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Temperature Dependence of DNA Elasticity and Cisplatin Activity Studied with a Temperature-Controlled Magnetic Tweezers System

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Understanding the elastic property of DNA is one of the most fundamental issues in DNA science. The elastic property of DNA has been much studied at a single molecule level by using various micromanipulation tools, but its temperature dependence has only been studied in a limited range of temperatures. Here, we report our results regarding the elastic property of a single DNA molecule at high temperature. In order to accomplish that, we designed a simple ITO-based temperature-control system with which the temperature of the sample chamber in magnetic tweezers could be tuned up to 90 °C. Soft silicone tubing that circulates water at a certain preset temperature was wound around the objective, a dominant heat sink in the system. For temperatures beyond 50 °C, the DNA molecule appeared longer than its original length, presumably due to thermal melting of DNA, while its persistence length (ξ) was gradually decreased. In addition, we found that the activity of cisplatin (DNA-binding anti-cancer drug) on DNA was enhanced at high temperatures, resulting in more efficient DNA condensation.

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I. INTRODUCTION

Single-molecule biophysics has revolutionized the field of biophysics because it helps us understand the physical properties of various biological systems, which were not available or accessible with traditional biophysical and biochemical methods [1]. Single-molecule biophysical techniques enable us to follow the reaction by individual biological molecules in real time while bulk biochemical methods only allow us to collect experimental results via a population average and frequently observe them after relevant reactions have already been completed. Considering the fact that in biology, a small number of protein molecules can trigger a particular biological process and some reactions may consist of several intermediate steps, typical biochemical approaches like gel electrophoresis only yield insufficient information because the intrinsic heterogeneity of biological molecules will be smeared out, so the fast dynamics of multi-step reactions will not be detected in such approaches. This will then hamper physical understanding of the detailed mechanisms behind biological phenomena. Single-molecule biophysics can overcome limitations in traditional methods by directly measuring physical quantities associated with biological systems at a single-molecule level.

Among many biophysical methods, magnetic tweezers are one of the most versatile tools in manipulating single biological molecules, especially DNA, because they can afford excellent control of both force and position and apply a physiologically relevant level of force to biological molecules and systems [2,3]. Other advantages of magnetic tweezers are the ability to apply precise amounts of torque and sub-pN forces to biological molecules, low thermal drift, compatibility with fluorescence microscopy and simplicity of design. Therefore, a broad range of interesting biophysical issues, such as DNA elasticity and DNA supercoiling, have been examined with this technique [3].

Since biological systems are working in perpetually fluctuating thermal environment and utilize thermal energy from the thermal reservoir, the temperature, which is a good measure of the fluctuation of a system, is one of the most critical parameters to control. In most single-molecule experiments so far, the temperature of the system was, however, not controlled; the experiments were usually conducted at room temperature (T_R). Noting that the structure, stability and function of biological molecules are governed by temperature and that their

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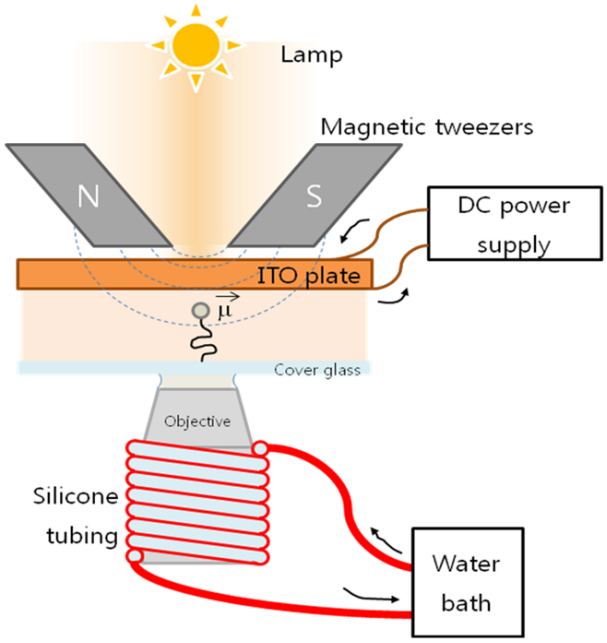


Fig. 1. Schematic of the temperature control system. The sample chamber was made by stacking an ITO plate on a cover glass. A DC power supply was connected to the plate to generate ohmic heat and a water bath circulated water at a preset temperature through the silicone tubing to warm the oil-immersion objective. DNA molecules attached to a magnetic bead were fixed in the sample chamber and were manipulated with a pair of magnets while being imaged with a bright field microscope.

activities in nature are optimized for a particular temperature (*i.e.*, our body temperature), the temperature in single-molecule measurements needs to be controlled to simulate relevant physiological conditions. Several previous single-molecule works already attempted to control the reaction temperature [4,5]. Here, we show our simpler approach to control the temperature in our single-molecule experiments with magnetic tweezers. Instead of using water-circulating metal jackets in order to warm the objective lens, we wound a coil of water-circulating soft silicone tubing around the objective and we used an ITO plate, as the top plate of a sample chamber, to directly heat the sample chamber. The upper side of the plate was coated with ITO. The temperature of the chamber (T_C) was calibrated against two control parameters: the temperature of a water bath (T_B) connected to the coil of tubing and the power supplied to the ITO plate. T_C could be easily determined once a calibration had been made. In our scheme, we can easily increase T_C up to 90 °C, much higher than the maximum temperature used in previous works. With our technique, it is possible to study various phenomena taking place at high temperatures.

In order to demonstrate the utility of our temperature-controlled magnetic tweezers, we carried out two experiments. First, we measured the temperature dependence of the DNA persistence length, a characteristic parameter of DNA elasticity in the worm-like-chain (WLC) model and found that the persistence length decreased significantly as the temperature was increased to 65 °C. Besides that, we also observed that the total length of DNA increased much at high temperature, presumably because DNA lengthens even at low force due to thermal melting. Second, we observed a temperature-dependent activity of cisplatin, a well-known anti-cancer drug, which binds to a duplex DNA molecule and bends it by 40° towards its major groove [6]. DNA molecules kinked by cisplatin binding were directly visualized with atomic force microscopy (AFM) before [7]. Due to that bend, a cisplatin-bound DNA can have a persistence length markedly different from that of natural DNA. The mechanical property of a single cisplatin-bound DNA was once investigated using single-molecule force spectroscopy with AFM [8]. By measuring the persistence length of DNA, we can estimate the degree of cisplatin binding [9]. Here, we tested whether the temperature could also affect the activity (binding) of cisplatin on DNA. We found that its activity increased dramatically as the temperature was increased from T_R to 45 °C. These examples demonstrate that temperature-controlled magnetic tweezers are very useful and effective in studying physical properties of biological molecules at a single-molecule level.

II. EXPERIMENTAL METHODS

1. Temperature-controlled Magnetic Tweezers System

The temperature control functionality was added to existing magnetic tweezers with two simple modifications (see Figure 1). First, the sample chamber was constructed by sandwiching a parafilm spacer between a 1-oz. cover glass (Fischer Scientific) and a 1-mm-thick ITO plate. The cover glass had two holes, one of which was an inlet for buffer and the other an outlet. A long aperture cut in the spacer film served as a channel that connects the inlet and the outlet inside the sample chamber. By applying a current to the ITO plate with a power supply (CE power supply, DC 12 V, 0.18 A), we could heat the whole chamber because the heat generated was proportional to the power consumption in the resistive ITO plate. In order to measure T_C directly, we embedded a temperature sensor in the chamber when T_C was calibrated.

Second, the objective lens in the magnetic tweezers was surrounded by a coil of soft silicone tubing carrying water with a preset temperature. Since the sample chamber was in direct contact with the objective lens made of metal, the lens often behaved as a dominant heat sink. In order to control T_C precisely and avoid considerable convection inside the chamber due to the temperature

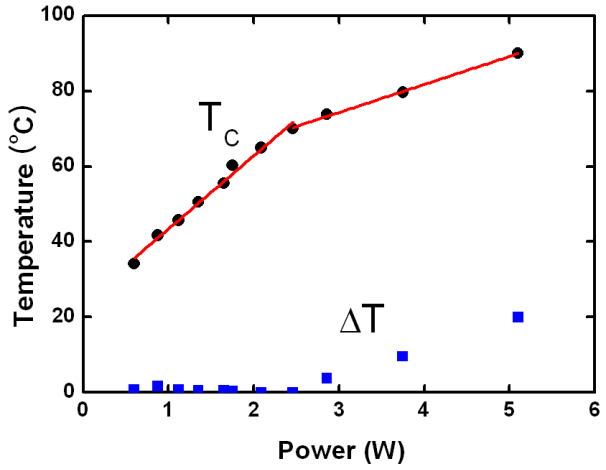


Fig. 2. Calibration of T_C. When the indicated amount of power was applied to the ITO plate, T_C (black) matched T_B well, as shown by the small $\Delta T = T_C - T_B$ (blue) up to 70 °C. Red lines are line fits to reveal the linearity of the data in the two temperature regions.

gradient through the chamber, we decided to control the temperature of the objective as well. In previous works, the objective lens was either topped with a copper water jacket circulating water [5] or surrounded by a coil of copper tubing [4]. In our study, we implemented the same idea by using soft silicone tubing and winding it around the objective. By circulating water preheated to a preset temperature in a temperature-controlled water bath (Cole Parmer, 12101-10), we could adjust the temperature of the objective. Silicone tubing normally maintains good thermal contact with metal. Since the manufacturer (Zeiss) did not recommend us to use the objective at high temperature, we set the maximum temperature of the water in the tubing to be less than 70 °C. T_C measured with the temperature sensor was directly calibrated against two control parameters: the temperature (T_B) of a water bath connected to the tubing and the power supplied to the ITO plate. The calibration result obtained when the ambient temperature was 24 °C is shown in Figure 2 and is discussed in Section III.

2. Sample Preparation

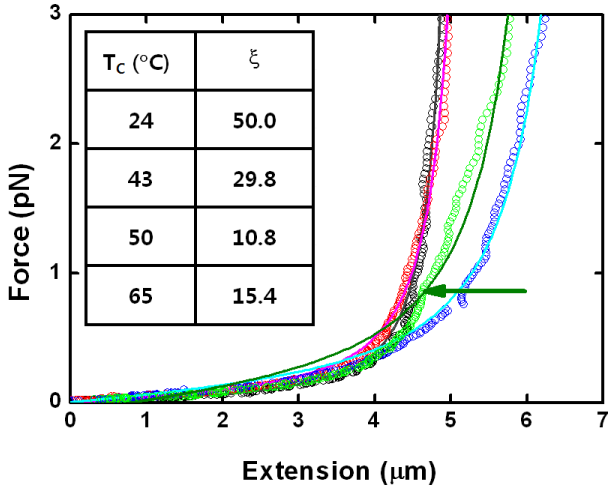
The DNA molecules (~5 μm) and the sample chamber for experiments with magnetic tweezers were prepared as described elsewhere [10,11]. While incubating DNA molecules in phosphate buffered saline (PBS) with Streptavidin-coated beads (Dynal) for 10 minutes, we attached one end of the molecule labeled with Biotin to the beads. The DNA molecules were then introduced into an anti-Digoxigenin (anti-Dig)-coated sample chamber, to which the other end of the molecule labeled with Dig would be bound. Via these specific attachments, more suitable than other methods [12], DNA tether molecules were prepared for single-molecule manipulation.

The cisplatin solution was prepared as described elsewhere [10,13]. Since its activity is strongly suppressed at high salt concentration, cisplatin was dissolved in a solution with a relatively low salt concentration ($[Na^+]$ = 30 mM), but the concentration was still high enough to keep binding of cisplatin low when the concentration of cisplatin was 200 μg/mL. We chose these concentrations as an optimal condition to set the baseline of the reaction in our experiment. If a considerable amount of cisplatin binds to DNA due to the thermal effect, the binding will be reflected as deviation of the persistence length from that of natural DNA because under that condition without heat, DNA appears as natural DNA.

III. RESULTS AND DISCUSSION

1. Temperature Calibration of the Sample Chamber

T_C is affected by both the temperature of the objective beneath the chamber and the power consumed in the ITO plate. Since the ambient temperature can also affect T_C, we kept the ambient temperature around 24 °C. Once T_C is calibrated against the power consumption at the ITO plate and T_B, the calibration data can be used to estimate T_C as long as the power consumption and T_B are known. In our experiment, we ramped up T_B to 70 °C and adjusted the power to the ITO plate to approximately match T_C to T_B. T_C nearly coincides with T_B for the applied powers shown in Figure 2. If a proper amount of power is provided to the ITO plate, $T_C - T_B$ (ΔT: blue) remains small and the power necessary to minimize ΔT is in fact quite linear with T_C ($T_C = 19.4\ P + 24$ °C: P (in Watt) is the power consumed in the ITO plate). Over 70 °C, we measured T_C when the indicated amount of power was supplied. As the power was increased, T_C departed from 70 °C, but increased almost linearly with the power ($T_C = 7.4\ P + 52$ °C). Owing to the linearity, one can easily estimate T_C, even for a power value not used in the calibration, simply by interpolating the curve. Once we have the calibration data, we only infer (estimate) T_C from T_B and the power to the ITO plate with no need to measure T_C. After the calibration, the temperature sensor was, therefore, not inserted in the sample chamber. In our scheme, we can easily increase T_C beyond 90 °C, the range of which was not reached in the previous single-molecule experiments controlling the reaction temperature. The range of the temperature they used was below 60 °C. Most interesting and biologically relevant phenomena occur in this low-temperature range; therefore, the limited tuning range of the temperature usually does not forbid studying thermal effects on most of biological systems. Still, the increased range of



T_C (°C)	ξ
24	50.0
43	29.8
50	10.8
65	15.4

Fig. 3. Temperature-dependence of the DNA persistence length. Force-extension curves for bare DNA at T_C = 24 °C (black, ξ = 50.0 ± 0.5 nm), 43 °C (red, ξ = 29.8 ± 0.5 nm), 50 °C (green, ξ = 10.8 ± 0.5 nm) and 65 °C (blue, ξ = 15.4 ± 0.5 nm). Black, magenta, dark green and cyan curves are fits obtained by using the WLC model for black, red, green and blue data, respectively. [Na^+] = 30 mM here. The inset presents a table of the DNA persistence lengths at the indicated temperatures.

the temperature with such minimal modifications, available in single-molecule experiments, would be beneficial because one can characterize the physical properties of biological molecules and samples at high temperatures and investigate at a single-molecule level various issues related to thermophiles, organisms which thrive at relatively high temperatures.

2. Temperature-dependent DNA Persistence Length

As mentioned, we carried out two experiments to demonstrate the utility of temperature-controlled magnetic tweezers. First, we measured the persistence length of DNA by obtaining the force-extension curve. It is well-established from a large number of theoretical and experimental works that the elasticity of DNA can be quantitatively explained by using the Worm-Like-Chain (WLC) model [14]. The model is expressed as the following equation: $\frac{F\xi}{k_BT} = \frac{1}{4(1-\frac{z}{L})^2} + \frac{z}{L} - \frac{1}{4}$, where F is the applied force, ξ the persistence length, k_BT the thermal energy, z the extension of DNA tether and L the contour length of DNA tether. The most important parameter in this model is the persistence length. The more flexible a polymer chain is, the smaller the persistence length of the chain will be. We can, therefore, determine the persistence length of DNA by using force-extension measurements with magnetic tweezers.

It is not, however, well-known how the persistence length changes as the temperature changes. Using our temperature-controlled magnetic tweezers, we measured the DNA persistence length for four different values of T_C. As shown in Figure 3 and its inset, the persistence length of DNA decreases substantially as T_C increases. The persistence length at T = 43 °C is 29.8 nm and that at T = 65 °C is 15.4 nm. At T = 50 °C, the fit by using the WLC model is not so satisfactory, presumably because both double-stranded and single-stranded forms may exist at that temperature. In the low-force range (<1 pN), the double-stranded form seems dominant. As the force increases, the double-stranded structure begins to be denatured to the single-stranded one, resulting in extra lengthening of DNA. Thus, there is an inflection point near 1 pN on the force-extension curve obtained at T = 50 °C (marked by an arrow in Figure 3) and the "effective" persistence length for T = 50 °C is just obtained as a best fit over the two distinct states (although it can be fit separately for these two regions, further theoretical consideration may be involved). In the case of T = 65 °C, no such transition was observed, presumably because the DNA was mostly denatured and uniform. The reduction in the persistence length indicates that DNA becomes more flexible as the temperature increases, which is very reasonable.

Besides the reduction in the persistence length, the total extension of the DNA tether measured under a tension of ∼3 pN appeared to be 3 %, 18 % and 27 % longer at 43 °C, 50 °C and 65 °C, respectively. The lengthening of DNA at 65 °C is due to thermal melting because the melting temperature of normal DNA is about the same temperature. The increases in extension for DNA at 43 °C and 50 °C must be due to softening of the chain by increased temperature. These might also be due to low-temperature melting possible when external tension is applied [5]. To our knowledge, a full theoretical understanding of this issue is yet to come.

3. Temperature-dependent Activity of Cisplatin on DNA

Cisplatin is one of the most famous anti-cancer drugs. It is proven to be effective in various kinds of solid tumors. Cisplatin is known to bind to a consecutive GG sequence of DNA and to bend the molecule at that site by 40° towards its major groove. It has been found in our laboratory that at a physiological salt concentration (the concentrations of monovalent cations, Na^+ and K^+ inside a cell are about 50 mM and 150 mM, respectively), cisplatin activity is strongly suppressed [13]. In order to see whether cisplatin activity also depends on temperature, we tested its activity on DNA, revealed as change in the persistence length of the reacted DNA, with varying T_C. Figure 4 shows the force-extension curves of cisplatin-bound DNAs. Here, we chose that [cisplatin] = 200 μg/mL when cisplatin reacted with DNA in the sam-

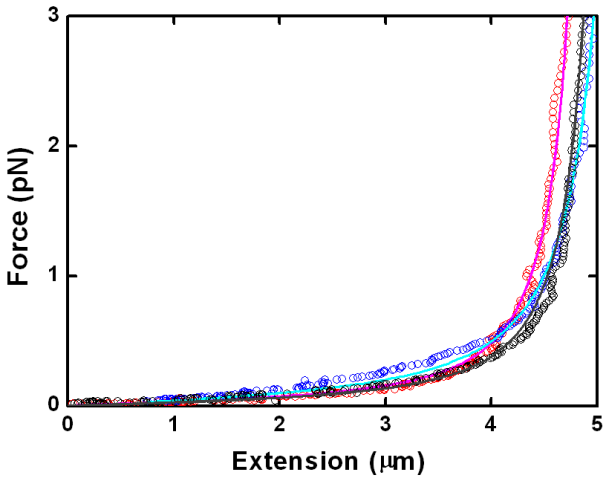


Fig. 4. Temperature-dependent activity of cisplatin. Force-extension curves for bare DNA at T_R (black) and cisplatin-bound DNA (red and blue). DNA that reacted with cisplatin (200 μg/mL; [Na^+] ξ = 30 mM) at 45 °C (blue) has ξ = 29.5 ± 0.8 nm while DNA that reacted with cisplatin (200 μg/mL; [Na^+] ξ = 30 mM) at 24 °C (red) has ξ = 45.0 ± 0.5 nm. Black, magenta and cyan curves are fits obtained by using the WLC model for black, red and blue data, respectively.

ple chamber. When the reaction took place at T_R, the persistence length of the reacted DNA became 45 nm, 90 % of the value from a bare DNA at physiological condition (Figure 4). When the same reaction took place at an elevated temperature of 45 °C, ξ decreased to 29.5 nm. According to our results in Ref 13, this value is close to a saturated (ξ = 25 nm) value of the persistence length of DNA when it reacted with cisplatin in the buffer with [Na^+] = 30 mM. That is, when DNA reacted with cisplatin (1 mg/mL and 0.5 mg/mL) at [Na^+] = 30 mM, ξ was about 25 nm and 35 nm, respectively. It is a rather surprising observation because an increase in the reaction temperature of only ∼20 °C can enhance the reaction as if DNA reacted with cisplatin whose concentration was ∼4 times more, which would have an important implication: Because this drug is administered to human whose body temperature is about 13 °C higher than T_R, this drug can be more effective than observed from an *in vitro* assay done at T_R.

IV. CONCLUSION

In this report, we described our temperature-controlled magnetic tweezers system. The scheme we took here is very simple to implement in an existing instrument, but still enables control of the temperature of the sample chamber with reasonable ease and accuracy. In addition, the temperature range it can cover is higher (up to 90 °C) and broader than that reported in previous works. With this instrument, we characterized the temperature dependence of the DNA persistence length and the temperature-dependent activity of cisplatin binding to DNA. Interestingly, we found that the DNA persistence length decreased significantly as the temperature was increased, which was expected from the increased flexibility of DNA at high temperatures. We also found that cisplatin activity was much enhanced when the temperature was increased by only about 20 °C. Since these results for the thermal effect can be obtained with a temperature-controlled instrument, they demonstrated the utility of the instrument. This instrument will also prove to be useful for studying various biological phenomena occurring at high temperatures such as enzymatic activities by proteins originating from thermophiles.

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