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Forces-induced pinpoint denaturation of short DNA

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ABSTRACT

A method that can pinpoint control DNA denaturation is reported. In the single molecule experiment using spFRET, DNA adhered on a quartz surface is acted upon by both a weak laser field force and a fast temporal mechanical force. The experiment showed that increasing strengths of laser power result in increasing percentage of denatured DNA; different mechanical forces produce different numbers of DNA opening. Besides the method's simplicity and convenience for DNA melting, its crucial advantage and potential application is the ability to denature DNA at specified locations, i.e., a weak laser and a fast temporal mechanical force can be used in pinpoint denaturation of short DNA.

Introduction

One of the most important physicochemical properties of Deoxyribonucleic acid (DNA) is its denaturation, a process in which double-stranded DNA (dsDNA) unwinds and separates into single-stranded DNA (ssDNA) by breaking the hydrogen bonds between bases. Previous experimental and theoretical works have shown that stability of dsDNA depends on interaction between complementary pairs in two chains and stacking interaction connecting the two neighboring base pairs in one chain [1–6]. Many factors both in vivo and in vitro can alter DNA solution and break the hydrogen bonds, including temperature, ionic concentration, molecular crowding condition, microchannel laminar flow and other mechanical forces [7–16]. The simplest method of DNA denaturation is to heat DNA up to a temperature above its melting point, but, one of the shortcomings of this method is its inability to be implemented in experiments in vivo because of the possibility in damaging bioactivity of the system [17,18].

Laser application is one of the most powerful tools used in experiments of biology [19–22]. Since it is contra-indicated to use lasers only in experiments with DNA denaturation, we propose a method that combines a mechanical force with a weak laser field in order to minimize risk of causing unnecessary exterior damages.

In this study, one strand of dsDNA is immobilized on a polyethyleneglycol (PEG)-coated quartz surface. An experimental platform is specifically designed to produce a fast temporal force. Laser power is set between 0.5 mW and 5 mW. Single pair Fluorescence Resonance Energy Transfer (spFRET) is employed to detect the distance between Cy3 and Cy5 to determine if a DNA molecule has been opened. It has been illustrated that the weak laser and mechanical force facilitate DNA denaturation.

Materials and methods

DNA preparation. The sequences of oligos are as follows, OligoA: 5′-Cy3-ACTCTGCTCGACGGAT-3′-Biotin; OligoB: 5′-ATCCGTCGAG CAGAGT*TTTTTTT-3′ (where T* stands for an aminemodified dT with Cy5 labeled).

ssDNA molecules labeled by dye and biotin are purchased from SANGON (Shanghai). A sample of dsDNA, containing 25 μM OligoA and 75 μM OligoB, is heated to 90 °C and annealed for 2 h at room temperature. Superfluous OligoA joins the reaction to ensure that dsDNA makes up majority of DNA with biotin [23].

Equipment. In Fig. 1, coated with polyethyleneglycol (PEG) (5000, Nektar Therapeutics), a quartz plate is adhered on a clean coverslip by double-slided tape (100 μm thick, 3 M). A groove is slipped on the double-slided tape to form a 20 μL volume sample chamber between the quartz plate and the coverslip. Epoxy is used to seal the boundaries. This plate has two holes each having a diameter of 0.8 mm. DNA is immobilized on the quartz surface by the biotin–Straptavidin binding between biotinylated DNA, Straptavidin, and biotinylated polymer PEG (3400, Nektar Therapeutics). DNA is observed in a standard solution with an oxygen scavenging system: 100 mM NaCl, 25 mM Tris–HCl at pH 7.5, 1 mg/mL glucose oxidase, 0.4% (w/v) D-glucose, 0.04 mg/mL catalase, and 1% (v/v) 2-mercaptoethanol at room temperature (22 ± 1) °C. The laser power can be adjusted and quantified by a laser detector before it reaches the prism

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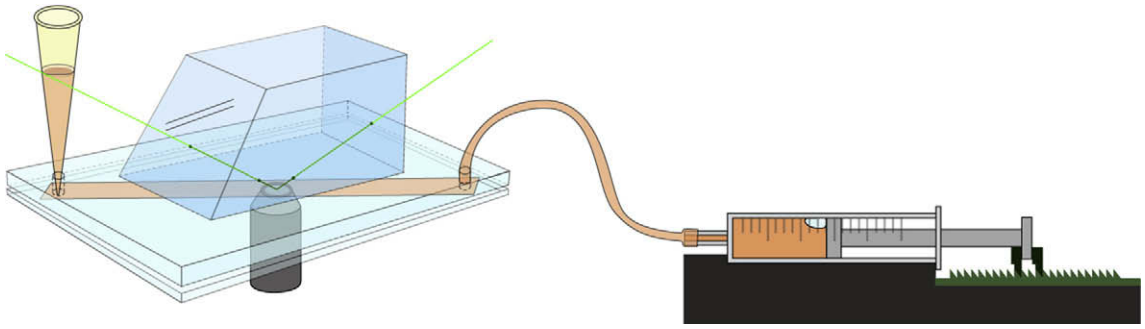

Fig. 1. Schematic illustration of the experimental instrument. A quartz surface with two holes is adhered to a coverslip to form a chamber. A total inner reflection with the aid of a prism is set up at the bottom surface of the quartz. The fast temporal mechanical force is resulted from sliding the injector.

[24,25]. Equipment for obtaining mechanical force is attached to the channel by a pipette tip, tubing, and an injector controlled by a pump. A bubble of 1 mL is formed inside the injector, so that a fast temporal mechanical force acted upon the DNA molecules by sliding the injector.

Reaction condition. Cy3 on DNA is excited by an Nd:YAG laser (532 nm, Coherence) via total internal reflection. The fluorescence signal from Cy3 and Cy5 collected by a water immersion objective lens (60×, Olympus) goes through a 550 nm long-pass filter to block out laser scattering and then is separated by a 630 nm dichroic mirror to be detected by an i-CCD camera (Prinston) [26]. The fluorescence signal is amplified prior to the camera readout; therefore, the recorded fluorescence intensity is in an arbitrary unit. The signal is recorded by using software MetaMorph.

Results and discussion

DNA denatured by cooperation of weak laser field and fast temporal force

As shown in Fig. 2, fluorescence signals of donor and acceptor molecules were described as green and red spots, respectively. Fig. 2(A) is an image acquired when laser of 3 mW is applied to the detection system. Light spots of Cy5 are much brighter than those of Cy3, so most DNA molecules were still dsDNA. While laser power of 3 mW was maintained to excite Cy3, a fast temporal force is added to the system by sliding the injector. After this operation, another image was obtained as depicted in Fig. 2(B). Obviously, most red spots disappeared. Based on the principle of FRET [24], it could be demonstrated that each disappeared red point indicated an additional denatured DNA. Compared with Fig. 2(A), the light of green spots increased, showing that the single strands modified with Cy3 still remained on the surface due to their inability to transfer the energy of Cy3 to coterminous Cy5. In order to demonstrate DNA denaturation process in single molecular level, light intensity of Cy3 and Cy5 of a molecule was recorded by CCD resolution of 200 ms. The lights of Cy3 (green line in Fig. 2(C)) and Cy5 (red line in Fig. 2(C)) modified on the same DNA were separated. After adding the fast temporal force, intensity of Cy3 (I_{donor}) increased while that of Cy5 ($I_{acceptor}$) decreased. According to the above observation, FRET efficiency (E_{FRET}) between the donor and acceptor was calculated by a simple function, $E_{FRET} = I_{acceptor}/(I_{acceptor} + I_{donor})$ as shown in Fig. 2(D) [26]. From both intensity and E_{FRET} chart in Fig. 2(C) and (d), we can understand clearly the denaturation process of one dsDNA molecule.

Neither a weak laser field nor a fast temporal force can induce DNA denaturation individually

A series of comparative experiments were performed to confirm the necessity of combination of a fast temporal mechanical force and a weak laser field for DNA opening rather singular efforts. Only applying laser power (0.5–5 mW) to illuminate the DNA sample for a certain period of time did not result in any DNA denaturation. By adding mechanical force without opening the laser, all dsDNA molecules retained their double-stranded structures as expected.

The percentage of denatured DNA increases with laser power in the range

Laser with power ranging from 0.5 mW to 3 mW was employed to study the percentage of denatured DNA under the same mechanical force. The process below was repeated five times under each laser power to test the effect of laser power. (1) Excite the solution and acquire an image immediately. (2) Adjust the laser to required power and open it. (3) Push the injector to create a fast temporal mechanical force. (4) Excite the solution again and catch another image immediately. All images were taken while the laser power was sustained at 3 mW. The exact numbers of un-denatured DNA were recorded by counting the light spots in Cy5 side. Set A as the dsDNA number of the first image and B as the number of the second image. Ignoring the reduction number caused by florescence bleaching, the proportion of denatured DNA was calculated as $(A - B)/A$, As shown in Fig. 3, the percentage of denatured DNA increased proportionally with increasing laser power, demonstrating an almost positive linear relationship between denatured DNA and laser power between 0.5 mW and 3 mW. As a result, it was shown that DNA could be denatured in a suitable ratio between the laser field and mechanical force.

Different percentage of denatured DNA for pushing and pulling the injector

As illustrated in Fig. 4, obvious differences of the percentage of denatured DNA were recorded by changing the way mechanical force was produced. This change came from the different forces acting on DNA molecules. Due to the bubble within the injector as shown in Fig. 1, the force that acted on DNA by pushing the injector was larger than by pulling. Therefore, we could conclude that under identical laser power, larger mechanical force could facilitate DNA denaturation.

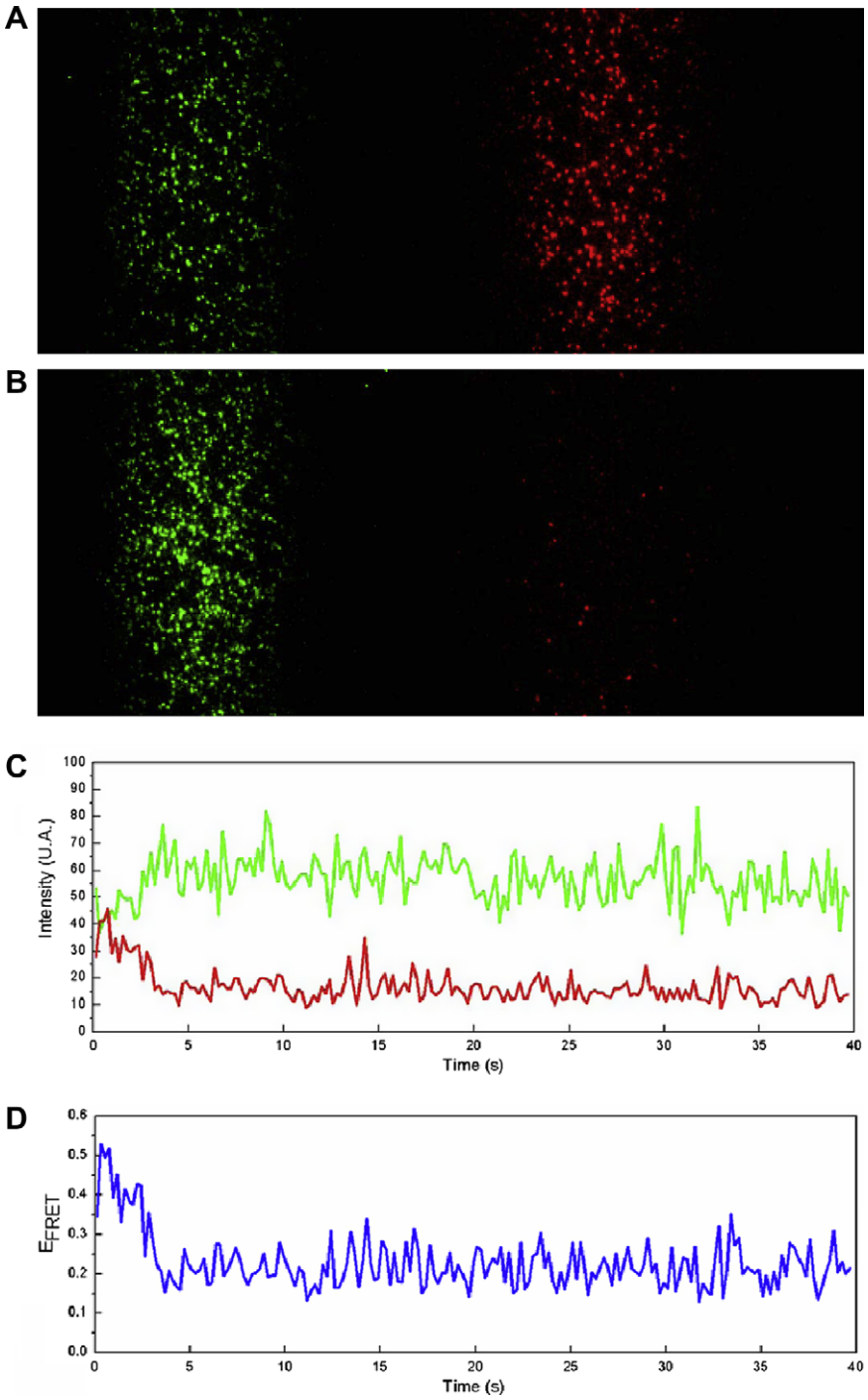

Fig. 2. An image taken by the spFRET system, the left side comprised of green is the donor (Cy3) light, while the right side comprised of red is the acceptor (Cy5) light. (A) Pattern of DNA before the fast temporal mechanical force is applied, dsDNA is dominated composition (B) pattern of DNA after the fast temporal mechanical force is applied, the main composition is ssDNA. (C) The intensity varied with different times. Green line indicates the optical intensity of a Cy3 molecule, red line the optical intensity of a Cy5 molecule. Cy3 and Cy5 are attached to the same DNA molecule. (D) E_{FRET} as a function of time. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

It has been revealed that hydrogen-bonding energy and stacking energy are the two main interactions for the stability of dsDNA [27,28]. In our investigation, the external forces consisted of a laser field and a mechanical force. The laser energy absorbed by DNA molecules can be denoted as EL, and the mechanical energy as EF. If the total absorbed energy (EL + EF) is more than a certain threshold energy, the dsDNA molecule will be denatured to two ssDNA molecules.

In summary, spFRET was relied upon to find a method of DNA denaturation. A weak laser field together with a fast temporal mechanical force easily breaks the hydrogen bond of dsDNA. In the single molecular experiment, the same laser that induces the DNA denaturation is used to excite the donor (Cy3). This method presents a potential application for the DNA melting because dsDNA can be easily melted by this physical method in an available ratio of the mechanical energy to the energy of a weak laser. An-

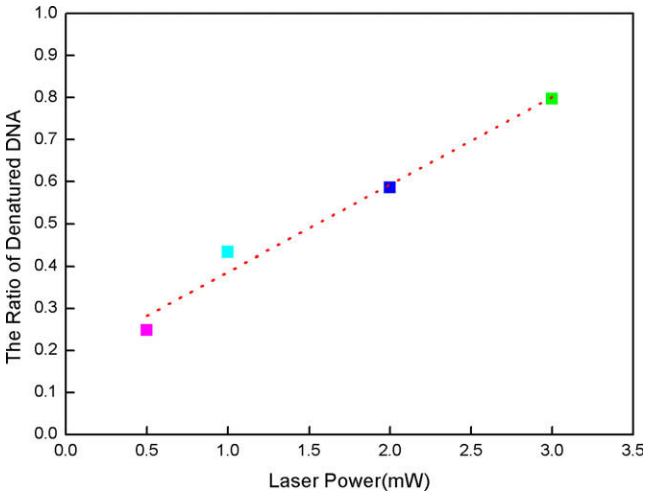

Fig. 3. Relationship between percentage of denatured DNA and laser power.

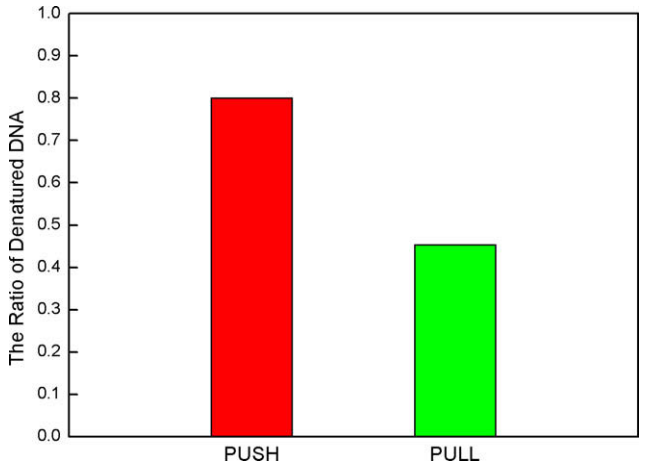

Fig. 4. Different percentages of DNA denaturation by pushing and pulling the injector.

other important advantage of this method is its capability to denature DNA in special locations via controlling laser to pinpoint at the location.

Acknowledgments

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