Force spectroscopy unravels the role of ionic strength on DNA-cisplatin interaction: Modulating the binding parameters

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In the present work we have gone a step forward in the understanding of the DNA-cisplatin interaction, investigating the role of the ionic strength on the complexes formation. To achieve this task, we use optical tweezers to perform force spectroscopy on the DNA-cisplatin complexes, determining their mechanical parameters as a function of the drug concentration in the sample for three different buffers. From such measurements, we determine the binding parameters and study their behavior as a function of the ionic strength. The equilibrium binding constant decreases with the counterion concentration ([Na]) and can be used to estimate the effective net charge of cisplatin in solution. The cooperativity degree of the binding reaction, on the other hand, increases with the ionic strength, as a result of the different conformational changes induced by the drug on the double-helix when binding under different buffer conditions. Such results can be used to modulate the drug binding to DNA, by appropriately setting the ionic strength of the surrounding buffer. The conclusions drawn provide significant new insights on the complex cooperative interactions between the DNA molecule and the class of platinum-based compounds, much used in chemotherapies.

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I. INTRODUCTION

Cisplatin [*cis-diamminedichloridoplatinum(II)*] is one of the most important chemotherapeutic drugs, being used in the treatment of testicular, ovarian, breast, bladder, cervical, lung, head, and neck cancers, as well as in neuroblastomas and in some brain tumors [1]. Along the past years, researchers have investigated the molecular mechanism of action of cisplatin, and in particular its interaction with the DNA molecule, which is the main drug target inside cells [2–14]. Figure 1 shows the chemical structure of the cisplatin molecule. In aqueous solutions, the two chloride ions dissociate and the compound incorporates two water molecules, reaching its bivalent (2+) active state, which binds to DNA [3].

The active state of cisplatin binds covalently to the DNA double-helix forming different types of adducts, which include intrastrand and interstrand crosslinks, functional monoadducts, and DNA-protein crosslinks [3]. Such adducts induce strong structural perturbations on the conformation of the double-helix, especially sharp bends and unwinding [4,15], and sometimes local strand breaks [16,17]. These structural perturbations affect significantly the mechanical properties of the DNA molecule, such that the DNA-cisplatin interaction can be monitored by measuring the changes of such properties as the drug binds to the double-helix [18].

Despite the number of studies concerning the DNA-cisplatin complexes, none has focused on the effects of the ionic strength on the binding parameters. We will show here that such aspect is fundamental for a complete and deeper understanding of the present interaction, studying the DNA-cisplatin binding at single molecule level for different ionic strengths. First, we measure the mechanical properties of the DNA-cisplatin complexes as a function of the ionic strength of the surrounding buffer, using optical tweezers to perform single molecule force spectroscopy. Then, the physical chemistry of the DNA-cisplatin interaction was determined [18] at each buffer condition from such measurements. Finally, the behavior of the physicochemical (binding) parameters as a function of the buffer ionic strength was analyzed and discussed.

II. MATERIALS AND METHODS

A. Sample preparation

The samples here consist of λ-DNA molecules (New England Biolabs) end-labeled with biotin in a phosphate-buffered saline (PBS) solution. One end of the DNA molecules is attached to a microscope coverslip surface, which is coated with streptavidin, while the other end is attached to a streptavidin-coated polystyrene bead with a diameter of 3 μm (Bangs Labs). The measurements were performed under three different ionic strengths, using PBS solutions with different compositions that are detailed in Table I.

B. Experimental procedure

The optical tweezers setup used in this work consists of a 1064-nm solid-state laser (Altechna) operating in the TEM_{00} mode, mounted on a Nikon Ti-U inverted microscope with a 100× NA 1.4 objective. The tweezers is previously calibrated before the experiments, by using the Stokes force calibration. The apparatus is then used to trap the polystyrene bead attached to a DNA molecule. By moving the microscope stage using a piezoelectric actuator (Newport Corp.), we stretch the DNA while monitoring the changes of the bead position in the tweezers' potential well, using videomicroscopy. To guarantee that the chemical equilibrium of the DNA-cisplatin complexes formed will not be much disturbed by the mechanical manipulation imposed, we limit the maximum stretching forces to ∼2 pN, working only within the entropic regime. The Marko-Siggia wormlike chain (WLC) [19] formula for the entropic force was used to fit the experimental data and to extract the two basic mechanical parameters of the

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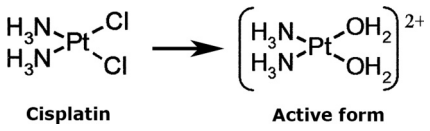

FIG. 1. Chemical structure of the cisplatin molecule. In aqueous solutions, the two chloride ions dissociate and the compound incorporates two water molecules, reaching its bivalent (2+) active state, which binds to DNA.

DNA-drug complexes: the persistence length A and the contour length L. Some exemplifying force-extension curves of the DNA-cisplatin complexes, along with the WLC fittings, are presented in the Appendix.

Before adding cisplatin in the sample chamber, the particular bare DNA molecule chosen to perform the experiments is carefully tested by performing a series of repeated stretching measurements to obtain the average values and the error bars (standard error of the mean) of the mechanical properties. The results obtained were the expected ones for the λ-DNA. For the two highest ionic strengths used (154 and 14 mM), we find $A_0 \sim 45$ nm and $L_0 \sim 16.5$ μm. For the lowest ionic strength used (1.4 mM), we find $A_0 \sim 61.5$ nm and $L_0 \sim 16.5$ μm. Such results are in excellent agreement with previous reports [20]. Cisplatin is then introduced in the sample chamber by using micropipettes, maintaining the same DNA molecule tethered by the tweezers. We wait ∼30 min before performing the subsequent measurements, which is the time interval required for the DNA-cisplatin complexes to achieve the chemical equilibrium [8,21]. Then, we determine the average mechanical properties and the error bars of this particular λ-DNA-cisplatin complex at the chosen drug concentration, by performing a series of repeated stretching experiments. Next, the series of stretching experiments were repeated scanning various cisplatin concentrations. Finally, to evaluate the variability of the results over different DNA molecules, we use different samples to repeat the entire experiment (scanning all the drug concentrations) for various different DNA molecules. The results and error bars reported here for the mechanical properties are averages over some different DNA molecules. All experiments were carried out at room temperature (25 °C). More details about the experimental methods and procedures can be found in Ref. [22].

C. Theoretical model

Recently, we have developed a quenched-disorder statistical model that allows one to determine the physicochemical (binding) parameters of a DNA-ligand interaction from the data of the persistence length as a function of the ligand concentration in the sample [23]. Here we will expose briefly this approach. The complete details can be found in Ref. [18].

For ligands that induce a monotonic decay on the persistence length, which is the case of cisplatin (see the results in the next section), the effective persistence length A_E of the DNA-ligand complexes can be written as [18]

$$\frac{1}{A_E} = \frac{1 - r/r_{\max}}{A_0} + \frac{r/r_{\max}}{A_1}, \tag{1}$$

where A_0 is the persistence length of the bare DNA molecule, A_1 is the local persistence length induced by the ligand upon binding on a site (or equivalently, the persistence length at bound ligand saturation), r is the bound site fraction (fraction of DNA base-pairs occupied by the bound ligands), and $r_{\max}$ is the saturation value of r [18].

The effective persistence length A_E can be connected to the binding parameters of the interaction by choosing an appropriate binding isotherm that captures the physical chemistry of the system. In the case of the DNA-cisplatin interaction, an appropriate binding isotherm is the Hill model [18,24], since this drug in general exhibits cooperativity upon binding to the double-helix [8,21]. The Hill model is the simplest binding isotherm capable to give an estimation of the cooperativity degree of a binding reaction. It reads

$$\frac{r}{r_{\max}} = \frac{(KC_f)^n}{1 + (KC_f)^n}, \tag{2}$$

where C_f is the free (not bound to DNA) ligand concentration, K is the equilibrium association binding constant, and n is the Hill exponent, a parameter usually used to measure the degree of cooperativity of binding reactions. If $n > 1$, the interaction is positively cooperative, i.e., a bound ligand molecule increases the effective affinity of DNA for subsequent ligand binding. If $n < 1$, otherwise, the interaction is negatively cooperative and a bound ligand molecule decreases the effective affinity of DNA for subsequent ligand binding. If $n = 1$, the interaction is noncooperative and the effective affinity is independent on the number of bound ligand molecules.

Equation (2) can be plugged into Eq. (1) to fit the experimental data of the persistence length. The binding parameters are left as adjustable parameters and can be determined from the fitting process. The complete details about this fitting procedure can be found in Ref. [18].

TABLE I. Composition of the different PBS buffers used

[Na] / Ionic strength	NaCl	Na_2HPO_4	NaH_2PO_4
150 mM/154 mM	140 mM	4.375 mM	1.25 mM
10 mM/14 mM	0	4.375 mM	1.25 mM
1 mM/1.4 mM	0	0.4375 mM	0.125 mM

III. RESULTS AND DISCUSSION

A. Persistence length data and model fitting

In Figs. 2–4 we show, respectively, the persistence length A of the DNA-cisplatin complexes as a function of the drug concentration in the sample C_T, for the three ionic strengths used, $I_1 = 154$ mM ([Na] = 150 mM), $I_2 = 14$ mM ([Na] = 10 mM), and $I_3 = 1.4$ mM ([Na] = 1 mM). The fittings of the persistence length data to our theoretical model are also shown as *solid lines*.

The results shown in Fig. 2 for [Na] = 150 mM reproduces very well previous experiments from our group, which were performed using a different sample preparation and a different experimental procedure [8,21]. These measurements were repeated in the present work to guarantee that the three sets of experiments performed at the three different ionic strengths

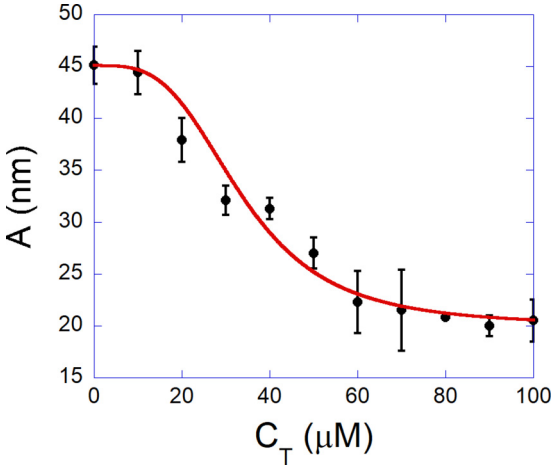

FIG. 2. Persistence length A of the DNA-cisplatin complexes as a function of the drug concentration in the sample C_T for [Na] = 150 mM. Observe that for such ionic strength the persistence length decreases as a function of the drug concentration, and the data present a sigmoidal shape, indicating that significant cooperativity is present in the interaction at such situation. In addition, the relevant concentration range in which the persistence length changes is $0 < C_T < 100$ μM.

will be compared under exactly the same experimental conditions. Observe that for such ionic strength the persistence length decreases as a function of the drug concentration, and the data present a sigmoidal shape, indicating that significant cooperativity is present in the interaction at this ionic strength [18,24]. Observe also that, in the present case, the relevant concentration range in which the persistence length changes is $0 < C_T < 100$ μM.

To advance further on the analysis, we have fitted the experimental data to our quenched disorder statistical model,

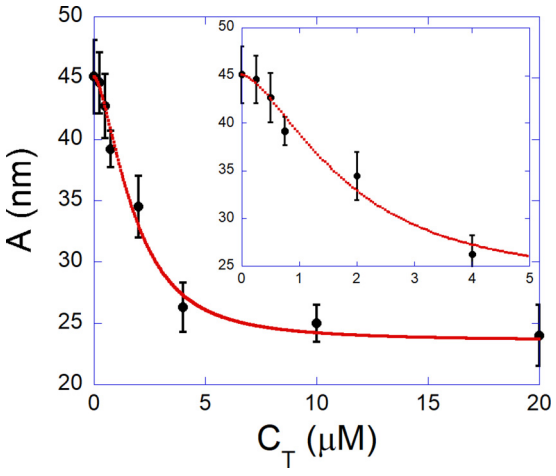

FIG. 3. Persistence length A of the DNA-cisplatin complexes as a function of the drug concentration in the sample C_T for [Na] = 10 mM. The sigmoidal shape of the persistence length curve is much less evident, indicating that a considerable decrease in the cooperativity degree of the binding reaction has occurred. The *inset* evidences the low concentration range. The relevant concentration range in which the persistence length changes is $0 < C_T < 20$ μM.

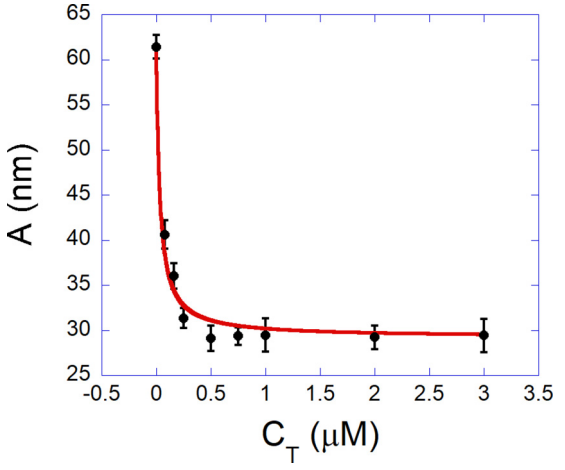

FIG. 4. Persistence length A of the DNA-cisplatin complexes as a function of the drug concentration in the sample C_T for [Na] = 1 mM. In this case, the persistence length strongly decreases even for very small drug concentrations, and there is no evidence of sigmoidal shape on the decay curve. The relevant concentration range in which the persistence length changes is $0 < C_T < 3$ μM.

above described. Such fitting returns the following binding parameters for the DNA-cisplatin interaction in the present case: $K = (2.4 \pm 0.4)\times 10^4$ M^{-1} and $n = 3.4 \pm 0.4$. In addition, the saturation persistence length returned by the fitting was $A_1 = (20 \pm 1.5)$ nm. All these results are in good agreement with the previous work mentioned [8,21], despite the differences in the sample preparation and in the experimental procedure. The relatively small value found for the equilibrium binding constant reflects the fact that a large amount of cisplatin ($\sim$100 μM) is needed to saturate the DNA molecule. On the other hand, the relatively high value found for the Hill exponent ($\sim$3.4) confirms that the binding reaction is positively cooperative, as mentioned before. In Refs. [8,21] we have interpreted this positive cooperativity as related to the structural changes induced on the DNA double-helix upon drug binding, which includes the formation of crosslinks and loops [4–6]. Such structures approximate different strand segments as more cisplatin binds to the double-helix, increasing the probability of forming even more crosslinks and loops [8].

The results shown in Fig. 3 for [Na] = 10 mM show that the sigmoidal shape of the persistence length curve is much less evident, indicating that a considerable decrease in the cooperativity degree of the binding reaction has occurred at this ionic strength. An *inset* showing the low concentration range is presented in the figure as evidence that the sigmoidal shape of the curve is still present here. Observe in addition that the relevant concentration range in which the persistence length changes ($0 < C_T < 20$ μM) has considerably decreased here, indicating an increase on the equilibrium binding constant. In fact, the model fitting now returns the parameters $K = (5.3 \pm 0.8)\times 10^5$ M^{-1}, $n = 1.8 \pm 0.4$ and $A_1 = (23.5 \pm 1)$ nm, which confirms the previous interpretations that the cooperativity had decreased and that the binding affinity had increased for this lower ionic strength.

Finally, in Fig. 4 we show the results obtained for [Na] = 1 mM. In this case, the persistence length strongly decreases

TABLE II. Physicochemical parameters obtained from model fitting for each ionic strength

[Na] (mM)	K (M^{-1})	n	A_1 (nm)
150	$(2.4 \pm 0.4)\times 10^4$	3.4 ± 0.4	20 ± 1.5
10	$(5.3 \pm 0.8)\times 10^5$	1.8 ± 0.4	23.5 ± 1
1	$(1.5 \pm 0.4)\times 10^7$	1.0 ± 0.2	29.2 ± 2

even for very small drug concentrations, and there is no evidence of sigmoidal shape on the decay curve. The relevant concentration range in which the mechanical parameter varies ($0 < C_T < 3\ \mu$M) has again decreased here. The model fitting returns the parameters $K = (1.9 \pm 0.5)\times 10^7$ M^{-1}, $n = 1.0 \pm 0.2$, and $A_1 = (29.2 \pm 2)$.

In Table II we summarize the results obtained from the model fitting, for the three ionic strengths used. The error bars reported for the binding parameters are given by the least-squares fittings. It is evident that the equilibrium binding constant K increases strongly as the ionic strength is lowered, while the cooperativity degree of the binding reaction, measured here as a Hill exponent (n), decreases accordingly. The saturation persistence length A_1, on the other hand, increases as the ionic strength is lowered.

B. Dependence of the binding parameters on the ionic strength

The active form of cisplatin, which binds to DNA, is positively charged (+2). Thus, it really is expected that the equilibrium binding constant depends strongly on the ionic strength, because the strong electrostatic interaction between the positively charged ligands and the negative phosphate backbone of the DNA double-helix is more screened as more counterions are present in solution. Although such effect is well studied for many DNA binding proteins, which in general have a high positive charge [25], there are few works that have addressed quantitatively such effect for small DNA binding drugs [22,26–29]. In the case of cisplatin, there is also another important effect here: the lower the concentration of chloride ions [Cl^-] in the buffer, the higher the dissociation desired for cisplatin to reach its active state (see Fig. 1) [15]. In other words, the concentration of cisplatin in the active state is higher in buffers with small concentrations of chloride ions, which results in an effective increase on the drug binding to DNA.

The quantitative behavior of the equilibrium binding constant as a function of the counterion concentration ([Na]) can be understood on the basis of the Record-Lohman model, which states [30]

$$\frac{\partial(\log K)}{\partial(\log[Na])} = -z\psi, \tag{3}$$

where z is the charge of the ligand and ψ is the fraction of counterion thermodynamically associated per phosphate in the absence of the ligand [25,30]. For double-stranded B-DNA in buffer solutions containing only monovalent counterions, we have $\psi = 0.88$ [25,30].

In Fig. 5 we plot the data of Table II for the equilibrium binding constant as a function of the counterion concentration. A fitting to Eq. (3) (*dashed line*) is also shown. Observe that the Record-Lohman model explains well our experimental data,

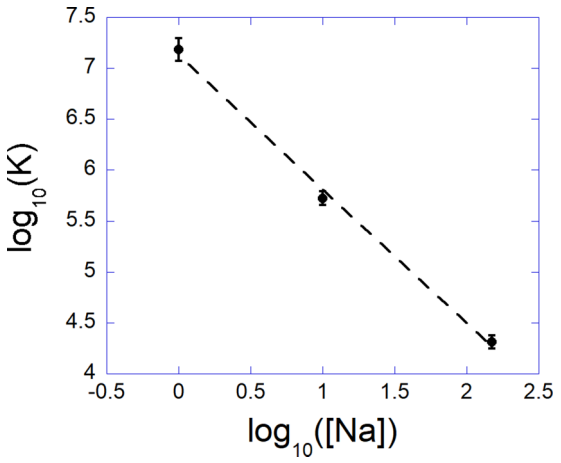

FIG. 5. Equilibrium binding constant K as a function of the counterion concentration. A fitting to Eq. (3) (*dashed line*) is also shown. Observe that the Record-Lohman model explains well our experimental data, and from the fitting we obtain $z = (1.5 \pm 0.1)$, which is relatively close to the expected value of the cisplatin charge (+2).

and from the fitting we obtain $z = (1.5 \pm 0.1)$, which is relatively close to the expected value of the cisplatin charge (+2). This result shows that Eq. (3) works well in the present case, and that our quenched-disorder statistical model used to extract the physical chemistry of the DNA-cisplatin interaction from the mechanical (persistence length) data returns consistent results. In the literature, most of the experimental studies performed with charged ligands and nucleic acids have in fact found that $\partial(\log K)/\partial(\log[Na]) < -z\psi$ [25], in agreement with the present work. In our opinion, this fact is due to: (a) an intrinsic limitation of the model (which loses accuracy for high ionic strengths), and (b) in practice, in solution one never gets 100% of the binding ligand molecules fully ionized, which reduces the net effective charge obtained from experimental data.

In Fig. 6 we plot the data of Table II for the persistence length at saturation A_1 (*blue squares*) and the Hill exponent n (*black circles*) as a function of the counterion concentration. The dashed lines presented in the figure are only guides to the eyes.

The fact that A_1 decreases with the increase of the ionic strength indicate that cisplatin is more efficient in reducing the DNA bending flexibility at higher ionic strengths, a result correlated to the DNA compaction promoted at such situation that will be discussed below.

The Hill exponent reflects the cooperativity degree of the binding reaction. Figure 6 shows that this parameter increases significantly as a function of the ionic strength. Such behavior can also be understood on the basis of the screening of the relevant electrostatic interactions. In fact, cooperativity is directly associated to ligand-ligand correlated interactions [31]. Since cisplatin is a bivalent cationic compound, the electrostatic repulsion between the drug molecules will play a significant role at low ionic strengths, thus hindering cooperativity. A nearly similar result was recently verified by us for other cationic drugs that exhibit a cooperative behavior in their interactions with the DNA molecule [22,28].

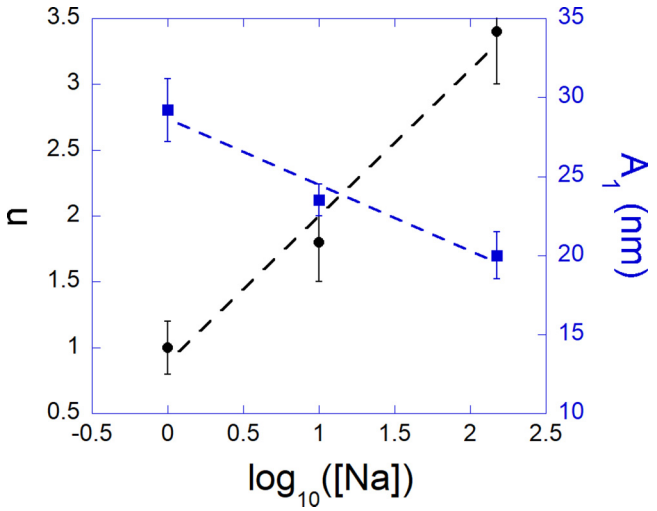

FIG. 6. Persistence length at saturation A_1 (*blue squares*) and Hill exponent n (*black circles*) as a function of the counterion concentration. The dashed lines presented in the figure are only guides to the eyes.

In the case of cisplatin, the cooperativity observed is related to the structural changes induced on the DNA double-helix upon drug binding, as mentioned before. The drug induces crosslinks and loops [4–6] that approximate and link different strand segments, resulting in DNA compaction for very high cisplatin concentrations [5,8]. A positive cooperativity should really be expected in this scenario, because the crosslinks and loops induced on the double-helix approximate different strand segments, therefore increasing the probability of forming even more crosslinks and loops as more cisplatin binds, due to the segment-segment proximity [8,21]. A similar conclusion was recently observed for the H-NS binding protein by Dame *et al.*, which have shown that a cooperative behavior in this case arises as an intrinsic property of DNA bridging due to duplex proximity [32]. The contour length data extracted from the WLC fittings confirm such interpretation, as we show in the next section.

C. Contour length data

In Figs. 7–9 we show the corresponding contour lengths L (normalized by the bare DNA value $L_0 \sim 16.5$ μm) of the same DNA-cisplatin complexes shown in Figs. 2–4, respectively. Observe that, for the highest ionic strength used (Fig. 7), the contour length decreases as a function of the drug concentration, a result obtained previously in Ref. [8]. As discussed in detail by Hou *et al.*, such decrease is related to the DNA compaction process induced by the formation of crosslinks and loops on the double-helix upon drug binding [5], which decreases the apparent contour length measured at the low force entropic regime [8,18]. For the lower ionic strengths used here, however, such compaction was not verified. On the contrary, we have measured an effective increase on the DNA apparent contour length as the drug concentration increases (see Figs. 8 and 9). Such increase is probably related to DNA unwinding [4,15] and/or local strand breaks [16,17] induced by cisplatin binding, which will certainly become evident if the drug does not compact the DNA.

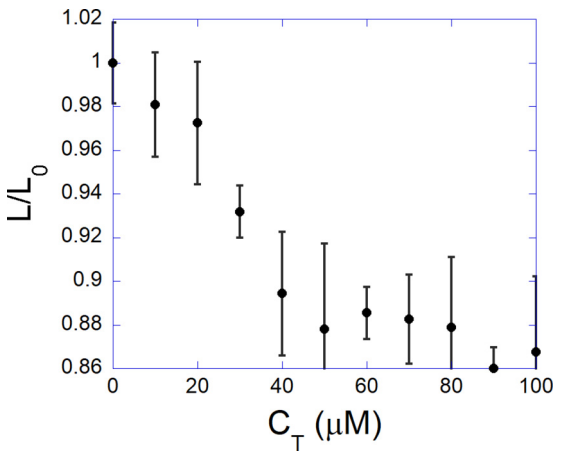

FIG. 7. Normalized contour length L/L_0 of the DNA-cisplatin complexes as a function of the drug concentration in the sample C_T for [Na] = 150 mM. Observe that this mechanical property here decreases as a function of the drug concentration. Such behavior is related to the DNA compaction process induced by the formation of crosslinks and loops on the double-helix upon drug binding, which decreases the apparent contour length measured at the low force entropic regime.

These results explicitly show that DNA compaction by the action of cisplatin does not occur at low ionic strengths, as a consequence of the small cooperativity present in the DNA-cisplatin system under these conditions. In other words, a high cooperativity is needed in order for DNA compaction to take place, which occurs only at relatively high ionic strengths, at least under our experimental conditions. In addition, the fact that the local persistence length A_1 decreases for higher ionic strengths is probably correlated to such compaction, since this process is facilitated for polymers with a lowered bending stiffness.

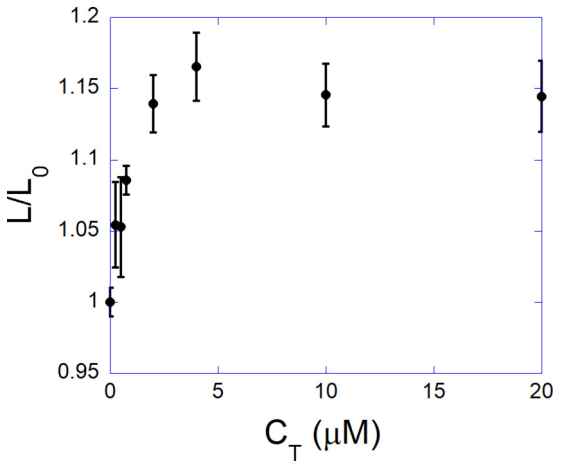

FIG. 8. Normalized contour length L/L_0 of the DNA-cisplatin complexes as a function of the drug concentration in the sample C_T for [Na] = 10 mM. Here an effective increase on the DNA apparent contour length was obtained, which is probably related to DNA unwinding and/or local strand breaks induced by cisplatin binding. Such changes on the double-helix structure become evident when the drug does not compact the DNA.

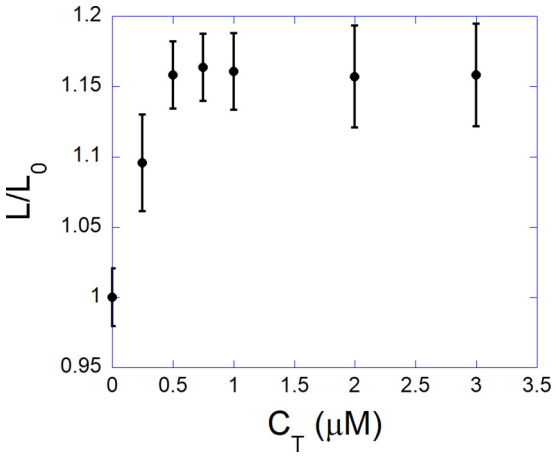

FIG. 9. Normalized contour length L/L_0 of the DNA-cisplatin complexes as a function of the drug concentration in the sample C_T for [Na] = 1 mM. The behavior here is very similar to the one found for [Na] = 10 mM.

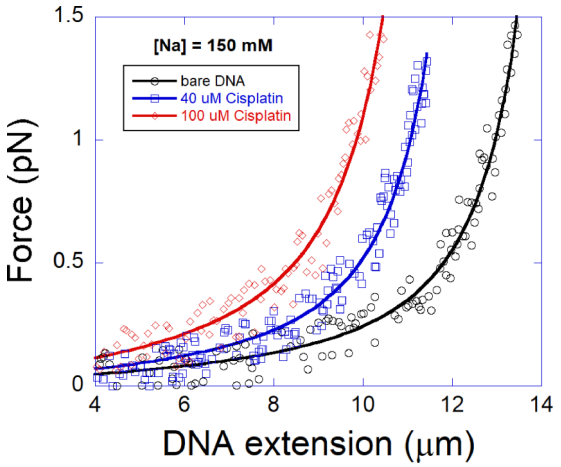

FIG. 10. Exemplifying force-extension curves of some DNA-cisplatin complexes obtained for [Na] = 150 mM. *Black circles*: bare DNA; *Blue squares*: 40 μM of cisplatin, i.e., an intermediate drug concentration; *Red diamonds*: 100 μM of cisplatin, i.e., a drug concentration corresponding to the saturation regime.

Finally, at this point one should ask if it is possible to extract the binding parameters also from the contour length data, to compare with those obtained from the persistence length analysis. To rigorously extract the binding parameters from a mechanical property, one needs a model able to connect such mechanical parameter to a binding isotherm. The model used here to perform such connection for the persistence length is general and valid (in principle) for any type of interaction (intercalation, groove binding, covalent binding, etc) [18,23]. However, for the contour length, the unique model present in the literature that can be used to perform such type of connection with a binding isotherm is valid only for intercalators [18]. There is not a model for the contour length which can be used to extract the binding parameters in the present case. The formulation of such a model is far from trivial, since cisplatin can induce a lot of different effects on the double-helix structure (crosslinks, loops, local strand break, unwinding, etc.), which affect the contour length by different ways. Surely, more experimental and theoretical research is needed to clarify this intricate behavior of the contour length of the DNA-cisplatin complexes.

IV. CONCLUSION

Here we have shown explicitly that the formation of DNA-cisplatin complexes is strongly dependent on the ionic strength, determining the binding parameters that characterize the interaction for three different buffers. It was found that the equilibrium binding constant decreases with the counterion concentration ([Na]), and such decrease can be used to estimate the effective net charge of cisplatin in solution. The cooperativity degree of the binding reaction, on the other hand, increases with the ionic strength, as a result of the different conformational changes induced by the drug on the double-helix when binding under different buffer conditions. These results provide significant new insights on the complex cooperative interactions between the DNA molecule and the class of platinum-based compounds. Such class of compounds is one of the most used in chemotherapies, such that the new findings reported here provide clues to improve the efficiency of such treatments and/or to help development of more efficient compounds.

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APPENDIX

Here we present some exemplifying force-extension curves of the DNA-cisplatin complexes at the three ionic strengths used: Fig. 10 for [Na] = 150 mM, Fig. 11 for [Na] = 10 mM, and Fig. 12 for [Na] = 1 mM. *Solid lines* are fittings to the Marko-Siggia wormlike chain (WLC) formula [19]. Observe that the WLC model fits well to our experimental data, allowing the determination of the mechanical parameters (persistence and contour lengths) with accuracy.

In Fig. 10 we show the following cisplatin concentrations along with the WLC fittings performed for these particular curves. *Black circles*: bare DNA (fitting results $A = 43.5$ nm, $L = 16.3$ μm); *Blue squares*: 40 μM of cisplatin, i.e., an intermediate drug concentration (fitting results $A = 36.7$ nm, $L = 14.3$ μm); *Red diamonds*: 100 μM of cisplatin, i.e., a drug concentration corresponding to the saturation regime (fitting results $A = 23.7$ nm, $L = 13.7$ μm). Observe that both the persistence and contour lengths decrease as the drug concentration increases in the sample, a behavior anticipated in Figs. 2 and 7.

In Fig. 11 we show the following cisplatin concentrations along with the WLC fittings performed for these particular curves. *Black circles*: bare DNA (fitting results $A = 55$ nm, $L = 16.4$ μm); *Blue squares*: 4 μM of cisplatin, i.e., an intermediate drug concentration (fitting results $A = 36.6$ nm, $L = 18.4$ μm); *Red diamonds*: 10 μM of cisplatin, i.e., a drug concentration corresponding to the saturation regime (fitting

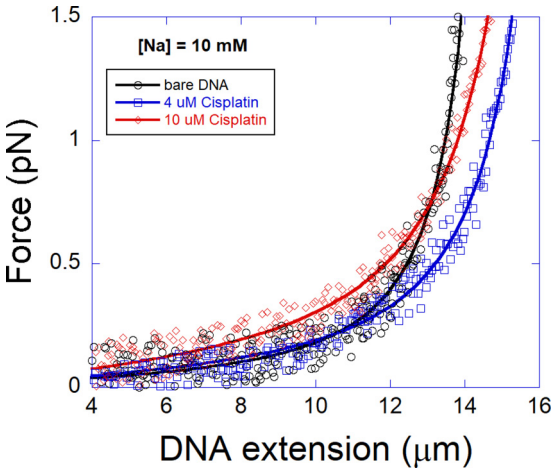

FIG. 11. Exemplifying force-extension curves of some DNA-cisplatin complexes obtained for [Na] = 10 mM. *Black circles*: bare DNA; *Blue squares*: 4 μM of cisplatin, i.e., an intermediate drug concentration; *Red diamonds*: 10 μM of cisplatin, i.e., a drug concentration corresponding to the saturation regime.

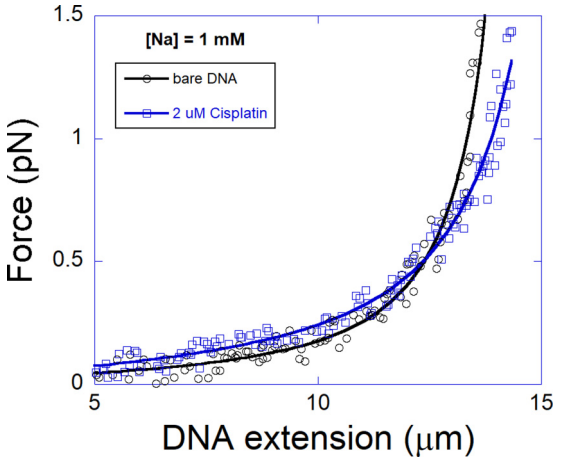

FIG. 12. Exemplifying force-extension curves of some DNA-cisplatin complexes obtained for [Na] = 1 mM. *Black circles*: bare DNA; *Blue squares*: 2 μM of cisplatin, i.e., a drug concentration corresponding to the saturation regime.

results $A = 22.6$ nm, $L = 18.6$ μm). Observe that while the persistence length decreases as the drug concentration increases, the contour length exhibits the opposite behavior. Such behavior was anticipated in Figs. 3 and 8.

Finally, in Fig. 12 we show the following cisplatin concentrations along with the WLC fittings performed for these particular curves. *Black circles*: bare DNA (fitting results $A = 60.7$ nm, $L = 16.1$ μm); *Blue squares*: 2 μM of cisplatin, i.e., a drug concentration corresponding to the saturation regime (fitting results $A = 31.8$ nm, $L = 17.8$ μm). Observe that while the persistence length decreases as the drug concentration increases, the contour length exhibits the opposite behavior. Such behavior was anticipated in Figs. 4 and 9.

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