Fractal aggregation of DNA after thermal denaturation

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Abstract

DNA thermal denaturation was observed by atomic force microscopy (AFM) on the surface of newly cleaved mica. It was found that at temperatures higher than the melting point, denaturation of DNA molecules took place and globular particles with size distribution were formed, and these particles could aggregate together to form fractal structures, which followed the diffusion limited aggregation (DLA) model. At 100 °C, degradation of DNA took place and small particles of about 20 nm in size were formed, and they also aggregated in fractal structures with a lower dimension. Evaporating speed of water affects the fractal dimension.
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1. Introduction

Fractal phenomena in nature have attracted interests of many scientists and have been widely studied. DNA molecules as genetic material show fractal properties both in shape and sequence [1–3]. Self-similarity has recently been found in DNA sequences [4,5], and a simple random fractal, the random walk, is used to represent DNA sequences. Glazier et al. [6] used the multifractal spectrum approach to reconstruct the evolutionary history of organisms from m-DNA sequences. They concluded that DNA sequences displayed fractal properties, and that these could be used to resolve evolutionary relationships in animals.

DNA molecule is a double strands polymer which is easily denatured or condensed by change of environment. This could damage the structure of DNA and form collapsed structures, and then self-organization or aggregation takes place easily. Chandra et al. [7] studied the aggregative behavior of DNA under uv-irradiation and found that under irradiation DNA degraded into several fragments and they formed beautiful fractal structures as a result of a random walk-related diffusion limited aggregation.

DNA denaturation is a basic question that had been widely studied [8–11]. Denaturation of DNA is induced by reducing the interactions within the double strands structure to separate into two single strands. Generally, DNA can be denatured by heat and chemical treatments such as pH, competitive denaturants [12]. For thermal denaturation, base pairs of DNA are opened at a high temperature. The opened strands have chance to renature or take a coil-to-globule transition. At a very high temperature such as 100 °C, DNA also have chance to degrade and form little fragments. Do those anamorphic structures easily form fractal aggregates? What is the effect of temperature? All of these questions will be discussed in this paper.

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2. Experimental

PFA-ATF$_2$ plasmid DNA (4.6 kbp) with concentration of 1.0 µg/ml (Takara Shuzo Co., Ltd., Japan) was used in this study. It was diluted with purified water (>18 MΩ, TW-300Ru, Nomura Micro Science Co. Ltd., Japan) to 50 ng/ml before used.

For thermal denaturation experiment, DNA solution was heated in a water bath with a temperature controller. New cleaved mica was also heated synchronously in a little dish in the same water bath, so were pipette tubes. The solution was heated from room temperature and balanced at a certain temperature for about 5 min, then 20 µl solution was transported onto the surface of mica by the pipette, and it was dried in water bath at the temperature. After dried the samples were stored in a sealed sample box and then measured by AFM.

A Nanoscope IIIa multimode AFM (Digital Instruments, Santa Barbara, CA) was used for imaging DNA in air. Commercially available silicon cantilever (model TESP) with 125 µm length was used for tapping mode AFM. The scanning rate was changed from 0.5 to 1.0 Hz, and 512×512 pixels images were record.

For fractal analysis, software WSxM 3.0 (Nanotec Electronica S.L., Spain) was used.

3. Results and discussion

Fig. 1(a)–(c) show typical AFM images of plasmid DNAs at room temperature: there show two kinds of structures, open circular (a) and supercoiled (b and c), but most of them are supercoiled structure with different interwoven degrees. These are in good agreement with the previous report that at room temperature plasmid DNA showed supercoiled and open circular structures on mica surface, the conformation being similar to natural DNA in an ideal two-dimensional solution [13]. Rivetti [14] suggested that DNA molecules were able to equilibrate on the surface of freshly cleaved mica and the molecules appeared to be extended with very few crossovers. Fig. 1(b) and (c) show that there are two kinds of supercoil structures: one with strands wind loosely (b) and another tightly (c). The height and the
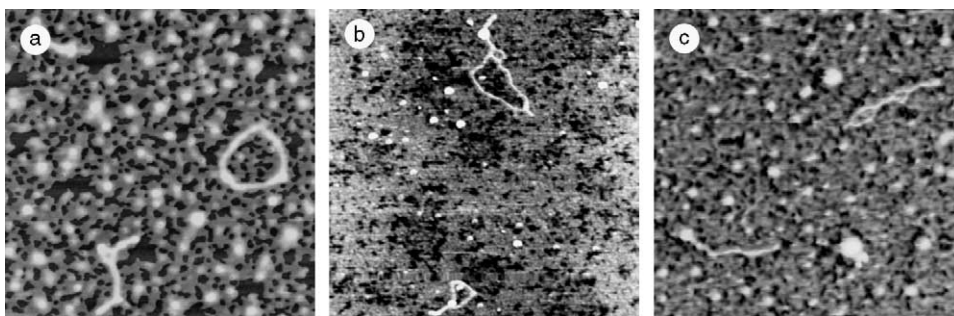

Fig. 1. Representative AFM images of plasmid DNA on the surface of newly cleaved mica at room temperature: open circular structure (a) (2 µm×2 µm), supercoiled structure (b) (2.5 µm×2.5 µm) and relaxed supercoil (c) (2 µm×2 µm).
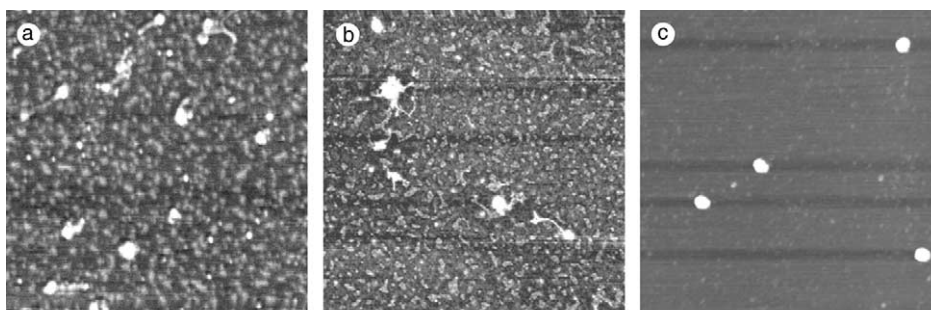

Fig. 2. Representative AFM images of plasmid DNA on the surface of mica after heated at 80 °C (a), 84 °C (b) and 90 °C (c). In both (a) and (b) circular structure disappear and formed globule structure with undenatured tail. (c) Only globule structure appear, which correspond to whole denaturation of DNA. Scale bar 500 nm for all images.
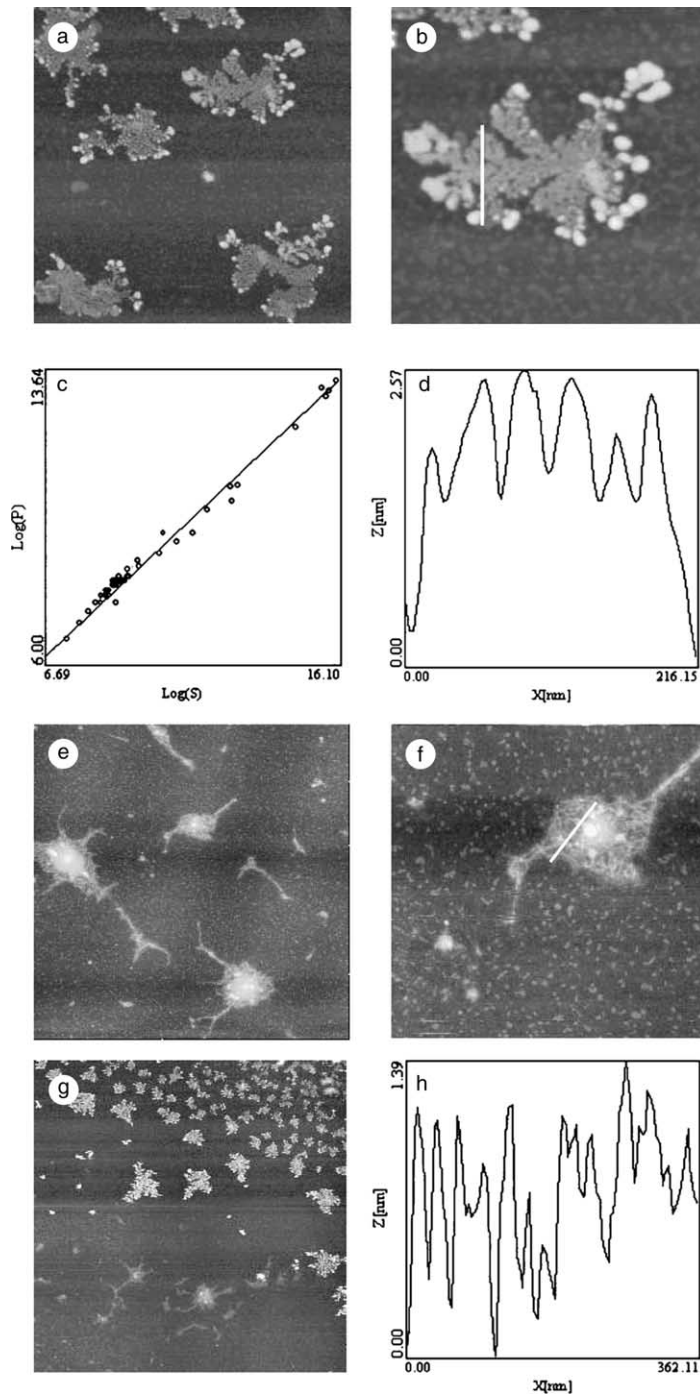
Fig. 3. AFM images and fractal analysis of DNA aggregates on the surface of newly cleaved mica prepared at 88 °C. (a) Fractal aggregates of denatured DNA (1.45 μm × 1.45 μm); (b) enlarged image of a selected aggregate in (a) (670 nm × 670 nm); (c) fractal analysis curve of aggregates in (a); (d) cross section curve of one aggregate in (b) indicated by a line; (e) another kind of aggregate co-existing on the surface of the sample (4.5 μm × 4.5 μm); (f) enlarged image of a selected aggregate in (e) (4.5 μm × 4.5 μm); (g) an image of a region where both kinds of aggregates co-exist (10 μm × 10 m); (h) cross section curve of aggregate in (e) indicated by a line.

width of the supercoiled DNA are about 0.45 and 15 nm, respectively, which are consistent with the values reported by other researchers [15,16].

Usually, in many textbooks, DNA denaturation temperature is around 85 °C. However, we found DNA begin denaturation even at 55 °C [11]. When we increased temperature up to 80 °C and find that the circular structure disappear and there shows some grains with little tail in Fig. 2, which means most of the double strands were separated and collapsed. Similar result appeared at 84 °C. When the temperature arrive 90 °C, only globular structure appear and it can be said all the DNA base pairs were denatured.

Fig. 3 shows AFM images of the sample prepared at 88 °C. In Fig. 3(a) there appear typical fractal structure of DNA aggregates, Fig. 3(b) shows one of the fractal structures in Fig. 3(a) indicated by a square. It can be seen that the fractal structure is composed of two different basic units, one is bigger particles located at the outer side of the structure and the other is smaller particles at the inner side of the structure. Fig. 3(d) is the cross section along the line in Fig. 3(b). The height and the width of the inner particle are about 2.5 and 40 nm, respectively. The height is much higher than that of the supercoil DNA, so the inner particle maybe denatured DNA molecules with collapsed structure. The bigger particles maybe aggregate of smaller ones. Fig. 3(c) is the fractal analysis curve of Fig. 3(a), and we get the fractal dimension $D = 1.52$, close to the value expected for the diffusion limited aggregation (DLA) model [17]. The model assumes that the particles originate far away from the seed of the aggregation perform a random walk and once they encounter the body of aggregation they stick there. Chandra et al. [7] studied the fractal growth of DNA fragments under uv-irradiation and found that the fractal formed following a nonuniversal DLA model, which was used to study the aggregation of particles with size distribution. Their results showed that smaller particles easily formed fractal aggregates first and then the bigger ones stick on the branches of the structure. In our case, we consider that DNA molecules were denatured by heating at 88 °C and transformed to collapsed structures with a narrow size distribution. They aggregated first and formed core of the fractal structure and then the bigger particles that were formed by aggregation of the collapsed DNA molecules attached at the outer side of the fractal structure.

However, at different positions of the sample there appears another kind of aggregate as shown in Fig. 3(e). The aggregate consists of many DNA chains with different length. Fig. 3(f) shows one of the aggregates, and Fig. 3(h) is the cross section curve along the line in Fig. 3(f). It can be seen that the height of the chains is about 1 nm and width about 20 nm. This means that the chains are still DNA without thermal denaturation or renatured again. It is interesting that in the center of near every aggregate there is a particle, which should be the denatured and collapsed DNA.

These two kinds of aggregates shown in Fig. 3(a) and (e) take place in the same sample and we even get an image of both structures side by side as shown in Fig. 3(g). By some reason, nucleation sites are denser at the upper right region and the fractals are smaller there. The fact that there exist two kinds of aggregates at the same time indicates incomplete denaturation or renaturation of DNA molecules at 88 °C.

Fig. 4 shows the sample prepared at 100 °C. There also appears fractal structure (Fig. 4(a)) which is different to those shown in Fig. 3; its shape is dendritic with lower fractal dimension. Fig. 4(b) shows the fractal analysis of Fig. 4(a) and gives value of dimension $D = 1.47$. It is known that at 100 °C DNA molecule easily degraded and transformed into many little fragments. Fig. 4(b) shows that the aggregate consists of many little particles of about 1.0 nm in height and 20 nm in width. They are fragments of degraded DNA. The lower fractal dimension indicates a quicker aggregation process at 100 °C, according to the DLA model [17].
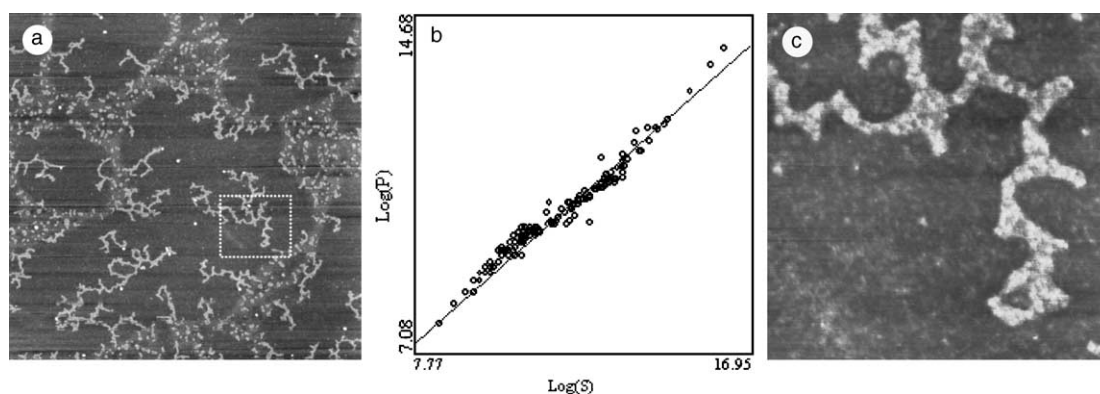

Fig. 4. Typical AFM images of DNA on the surface of newly cleaved mica prepared at 100 °C. (a) AFM image of fractal aggregates of degraded DNA fragments (5.5 μm × 5.5 μm); (b) relative fractal analysis curve; (c) enlarged image of selected area in (a).

4. Summary

DNA solution was heated at temperatures higher than the melting temperature, which made DNA double strands denaturation and then collapse or renature. At 88 °C, part of DNA took place a coil-to-globule transition to form little particles, then these particles formed fractal aggregates following nonuniversal DLA model for particles with size distribution. There found another kind of aggregate which consisted of DNA chains. At 100 °C, only one kind of fractal aggregate appeared, which consisted of particles with size of about 20 nm. The little particles are fragments of DNA degraded at the high temperature.

Acknowledgements

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