Direct Observation of DNA Catenanes by Atomic Force Microscopy

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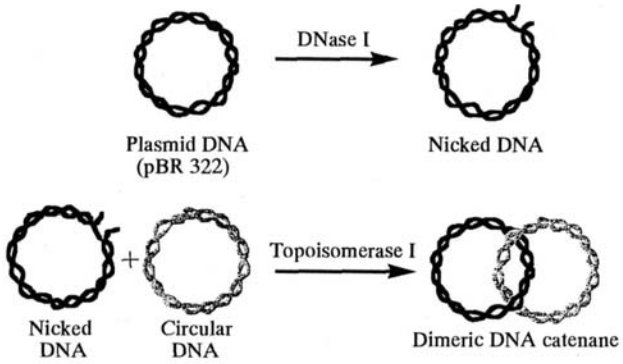
Single molecular images of DNA catenanes have been clearly observed for the first time by AFM using a tapping mode at room temperature and in an ambient atmosphere.

Atomic force microscopy (AFM)[1] is a useful technique for imaging biorelated materials on a nanometer scale under conditions close to their natural environments without complicated sample preparation techniques, such as staining, used in transmission electron microscopy.[2-5] Previously, we succeeded in observing antibody molecules[6] and supramolecules between antibodies and porphyrin dimers[7] at room temperature by contact mode AFM using a graphite as a base and high concentrations of salts to prevent coaggregation of immunoglobulins. Although AFM images of proteins and DNA are larger than the real structure because the images are created by the convolution of the protein or DNA itself and radius of the curvature of the tip, the effect is negligible for length measurements in the case of DNA whose length is much greater than the curvature of the tip.[8-12] Thus AFM is expected to be a powerful tool for investigating microscopic structures of DNA and complicated DNA supramolecules by taking advantage of the height information.

Catenated DNA molecules, i.e., multiply linked DNA rings, are frequent intermediate products of basic biological processes such as DNA replication, recombination, and topoisomerase action.[13] It is crucial to determine the complex structure of catenanes in order to provide important information about the processes that generate them and the structure of DNA. [n]Catenanes can be expected to have novel physicochemical properties. We report here direct observation of DNA catenanes by tapping mode AFM at room temperature.

The two main methods used for determining the topology of DNA catenanes are agarose gel electrophoresis and AFM. Although only AFM gives the complete stereostructure of individual molecules, electrophoresis provides a more rapid and quantitative overview of the population. Therefore, it is best to use both methods in combination.

DNA catenanes were prepared[14] by the addition of topoisomerase I to the mixture of nicked DNA and pBR322 plasmid DNA as shown in Scheme 1. Nicked DNA was synthesized by the addition of DNase I to a solution of plasmid pBR322. Catenated DNA molecules were prepared by the reaction of topoisomerase I with nicked DNA. Catenation reactions were monitored by the agarose gel electrophoresis. Reaction products were analyzed by 1.0% agarose gel electrophoresis using Tris/Acetate/EDTA buffer at 5 V/cm for 90 minutes. Gels were stained with ethidium bromide (20 mg/ml) and photographed using ultraviolet illumination (312 nm). DNA was extracted from the band of 1.0% agarose gel by using a centrifugal filter device, Ultrafree-DA (MILLIPORE), spinning at 6000 x g for 10 min. A total 4 μl of DNA solution (0.5 ng/ml in 10 mM Tris-HCl, 1 mM EDTA, 10 mM $MgCl_2$, 10 mM HEPES) was



Scheme 1.

placed onto freshly cleaved mica for 15 min and rinsed with deionized water, and then dried for 6 h in a desiccator with $CaCl_2$. The sample surface was observed by tapping mode AFM. All AFM measurements were taken on a multimode Nanoscope IIIa (Digital Instruments, Santa Barbara, CA). The line scan speed was 2 Hz with 512 pixels per line. The typical tapping frequency was 340-380 kHz.

Figure 1 shows the results of electrophoresis of the reaction products. There are only two bands (lane 1 and lane 2) in the presence of 0.05 units/μl of topoisomerase I. However, a new band is observed in the presence of two-fold amounts of topoisomerase I to the previous conditions. The slower migrated band appeared in lane 3 is considered to be a [2]catenane.

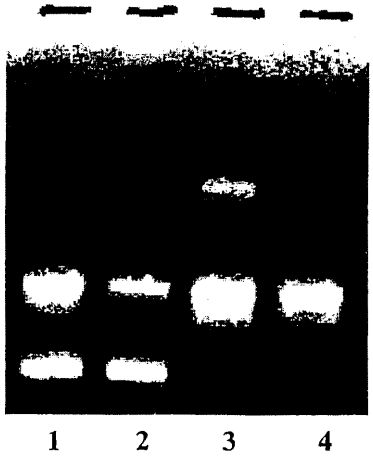


Figure 1. Agarose gel electrophoresis of the reaction mixture between nicked DNA and circular DNA in the presence of topoisomerase I. [Topoisomerase I]: 0.05 units/μl (lane 1 and lane 2), 0.1 units/μl (lane 3 and lane 4). Lane 1 and lane 3 include nicked DNA. The concentration of circular DNA was fixed at 14 ng/μl.

Figure 2 (a) shows the DNA molecular images extracted from the slower migrated band appeared in lane 3 in Figure 1. Parts of two circles fused over a distance of ~ 90 nm can be seen. Each circle is assigned to be pBR322 plasmid whose length is about 1.7 μm comparable in the length to the 4361 bps (Figure 2 (b)). This molecular image is ascribable to be dimeric DNA catenane by the following reasons; (1) this fused DNA is constituted by the two plasmid DNA and (2) the molecules seen in Figure 2 (a) was extracted by the higher molecular weight band in electrophoresis, including no DNA monomer. The overpassing and underpassing strands at crossings can be seen. The interlocked structures of DNA can be differentiated from the overlapped structures by the section analysis of the molecular image in Figure 2 (a).

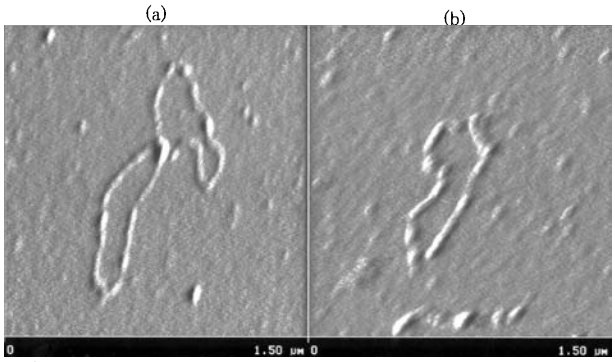


Figure 2. The tapping mode AFM images of dimeric DNA catenane (a) and plasmid DNA (DNA monomer) (b).

Cozzarelli et al. reported for the first time the topological structures of DNA catenanes by using an electron microscope.[14] However, the resolution of the segments at the junction is poor in electron microscopic examination of conventionally strained and shadowed DNA. To overcome this problem, they used a protein (RecA) coating method to enhance visualization of the crossings.

In conclusion the topological structures of DNA catenanes have been studied by AFM and electrophoresis. The single molecular images of DNA catenanes are clearly observed by AFM using a tapping mode at room temperature and in an ambient atmosphere. The topological structures of *naked* DNA catenanes are directly and successfully observed by AFM without any coating. Our experiment is the first example of the direct observation of DNA catenanes.

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