and are more easily moved than molecules in an array, because more of the tip interacts with the isolated molecules.

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molecules in these arrays was only 2 nm, comparable with the expected width of acetan helices. In contrast, the measured width of isolated acetan molecules was 10 nm. This increase in width is due to the convolution of the tip width with the width of the polysaccharide helix. When the tip scans close-packed arrays of helices, much less of the tip interacts with the helices. Isolated molecules are also more easily moved by the AFM tip, which reduces resolution (see Fig. 1).

Helix turns have been seen only rarely in AFM images of double-stranded DNA (Hansma et al., 1995), which does not form ordered arrays on mica with the methods for preparing AFM samples that have been used to date, including the method used by Kirby and co-workers. Hopefully, helix-turn resolution of polysaccharides will soon become routine with the AFM, because ordered arrays can be formed. In addition, the AFM should be good for imaging branching of polysaccharides, probably on samples with well spread molecules.

Polysaccharide research is an important area of materials science. Industrial uses for polysaccharides include not only products for the food industry but also such diverse products as mucilages and other adhesives, detergent additives for preventing the redeposition of dirt, sizing agents for paper and textiles, and thickeners for fire-fighting fluids (Aspinall, 1983). Thus, a useful area of materials research is to understand further the structure and properties of this prolific class of biopolymers. The AFM is a new tool capable of investigating the structure and, sometimes, the function of biological macromolecules (Hansma and Hoh, 1994). Kirby and co-workers are bringing the power of AFM-imaging to the analysis of polysaccharide structure.

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