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The Uncoiling of Supercoiled Plasmid DNA Over Time Observed by Atomic Force Microscopy

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Abstract

Long term stability of DNA structures in a cell is critical to sustaining life. The DNA structures could be degraded biologically (e.g. enzymes), chemically (e.g. drugs), and physically (e.g. thermal agitation process) with time. The DNA structures are maintained by being regenerated and/or being recovered by proteins within a cell. However, even though it is important, it is difficult to observe the time-evolution of DNA structures for extended periods at a molecular resolution. Here, we observed the time evolution of DNA structures for two months, in order to understand the long term stability of DNA structures. For this study, we used purified plasmid DNA molecules extracted from Escherichia coli (E-coli) as a sample. We also employed atomic force microscopy (AFM) to observe the plasmid DNA structures at a molecular resolution. The purified plasmid DNA molecules were diluted with pure water, deposited on a mica surface, and observed by an AFM on a regular basis in an ambient environment for two months. The sequential AFM images show the plasmid DNA formed globular structures at the beginning and transformed into uncoiled plasmid DNA network structures after two months. The globular structures appeared to be the supercoiled state of plasmid DNA, a well-known strategy to store genetic information in a confined space for bacterial systems. The observed DNA network structures are believed to be results of long periods of unwinding and rejoining processes of the supercoiled plasmid DNA. The unwinding and rejoining processes would have been caused by small residual proteins (or enzymes) possibly present in the plasmid DNA solution. This study reveals DNA stability is dramatically influenced by prolonged (~ a few months) exposure to small amounts of residual proteins (or enzymes). The result also suggests the AFM is a powerful tool in observing the biological process at the molecular level over extended periods of time.

The Uncoiling of Plasmid DNA over Time

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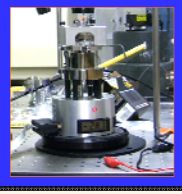


1. Abstract

Long-term stability of DNA structures in a cell is critical to sustaining life. The DNA structures can be degraded biologically (e.g. enzymes), chemically (e.g. drugs), and physically (e.g. thermal agitation process) with time. The DNA structures are maintained by being regenerated and/or being recovered by proteins within a cell. However, even though it is important, it is difficult to observe the time-evolution of DNA structures for extended periods at a molecular resolution. Here, we observed the time evolution of DNA structures for two months, in order to understand the long term stability of DNA structures. For this study, we used purified plasmid DNA molecules extracted from Escherichia coli (E-coli) bacteria as a sample. We also employed atomic force microscopy (AFM) to observe the plasmid DNA structures at a molecular resolution. The purified plasmid DNA molecules were diluted with pure water, deposited on a mica surface, and observed by an AFM on a regular basis in an ambient environment for two months. The sequential AFM images show the plasmid DNA formed globular structures at the beginning and transformed into uncoiled plasmid DNA network structures after two months. The globular structures appeared to be the super-coiled state of plasmid DNA, a well-known strategy to store genetic information in a confined space for bacterial systems. The observed DNA network structures are believed to be results of long periods of unwinding and rejoining processes of the super-coiled plasmid DNA. The unwinding and rejoining processes would have been caused by small residual proteins (or enzymes) possibly present in the plasmid DNA solution. This study reveals DNA stability is dramatically influenced by prolonged (~ a few months) exposure to small amounts of residual proteins (or enzymes). The result also suggests the AFM is a powerful tool in observing the biological process at the molecular level over extended periods of time.

2. Introduction

The stability of DNA structures is vital to the survival of a cell, and there are many ways in which the DNA structures of cells can be degraded, therefore jeopardizing the cell. One of the ways DNA structures can be degraded is through the actions of enzymes, an example being topoisomerase. It is very important to study the degradation of DNA structures, even though it can be difficult to observe the time-evolution of DNA structures for extended periods at a molecular resolution. Here, in order to understand the long-term stability of DNA structures, we observed the time-evolution of plasmid DNA structures for 47 days with an Atomic Force Microscope (AFM). An AFM is a microscope which utilizes an atomically-sharp tip attached to a cantilever to create an image of the surface of a sample. An illustration of the basic workings of the AFM is displayed in Figure 3. Plasmid DNA's natural state is to be super-coiled, because in certain organisms, such as E-Coli, DNA must be super-coiled to function inside such a minute organism. If the plasmid DNA structure is degraded in any way, the structure will "uncoil" into a less tightly super-coiled state. This process is demonstrated in Figure 2. According to Fundamentals of Biochemistry 3rd Edition: Life at the Molecular Level, a single nick in a super-coiled strand of DNA is enough to relax the strand into an uncoiled state. A nick in a strand of a DNA could be the result of some residual proteins or enzymes breaking down the DNA strand.

While both the electron microscope and AFM have many biological uses, the AFM has distinct advantages over the electron microscope in imaging plasmid DNA. With an electron microscope, the DNA is imaged in a vacuum. If the DNA is imaged using an AFM, the DNA can be imaged in a more natural environment: fluid. Also preparing samples for AFM imaging is less time consuming than preparing samples for imaging by an electron microscope.

The AFM also has a disadvantage to the electron microscope in that the diameter of the AFM tip determines the resolution and amount of detail in the AFM image. Figure 4 demonstrates if the AFM tip is too large, the smaller details in the sample will not be detected.

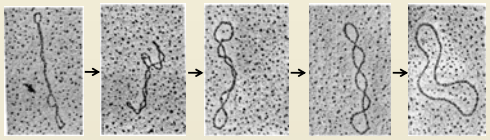
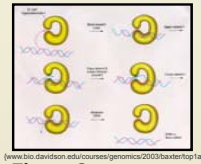


Figure 1: Electron Micrographs of DNA: Tightly Super-coiled (Far left) to Uncoiled (Far right) {Courtesy of Fundamentals of Biochemistry 3rd Edition}



{www.bio.davidson.edu/courses/genomics/2003/baxter/top1activity.gif}

Figure 2: Topoisomerase cutting and rejoining DNA strands

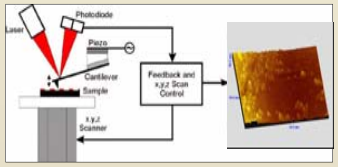


Figure 3: Diagram of AFM

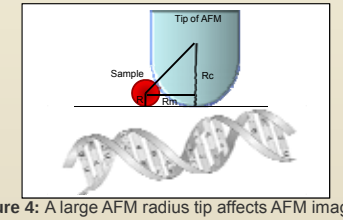
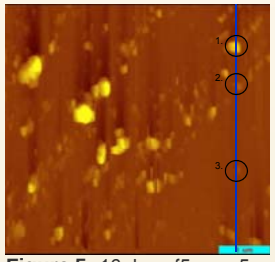


Figure 4: A large AFM radius tip affects AFM image resolution. Rm is the radius measured by the AFM.

3. Experimental Procedure

For this 47 day project, purified plasmid DNA molecules obtained from E-Coli were used to observe the time-evolution of the structure of the plasmid DNA at a molecular resolution. The plasmid DNA solution was a 25mL water to 2mL plasmid DNA molecules solution obtained from Dr. Ken Cornell. The plasmid DNA solution was kept at 5°C in a covered glass container. For imaging, the plasmid DNA solution was placed on a mica surface, air dried, and imaged with an AFM.

4. Results



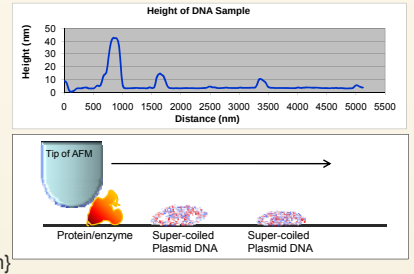
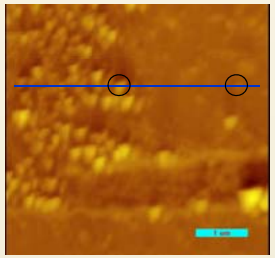


Figure 5: 16 days {5μm x 5μm}



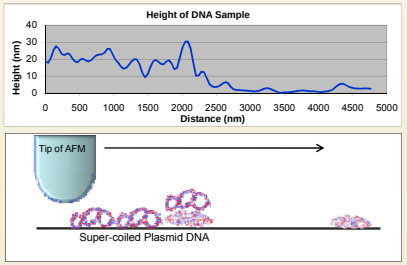
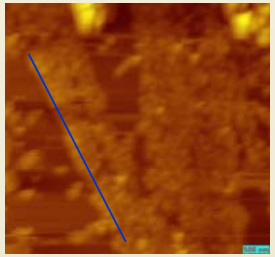


Figure 6: 27 days {5μm x 5μm}



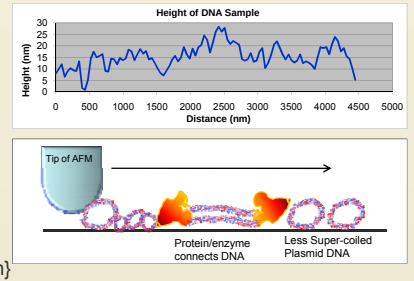
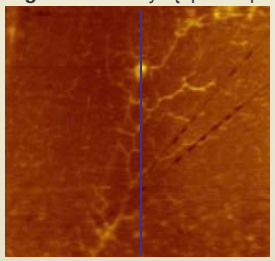


Figure 7: 47 days {5μm x 5μm}



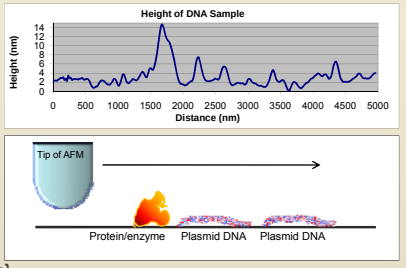


Figure 8: 47 days {5μm x 5μm}

5. Analysis and Discussion

In Figures 5 through 8, the results of the study can be seen. At 16 days (Figure 5), the plasmid DNA remained in globular structures. These globular structures appear to be the super-coiled state of the plasmid DNA. To determine if the globular structures observed in Figure 5 were actually super-coiled plasmid DNA, the height of certain areas in the AFM image were graphed using XPM Pro 2.0, a program used to analyze AFM images. The circles on the AFM images indicate areas of interest. It should be noted that super-coiled plasmid DNA is usually between 2nm to 15nm in height and 250nm in width. Along the blue line in Figure 5, Circle 1 appears to contain a residual protein/enzyme, and Circle 2 and Circle 3 seem to contain super-coiled plasmid DNA.

This is supported by the graph to the right of the AFM image, which shows that the height in Circle 1 is approximately 43nm, which is considerably larger than most super-coiled plasmid DNA molecules. Residual proteins/enzymes are usually much larger than super-coiled plasmid DNA. Circles 2 and 3, however, have heights of 15nm and 11nm, respectively. These heights are within the height parameters of super-coiled plasmid DNA. At 27 days (Figure 6), there are two regions of interest along the blue line. One region has a maximum height of about 30 nm, while the other region has a maximum height of 6 nm along the blue line. This difference in height might be due to the partial uncoiling and stacking of the plasmid DNA. This concept is illustrated in the diagram next to Figure 6. The super-coiled plasmid DNA appear to be uncoiling and clumping together. The higher region appears to be the region where the less super-coiled plasmid DNA has clumped together. On the 47th day (Figures 7 and 8), the plasmid DNA solution was imaged twice. Both AFM images seem to contain less super-coiled plasmid DNA. In Figure 7, the height along the blue line fluctuates between about 28nm and 7nm. The concept of less super-coiled plasmid DNA being linked together by the residual proteins/enzymes may explain the height fluctuation. In addition, plasmid DNA has a maximum length of about 2μm, but the structures in Figure 7 were much longer, indicating that the plasmid DNA was joined with proteins. In Figure 8, an uncoiled plasmid DNA network structure appears to be present, along with small globular structures. The small globular structures might be less super-coiled plasmid DNA. The uncoiled plasmid DNA network seemed to be joined to another such network by a residual protein/enzyme. The joining point is 14 nm high, which is much higher than the rest of the selected area. The possible less super-coiled plasmid DNA was still observed in Figure 8, even though an uncoiled plasmid DNA network structure was present. The presence of less super-coiled plasmid DNA in Figure 8 may be attributed to the fact that the residual proteins might not have been equally distributed in the plasmid DNA solution.

After a 47-day exposure to the residual proteins and/or enzymes in the plasmid DNA solution, the plasmid DNA molecules appear to have gone from globular, tightly super-coiled plasmid DNA structures to uncoiled plasmid DNA network structures, as illustrated in Figure 9. The plasmid DNA network structures are believed to be the result of unwinding and rejoining processes of plasmid DNA molecules, most likely caused by an enzyme like topoisomerase. These processes "uncoil" or degrade the plasmid DNA leaving it less super-coiled than before. The residual proteins and/or enzymes behave similar to the enzyme topoisomerase depicted in Figure 2. The enzyme nicks one of the super-coiled DNA strands, unwinds the DNA strands, and rejoins the strands, leaving the plasmid DNA super-coiled structure more relaxed and less coiled.

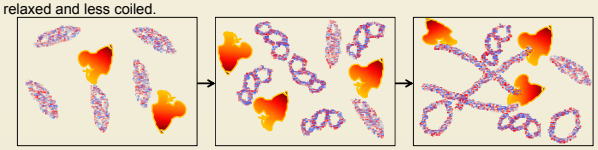


Figure 9: The uncoiling of super-coiled plasmid DNA Enzymes/Proteins (Orange-Red), Plasmid DNA (Red/Blue)

6. Conclusion

DNA structures can be degraded in a variety of ways, including by enzymes. The study of the degradation of DNA structures is important, because in a cell, long-term stability of the DNA structures is necessary for the cell's survival. It is often difficult to observe the time-evolution of DNA structures at a molecular resolution. The AFM made it possible to study the DNA structures for extended periods of time in a more natural environment than would be allowed by the electron microscope. In this study, we observed the changing of DNA structures over time for roughly two months, with the intention of understanding the long-term stability of DNA structures. Plasmid DNA's natural state is to be super-coiled. Degradation or "uncoiling" of the plasmid DNA occurs when an enzyme and/or protein is able to relax the plasmid DNA through unwinding and rejoining processes. In the beginning of the study, we observed globular structures, which when measured, some appeared to be super-coiled plasmid DNA structures, while the others seemed to be residual proteins/enzymes. After 47 days, the globular structures had transformed into uncoiled plasmid DNA network structures, probably due to the unwinding and rewinding processes of an enzyme such as topoisomerase. The results of this study reveal DNA stability is strongly influenced by prolonged exposure to residual amounts of proteins and/or enzymes. This study also suggests the AFM is a powerful tool in observing biological processes, such as the "uncoiling" of super-coiled plasmid DNA, over extended periods of time at a molecular resolution.

7. Acknowledgements

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• Dr. Ken Cornell (BSU) provided the Plasmid DNA solution for the research.

8. References

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•Fundamentals of Biochemistry 3rd Edition: Life at the Molecular Level by D. Voet, J. Voet, C. Pratt