Direct Visualization of DNA Decondensation on Mica by Atomic Force Microscopy

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Lambda DNA/Hind III solution in the presence of cations was gradually diluted into four different concentration, namely 50, 25, 10, 1 ng/μl. The morphology change during this process was observed by atomic force microscopy. Images reveal that at the highest concentration, a flower-like structure with multiple loops crossing at the same point was exhibited on mica. Following it are some irregularly planer "layers" accompanied by occasional toroids. When DNA concentration was diluted to 10 ng/μl, a large complex network structure appeared, which was eventually replaced by the extended and isolated DNA molecules at 1 ng/μl. This structural transition may be helpful for us to understand the molecular mechanism of DNA decondensation.

DNA condensation is relatively universal in living organisms. It not only can decrease the occupied volume of DNA in vivo, but also can accelerate a large number of biological processes. Hence, increased attention has been devoted to this phenomenon. Although much was now known about physical properties and structures of the condensed DNA, the kinetic pathway leading to condensation or decondensation is only sketchily understood. It is clear that the condensed DNA must change into an extended form during transcription, replication and DNA repair, otherwise enzymes and regulatory molecules can not access to the underlying DNA template. While upon cessation of these processes, the extended structure should also be able to revert to its inactive compacted state in order to keep the stability and occupy a much smaller space. This folding and unfolding cycle is crucial for organisms to maintain the regular activities, but the exact molecular mechanisms concerning the transition remain unclear. Thus, much effort was made to approach this problem.[1,2] In this experiment, we also provide some interesting results about DNA decondensation.

λ-DNA Hind III Markers (Promega U.S.A.) were diluted with Milipore purified water to 100 ng/μl, 50 ng/μl, 20 ng/μl, 2 ng/μl respectively, and then mixed with an equal volume of a 5 mM $MgCl_2$ solution. After incubated at room temperature for 10 min, a 2 μl of this mixed solution was deposited onto freshly cleaved mica and dried under an infra-red lamp (a slice of filter paper was used to absorb the residual salt during this process) prior to the AFM (NanoscopeIII, Digital Instrument) observation. All images shown are height images. Scan parameters were optimized for each experiment, but frequencies were typically 2.5-4.0 Hz.

Base on previous studies,[3] four different concentrations were chosen in this experiment. At the highest concentration examined, a quite unusual structure (shown in Figure 1A) was exhibited on mica. The shape and geometry of this structure are neither similar to the isolated and extended DNA, nor to the usually observed condensates such as toroids and rods. Although DNA fragments with different sizes are contained in the aqueous solution, the morphologies shown on mica tend to be uniform. Most of them are characterized by a flower-like structure with multiple loops crossing at the same point. Of course, other structures with somewhat variable appearance are also observed at the same time. This may reflect differences in the regularity of organization of the DNA or variation in the physical properties of individual structures. Nevertheless, the co-existence of these structures gives the implication that they are either thermodynamically equivalent terminals in the pathway of "flower" formation or the trapped kinetic intermediates in the different stages during this process. So far, we have no evidence to determine which hypothesis is right and rule out other possibilities. Figure 1B depicts a single "flower" normally seen in such conditions. Close scrutiny reveals that only four main loops radiate from the central point, other small loops and nodules are actually formed by the segments of these large ones. Whatever size the loops are, they usually bend sharply, bringing the strand of the loop into close, nearly parallel juxtaposition. Such acute bends have a large elastic energy, which must be compensated in order to keep their stability. In our case, they are likely the result of cationally catalyzed collapse,[4] since cations can reduce the effective diameter of DNA,[5] permitting the close approach of different segments.

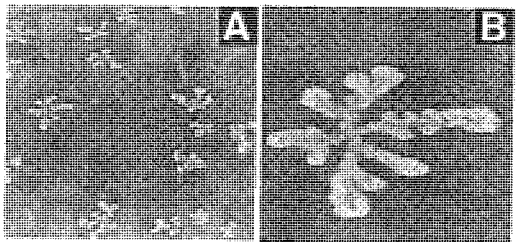

Figure 1. AFM images of λ-DNA / Hind III at 50 ng/μl, the scan size of (A) and (B) is 20x20 μm, 4x4 μm respectively.

Dimensional statistics shows that the average measured height of the strands along the loops was 13±5 nm, much larger than the height of duplex DNA in AFM image,[6] indicating a multi-molecular organization was involved in their formation. But to our surprise, there are no obvious free ends appearing in the structure. This leads us to believe that the intra or intermolecular contacts must be related to the ends. Several possibilities have been proposed for such contacts.[1] These include partial overlaps between molecules, end to end interactions and termination of free ends at the crossover point. As to which type interaction most likely occurs, further research is needed.

With the decreasing of DNA condensation, a significant morphology change has taken place. As shown in Figure 2A, the "flower" condensate formed at 50 ng/μl DNA disappeared completely under this condition, instead of which some irregularly planar aggregates were exhibited on mica. The aggregates seem to consist of several layers. In each layer,

different DNA molecules were put into close proximity so we can hardly identify any single one of them. The rough sketch of these layers usually approximate to a ring, giving the implication that they are probably developed from a structure with central point. This speculation was in good agreement with the results observed in the previous section. Also worth mention is a single toroid shown in the center of the image. By measurement, it was found the outer diameter is 440 nm, four times larger than that frequently observed. This discrepancy may be related to the concentration of DNAs and the reaction time, which can influence the toroidal structure, changing the outer diameter size from 100 to 600 nm or even larger.[7] Height measure reveals that its thickness is 10.2 nm, corresponding to four or five turns coiled up.[8] Based on these, the total DNA in this toroid was estimated to contain 18 kbp at least, since one turn of a toroid with outer radius 50 nm contains about 930 bp.[9] So only the largest DNA fragment in the sample can meet this requirement, this may account for its rareness. Figure 2B is another AFM image observed under this condition. Here it can be seen that a network was formed by the individual molecules branching out from the periphery of the "layer". This structure is a good illustration that DNAs tend to disassociate as the local concentration decreases.

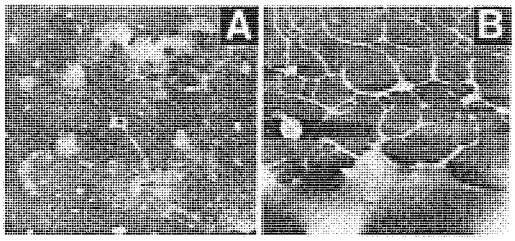

Figure 2. AFM images of λ-DNA / Hind III at 25 ng/μl, the scan size of (A) and (B) is 10x10 μm.

The network structures become very prevalent when DNA concentration was diluted to 10 ng/μl. As shown in Figure 3, the network is extremely large and may be composed of hundreds of DNA molecules, strands of which alternately associate and separate, forming a complex fenestrated structure. Meshes, as the basic units of the structure, vary not only in morphology and size, but also in the height of the strand, this may reflect the differences in the interaction of some DNA molecules with others or mica. To have a better understanding of the details, a model proposed by Duguid[10] was borrowed to our experiment. In this model, the divalent cations initially interact with the DNA at phosphate and/or base sites, and then the metal-base interaction will destabilize the duplex DNA, causing some of the hydrogen bonds to rupture and base pairs to swing open. This allows the divalent metals to bind to additional sites on the DNA bases. Once the DNA bases have swung open, DNA strands that are close to each other can be linked by metal ion bridges, hydrogen bonding, and base stacking, eventually forming a network.

At the lowest DNA concentration examined, a large majority of the molecules show an extended and isolated morphology (Figure 4A), but there are also some coiled and tangled molecules (Figure 4B), which are presumed to be the "trace" of

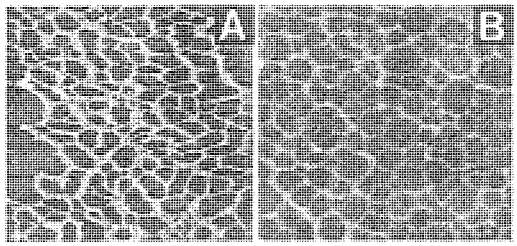

Figure 3. AFM images of λ-DNA / Hind III at 10 ng/μl, the scan size of (A) and (B) is 10x10 μm, 5x5 μm respectively.

the previous structure network. Dimensional measurements show that the average height of the strands is 0.7±0.2 nm, much smaller than the expected value 2.6 nm (which is the helix diameter of A-DNA).[6] This discrepancy is probably due to the compression of DNA by tip or shrinkage in the thickness. In addition, it should be pointed out that the heights along the strand do not always keep constant. There are some bead-like structures with obvious larger heights. These structures are probably formed by self-coiling of the strand or by end to end aggregation of the different molecules. Studies have shown the end to end aggregation is favored by the free energies of base stacking, which are comparable to kT (0.6 kcal/mol),[11] so even blunt-ended DNA molecules can be joined up.[12]

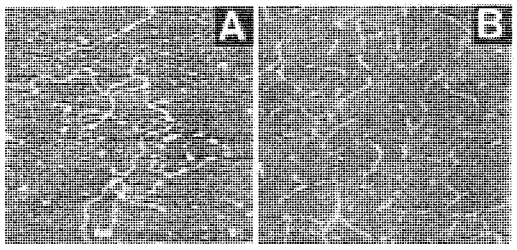

Figure 4. AFM images of λ-DNA / Hind III at 1 ng/μl, the scan size of (A) and (B) is 5x5 μm, 10x10 μm respectively.

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References and Notes

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