Elasticity of Cisplatin-Bound DNA Reveals the Degree of Cisplatin Binding

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Cisplatin was incidentally discovered to suppress cell division and became one of the most successful antitumor drugs. It is therapeutically active upon binding to DNA and locally kinking it. We demonstrate that after a bimodal modeling, the degree of platination of a single DNA molecule can be consistently and reliably estimated from elasticity measurements performed with magnetic tweezers. We predicted and measured for the first time two separate persistence lengths of kinked DNA at high and low tensions. We also directly observed that the degree of platination of DNA strongly depends on the concentration of sodium chloride as required for cisplatin's intracellular activity. Our study shows that micromanipulation techniques accurately reveal the degree of chemical modification of DNA which can be used for a new type of structure-sensitive biosensors.

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Cisplatin has long been a subject of intensive research in biochemistry and medicine [1] and became one of the most widely used antitumor drugs (under the brand name of *platinol*). Its therapeutic activity has been attributed to the formation of cisplatin-DNA adducts (kinks along the DNA molecule) [2] (Fig. 1). A great deal of effort has been made to collect precise thermodynamic and structural information on platinated DNA and to understand its working mechanism. It is known that in water, cisplatin $Pt(NH_3)_2(Cl)_2$ is hydrolyzed into monovalent or divalent cations by losing chloride ions. Intrastrand binding of a divalent cisplatin cation on two neighboring guanine bases is the dominant binding mode to DNA. Upon binding, the adduct bends a DNA helix by $\theta_k = 2\gamma = 40°$ and unwinds it by 13° [3] [Figs. 1(b) and 1(c)]. Such modification of DNA structure eventually leads to cell death, more seriously for cancer cells. The extensive reviews by Sherman and Lippard [4] and Jung and Lippard [5] summarized current understanding on the mechanism of cisplatin activity. Despite all the progress made in this field, direct biophysical characterization of cisplatin activity at the molecular level is still missing. Gaub's group had first investigated the effect of cisplatin binding on the structural changes such as the B-DNA–S-DNA and the melting transitions. These transitions occur at rather extreme tension, beyond 60 pN [6]. Here we focus on the effect of cisplatin binding on the elasticity of DNA under a physiologically relevant tension (<6 pN). For this purpose, we directly measured the elasticity of cisplatin-bound DNA at such moderate tension, and observed that there are two distinct regions in each of which the force-extension curve of DNA should be fitted with a separate persistence length. With the development of a bimodal model that properly describes the elasticities of DNA in the two regions, we showed how the degree of platination can be extracted consistently from both small and large force parts of the force-extension data (yet <6 pN). We also found that the degree of platination strongly depends on the concentration of sodium chloride and ascertained that our model can explain the elasticity of DNA for a broad range of DNA platination.

As our experiments were performed far from any structural transitions, DNA chains can be considered as otherwise featureless semiflexible chains and described by the wormlike chain (WLC) model. All microscopic details go into a single parameter, the persistence length l_p over which the orientational correlations along the chain decay.

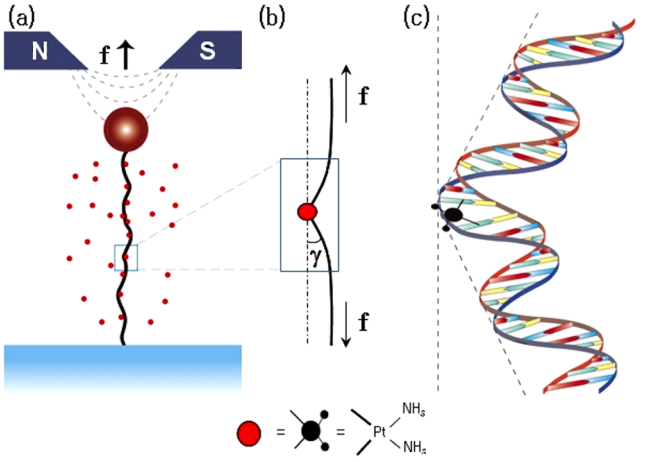

FIG. 1 (color online). (a) Platination of DNA under external force f. (b) The part of kinked DNA with a half-kink angle $\gamma = 20°$. (c) Schematic illustration of the kink caused by a cisplatin binding.

The force-extension curve of a WLC can be described in terms of the applied tension f, the contour length L, and l_p by the formula [7]

$$\frac{f l_p}{k_B T} = \frac{x}{L} + \frac{1}{4(1 - x/L)^2} - \frac{1}{4}, \tag{1}$$

which interpolates over the range between the well-known low-tension linear response regime controlled by the equilibrium fluctuations of the extension x and the high-tension regime characterized by the suppression of thermal fluctuations around the stretched configuration. In the linear regime ($x/L \ll 1$), Eq. (1) reduces to the general form $\langle x \rangle = \frac{\langle x^2 \rangle_0}{k_B T} f$. At large force, the full extension L is asymptotically approached according to $L - \langle x \rangle \propto 1/\sqrt{f}$. In summary,

$$\langle x \rangle = \begin{cases} \frac{\langle x^2 \rangle_0}{k_B T} f \text{ with } \langle x^2 \rangle_0 = \frac{2 L l_p}{3} & (f l_p < k_B T) \\ L - \frac{L}{2}\sqrt{\frac{k_B T}{f l_p}} & (f l_p > k_B T). \end{cases} \tag{2}$$

In order to directly measure the effect of cisplatin binding on DNA elasticity at a physiologically relevant tension, we utilized the micromanipulation technique of single DNA molecules with magnetic tweezers [8]. DNA molecules constructed by ligating a ~14.8 kb DNA insert with short biotinylated and digoxigenin-labeled DNA linkers at each end were fixed in the sample chamber via their specific attachments to a streptavidin-coated magnetic bead [MyOne (diameter 1 μm), Dynal, Invitrogen] and the antidigoxigenin-coated glass surface [see Fig. 1(a)]. The force on the DNA molecule was measured from the equipartition theorem by tracking Brownian fluctuations of the bead, and the extension of the molecule was deduced from the diffraction pattern cast by the bead once the relation between the diffraction pattern and the bead height was calibrated. The details on the sample preparation and experimental procedures can be found elsewhere [9]. For all our experiments, we used sufficiently high salt concentration ([NaCl] > 10 mM) at which the persistence length of DNA was checked to remain at the well-known value for bare DNA, $l_p = 51$ nm unless cisplatin is present [Fig. 2(a)]. After DNA tether molecules were incubated with cisplatin (1000 μg/ml ~ 3.3 mM) for ca. 30 min under ~6 pN tension, cisplatin was washed out thoroughly from the chamber and the elastic response of the DNA was then measured by varying the tension.

As illustrated in Fig. 2(a), cisplatin-bound DNA showed a significantly different force-extension curve from bare DNA. Kinks introduced to the DNA molecule upon cisplatin binding strengthen the DNA chain (that is, make it more resilient against pulling). Interestingly, its force-extension curve cannot be fitted with a single persistence length, i.e., the parts of the curve where the tension is higher or lower than ~0.4 pN should be fitted with separate persistence lengths, which indicates that the simple WLC fails in describing the elasticity of DNA over the entire curve. Furthermore, the effective persistence length under high tension differs from that of bare DNA, which necessitates a new theoretical approach as noted below.

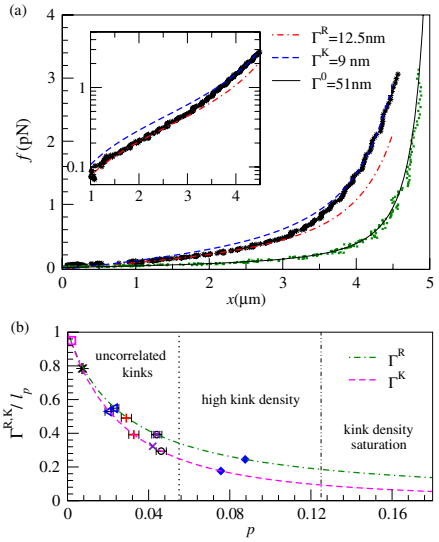

FIG. 2 (color online). Bimodal fit for the force-extension data of platinated DNAs. (a) Symbols indicate the force-extension curve of cisplatin-bound DNA. The molecule was incubated with 3.3 mM cisplatin in [NaCl] = 20 mM. The effective l_p's were deduced from the two regimes: $\Gamma^K = 9$ nm ($p = 0.076$) and $\Gamma^R = 12.5$ nm ($p = 0.087$) for high and low tensions. The inset shows the same data in semilog scale. The force-extension of bare DNA (fitted with $\Gamma^0 = 51$ nm) is also shown for comparison. (b) The effective persistence lengths versus the degree of cisplatin binding. The upper (dot-dashed) line is from the random flight model suitable for the low-tension regime and the lower (dashed) line from the aligned kink model for the high-tension regime. Symbols represent the experimental data under various conditions (batch I: ◇, ×, +, *, □ for [NaCl] = 20, 40, 60, 90, and 180 mM, respectively, and the concentration of cisplatin is 3.3 mM for all the data; batch II: ○, △ for [NaCl] = 10 and 30 mM, respectively [16]). The p values for each experimental data set were obtained by reading them from the theoretical curves relating p to $\Gamma^{R,K}$. Ideally, identical symbols should be positioned vertically as p should be the same for both. The discrepancy indicates the precision of determining cisplatin occupancy with this method. The theoretical maximum of p (= 1/8) is marked as a double dotted line. At the high kink density, kinks remain correlated even at 5 pN and, consequently, the accuracy of Γ_K is compromised there.

Although theories for DNA chains kinked by bound chemicals or proteins have been provided by several authors in recent years, none of them is suitable for our experimental situation. Besides numerical studies on a permanent kink by Li *et al.* [10], annealed kinks with variable kink angles were considered by Wiggins *et al.* [11], chemicals inducing kinks with a fixed angle in full equilibrium with the embedding solution by Popov and Tkachenko [12], and kinks produced by sliding loops by

Kulić *et al.* [13]. In these cases [11–13] the behavior of bare DNA is recovered under high tension. There is no evidence for unbinding of cisplatin (or kink angle flattening) in our experiments. As also mentioned in Ref. [5], double-bound cisplatin is unable to desorb or to efficiently move along DNA during the measurement.

Platination strengthens DNA and affects the force law shown in Eq. (1). As seen shortly, the strengthening induced by cisplatin binding behaves differently for different levels of tension and the force law with a single persistence length cannot provide a satisfying description for the entire curve. Here we distinguish two regimes based on whether neighboring kinks are correlated or not, which is determined by kink spacing and applied tension.

In order to handle the regime of uncorrelated kinks, let us begin with a single kink which imposes a local angle γ with respect to the principal direction of the DNA chain [Fig. 1(b)]. The chain orientation will approach the principal direction over a length Λ set by equating the bending energy E_b around a kink (aiming at a smooth profile and hence favoring large Λ) to the work E_f against the tension (minimizing nonstraight regions around the kink and hence favoring small Λ). Estimating the curvature and the chain shrinkage upon kinking to be γ/Λ and $\Lambda\gamma^2$, respectively, we get $E_b \sim k_B T l_p(\gamma/\Lambda)^2\Lambda$ and $E_f \sim \Lambda\gamma^2 f$, which leads to $\Lambda = \sqrt{k_B T l_p/f}$ and to the effective shrinkage per kink $\sim\gamma^2\sqrt{k_B T l_p/f}$. Kinks separated by a spacing S do correlate if they are closer than 2Λ, otherwise they are independent. The correlation length Λ makes sense only if it is shorter than $l_p/2$, i.e., the tension is large enough to align the chain [$f l_p > 4(l_p/S)^2 k_B T$]. Otherwise the correlation length is just $\sim l_p$. There always exists a regime of high tension where the kinks are uncorrelated. The criterion for the kink correlation at maximum tension ($\sim$5 pN) is indicated in Fig. 2(b) as a dotted line. These qualitative arguments on high-tension regime can be substantiated by solving the corresponding Euler equation [14]. This leads to the relaxation profile of the kink angle, $\tan[\theta(s)/4] = \tan(\gamma/4)\exp(-s/\Lambda)$, where s is the curvilinear coordinate along the chain and $\Lambda = \sqrt{k_B T l_p/f}$ as expected. For practical purposes, the tangent appearing in this formula is not essential, and a purely exponential relaxation of the kink angle is sufficiently accurate. It is then straightforward to calculate the chain shrinkage per kink as $8\Lambda\sin^2(\gamma/4)$ (or $\Lambda\gamma^2/2$ after expansion).

In the high-tension regime of uncorrelated kinks, kinks can be treated as localized noninteracting defects separated by stretched segments. Similarly to bare DNA, the configurations are subject to thermal fluctuations, which are only weakly affected by well separated kinks. In a linear approximation, the length stored in fluctuations under a given tension in kinked DNA is the same as for a bare DNA without kinks [see Eq. (2)]. The lengths pulled out of both thermal fluctuations and the frozen-in kinks increase with tension as $-1/\sqrt{f}$ and they add up to $\langle x\rangle = L - \frac{L\Lambda}{2l_p} - \frac{L}{S}\times\frac{\gamma^2\Lambda}{2}$. The force law for platinated DNA in the high-tension regime then corresponds to that of a WLC with a reduced persistence length Γ^K given by

$$\frac{1}{\sqrt{\Gamma^K}} = \frac{1}{\sqrt{l_p}}\left(1 + \frac{\gamma^2 l_p}{S}\right) \quad \text{(high tension)}. \tag{3}$$

We call the model valid for high tension the ''aligned kink model.''

In the absence of external force, however, kinks due to cisplatin adducts deflect the chain configuration and effectively reduce the average coil size. According to the linear response theory, the reduction of the end-to-end fluctuation strengthens the chain in the low-tension regime as observed in Fig. 2(a). The corresponding physics is captured by the ''random flight model'' detailed below [15]. Around a single kink the tangent angle changes by $\theta_k = 2\gamma$ within a base-pair distance $a = 3.4$ Å along the strand. We define s_k as the effective curvilinear length associated with a bending by θ_k, $\cos\theta_k = e^{-s_k/l_p}$. The kink angle $\theta_k = 40°$ corresponds to $s_k \approx 0.27 l_p \approx 13.7$ nm. If the probability of platination per base is p (we also call it cisplatin occupancy), the tangent-vector correlation over the base-pair length a is given by $K \equiv \langle\cos\theta\rangle = (1-p)e^{-a/l_p} + pe^{-a/l_p - s_k/l_p}$. Two bond vectors b_n and b_{n+1}, expressed in the spherical coordinates of a unit sphere (θ_n, ϕ_n) and $(\theta_{n+1}, \phi_{n+1})$, respectively, have an angle β between them: $\cos\beta = \cos\theta_n\cos\theta_{n+1} + \sin\theta_n\sin\theta_{n+1}\cos(\phi_n - \phi_{n+1})$. Assuming that azimuthal angles are not correlated, the angular deviation between the bond vectors m base pairs apart is $\langle\cos\theta_m\rangle = K^m$. For small $p \ll 1$, we can define the effective persistence length Γ^R by $\Gamma^R = -a/\ln(K)$. From the definition of s_k, we find the effective persistence length Γ^R for cisplatin-bound DNA:

$$\frac{1}{\Gamma^R} = \frac{1}{l_p} + \frac{p}{a}(1 - e^{-s_k/l_p}) \quad \text{(low tension)}. \tag{4}$$

We should note that $p = 1/8$ is the saturation limit of cisplatin binding. (The probability of having two neighboring guanines or cytosines on one strand is $1/16$ each.) At the maximum cisplatin occupancy, we then obtain $\Gamma^R \approx 27.8a \approx 9.5$ nm, which is markedly larger than the value predicted by Eq. (3) for the strong stretching limit, $\Gamma^K \approx 4.7$ nm. This supports our idea of two separate persistence lengths in the high- and low-tension regimes. For a semiflexible chain carrying $n = p(L/a)$ kinks, the force-extension relation in the small (large) force regime can still be described by Eq. (1), where the persistence length l_p is now replaced with Γ^R (Γ^K). The random flight model is valid when the tangent angles at kinks are not correlated with the direction of the external force. The elastic response in this regime is entropic and we fit the data with Eqs. (1) and (4). In the high-tension regime, the stored

length in the kinks is pulled out and thermal fluctuations are suppressed as described by Eqs. (1) and (3).

As shown in Fig. 2(a), the effective persistence lengths of DNA incubated with cisplatin in 20 mM NaCl are $\Gamma^K = 9$ nm in the high-tension regime and $\Gamma^R = 12.5$ nm in the low-tension regime. The crossover from the low- to high-tension regime occurs around $f \approx 0.4$ pN, which is consistent with the condition $2\Lambda \approx l_p$. The corresponding cisplatin occupancy is $p^K (= a/S) \approx 0.076$ [inferred from Eq. (3)] and $p^R \approx 0.087$ [inferred from Eq. (4)]. This measurement was performed at the cisplatin occupancy which is about $2/3$ of the theoretical saturation value where our previous assumption of noninteracting kinks is unlikely to be accurate. At [NaCl] = 60 mM [+ in Fig. 2(b)], we obtain $\Gamma^K = 20$ nm and $\Gamma^R = 25$ nm, which yield $p^K = 0.032$ and $p^R = 0.029$. These values are in good agreement and the estimate of true cisplatin occupancy would be $p = 0.031 \pm 0.002$. At [NaCl] = 90 mM [* in Fig. 2(b)], the effective persistence lengths in the two different regimes (Γ^R and Γ^K) converge to $\Gamma^K \simeq \Gamma^R = 40$ nm and cisplatin occupancy is reduced ($p = 0.0073$). At even lower platination, as is the case for [NaCl] = 180 mM [□ in Fig. 2(b)], both persistence lengths ($\approx$50 nm) are basically the same as the persistence length of bare DNA. As seen here, the concentration of NaCl in the reaction buffer influences the cisplatin binding dramatically. The platination of DNA is far less efficient at high salt concentration ($>$90 mM), while it is nearly saturated at low salt concentration ($<$10–20 mM).

Our observation supports the following physiological requirements. The majority form of the *intercellular* cisplatin complex should be neutral to facilitate passive transport through the cell membrane. The neutral form is, however, unable to bind to DNA. Inside a cell, the cationic form should prevail as the concentration of chloride ion is low (4–10 mM). This form is able to bind to DNA and unable to passively diffuse back across the membrane out of the cell. From the viewpoint of chemical equilibrium, the active cationic form of cisplatin is favored under the low chloride concentration inside a cell. From the biological point of view, however, the passive transport scenario is not complete as active transport through the membrane is possible and other anions (e.g., phosphates) present in the cell could also impede activation of cisplatin [5]. To gain insight on how cisplatin interacts with DNA *in vivo*, we have extended our study as follows. To mimic the physiological conditions inside a cell, we have designed and tested a buffer the composition of which is consistent with the concentrations of various ions inside the cell and investigated the effect of histones on cisplatin binding to DNA.

We showed that the elasticity of DNA measured with magnetic tweezers reveals the degree of DNA platination with the help of the bimodal modeling. The cisplatin-bound DNA cannot be described as a WLC with a single persistence length but with two separate persistence lengths provided by the random flight model and the aligned kink model for low- and high-tension regimes, respectively. The independently measured persistence lengths should indicate the same value for cisplatin occupancy. This consistency check gave us an assurance about the results. Our study demonstrated that the micromanipulation technique not only probes the elasticity of platinated DNA but also, together with a proper modeling, characterizes the extent of DNA modification by drugs, which could become a basis for a new type of structure-sensitive biosensors.

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