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Confined Water Nanofilm Promoting Nonenzymatic Degradation of DNA Molecules

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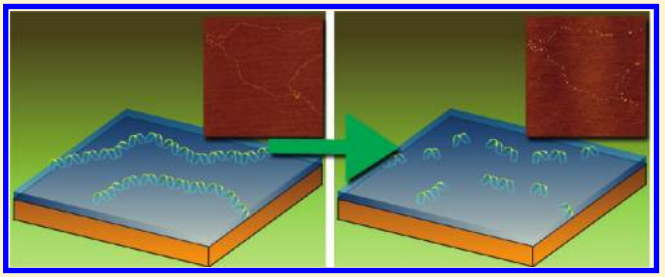
Supporting Information

ABSTRACT: Many important physical, chemical, and biological processes taking place in nanoscopic confined water environments may behave very different from that in bulk. In this paper, we report an unusual nonenzymatic degradation of DNA molecules in the confined water nanofilm on mica substrate. We found that the half-time of DNA in the degradation process in the water nanofilm is about several hours under high RH at temperatures ranging from 10 to 50 °C, which is much faster than that in bulk water, ~10^{11} years at 25 °C. Careful analysis indicated that it was the formation of a water/vapor interface in the water nanofilm that played a major role in promoting DNA degradation.



1. INTRODUCTION

The interfacial water confined in nanoscopic spaces usually exhibits unconventional properties not seen in bulk and plays unique roles in many important physical, chemical, and biological processes.[1−7] The water nanofilms spontaneously formed on solid substrates under ambient conditions are typical two-dimensional (2-D) interfacial water systems which have renewed wide attention[8−14] and been used to study many important physical/chemical processes in varied scientific areas.[15,16] The interfacial 2-D water nanofilm has also been found to be widely exist in biological system,[17] typically in a state that surrounds biomolecules in crowding environments in a in vivo environment,[18−21] and is recently considered to have unique functions in biological processes.[5,22−24] However, the in vivo system is so complex that there is a lack of means to study it directly. Therefore, research tends to find much simpler systems to clarify how such a 2-D confined water environment influences the behavior of biomolecules in it. The 2-D water nanofilm on a flat solid substrate can serve as a model system for investigation of biomolecular processes in 2-D water. Recently, Li et al. found that the self-assembling behaviors of some disease-related peptides incubated in a 2-D ambient water nanofilm on a mica surface are totally different from those in a bulk water system.[4] This difference may be ascribed to the modified weak interactions in the water nanofilm, including H-bond and hydrophobic interactions.[25]

Is it possible that the 2-D confined water influences the strong interactions such as the stability of covalent bonds of the reagents participating in a biological reaction, i.e., the covalent bonds in DNA backbone, an important issue in biology and biotechnology? Some hints come from investigations on the unique reactivity of organic compounds "on water" that revealed that water interfaces can affect a lot of reaction pathways.[26,27] It is well known that the phosphate diester bonds in DNA are very stable in pure bulk water with a half-life of about 10^{11} years at 25 °C.[28,29] Therefore, to break DNA one usually has to seek the help of natural enzymes or humanmade catalysts[30] or take special physical/chemical treatments under extreme conditions, such as ionizing radiation[31] and acidic/basic reagents.[32] However, here we report unusual, nonenzymatic degradation of DNA in such 2-D water nanofilm where the half-time of the DNA backbone decreases to the hour's level.

2. EXPERIMENTAL SECTION

DNA Sample Preparation. Freshly cleaved mica surface was treated with 10 mM $Ni(NO_3)_2$ solution for 1 min to make it positively charged. Then it was rinsed with pure water three times and dried under N_2 gas. One drop of 10 μL of an aqueous solution of linear lambda DNA molecules with a concentration of 20 ng/μL was placed on the salt-treated mica surface for DNA binding for 1 min. The sample was rinsed with water again and dried with a N_2 flow. After incubation, all samples were kept under a dry atmosphere until they were studied with atomic force microscopy (AFM) (Figure 1A−D).

Sample Incubation in Humidity Controlled Chamber. For the DNA samples incubated under a relative humidity (RH)

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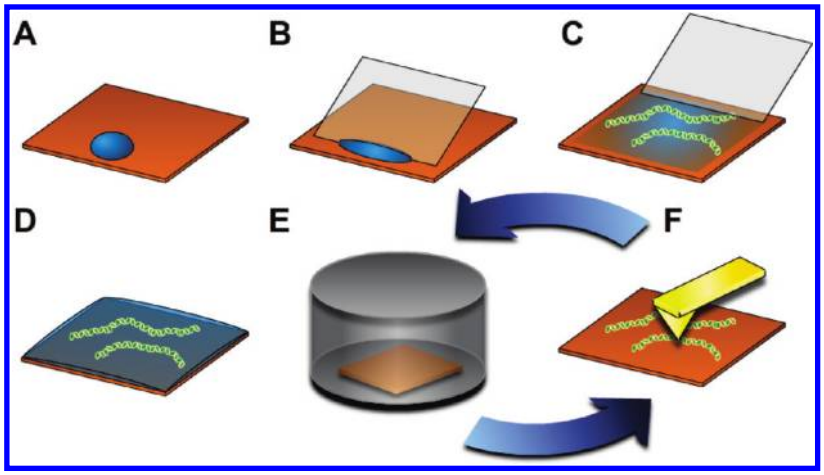

Figure 1. Schematics of the experimental setup and detailed procedure. (A−D) DNA sample preparation. (E) Sample incubation in a chamber with controlled temperatures and RH. (F) AFM ex situ observation before and after step E.

lower than 90%, we used a chamber (SDH-01N, Shanghai Jianheng Instrument Co.) to control the incubation temperature and RH. The thicknesses of the water nanofilms were controlled by adjusting the RH and temperature in the chamber with an accuracy of ±5% and ±0.1 °C, respectively. For the DNA samples incubated under 100% RH, we used an extra sealed box half-filled with pure water, which was preincubated in the chamber with a setting temperature. After the DNA samples were placed in this box, the box was sealed again and remained in the chamber for a certain time from several to dozens of hours. After incubation, the samples were kept in a drybox until they were studied with AFM (Figure 1E).

Atomic Force Microscope (AFM) Observation. DNA samples were investigated with an AFM (Multimode Nanoscope IIIa, Vecco/Digital Instruments, Santa Barbara, CA) equipped with a J scanner. Commercial NSC11 silicon cantilevers (MikroMasch Co.) with a force constant of ∼48 N/m and resonant frequency of ∼330 kHz were used. All AFM operations were carried out in air at room temperature under a RH of 40%. DNA samples were transferred to the chamber for incubation after AFM imaging. To relocate the DNA molecules after incubation, a marker was generated on the sample surface near the DNA molecules by scratching the surface with the AFM tip, which was pushed against the surface by manually adjusting the motor control switch. The scratch was visible in the optical microscope, and its relative position to the preimaged DNA molecules served as a direction for DNA relocation (Figure 1F).

DNase I Control Experiments. The DNase I was purchase from Fermentas International Inc. (China) with an original concentration of 1 U/μL. Before use, it was diluted with pure water to a concentration of 0.001 U/μL. One drop of 10 μL of diluted DNase I was deposited on the mica substrate with DNA sample and incubated for several minutes. Then the solution was blown off from the substrate. From an intuition point of view, the amount of enzyme molecules remaining on the sample should be much more than those from the environment. After that the samples were transferred to a box and incubated at a RH of 100% and temperature of 37 °C. Then the sample was imaged with AFM.

Statistic Analysis. Measurement of the length of DNA on the AFM images was carried out with image process software, Image J, which is an open source software provided by the National Institutes of Health (NIH). The calculation is based on the guide of Image J software in the "Measuring DNA Contour Lengths" section. Furthermore, the lengths of DNA in the shrink dots of the DNA chains at the broken ends were also estimated and added to the count. After comparing the DNA lengths on the AFM images obtained before and after sample incubation in the chamber, a proportion of DNA residue can be obtained. For details of the estimation of the "compression" effect in the statistic work, please refer to the Supporting Information.

3. RESULTS AND DISCUSSION

In experiments, lambda DNA samples were first deposited on a mica surface pretreated with Ni^{2+}. Then the samples were incubated in a chamber in which the temperature and RH are well controlled. With this control, water nanofilms on the mica surfaces in the chamber could be generated and the film thicknesses were modulated from less than 0.3 nm (less than one full covered molecular layer) to several nanometers as reported previously.[15,33] The experimental setup and procedure are shown in Figure 1. By comparing ex situ AFM images of the individual DNA molecules before and after incubation in the chamber, we found that the DNA strands were broken on the surface under high RH conditions.

Figure 2 shows several typical examples. When DNA molecules were deposited on the Ni^{2+}-treated substrate, they were in an extended linear shape (Figure 2, upper panel) and were almost intact at their original positions, even for a long incubation time of 120 h under a RH of 30−80% (Figure 2A2), consistent with early reports.[34,35] However, after incubation under 100% RH at 37 °C for 10 h, linear DNA molecules were broken into short fragments (Figure 2B2). When the incubation temperature increased, a higher degree of DNA degradation was found (Figure 2C). For example, after incubation at 50 °C under 100% RH for 10 h, linear DNA molecules were broken into many small pieces with a dot-line shape (Figure 2C).

A statistic analysis has been done on the apparent degradation ratio of DNA molecules on a mica surface incubated at 37 °C under 100% RH. As shown in Figure 3A, in the first 3.3 h the degradation ratio is around 12% ± 6%. When the incubation time was extended to 16.6 h, the degradation ratio reached 74% ± 8%. From Figure 3A it could be concluded that the half-time of the degradation process is around 12 h under such temperature and humidity conditions. It is much faster than that in a bulk

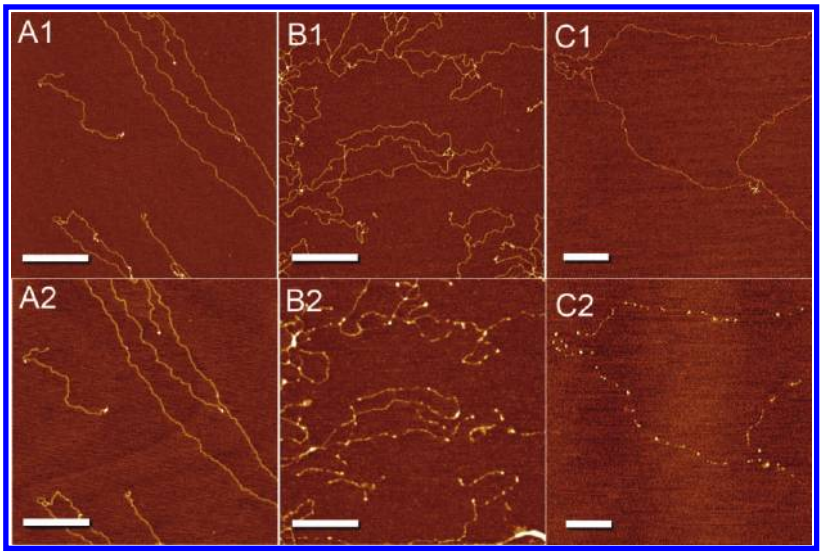

Figure 2. Typical AFM height images of lambda DNA molecules before (upper panel) and after (lower panel) incubation in a high RH environment: (A) 37 °C for 120 h, 80% RH; (B) 37 °C for 10 h, 100% RH; (C) 50 °C for 10 h, 100% RH (the scale bars in each image are all 500 nm).

water environment. Furthermore, we systematically investigated the DNA degradation under various humidity and temperatures. We found that the degradation could only be observed under high RH conditions. As shown in Figure 3B, DNA degradation only happened when the RH was higher than 85% at temperatures ranging from 10 to 50 °C.

In order to prove that the DNA degradation is not caused by DNA enzyme from the environment during the processes, we deliberately introduced DNase I on the substrate with DNA molecules in a control experiment. No enhancement of the DNA degradation was observed. In addition, other control experiments indicated that there was no DNA degradation to be found on mica surfaces under bulk water even for a time period longer than 20 h, consistent with previous reports by many researchers.[36]

The experiments also have been done on Mg^{2+}- and Cu^{2+}-modified mica surfaces. It was found that DNA molecules on such surfaces were stable if they were incubated at low RH (e.g., 30−80%). However, on the Mg^{2+}-modified mica surfaces, DNA molecules aggregated into bundles after incubation at a RH of 100% in less than 1 h (Figure 4), probably because of a poor binding ability of the substrate to the DNA.[36] Therefore, it is difficult to exactly detect the broken points in the DNA bundles with AFM observation. The DNA degradation on a Cu^{2+}-modified mica surface under high RH was very similar to that on the Ni^{2+}-modified substrate, which indicated that DNA degradation was not a result of a specific metal ion.

These results indicate that the enhanced DNA degradation in high RH conditions indeed originates from the ambient interfacial water nanofilm on mica with a several nanometer thickness.[25,33,37−40] One may be interested in what characteristics of this water nanofilm promoted DNA degradation. When the RH is less than 85%, the thickness of the water nanofilm on mica surface is usually smaller than 1 nm. When the RH is close to 100%, however, its thickness reaches a nanometer scale and the DNA backbone is immobilized in this water nanofilm confined between two closely adjacent interfaces: the hydrophilic water/mica interface and hydrophobic water/vapor interface. According to current knowledge, both this water nanofilm and the water/vapor interface are bulk-like.[25,41] Obviously, the bulk-like water nanofilm itself cannot promote DNA degradation. In contrast, it has been reported that the nanoscopic confined water film between the mineral surfaces increases the stability of the conformation of DNA molecules.[42] Since it has also been proved that the degradation cannot be promoted at the water/mica interface in bulk water, the water/vapor interface on the water nanofilm is the only suspect left, which must be the cause.

The question is now focused on how the water/vapor interface promotes DNA degradation. Intuitively, the stretching of the DNA backbone caused by the surface tension of the water interfaces should be considered first. Recently, Sheiko et al. reported that adsorption of brush-like polymers to a substrate resulted in a side chain−substrate interaction that provides a force to mechanically rupture the covalent bonds in the backbone of the polymers.[43] However, the situation in our system is evidently different since DNA molecules have no side chains and, as a whole, the DNA backbone is very elastic and reamins stable in bulk water, even being stretched to a sufficient ratio.[44,45] We think that the adsorption of DNA onto the mica substrate may induce stress locally in the long DNA chains. Combining this stress with the surface tension induced by the water/vapor interface, it then may be strong enough to break the long DNA chains. From our experiments, at the early stage of the DNA degradation process the widely existing shrink dots of the DNA chains at the broken points are direct evidence. Therefore, mechanical forces at interfaces may distort the local steric conformation of DNA molecules and become the main force in generating gaps in long DNA molecules in such conditions.

However, the tension at the interfaces should not be the single reason for such a degradation process. After a long time (more than 15 h) incubation at 37 °C under 100% RH, most of the DNA molecules degraded into very small fragments along the primary deposited positions. In a 50 °C temperature experiment, the same situation could also be observed in Figure 2 C2. Furthermore, coiled DNA and straight-line-shaped DNA only showed a small difference in the apparent degradation ratio (Supporting Information). In addition, our control experiments in which DNA was incubated with wet−dry cycles (100−30% RH, 4 cycles, 15 min for each RH in a cycle) showed that DNA degradation did not speed up by the repeated wet−dry process, indicating DNA degradation mainly happens during incubation

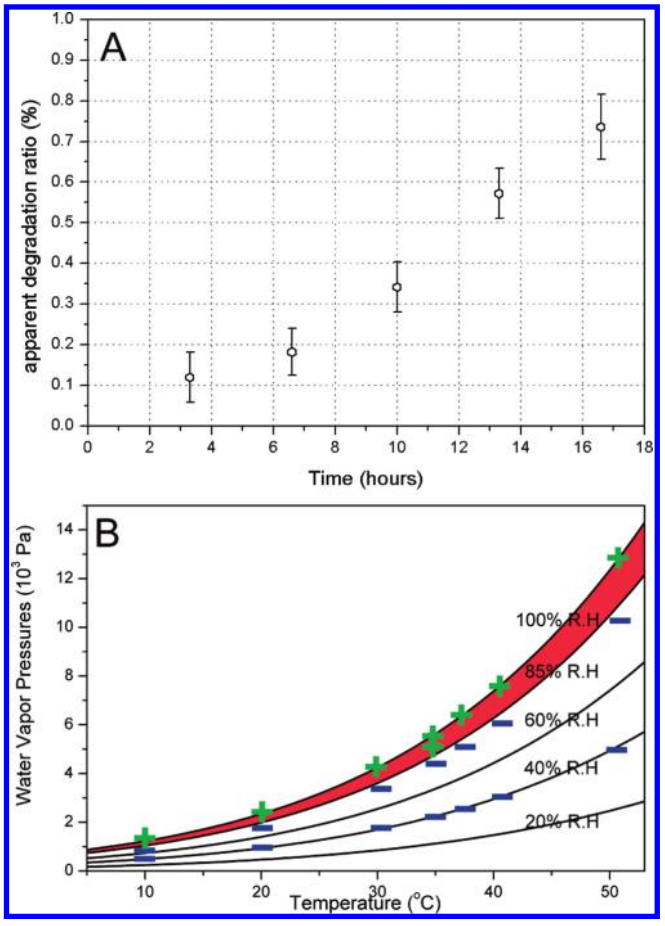

Figure 3. (A) Plot of the apparent degradation ratio of the DNA molecules to the incubation time under 37 °C, 100% RH. The half time of the degradation process of DNA molecules under such conditions is around 12 h. For each data dot, the lengths of all DNA strands in five randomly selected images, which have a scanning area of 5 μm × 5 μm, from five independent samples were measured before and after incubation. The proportions of the degraded DNA in the five samples were averaged. Standard deviation of these proportions was used as the error bar. (B) Plots of the absolute water vapor pressure versus temperature. AFM analysis of the DNA molecules on mica surface showed that no degradation was found at low RHs (20%, 40%, 60%) at temperatures ranging from 10 to 50 °C, as indicated with the minus sign "−". Only in the red area (RH 85% or higher) could the degradation be observed, as indicated with plus sign "+".

under a high RH environment. Therefore, these results could not be simply explained by the tension in the backbones of DNA molecules, and other origins should also be responsible for DNA degradation. In fact, the water/vapor interface exhibits many eccentric properties which can serve as a special "catalyst" for many chemical reactions. For example, it has been reported that the cycloaddition reaction at a hydrophobic water interface can be increased by 5 orders.[26,27] Note that the water/vapor interface is usually considered to have similar properties with the water/hydrophobic material interfaces.[46] Although the detailed mechanisms are not clear, we doubt that in our system the enrichment of the hydronium and hydroxide at different sides of the water/vapor interface[47−49] may play an important role in the increase of the efficiency of the nucleophilic attack at the phosphorus center.[50]

One may wonder why a similar result has not been observed at the water/vapor interface in a bulk system. Since the interfacial water/vapor layer only occupies a very small volume proportion in a bulk system, the hydrophilic DNA molecules are usually dissolved in the bulk water and have little chance to stay at the hydrophobic top layer. In addition, it will be difficult to detect the degradation of such small amount of DNA caused by the hydrophobic vapor/water interface, if there is any. In our system, however, each DNA molecule is fixed by the mica substrate and could stay at the water/vapor interface for many hours, making it possible for the enhanced degradation to be observed by AFM ex situ imaging.

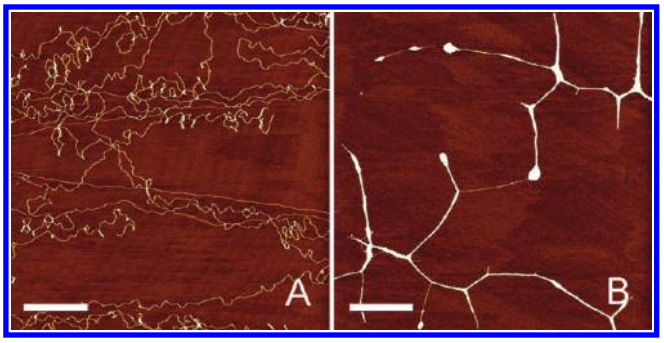

Figure 4. AFM ex situ observation of the λ DNA molecules deposited on Mg^{2+} molecules treated in 35 °C, 100% RH for 1 h. The result in B showed that almost all the DNA molecules had aggregated into rods structure, and it is difficult to estimate the degradation degree at the single-molecule level (the scale bars in each image are all 400 nm).

4. CONCLUSIONS

In summary, we found that DNA molecules absorbed on an ambient mica surface undergo an unusual degradation process under a near saturated water vapor environment. Careful analysis indicated that it was the formation of a water/vapor interface in the water nanofilm that played a major role in promoting the DNA degradation. Since the interfacial 2-D water including water/hydrophilic and water/hydrophobic interfaces widely exists in biological systems, we think that the current study may help us understand how the water microenvironment influences the stability of DNA backbones. Furthermore, it has been reported that the mica may play an important role in the origin of life.[51] Since the formation and degradation of biopolymers on mineral surfaces are believed to be the key steps in the early stage of life,[52] we think this study could provide helpful information for understanding the origin of life.

■ ASSOCIATED CONTENT

Supporting Information. Statistic analysis method and comparison on the degradation degree between coiled DNA and the straight-line-shaped DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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