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# The Elastic Property of a Single DNA Molecule Cross-Linked by Cisplatin: a Magnetic Tweezers Study

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We observed that the elastic property of a DNA molecule was dramatically changed when the molecule reacted with an anticancer drug, cisplatin. It has been known from bulk assays that cisplatin induces the formation of intra- and inter-strand crosslinks, especially at two neighboring purine sites. From micromanipulation of a single DNA molecule with a magnetic tweezers apparatus, we obtained kinetic information on the interaction between a single DNA molecule and cisplatin, and on the conformation and elastic property of a cisplatin-bound DNA molecule: a single DNA molecule under a constant force was gradually shortened as it interacted with cisplatin, and the elasticity of the DNA molecule revealed by the force vs. extension data was dramatically altered, which indicates that DNA crosslinking by cisplatin is responsible for the changes in the conformation and elasticity. We demonstrated that magnetic tweezers are a versatile tool in studying the mechanical properties of an individual DNA molecule, which have a significant impact on the function of DNA and, therefore, on the anti-cancer effect of cisplatin.

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## I. INTRODUCTION

In the past decade, the field of single molecule biophysics has attracted much attention because it allows us to obtain physical information regarding various biological systems, which was not available or accessible with traditional biochemical methods. With single molecule biophysical techniques, we can follow the activity of an individual biological molecule in real time (for a recent review of this subject, see Ref. [1] and references therein) while, with bulk biochemical methods, we only collect experimental results obtained by using the population average, and frequently observe them after relevant reactions have already been completed. In biology, only a few protein molecules are often sufficient for a particular biological process and the reaction involved in such a process may consist of many intermediate steps. With a typical biochemical approach like gel electrophoresis, one cannot obtain valuable information on individual biological molecules or on the dynamics of such multi-step reactions, which limits our understanding of the detailed mechanisms behind biological phenomena.

In order to study the activity of a single biological molecule in real time, a number of new biophysical methods have been developed. Fluorescence microscopic techniques, such as total internal reflection fluorescence microscopy and confocal microscopy, have been successfully employed to visualize and track individual fluorescently-labeled molecules [2,3]. Scanning probe microscopic techniques, such as scanning force microscopy and near-field scanning optical microscopy, have been able to provide detailed information on structural and conformational features of individual biological molecules at nanometer resolution or better [3]. Although these methods are very powerful in their advantages, they are usually unable to manipulate biological molecules and are often limited to passive visualization of these molecules. Single-molecule micromanipulation techniques, such as magnetic tweezers and optical tweezers, fill the bill [4]. These two micromanipulation methods are versatile and afford excel-

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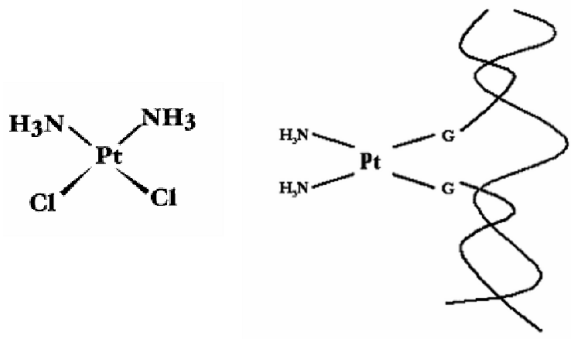


Fig. 1. Chemical structure of cisplatin and its proposed interaction with DNA (from Ref [8]).)

lent control of both force and positioning. In comparison to atomic force spectroscopy, these methods can apply a physiologically relevant level of force to biological molecules and systems. Unlike hydrodynamic manipulation, these tweezers can manipulate biological molecules with well-defined forces and with superior control of positioning [4].

The specific advantages of magnetic tweezers over optical tweezers include 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, in a broad range of biophysical experiments, such as DNA elasticity, DNA supercoiling, and manipulation of intracellular organelles, magnetic tweezers have been a primary choice for single molecule micromanipulation.

As many previous experimental works suggested, magnetic tweezers are an ideal tool to study the conformation of a single DNA molecule [5–7]. An interesting conformational change of DNA was discovered when a potent anti-cancer drug, cisplatin, bound to a duplex DNA molecule. Cisplatin, a platinated inorganic molecule first synthesized more than one hundred fifty years ago, was discovered to be effective as an anti-cancer drug [8]. The mechanism for the anti-cancer activity was proposed as follows: Due to its high affinity to a purine base, cisplatin can crosslink two neighboring purine bases (See Fig. 1), and the crosslink is then sensed as a lesion to be repaired. Since the high-mobility-group proteins bind to the lesion, the repair machinery cannot remove the lesion and induce a programmed cell death (apoptosis). Since cancer cells are reproduced much more rapidly than normal cells, cisplatin is more deadly to cancer cells than normal ones. Since the current model suggests that cisplatin-bound DNA has kinks where the strands are bent approximately by 40° [9], the conformation and the elasticity of cisplatin-bound DNA should be markedly different from those of free naked DNA.

In our work, we directly measured the elastic property of an individual single DNA molecule by using a magnetic tweezers apparatus and discovered that the elasticity of cisplatin-bound DNA is dramatically altered

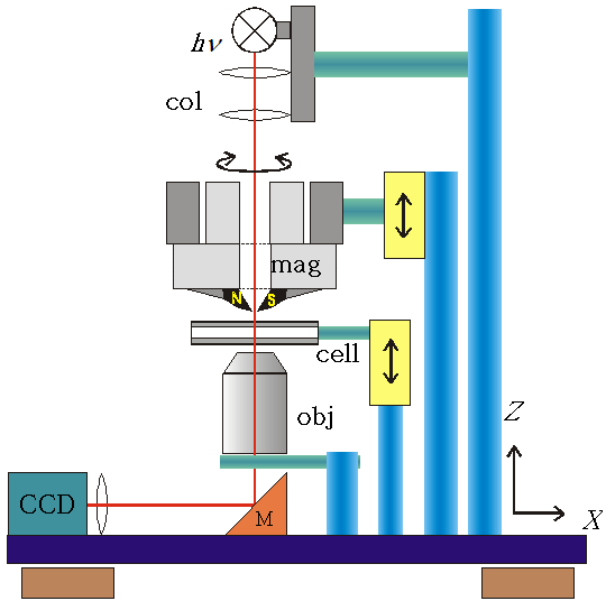


Fig. 2. Schematic diagram of the magnetic tweezers instrument (h$\nu$: light source, col: collimation optics, mag: magnets, cell: sample chamber, obj: objective lens, and CCD: charge-coupled device).

compared to that of naked DNA. The altered elastic property of cisplatin-bound DNA can be qualitatively explained by modifications of DNA due to cisplatin-mediated crosslinking.

## II. EXPERIMENTAL METHODS

A detailed description of the magnetic tweezers apparatus is given elsewhere [10] (for a schematic diagram of the magnetic tweezers, see Fig. 2), so only the essential parts will be described here because the design of the magnetic tweezers in our laboratory is very similar to the one described elsewhere, except for a few recent improvements that are described in the following. We used a better camera (Sensicam em: PCO) that has a significantly higher quantum efficiency (∼65 %) than a Pixelfly camera (PCO) and at least 4 times better spatial resolution at the same magnification. This camera is used to record the Brownian fluctuation for force measurement. In addition, we implemented a computer-controlled XY sample stage (SigmaKoki) so that we could position the sample chamber anywhere with a high positional repeatability (better than 10 microns - sufficient for our experiments because it was smaller than the dimension of one view field).

In order to study the crosslinking of a single DNA molecule by cisplatin, single DNA molecules, one end of which was labeled with biotin and the other end with digoxigenin, were attached to the sample chamber by binding the biotin-labeled end to a streptavidin-coated magnetic bead and the digoxigenin-labeled end to the

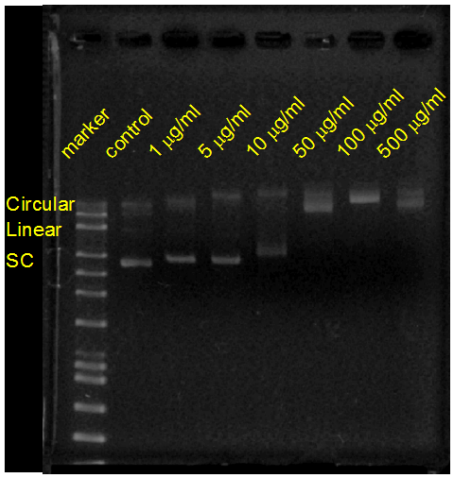


Fig. 3. In vitro assay of the cisplatin-induced mobility shift of DNA. $1^{st}$ lane: DNA ladder, $2^{nd}$ lane: control (the absence of cisplatin), $3^{rd}$ – $8^{th}$ lanes: pPIA2-6 incubated with the indicated concentration of cisplatin; the bands corresponding to linear, circular, and supercoiled pPIA2-6 are labeled as linear, circular, and SC.

anti-digoxigenin-coated bottom glass plate of the chamber. The DNA tether molecules derived from a pPIA2-6 (∼15 kb) [11] plasmid were prepared as described previously [10]. From the elastic and the torsional properties of the attached DNA molecules, we selected the DNA molecules that were singly tethered to the glass substrate.

In order to characterize the mechanical properties of a single DNA molecule, one should be able to measure the force exerted on and the length of the molecule (the end-to-end distance of the molecule). The force exerted on a DNA molecule can be determined by utilizing the Brownian fluctuation of the magnetic bead attached to the DNA molecule by the simple relation $F = k_BTl/<\Delta x^2>$, where $k_BT$ is the thermal energy, $l$ the length of the DNA tether, and $\Delta x$ the lateral fluctuation of the bead [6].

The magnetic tweezers apparatus is equipped with so-called hybrid supermagnets, which are based on hybrid magnet technology [12]. These new magnets can exert ∼10 pN to a magnetic bead with a 1.0-$\mu$m diameter. The details of the magnets are described elsewhere [10].

The vertical position of the bead, which yields information on the end-to-end distance of the tethered DNA molecule, can be determined by analyzing the diffraction patterns cast by the bead because the diffraction pattern is correlated to the distance between the bead and the objective. By using the Hilbert transformation to analyze the diffraction ring patterns, we could measure the Z position of the bead as fast as 20 Hz in real time, fast enough to follow the change in the length of the DNA molecule induced by cisplatin-mediated crosslinking. The details of the Hilbert transformation scheme are also described elsewhere [10].

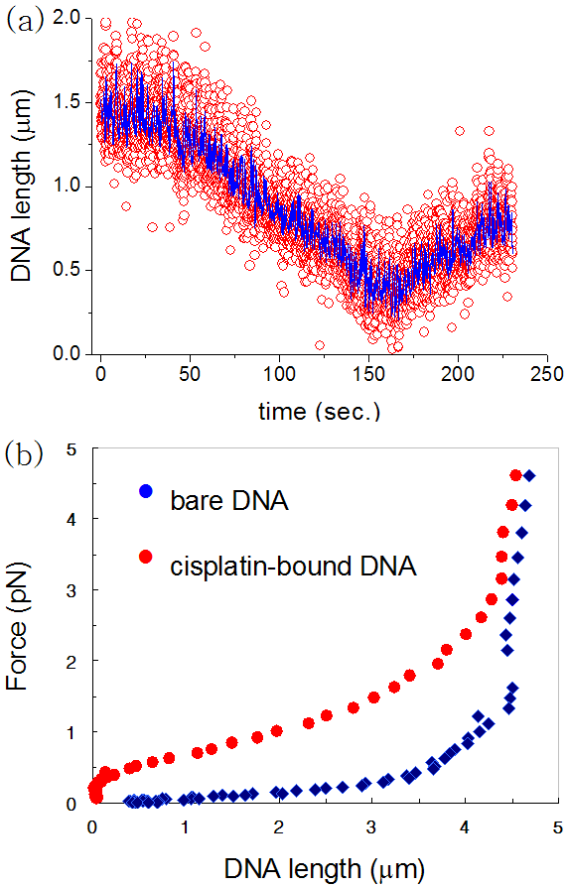


Fig. 4. (a) Real-time observation of DNA crosslinking induced by cisplatin. The experimental data are shown as red circles and the moving average as a blue line. (b) Altered elasticity of a cisplatin-bound DNA and naked DNA (no cisplatin).

In order to find the optimal concentration of cisplatin for single-molecule assay, we conducted an in vitro bulk assay of cisplatin-mediated crosslinking. Aqueous solutions of cisplatin were prepared with a variety of concentrations (1, 5, 10, 50, 100, and 500 mg/ml), and negatively supercoiled pPIA2-6 plasmids were incubated with the cisplatin solutions for 10 min. at 37 °C. Then, we ran a gel electrophoresis with the DNA samples for ∼1.5 h. at 50 V with 0.7 % agarose. The gel was then stained with ethidium bromide to visualize the DNA bands under UV illumination. Since in aqueous solution, cisplatin degrades in days, the activity of the cisplatin solution was checked via an in vitro assay before every single-molecule assay. Fig. 3 shows one such in vitro assay. The experimental procedures for single-molecule assays will be described in Results.

## III. RESULTS

As explained in "Experimental Methods," we carried out an in vitro assay to measure the effect of cisplatin on

pPIA2-6 plasmids. As shown in Fig. 3, the mobility of the plasmids incubated with cisplatin decreased as the concentration of cisplatin increased. This indicates that cisplatin modified the structure of the plasmid to one with lower mobility. At 50 $\mu$g/ml of cisplatin, the band corresponding to negatively supercoiled pPIA2-6 shifted towards that of nicked circular pPIA2-6. Interestingly, at even higher concentrations (100 and 500 $\mu$g/ml) of cisplatin, the reaction products appeared to move slightly faster than those for 50 $\mu$g/ml. This indicates that the plasmid turns into a slower species at an intermediate concentration and then into a somewhat faster species at high concentrations.

In order to obtain kinetic information on cisplatin-mediated crosslinking, we measured the cisplatin activity on a single DNA molecule. To avoid accidental formation of crosslinks between distal parts of one DNA duplex during the single-molecule assay, we pulled the DNA tether at a constant force (∼2 pN) as the cisplatin-containing buffer was introduced. By maintaining a constant tension on the DNA, we could avoid having DNA tethers being looped or tangled when they were incubated with cisplatin. Since we observed a dramatic effect due to cisplatin at a concentration of 50 $\mu$g/ml, we chose the same concentration for our single-molecule assay.

As soon as the cisplatin solution was introduced to the chamber, the bead attached to a DNA molecule was monitored, and the change in the length of the tether was recorded. Soon after the DNA tether was exposed to the cisplatin solution, the end-to-end distance of the DNA tether was shortened, as shown in Fig. 4(a), under constant force. The decrease in the length is much more than the fluctuation normally observed with a naked DNA molecule. Since the DNA molecule was under a constant tension (∼0.43 pN), two distal parts of the DNA strand were unlikely to be crosslinked, and the step, if there is any, was too minute to be resolved in our measurement. When the DNA tether was incubated in the chamber for a longer time (∼ an hour or so), the bead and DNA complex was stuck to the substrate so strongly that it was impossible to raise the bead from the surface of the substrate, presumably because multiple crosslinks were formed among distal sites in the strands.

After incubating the DNA molecule with cisplatin briefly, the force to the DNA was tuned between high (∼10 pN) and low (∼tens of fN) values to obtain the force vs. extension curve. As shown in Fig. 4 (b), the elasticity of a cisplatin-bound DNA is dramatically altered compared to that of a naked DNA. At a low force such as 0.1 pN, the cisplatin-bound DNA is almost at its smallest extension while a naked one has nearly half of its full extension [5]. It is evident that there were no crosslinks between distal parts of the strands because at high force, the maximum length of the cisplatin-bound DNA is approximately the same as the full length of a naked DNA although the former appears to be slightly less than the latter.

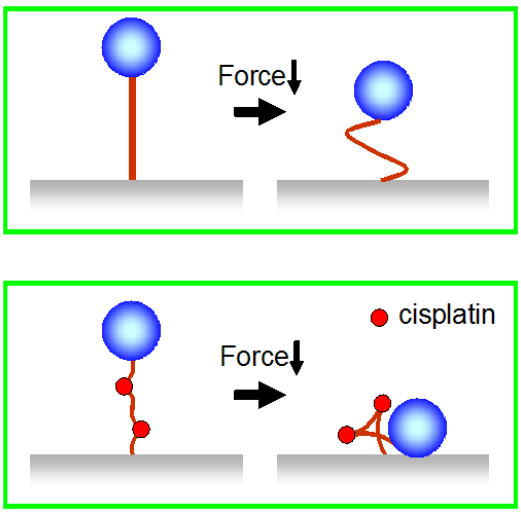


Fig. 5. Proposed mechanism for the altered elasticity and conformation of DNA.

## IV. DISCUSSION AND CONCLUSIONS

From the single-DNA-molecule measurements with a magnetic tweezers apparatus, we showed in real time that cisplatin is able to induce crosslinking of a single DNA molecule and that the cisplatin-bound DNA molecule has a markedly different elastic property revealed by the force vs. extension measurement under a varying external force. It was interesting to see the DNA molecule actively crosslinked by cisplatin in real time although there was no active enzyme able to induce bends in the DNA. Therefore, the DNA conformation suitable for crosslinking, a bend, should be prepared stochastically by thermal fluctuations. Then, due to the thermally induced bend, a cisplatin molecule, which is already bound to a purine, grabs another neighboring purine in the same or opposite strand when the purine approaches the cisplatin-bound purine.

To explain the altered elasticity measured with the single-molecule experiments, we propose the model shown in Fig. 5. When the DNA molecule is exposed to cisplatin, cisplatin molecules bind preferentially to purines. When one cisplatin binds to two neighboring bases, the duplex is bent by 40° at the crosslink site. Such crosslinks will occur in random locations on the strand, and the total number of the crosslinks is determined statistically. Therefore, as the incubation time increases, the degree of crosslinking will increase as well. Since DNA is a rather stiff polymer, it remembers the original orientation over the length scale of its persistence length, which is around 50 nm at an intermediate concentration of salt [13]. The end-to-end distance of a DNA molecule is then significantly reduced under a weak force if local bends of 40° exist. That is why a cisplatin-bound DNA shortens more easily than a naked one. Under high force, however, the force aligns the strand along the di-

rection of force except for the parts near the crosslinking sites; therefore, the total length of the DNA is approximately the same as the full length of the DNA molecule.

Cisplatin is a potent anti-cancer drug. It triggers apoptosis in affected cells. One of the mechanisms is to alter the conformation of DNA so drastically that DNA repair machinery cannot successfully fix the mutations [8]. In our magnetic tweezers study, we obtained kinetic information on cisplatin-induced crosslinking of a DNA molecule at a single-molecule level and investigated the molecular conformation and elasticity of a cisplatin-bound DNA molecule, which is not readily measurable with traditional bulk methods. One can then study the conformational and elastic modification of DNA with other DNA binding chemicals and drugs with single-molecule manipulation methods, especially the magnetic tweezers method.

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